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Study of the variability, correlation and importance of chemical and nutritional characteristics in cactus pear (*Opuntia* and *Nopalea*)

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Cactus pear is a widely cultivated plant in the Brazilian semiarid region that contributes significantly to the feeding of livestock, especially in times of drought. Because it has a great phenotypic variability among the varieties cultivated in Brazil, there is need to characterize the diversity of chemical and nutritional characteristics. The objectives of this study were to characterize the diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 20 chemical and nutritional characteristics, and to determine the correlation and importance of these characteristics in the variability among genotypes, using multivariate analysis techniques. The study was conducted at the IPA (disambiguation) experimental station in Arcoverde-PE, using randomly designed blocks with three replications. The materials IPA-100003, IPA-200016, IPA-200008, IPA-100004, IPA-200021, IPA-200205 and IPA-200149 were evaluated for 20 quantitative characteristics of the plants. The collected data were analyzed by analysis of variance by F test and means grouped by the Scott-Knott test ($p < 0.05$). The broad-sense heritability and correlation among characteristics were estimated. The diversity was estimated by multivariate methods. Analyses of variance and diversity revealed significant differences among genotypes, with the possible formation of three or four genetically distinct groups. The heritability values ranged from 78.04 to 99.99%. The content of flavonoids and potassium were the characteristics that contributed most to the divergence among the materials. These characteristics are significantly correlated with the nitrogen-free extract and phenolic compounds. The confirmation of variability among the cactus pear varieties studied serves as potential materials in breeding programs. Multivariate analysis techniques are effective in the study of diversity of species of the genera *Opuntia* and *Nopalea*.

Key words: Brazilian semiarid region, characterization of forage, food analysis, genetic distance, grouping, multivariate analysis.

INTRODUCTION

Cactus pear (*Opuntia* spp. and *Nopalea* spp.) is native in tropical and subtropical America, but currently is in a wide range of soil and climatic conditions in all continents (America, Africa, Asia, Europe and Oceania), both in cultivated and wild forms. These plants are used in these countries for various purposes: production of fruits and vegetables for human consumption; fodder for animal feed; soil conservation; biomass for energy (biogas and ethanol); production of cochineal for carmine production; and numerous by-products, such as drinks, vegetarian cheese, medicines and cosmetics (Dubeux-Junior et al., 2013).

In Brazil, there are currently about 600,000 ha cultivated with species *Opuntia ficus indica* (cv. Giant and Round) and *Nopalea cochenillifera* (cv. Small palm). Despite recent government efforts to diversify the use of this culture, most planted areas is still devoted to forage production. For being cactaceous, with numerous anatomical, morphological, physiological and chemical characteristics of adaptation to the ecological conditions of arid and semiarid environments, it has become one of the main forage plants used in ruminant feed, whether goats, sheep and cattle in the Brazilian semiarid. This region is characterized by having a high index of annual evaporation, higher than 2,000 mm, and annual average rainfall of less than 750 mm, concentrated in a single period of 3 to 5 months. In addition, many areas in the region are salinized (> 4, 0 $dS.m^{-1}$) (Araújo et al., 2005; Dubeux-Junior et al., 2013).

Research has revealed that cactus pear has a high content of various chemical compounds considered natural herbal remedies that can add value to their products. The cladodes, fruit, seeds, flowers and roots are used in the treatment of gastritis, fatigue, liver damage, digestion, general detoxifying, hyperglycemia, hyperlipidemia, acidosis, arteriosclerosis, wound healing, gastric ulcer, anti-inflammatory, neuroprotective, antimicrobial, antioxidant, etc. In those parts, hydrocolloid fibers, pigments, minerals, vitamins and bioactive substances are found with nutraceutical action (Nazareno, 2013; El-Mostafa et al., 2014).

By having a wide genetic diversity in genera *Opuntia* and *Nopalea* with about 300 species (Mondragón-Jacobo and Pérez-González, 2001), there is the need to characterize the genetic diversity of the varieties grown in Brazil. In the studies of the genetic diversity of plant species, researchers are interested in grouping similar genotypes, so that the greatest differences occur among the groups formed. In this respect, multivariate techniques, such as discriminant, principal component, coordinate and grouping analyses may be applied. The adoption of one of the techniques mentioned varies according to the desired pattern result, precision, ease of analysis and available information, whether a morphological, physiological, ecological, chemical or molecular characteristic (Ferreira et al., 2003).

The objectives of this study were to characterize the diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 20 chemical and nutritional characteristics, and to determine the correlation and importance of these characteristics in the variability between genotypes, using multivariate analysis techniques.

MATERIAL AND METHODS

Location of the experiment

The study was conducted at the experimental station of Arcoverde, of the Agronomic Institute of Pernambuco (IPA), located in Arcoverde-PE (8°25'S 37°05'W), altitude of 680.70 m, average annual temperature (22.90±1.68°C), average annual relative humidity (69.60±5.30%), average annual wind speed (3.92±0.48 m s^{-1}), average cumulative evaporation (1700.40 mm), average annual cumulative rainfall of 798.1 mm, microregion of sertão of Moxotó (Inmet, 2015).

Plant material and conducting the experiment

The materials used are listed in Table 1. The cladodes of the clones were planted on the 22 and 23 of April 2010, spaced 1.0 x 0.5 m; using one cladode per hole. The experimental design was a randomized block design with three replications. Each block consisted of three rows with eight plants of each variety. The experimental plot was composed by the middle row, with six useful plants, 3.0 m^2 of useful area. The soil was fertilized 30 days after planting, with 20 $t.ha^{-1}$ of manure spread between the lines. Periodically, cultural practices were carried out in the form of weeding with hoe in all the cultivated area. The collection of materials were held at 8:00 a.m. on February 19, 2013 (dry season). After collection, the material was cleaned, cut into small pieces (2 to 3 cm in length), and dried in a forced-air oven at 55°C, where it remained for 72 h until constant weight, in which the air-dried mass was obtained. The dried material was crushed in a Willey® type mill and packed in sealed plastic containers for the chemical and nutritional determinations.

Determination of chemical and nutritional characteristics

These were determined: the contents of total phenolic compounds (PC), total flavonoid (FLAV), total anthocyanins (ANT), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE).

The PC were determined according to the methodology described by Gulcin et al. (2004) using spectrophotometric method, and the results expressed in mg of GAE (gallic acid equivalent) per gram of dry mass (mg GAE g^{-1} DM). The FLAV were determined according to Pereira et al. (2009) using spectrophotometric method and the results expressed in mg of QE (quercetin equivalent) per gram of dry mass (mg QE g^{-1} DM). The ANT, according to Lemos (2008) using spectrophotometric method, the results were expressed in µg of QE (quercetin equivalent) per 100 gram dry matter (µg QE. 100 g^{-1} DM).

The N was determined by the Kjeldahl method; P by blue

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Table 1. Cactus pear varieties, genera *Opuntia* and *Nopalea*, used in the study and grown in the state of Pernambuco, Brazil.

Number	Varieties	Species	Common name
1	IPA-100003	*Opuntia fícus indica*	IPA-20
2	IPA-200016	*Opuntia stricta*	Elephant Ear Mexican
3	IPA-200008	*Opuntia atropes*	F-08
4	IPA-100004	*Nopalea cochenillifera*	Small palm
5	IPA-200021	*Nopalea cochenillifera*	F-21
6	IPA-200205	*Nopalea cochenillifera*	IPA-Sertânia
7	IPA-200149	*Opuntia larreri*	-

colorimetry molybdenum; K and Na by flame photometry of emission; S by turbidimetric; Ca, Mg, Fe, Cu, Zn and Mn by spectrophotometry atomic absorption of accordance with Malavolta et al. (1997) and the results were expressed in $g.kg^{-1}$ or $mg.kg^{-1}$. DM, MM, CP, EE, CF and NFE were determined according to the study of Messias et al. (2013) using physical and chemicals methods, and the results were expressed in %.

Statistical analysis

The data were initially evaluated by analysis of variance (ANOVA), and the means were compared by the Scott and Knott test (1974), at the level of 5% probability. The broad-sense heritability was calculated by the estimator: $h^2 = \sigma^2_g / \sigma^2_p \times 100$; where: σ^2_g = genetic variance and σ^2_p = phenotypic variance. The genetic variance was calculated by the estimator σ^2_g = MSTreat – MSRes / J; where: MSTreat = Mean square of the treatment; MSRes = Mean square of the residue and J = number of repetitions (Rêgo et al., 2011).

Diversity among varieties was estimated using a measure of dissimilarity expressed by the Mahalanobis distance (D^2) according to Cruz et al. (2012). The hierarchical grouping method according to Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Tocher optimization method (Rao, 1952) and the method of Principal Component Analysis (Cruz et al., 2012) were performed.

The relative importance of characters in relation to diversity was studied according to the methodology described by Singh (1981) and the principal component analysis (Cruz et al., 2012). The correlations among the characteristics were obtained as described by Rêgo et al. (2011), and the probability of 1 and 5% were tested by t-test. Data analyzes were performed with the help of the statistical program GENES®- Computer Application in Genetics and Statistics (Cruz, 2001) and Assistat® 7.7 (Silva and Azevedo, 2006).

RESULTS AND DISCUSSION

The analysis of variance by F test ($p \leq 0.01$) showed significant differences in the content of phenolic compounds (PC), total flavonoid (FLAV), anthocyanins (ANT), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), total protein (TP), ether extract (EE), nitrogen-free extract (NFE), and p ≤ 0, 05 for the nitrogen content (N), which confirms a variability among the varieties of cactus pear to most chemical and nutritional characteristics. There were no significant differences in the content of magnesium (Mg), sodium (Na), manganese (Mn), dry matter (DM), mineral matter (MM) and crude fiber (CF) in the plants (Table 2).

Guevara-Figueroa et al. (2010), studying the centesimal composition, content of cladode phenolic compounds of 10 cultivated and wild genotypes of *Opuntia* spp., reported differences among them in relation to PC, FLAV, CP, EE, CF, NFE and MM. Furthermore, Chahdoura et al. (2015) in a study about the nutritional composition of cladodes of *Opuntia microdasys* and *Opuntia macrorhiza* reported differences for EE, MM, NFE, CF, Cu, Ca, Mg, Na and K between them. These authors found no differences in DM, CP, Fe, Mn and Zn between these species.

Bensadón et al. (2010), studying the nutritional value of cladodes of *Opuntia ficus indica*, varieties Milpa Alta and Atlixco, found no difference between them in DM, CP, EE, MM, CF and PC. Furthermore, Batista et al. (2003), studying the chemical composition of cladodes of two varieties of the genus *Opuntia* (Giant palm and IPA-20) and one variety of genus *Nopalea* (Small palm) also reported no difference among them in relation to DM, CP, EE, MM, NFE, P, Mg, Fe, Cu and Zn. However, the researchers reported significant differences among these genotypes for Ca and Mn contents.

The genotypes showed an average variation in the content of PC (1.23 to 2.84 mg GAE g^{-1} DM), FLAV (1.52 to 3.02 mg QE g^{-1} DM), ANT (0.05 to 0.34 µg QE. 100 g^{-1} DM), N (6.41 to 13.73 $g.kg^{-1}$ DM), P (0.63 to 2.08 $g.kg^{-1}$ DM), K (1.83 to 7.74 $g.kg^{-1}$ DM), Ca (7.30 to 17.22 $g.kg^{-1}$ DM), Mg (4.63 to 7.09 $g.kg^{-1}$ DM), Na (0.11 to 0.14 $g.kg^{-1}$ DM), S (0.44 to 1.30 $g.kg^{-1}$ DM), Fe (14.68 to 69.27 $mg.kg^{-1}$ DM), Cu (2.07 to 93.02 $mg.kg^{-1}$ DM), Zn (23.33 to 63.79 $mg.kg^{-1}$ DM), Mn (246.12 to 598.45 $mg.kg^{-1}$ DM), DM (10.09 to 11.12%), MM (4.12 to 8.09% in the DM), CP (3.94 to 14.90% in the DM), EE (1.34 to 2.73% in the DM), CF (7.37 to 12.59% in the DM) and NFE (56.07 to 71.87% in the DM) (Table 3). Most of the values found in this study is in line with those reported in the literature (Batista et al., 2003; Bensadón et al., 2010; Dubeux-Junior et al., 2010; Guevara-Figueroa et al., 2010; Silva et al., 2012; Chahdoura et al., 2015).

The chemical components of plants vary both in

Table 2. Analysis of variance and estimates of the environmental variation coefficient (EV), ratio of genetic (GV) and environmental variation (EV) coefficients, broad-sense heritability (h^2), chemical and nutritional characteristics of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Mean squares											
VF	DF	PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S
Block	2	0.00	0.00	0.00	7.51	0.04	0.09	13.23	2.82	0.00	0.00
Varieties	6	0.95**	0.85**	0.03**	20.22*	0.75**	4.97**	39.16**	1.87ns	0.00ns	0.25**
Residues	12	0.00	0.00	0.00	4.44	0.05	0.83	1.08	1.51	0.00	0.05
EV (%)	-	1.93	0.05	4.43	20.19	21.38	24.86	8.15	21.03	20.63	21.45
GV/EV	-	15.03	514.56	19.77	1.09	2.12	1.29	3.43	0.28	0.10	1.22
h^2 (%)	-	99.85	99.99	99.91	78.04	93.12	83.29	97.25	19.31	2.96	81.77
Mean squares											
VF	DF	Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
Block	2	251.18	60.54	32.88	792.28	0.15	3.48	0.06	0.01	17.50	35.58
Varieties	6	1151.08**	4240.17**	547.01**	41180.40ns	0.60ns	4.25ns	43.35**	0.67**	11.98ns	68.46**
Residues	12	187.74	269.76	69.17	18222.31	0.36	1.46	0.67	0.04	8.19	7.54
EV (%)	-	35.07	31.19	23.77	33.15	0.67	19.73	12.09	11.03	29.03	4.24
GV/EV	-	1.31	2.22	1.52	0.65	0.46	0.80	4.59	2.17	0.39	1.64
h^2 (%)	-	83.69	93.64	87.35	55.75	39.29	65.73	98.44	93.37	31.66	88.99

PC total phenolic compounds, FLAV total flavonoids, ANT total anthocyanins, N nitrogen, P phosphorus, K potassium, Ca calcium, Mg magnesium, Na sodium, (S) sulfur, (Fe) iron, (Cu) copper, (Zn) zinc, (Mn) manganese, (DM) dry matter, (MM) mineral matter, (CP) crude protein, (EE) ether extract, (CF) crude fiber, (NFE) nitrogen-free extract, (ns) not significant, * significant at 5%, ** significant at 1% by F test.

composition and in content, and vary between species and within species. Factors that contribute to this difference in the genus *Opuntia* are genetic factors, environmental growth conditions, soil, cultivation, collection period, stress, age of the plants, order of the analyzed cladode, analyzed tissues, drying temperature of cladodes, extraction methods and differences in methodologies used in determinations (Santos-Zea et al., 2011; Bari et al., 2012).

Significant differences among the varieties, for some chemical components, were expected, knowing that they are plant materials that have diverging phenotypical characteristics, even they being seeded and grown in the same area and exposed to the same environmental conditions. This situation is a favorable indication for the study of genetic diversity.

The ratio between the genetic variation (GV) and environmental variation (EV) coefficients was above one for the characteristics PC, FLAV, ANT, N, P, K, Ca, S, Fe, Cu, Zn, CP, EE, and NFE with heritability values (h^2) between 78.04 and 99.99%, indicating high genetic control of these characteristics. For the characteristics Mg, Na, Mn, DM, MM and CF, the GV/EV was less than one, indicating possibly the influence of the environment on these characteristics (Table 2).

Given the existence of genetic variability among genotypes, we proceeded to the study of genetic divergence among the materials. For the characteristics in which the ANOVA was significant, differences were identified ($p \leq 0.05$) by the Scott-Knott test, and the formation of two (N, P, K, Fe and Zn), three (S, Cu, CP, EE, NFE), four (ANT and Ca), six (PC) and seven (FLAV) groups of means (Table 3). The Tocher grouping method gathered the seven varieties into three groups. The group I was represented by genotypes 3, 5, 4 and 7; group II by 2 and 6; and group III by genotype 1 (Table 4).

According to the dendrogram obtained by UPGMA hierarchical grouping method, the varieties of cactus pear were gathered into three groups, considering the cut off 45%, respectively, of the relative genetic distance (Cruz et al., 2012). This distribution is similar to that obtained by the Tocher method (Table 4 and Figure 1). The principal component analysis demonstrated that the use of the first three variables was sufficient to account for almost 77% of the total variation obtained in the seven genotypes (Table 5).

Thus, a reasonable description of the diversity of genotypes can be made by these components in two-dimensional or three-dimensional plane. According to Cruz et al. (2012), it is necessary that the first principal components should be close to 80% of the total value to explain the variability manifested among individuals, taking the interpretation of the phenomenon with considerable simplification of the characteristics in two-dimensional or three-dimensional plane. By analyzing the chart of score dispersions of principal component analysis, we observed the formation of four groups. The genotypes were divided into group I (2, 4 and 6), group II (5 and 7), group III (3) and group IV (1) (Figure 2).

The grouping of genotypes by the principal component

Table 3. Means of chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Characteristics	Varieties						
	1	2	3	4	5	6	7
PC	2.84[a]	1.84[e]	1.28[f]	1.93[d]	2.16[c]	1.23[f]	2.25[b]
FLAV	3.02[a]	1.57[f]	2.26[d]	2.51[b]	2.33[c]	1.52[g]	1.93[e]
ANT	0.16[b]	0.05[d]	0.05[d]	0.08[c]	0.07[c]	0.06[d]	0.34[a]
N	12.15[a]	12.93[a]	9.20[b]	9.21[b]	9.36[b]	6.41[b]	13.73 [a]
P	1.29[b]	0.74[b]	2.08[a]	0.86[b]	0.78[b]	0.63[b]	1.03 [b]
K	4.74[a]	4.35[a]	2.35[b]	2.88[b]	5.13[a]	1.83[b]	4.34 [a]
Ca	15.08[b]	13.46[b]	17.22[a]	10.00[c]	10.26[c]	15.75[a]	7.30 [d]
Mg	4.63[a]	6.15[a]	5.70[a]	5.58[a]	7.09[a]	5.36[a]	6.38 [a]
Na	0.11[a]	0.11[a]	0.14[a]	0.11[a]	0.11[a]	0.11[a]	0.11 [a]
S	0.89[b]	0.86[b]	1.30[a]	1.12[a]	0.44[c]	1.12[a]	1.18 [a]
Fe	58.49[a]	69.27[a]	44.49[a]	28.20[b]	23.08[b]	35.22[b]	14.68 [b]
Cu	2.07[c]	2.14[c]	86.96[a]	49.74[b]	59.26[b]	93.02[a]	75.28 [a]
Zn	63.79[a]	34.73[b]	31.06[b]	27.97[b]	23.33[b]	27.22[b]	36.75 [b]
Mn	598.45[a]	418.93[a]	246.12[a]	450.37[a]	294.64[a]	469.75[a]	371.77[a]
DM	10.09[a]	11.12[a]	10.60[a]	11.01[a]	10.30[a]	11.10[a]	10.14[a]
MM	6.20[a]	6.48[a]	8.09[a]	5.45[a]	6.18[a]	4.12[a]	6.26[a]
CP	14.90[a]	7.18[b]	4.17[c]	5.39[c]	4.86[c]	3.94[c]	7.08[b]
EE	2.32[b]	1.34[c]	1.70[c]	1.76[c]	2.73[a]	1.59[c]	1.95[c]
CF	10.40[a]	8.69[a]	12.59[a]	10.13[a]	11.96[a]	7.37[a]	7.82[a]
NFE	56.07[c]	65.18[b]	62.83[b]	66.24[b]	63.86[b]	71.87[a]	66.73[b]

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), potassium K ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ DM), Cu copper ($mg.kg^{-1}$ DM), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), ether extract EE (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM); * Means followed by the same letter on the line do not differ by the Scott-Knott grouping test ($p < 0.05$).

Table 4. Grouping of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on the chemical and nutritional characteristics, the Mahalanobis distance and Tocher optimization method.

Group	Varieties
I	3, 5, 4, 7
II	2, 6
III	1

method differed a little to that obtained by the UPGMA hierarchical method and Tocher method. It classified genotype 3 in a separate group, and genotype 4 shows similarity to 2 and 6 (Figure 1 and 2; Table 4). The grouping analysis (cluster analysis) identifies groups of similar individuals after estimation of a dissimilarity matrix. There are several grouping methods, example of Tocher, UPGMA and principal component that differ by the type of results, and the different ways to define the closeness between individuals or groups formed. In all cases, it is not known, a priori, the number of groups to be established and different methods give different results (Cruz et al., 2012).

The grouping methods are based mainly on hierarchical methods of optimization and ordination. In the hierarchical methods, there is the method of the average distance among groups (UPGMA), in which the groups are identified in the form of dendrograms, arranged on multiple levels and does not take into account the optimal number of groups. In optimization, there is the Tocher algorithm, which aims to achieve a partition of individuals that optimize (maximize or minimize) some predefined measure. It is based on the formation of groups in which the distances within the groups are smaller than the distances among groups, obtaining the optimal number of groups. In ordination, we highlight the principal component analysis technique that aims to reduce the dimensionality of the variables so that the new combination of resulting uncorrelated linear variables explain the structure of variance and covariance of the original set of variables, resulting in the grouping of subjects based on dispersions in relation to the Cartesian axes (Cruz et al., 2012).

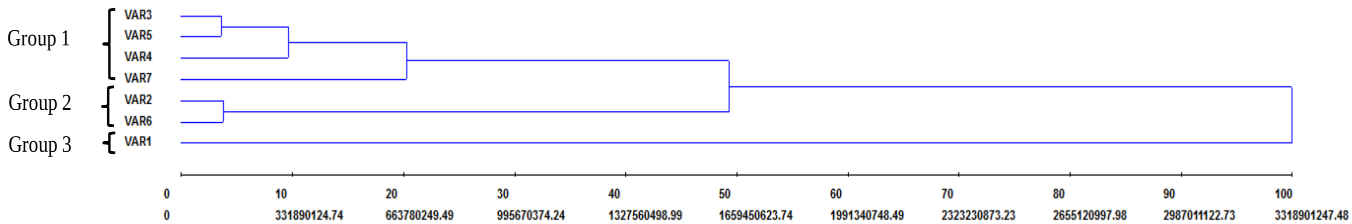

Figure 1. Representative dendrogram of the grouping by UPGMA of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on chemical and nutritional characteristics.

Table 5. Estimates of the eigenvalues associated with the principal components and relative importance (eigenvectors) for 20 chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Components	Root (eigenvalue)	Rood (%)	Accumulated (%)	Relative importance (eigenvectors)									
				PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S
1	7.07	35.34	35.34	0.35	0.26	0.16	0.25	0.04	0.29	-0.06	-0.08	-0.12	-0.14
2	4.43	22.13	57.47	-0.12	0.17	-0.16	-0.06	0.43	-0.15	0.33	-0.18	0.42	0.17
3	3.82	19.09	76.56	0.04	0.07	0.11	0.04	0.11	0.18	-0.25	0.40	0.11	-0.15
4	2.33	11.64	88.20	0.02	-0.02	0.51	0.18	0.19	-0.16	-0.15	-0.15	0.08	0.49
5	1.82	9.08	97.28	-0.05	-0.36	0.05	0.48	0.01	0.20	-0.07	0.26	0.06	0.03
6	0.54	2.72	99.99	-0.12	-0.45	0.12	-0.08	0.02	0.13	0.50	0.08	0.04	-0.27
7	0.00	0.00	99.99	0.03	0.07	-0.13	0.02	-0.06	-0.02	-0.01	-0.10	-0.06	0.01
-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	0.00	0.00	99.99	-0.17	-0.03	-0.29	0.02	-0.09	-0.19	-0.17	0.02	0.02	0.19
20	0.00	0.00	100.00	-0.02	-0.06	0.02	0.21	0.05	-0.06	-0.01	-0.11	0.04	0.23

Components	Root (eigenvalue)	Root (%)	Accumulated (%)	Relative importance (eigenvectors)									
				Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
1	7.07	35.34	35.34	0.10	-0.27	0.31	0.18	0.27	0.09	0.34	0.20	0.07	-0.32
2	4.43	22.13	57.47	0.19	0.02	0.10	-0.12	0.04	0.34	0.02	-0.05	-0.32	-0.23
3	3.82	19.09	76.56	-0.32	0.19	-0.21	-0.39	0.24	0.23	-0.18	0.30	0.26	-0.03
4	2.33	11.64	88.20	-0.26	0.26	0.14	0.03	0.24	0.02	0.05	-0.14	-0.26	0.09
5	1.82	9.08	97.28	0.31	-0.26	-0.27	-0.19	-0.13	0.34	-0.02	-0.37	-0.13	0.01
6	0.54	2.72	99.99	0.15	0.26	0.18	-0.07	0.36	-0.06	0.09	0.23	-0.24	0.05
7	0.00	0.00	99.99	0.14	-0.20	0.02	0.13	0.61	-0.02	-0.67	-0.17	0.01	0.06
-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	0.00	0.00	99.99	0.11	-0.20	-0.28	-0.04	0.44	0.05	0.52	0.14	0.03	0.35
20	0.00	0.00	99.99	0.18	-0.16	0.06	0.07	-0.26	0.10	-0.30	0.75	-0.11	0.19

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), potassium K ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ MS), Cu copper ($mg.kg^{-1}$ MS), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), EE ether extract (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM).

The use of multivariate techniques in the detection of diversity requires a certain degree of structure in the data. Therefore, it is important that different grouping criteria should be used, and that the consensus structure of most of them be considered as correct in order to assure that the result is not an artifact of the technique used (Arriel et al., 2006; Viana, 2013). The characteristics that contributed most to the total variance of the first component were PC and CP; in the second component, P and Na; in the third component, Mg and Mn (Table 5). The most important characteristics are those whose weighting coefficients (eigenvectors) are of greater magnitude, in absolute value, in the first principal components (Cruz et al., 2012). These, then, would be the most responsive characteristics in the selection process among cactus pear populations. The other

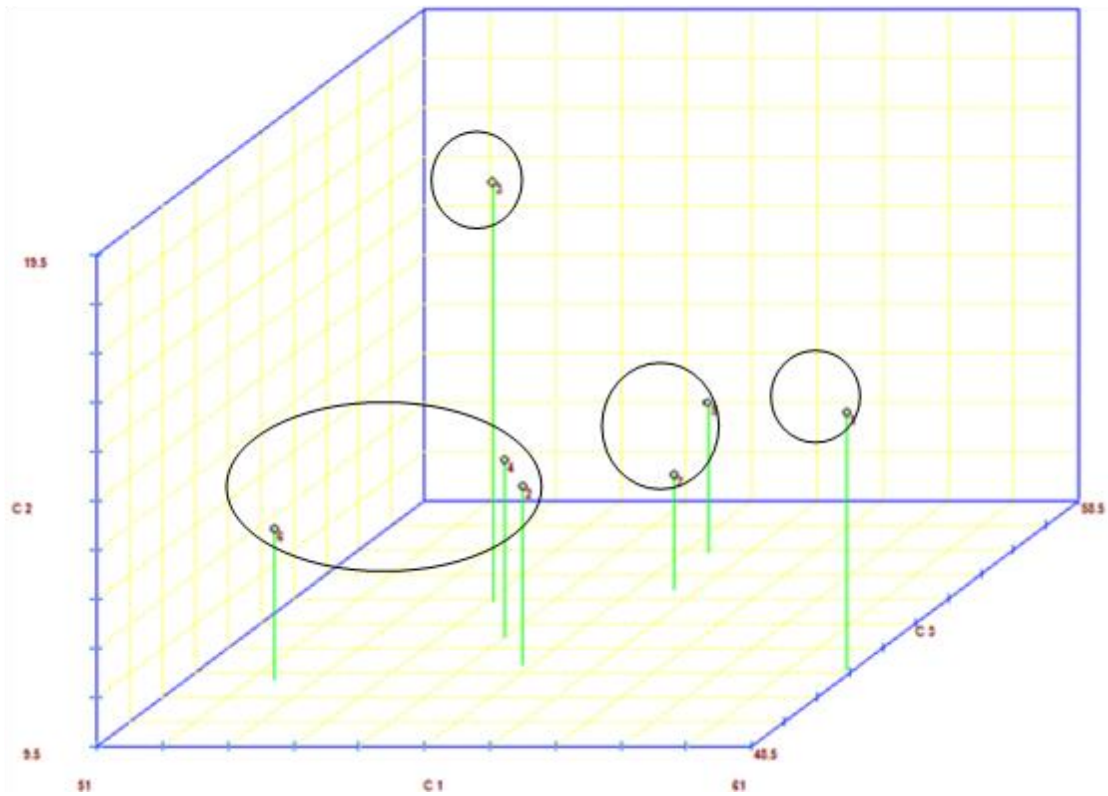

Figure 2. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third principal component (C1, C2, C3), based on twenty chemical and nutritional characteristics.

characteristics contributed little to the divergence among the genotypes and could be neglected (Rêgo et al., 2011).

The formation of three groups of cactus pear by UPGMA and Tocher methods, and four groups by the principal components method provide relevant information to the conservation of genetic material as a source for breeding programs. According to Silva et al. (2011), crossings between genotypes from different groups provide superior lineages for the improvement of characteristics of interest. Obtaining lineages from commercial varieties is a viable alternative because they are already-improved and tested genotypes in various growing environments. Considering hybrids, it is also possible to have a high proportion of different fixed loci, facilitating the selection and recombination of favorable alleles (Amorim and Souza, 2005).

Thus, as a suggestion for the breeding program with genera *Opuntia* and *Nopalea*, the breeder must consider not only the distance between groups as a criterion to guide the crossings, but also the genotype individual performance for each characteristic of agronomic and zootechnical interest (Ferreira et al., 2003). Besides, there is the possibility and ease of obtaining viable crossings between individuals of different genera or species. In many cases, it is not possible to obtain viable crossings between different species of plants by natural means, which is possible only by non-conventional methods or by biotechnology techniques (Paixão, 2012).

In the analysis of relative contribution of characteristics for the diversity among the seven varieties of cactus pear by Singh methodology (1981), the FLAV contributed with 46.30% and K with 14.07% for the variability among genotypes. These two characteristics contributed with 60.37% of the total variability among the materials. The characteristics that have contributed least to the divergence were Cu, EE, Mn and Mg (Table 6). The lower contribution variables are little informative in the characterization of variability, and can be discarded in diversity studies (Rêgo et al., 2011).

Most chemical and nutritional characteristics were not significantly correlated with each other, except for Na, that had a positive correlation ($p \leq 0.01$) with P (0.88) and Zn with CP (0.97) (Table 7). Sodium is not an essential nutrient for most plants. In fact, the greatest interest in the study of this ion is because its concentration in soils of arid and semiarid regions is high, hindering or even preventing the growth and development of plants. However, this ion plays an important role in osmotic adjustment of plants exposed to salt or water stress in these regions. In addition, it acts as an essential nutrient

Table 6. Relative contribution of 20 chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in semiarid region of Pernambuco through Singh methodology (1981).

Caracteristics	Relative contribution (%)
PC	7.33
FLAV	46.30
ANT	0.80
N	1.78
P	5.03
K	14.07
Ca	1.33
Mg	0.34
Na	0.00
S	8.06
Fe	0.71
Cu	0.08
Zn	2.42
Mn	0.19
DM	1.10
MM	0.63
CP	3.13
EE	0.12
CF	1.95
NFE	4.63

PC total phenolic compounds (mg GAE g^{-1} DM), FLAV total flavonoids (mg QE g^{-1} DM), ANT total anthocyanins (µg QE. 100 g^{-1} DM), N nitrogen ($g.kg^{-1}$ DM) P phosphorus ($g.kg^{-1}$ DM), K potassium ($g.kg^{-1}$ DM), Ca calcium ($g.kg^{-1}$ DM), Mg magnesium ($g.kg^{-1}$ DM), Na sodium ($g.kg^{-1}$ DM); S sulfur ($g.kg^{-1}$ DM), Fe iron ($mg.kg^{-1}$ MS), Cu copper ($mg.kg^{-1}$ MS), Zn zinc ($mg.kg^{-1}$ DM), Mn manganese ($mg.kg^{-1}$ DM), DM dry matter (%), MM mineral matter (% DM), CP crude protein (% DM), EE ether extract (% DM), CF crude fiber (% DM), NFE nitrogen-free extract (% DM).

for growth of some C4 and CAM plants, example of cactus pear (Epstein and Bloom, 2006).

Some C4 and CAM plants need this nutrient to make photosynthesis; however, it is not known whether this essential function has a biochemical or biophysical role. What is known is that this process focuses on the initial metabolism of four carbons that takes place in the mesophyll cells, or in the transport of this molecule between the mesophyll cells and vascular bundle sheath. Studies indicate that sodium facilitates the absorption of pyruvate by the chloroplasts of the mesophyll, perhaps activating the pyruvate carrier; it could still maintain the functional integrity of chloroplasts of the mesophyll (Epstein and Bloom, 2006). In addition, Na^+ is involved in the regeneration of phosphoenolpyruvate, and replaces K^+ in some functions in C4 and CAM plants (Taiz and Zeiger, 2013).

Phosphorus participates in reactions of photosynthesis and respiration as part in the molecules of NADPH, ATP, and various intermediates generated by these processes. The positive correlation between P and Na can be explained by the fact that cactus pear is a plant with CAM photosynthetic metabolism and need Na^+ to perform photosynthesis. Zinc is part of many proteins; in many enzymes, that metal ion is required in the active site (carbonic anhydrase, superoxide dismutase, alcohol dehydrogenase, glutamate dehydrogenase); in others, it is an integral component, not participating in the active site (Epstein and Bloom, 2006; Taiz and Zeiger, 2013). Thus, the positive correlation between Zn and CP. Positive correlations ($p \leq 0.05$) were also found in PC and K (0.82) or CP (0.83); and between P and MM (0.80) (Table 7).

Phenolic compounds belong to a class of secondary metabolites that includes a wide variety of structures, both simple and complex, and have at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group. Among these substances, there are structures as varied as the phenolic acids, coumarin derivatives, water-soluble pigments of flowers, fruits and leaves, lignins, tannins, etc. In addition, the phenolic compounds are part of the structure of proteins, alkaloids and terpenoids (Carvalho et al., 2010). Various functions are assigned to these compounds, from protection to biotic and abiotic stresses, pollinator attractants and seed dispersers, mechanical support, ultraviolet radiation protection, etc. (Taiz and Zeiger, 2013). Thus, there was a positive correlation between PC and CP for being part of the structure of various proteins.

Potassium, despite being the most abundant cationic mineral constituent of plants, and constituting up to 10% of the dry weight of a plant, is not an integral constituent of any metabolite that can be isolated from plant material. It is present in the cytosol and cell vacuoles as a free ion (K^+) in high concentrations. The main functions of this ion are the osmotic adjustment in plants exposed to salt or drought stress, activation of enzymes, stabilization of the functional configuration of macromolecules, participation in transport through the ion membrane, anion neutralization, maintenance of the osmotic potential and transport of organic and inorganic nutrients (Epstein and Bloom, 2006). Therefore, positive correlation between K and PC was present, since many of these substances are part of proteins or other macromolecules that needs to be chemically stabilized.

Ashes, or mineral matter, provide an indication of the wealth of the sample in mineral elements. The major elements found are the cations: calcium, potassium, sodium, magnesium, iron, copper, cobalt and aluminum; and the anions: sulfate, chloride, silicate and phosphate, which are absorbed from the environment, either by the roots or shoot of the plant (Messias et al., 2013). Therefore, was a positive correlation between the P and MM in the cactus pear samples. Negative correlations ($p \leq 0.05$) were found between FLAV with NFE (-0.81), and

Table 7. Correlations between the chemical and nutritional characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Caracteristics	PC	FLAV	ANT	N	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	DM	MM	CP	EE	CF	NFE
PC	1	0.65ns	0.53ns	0.65ns	-0.11ns	0.82*	-0.42ns	-0.09ns	-0.53ns	-0.45ns	0.04ns	-0.65ns	0.70ns	0.49ns	0.70ns	0.05ns	0.83*	0.61ns	0.05ns	-0.69ns
FLAV	-	1	0.11ns	0.13ns	0.40ns	0.33ns	-0.00ns	-0.36ns	0.06ns	-0.16ns	0.01ns	-0.34ns	0.56ns	0.28ns	0.59ns	0.28ns	0.60ns	0.62ns	0.61ns	-0.81*
ANT	-	-	1	0.63ns	0.02ns	0.37ns	-0.59ns	0.05ns	-0.28ns	0.22ns	-0.44ns	0.05ns	0.38ns	0.14ns	0.69ns	0.03ns	0.37ns	0.22ns	-0.38ns	-0.10ns
N	-	-	-	1	0.06ns	0.70ns	-0.42ns	0.12ns	-0.23ns	-0.07ns	0.22ns	-0.59ns	-0.53ns	0.14ns	0.48ns	0.42ns	0.58ns	0.03ns	-0.14ns	-0.46ns
P	-	-	-	-	1	-0.20ns	0.46ns	-0.26ns	0.88**	0.46ns	0.16ns	0.17ns	0.26ns	-0.35ns	0.38ns	0.80*	0.09ns	-0.00ns	0.62ns	-0.50ns
K	-	-	-	-	-	1	-0.48ns	0.39ns	-0.48ns	-0.73ns	0.06ns	-0.62ns	0.38ns	0.09ns	0.59ns	0.23ns	0.55ns	0.62ns	0.14ns	-0.56ns
Ca	-	-	-	-	-	-	1	-0.58ns	0.55ns	0.22ns	0.66ns	-0.01ns	0.22ns	0.10ns	-0.26ns	0.16ns	0.07ns	-0.31ns	0.23ns	-0.19ns
Mg	-	-	-	-	-	-	-	1	-0.09ns	-0.46ns	-0.46ns	0.23ns	-0.66ns	-0.73ns	0.03ns	0.18ns	-0.54ns	0.26ns	0.13ns	0.31ns
Na	-	-	-	-	-	-	-	-	1	0.49ns	0.12ns	0.41ns	-0.14ns	-0.59ns	-0.00ns	0.71ns	-0.32ns	-0.22ns	0.59ns	-0.14ns
S	-	-	-	-	-	-	-	-	-	1	-0.06ns	0.43ns	0.02ns	-0.01ns	-0.16ns	0.09ns	-0.17ns	-0.65ns	-0.25ns	-0.27ns
Fe	-	-	-	-	-	-	-	-	-	-	1	-0.70ns	0.49ns	0.37ns	-0.26ns	0.24ns	0.45ns	-0.38ns	0.05ns	-0.43ns
Cu	-	-	-	-	-	-	-	-	-	-	-	1	0.62ns	-0.56ns	-0.07ns	-0.11ns	-0.74ns	-0.05ns	-0.01ns	0.62ns
Zn	-	-	-	-	-	-	-		-	-	-	-	1	0.69ns	0.55ns	0.14ns	0.97**	0.18ns	-0.03ns	-0.76*
Mn	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.01ns	-0.55ns	0.72ns	-0.04ns	-0.46ns	-0.24ns
DM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.37ns	0.56ns	0.72ns	0.28ns	-0.63ns
MM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.08ns	0.06ns	0.67ns	-0.56ns
CP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.30ns	-0.02ns	-0.78*
EE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.47ns	-0.48ns
CF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.58ns
NFE	-	-	-	-	-	-	-	-		-	-	-	-	-	--	-	-	-	-	1

* and ** significant at 5 and 1% probability, respectively, by the t test; (ns) not significant.

between NFE with Zn (-0.76) or CP (-0.78) (Table 7).

In animal feed, nitrogen-free extract represents the nonstructural carbohydrates, soluble in acids and bases, usually consisting of starch, sugar and pectin. It indicates the energy value of a food, and is calculated by the difference of the remaining fractions of organic or dry matter (CF, EE, CP and MM) (Detmann et al., 2012; Messias et al., 2013). Therefore, there was a negative correlation between NFE and CP.

Several functions are attributed to flavonoids in plants. The most important include: protection against the incidence of ultraviolet and visible rays; protection against attacks of insects, fungi, viruses and bacteria; attraction of animals and insects for pollination and seed dispersal purposes; antioxidants; hormonal action control; allelopathic agents; enzyme inhibitors; protection against abiotic stresses, etc. (Zuanazzi and Montanha, 2010; Bartwal et al, 2013). Therefore, the flavonoids are the most abundant class of secondary metabolites, present in species of genera *Opuntia* and *Nopalea*.

The flavonoid content is influenced by environmental factors. The salt stress, water stress, temperature and light to which the plants of cactus pear are exposed, increase the content of these metabolites in the plant (Ramakrishna and Ravishankar, 2011; Rodziewicz et al., 2014). However, these stresses reduce the growth and development of cactus pear by negatively influencing the photosynthesis and, thereby, the production of carbohydrates (Bartwal et al., 2013).

As the primary metabolism (carbohydrate) is closely related to secondary metabolism (phenolic compounds), alterations in the first can profoundly affect the second. In addition, many secondary metabolites are formed by sequences of reactions analogous to those of primary metabolism. Thus, under stress conditions, the carbon that would be used in the production of carbohydrate is diverted to the production of secondary metabolites (Ramakrishna and Ravishankar, 2011; Santos, 2010). Then, the negative correlation between NFE and FLAV was found.

In addition, to be protected from abiotic stresses(drought, salt, temperature and high radiation), plants produce several antioxidant enzymes, such as the superoxide dismutase, which has zinc as one of its cofactors. Therefore, the negative correlation between Zn and NFE is found, once the carbon that would be used in the production of carbohydrate is diverted to the production of these enzymes (Epstein and Bloom, 2006; Taiz and Zeiger, 2013).

According to Cruz et al. (2012), the existence of significant correlations indicates the feasibility of indirect selection in order to obtain gains in the characteristic of greatest importance. Among the genotypes that stood out with the highest, overall means for the chemical and nutritional characteristics were 1, 3 and 5 (Table 3). Thus, crossings involving these genotypes could generate superior progenies in characteristics of agronomic, zootechnical or physiological interest.

These results suggest future work aimed to explore the variability found among the cactus pear genotypes studied, and the possibility of using other methods such as protein molecular markers, chemical markers (secondary metabolites), physiological and biochemical characteristics, and chromosomal variation for the determination of genetic variability, providing a complementary analysis to studies by chemical and nutritional characteristics.

Conclusions

The seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, present divergence. Multivariate methods used for divergence gather these genotypes into three or four groups. The characteristics that contribute most to the diversity among the varieties are the flavonoid and potassium contents. These characteristics are correlated with nitrogen-free extract and total phenolic compounds. Multivariate analysis techniques are effective in the study of diversity of species of the genera *Opuntia* and *Nopalea*.

Conflict of Interests

The authors have not declared any conflict of interests.

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