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

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The cactus pear is a widely cultivated plant in the Northeast of Brazil, contributing significantly to the feeding of livestock, especially in times of drought. Because of the high variation between phenotypic varieties grown in this region, it is essential to study the genetic diversity. The objectives of this study were to characterize the genetic diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 19 morphological and behavioral characteristics, and to determine the phenotypic correlation and importance of these characteristics to the variability among genotypes, using multivariate analysis techniques. The study was conducted at the experimental station of Agronomic Institute of Pernambuco (IPA), located in city of Arcoverde, State of Pernambuco, Brazil using randomized block design with three replications. The materials IPA-100003, IPA-200016, IPA-200008, IPA-100004, IPA-200021, IPA-200205 and IPA-200149 were evaluated for 19 quantitative characteristics of the plants. The collected data were analyzed by analysis of variance by F test, and the means grouped by the Scott-Knott test ($p<0.05$). The broad-sense heritability and phenotypic correlation characteristics were estimated. The genetic diversity was estimated by multivariate methods (unweighted pair group method with arithmetic mean-UPGMA, Tocher, principal component and canonical variables). Analyses of variance and genetic diversity revealed significant differences among genotypes, with the possible formation of two, three or four genetically distinct groups. The heritability values ranged from 79.6 to 97.0% for all 19 quantitative characteristics. The water content and cladode fresh matter are the characteristics that contributed most to the genetic divergence among the materials. Moreover, these characteristics are significantly and positively correlated with dry matter, width, length and cladode area. Thus, the genetic variability among the studied varieties of cactus pear and their potential use in breeding programs are confirmed. The uni and multivariate methods used for the genetic divergence differ and gather genotypes in two, three or four groups.

Key words: Brazilian semiarid, forage, genetic distance, grouping, multivariate analysis.

INTRODUCTION

The cactus pear (*Opuntia* spp. and *Nopalea* spp.) is a cactaceae originally from Mexico, which is exploited since the pre-Hispanic period, holding the greatest genetic diversity and one of the largest cultivated areas in the world with over 28.3 million hectares. The current distribution of these plants in the world includes different

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*	Little
5	IPA-200021	*Nopalea cochenillifera*	F-21
6	IPA-200205	*Nopalea cochenillifera*	IPA-Sertânia
7	IPA-200149	*Opuntia larreri*	-

environments and a wide range of species, which explains the high genetic variation that originates from the great ecological diversity of the areas where they are native (Barrios and Muñoz-Urías, 2001; López-García et al., 2001).

These plants are used for various purposes: human and animal food, energy production, medicine, cosmetics, chemical, and food industry. It is difficult to find a plant as distributed and exploited, especially in arid and semiarid areas, or as subsistence economy by producers of small animals, or as a culture focused on the industrial market (Barbera et al., 2001).

For their physiological, morphological, and chemical characteristics that enable these plants to tolerate arid and semiarid environments, especially with regard to absorption, recovery and use of water, they have adapted very well in Brazilian northeast. This region is characterized by having a high index of annual evaporation, greater than 2.000 mm, and average rainfall of less than 750 mm, concentrated in a single period of 3 to 5 months; in addition, in some years, the lack of rain is prolonged, resulting in the phenomenon of droughts (Araújo et al., 2005).

The species *Opuntia ficus indica* Mill. and *Nopalea cochenillifera* Salm Dyck. are widely cultivated, especially in the states of Pernambuco, Alagoas, Paraiba, Sergipe, Bahia and Ceará. It is estimated that the area cultivated in Brazil reaches about 550,000 ha. The main use of these plants in this region is based on food support for ruminants, mainly in the dry season, considering that this segment is strongly affected by the lack of forage plants during this period (Santos et al., 2006).

Most of the work involving selection and breeding of cactus pear uses statistical methods of univariate type, since they are focused on the analysis of the variation in a single random variable. However, the selection of plants based on various important variables may be more advantageous, especially when performed on a set of quantitative characteristics (Ferreira et al., 2003).

In the simultaneous handling of several characteristics, the multivariate techniques consider simultaneously a set of random variables, each of which has the same degree of importance. The multivariate techniques for diversity studies are applied from dissimilarity measures among the genotypes, such as the Mahalanobis distance. In addition, grouping analysis brings together individuals with similar characteristics in relation to the observed variables. Among the methods, we highlight the Tocher; the hierarchical, such as the unweighted average linkage (UPGMA), and principal component analysis and canonical variables (Cruz et al., 2012).

The objectives of this study were to characterize the genetic diversity in seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, through 19 morphological and behavioral characteristics, and to determine the phenotypic correlation and importance of these characteristics in the variability among genotypes.

MATERIALS AND METHODS

Location of the experiment

The study was conducted at the Experimental Station Arcoverde, from the Agronomic Institute of Pernambuco (IPA), located in city of Arcoverde, State of Pernambuco, Brazil (8°25'S, 37°05' W), 680.7 m altitude, average annual temperature 22.9±1.7°C, average annual relative humidity 69.6±5.3%, wind speed (annual average 3.9±0.5 m s^{-1}), accumulated evaporation (average 1700.4 mm), average annual accumulated rainfall of 798.1 mm, microregion of the 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 April 22 and 23, 2010, spaced 1.0 x 0.5 m; using one cladode per hole. The experimental design was a randomized complete block design represented by seven treatments and three

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replications. Each block consisted of three rows planted with eight plants of each variety, totalizing twenty four plants of each treatment. The experimental plot was composed by the middle row using with six plants and covering 3.0 m^2 of 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 measurements in the plant, and collection secondary and tertiary cladodes were held at 8:00 am on 19 February, 2013 (dry season). After the measurements, the cladodes was cleaned, weighed, 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 reaching constant weight, by which the dry matter (DM) of cladodes was obtained.

Determination of morphological characteristics and production

The evaluated variables were: the width (CW), length (CL), thickness (CT), area (CA), fresh matter (CFM), dry matter (CDM) and water content (H_2O) and total number of cladodes (NC); width (PW), height (PH), total photosynthetic area (TPA), cladode area index (CAI), fresh (FM), and dry mass production (DM) of plants. CW, CL, CT, PW and PH were measured with a caliper and measuring tape. The CFM and CDM were obtained with a precision scale (0.01 g). The H_2O was determined as described by Guimarães and Stone (2008) using the following formula: H_2O = [(CFM – CDM) / CDM] x 100. PH was estimated as described by Sales et al. (2013), using the following formula: CA = CL x CW x 0.632. The TPA was estimated by multiplying CA with NC. The cladode area index (CAI) was estimated according to Sales et al. (2013), by the ratio between TPA and the soil area occupied by the plant. The FM and DM was estimated by the NC multiplied by CFM and CDM.

Determination of behavioral characteristics

The varieties was visually assessed by the characteristics of Desirability (DESIR) - general appearance of the genotype, in which are considered: budding, productive aspect and plant health, infestation by the carmine cochineal (*Dactylopius opuntiae*) (CAR), infestation by cactus scale (*Diaspis echinocacti*) (SCA), disease incidence (DIS), and wilt index (WIL).

We used for DESIR, scores: 1 (high), 2 (average), 3 (low). For CAR, SCA, DIS and WIL, scores: 0 (absence), 1 (low), 2 (average), 3 (high) and 4 (highest). The observations were made in the twenty four plants of the three rows of each variety (Pereira et al., 2014).

Statistical analysis

The data were initially evaluated by analysis of variance (ANOVA), and the means were compared by the Scott and Knott's test at 5% (Scott and Knott, 1974).

The broad-sense heritability was calculated by the estimator: $h^2 = \sigma^2_g / \sigma^2_p \times 100$, where: σ^2_g is the genetic variance and σ^2_p is the phenotypic variance. The genetic variance was calculated by the estimator: σ^2_g = SMTreat – SMRes / J, where SMTreat is the square mean of the treatment, SMRes is the square mean of the residue, and J is the number of replicates. The phenotypic variance was calculated by the estimator: σ^2_p = SMTreat / J. The environmental variance was calculated by the estimator: σ^2_e = SMRes / J. The genetic variation coefficient was calculated by the estimator: (GV) = (σ_g / M) x 100, where M is the average of the characteristic. The environmental variation coefficient was calculated by the estimator: (EV) = (σ_r / M) x 100 (Alves et al., 2006; Rufino et al., 2010; Rêgo et al., 2011).

The genetic diversity among varieties was estimated using a measure of dissimilarities expressed by the Mahalanobis distance (D^2) according to Cruz et al. (2012). Grouping was performed by hierarchical method unweighted pair group method with arithmetic mean (UPGMA), Tocher optimization method (Rao, 1952), principal component and canonical variables analysis methods (Cruz et al., 2012).

The relative importance of characters in relation to genetic diversity was studied according to Singh (1981), canonical variable and principal components analysis (Cruz et al., 2012). The correlations of Pearson among the characteristics were obtained as described by Rêgo et al. (2011), and the probability of 1 and 5% by t-test.

Data analyzes were performed in the statistical software 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 among cactus pear varieties for width (CW), length (CL), area (CA), dry matter (CDM) of cladodes; number of cladodes (NC), infestation by *D. opuntiae* (CAR), infestation by *D. echinocacti* (SCA), wilt (WIL) of plants and ($p \leq 0.05$) for total photosynthetic area (TPA), cladode area index (CAI) and dry mass (DW) of plants. There were no significant differences among cactus pear varieties for thickness (CT), fresh matter (CFM), water content (H_2O) of the cladodes, width (PW), height (PH), fresh mass (FM), desirability (DESIR), and incidence of diseases (DIS) in the plants (Table 2).

Ferreira et al. (2003) and Neder et al. (2013), studying the genetic diversity of cactus pear (*Opuntia ficus indica* Mill.), reported significant differences among 38 accesses studied in relation to CT, CL, CW, NC, FM, DM, PH and PW. The failure to detect differences among the characteristics CT, CFM, H_2O, PW, PH, FM and DESIR among varieties of cactus pear, is probably related to the time of data collection (dry season) after a large water deficit suffered by plants, approximately 13 months. This may have affected the results of the measurements, since the plants were not in their full turgor, besides, some genotypes lost some cladodes and these could not be accounted for in the measurements. The phenotypic difference among varieties in the rainy season, when the plants are fully turgid is clear (Alves et al., 2013).

The genotypes showed an average variation in CW (8.50 to 22.33 cm), CL (19.90 to 32.33 cm), CT (0.36 to 0.76 cm), CA (107.40 to 434 , 90 cm^2), CFM (59.46 to 401.66 g), CDM (20.20 to 81.06 g), H_2O (39.26 to 320.60 g), NC (12.16 to 79.83 units), PW (65.66 to 114.11 cm), PH (52.33 to 80.33 cm), TPA (2,949.90 to 9,898.06 cm^2), CAI (0.59 to 1.97), FM (2164.58 to 8356.25 g), DM (611.55 to 2293.86 g), DESIR (2.33 to 3.00), CAR (0.00 to 1.66), SCA (0.00 to 2.00), DIS (1.00 to 2.00) and WIL (1.66 to 3.00) (Table 3). These values are consistent with found in the genera *Opuntia* and *Nopalea*. Researchers relate this variation to genotypic variability, plant age, soil, management, crop treatment and environmental factors (Paixão, 2012; Amorim, 2011; Ferreira et al., 2003;

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), morphological and behavioral characteristics of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Mean squares												
S.V	D.F	CW	CL	CT	CA	CFM	CDM	H_2O	NC	PW	PH	TPA
Block	2	16.10	44.08	0.02	18493.82	41172.76	1082.57	28904.64	346.30	280.61	228.00	35062515.45
Varieties	6	71.47**	66.54**	0.07ns	42112.81**	47023.10ns	2014.03**	30611.97ns	1609.96**	773.35ns	222.41ns	16513675.79*
Residues	12	2.13	2.96	0.04	1456.89	20190.56	411.08	15291.76	80.30	288.31	149.39	5385859.75
EV (%)	-	9.99	6.67	32.92	15.17	75.92	41.66	89.29	23.30	19.38	18.93	29.52
GV/EV	-	3.30	2.67	0.54	3.05	0.67	1.14	0.58	2.52	0.75	0.40	0.83
h^2 (%)	-	97.02	95.55	46.77	96.54	57.06	79.59	50.05	95.01	62.72	32.83	67.39

Mean squares										
S.V	D.F	CAI	FM	DM	DESIR	CAR	SCA	DIS	WIL
Blocks	2	1.40	26736681.39	1067008.28	0.33	0.05	0.00	0.90	0.76
Varieties	6	0.66*	14134953.97ns	836550.51*	0.22ns	1.19**	1.97**	0.38ns	0.75**
Residues	12	0.22	7906517.59	266899.74	0.22	0.05	0.11	0.40	0.15
EV (%)	-	29.52	52.93	35.24	0.00	91.65	58.33	0.00	18.53
GV/EV	-	0.83	0.5124	0.84	0.00	2.83	2.36	0.00	1.15
h^2 (%)	-	67.39	44.06	68.10	0.00	96.00	94.35	0.00	79.79

CW Cladode width, CL cladode length, CT cladode thickness, CA cladode area, CFM cladode fresh matter, CDM cladode dry matter, H_2O water content of cladode, NC total number of cladodes, PW plant width, PH plant height, TPA total photosynthetic area of the plant, CAI cladode area index, FM fresh matter production of the plant, DM dry matter production of the plant, DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt index. *, ** significant at 5% and 1%, respectively or ns not significant, by Fisher's test.

Sales et al., 2003).

The ratio between the coefficient of genetic (GV) and environmental variation (EV), was above one for the characteristics CW, CL, CA, CDM, NC, CAR, SCA, WIL, with heritability values (h^2) between 79.6 to 97.0%, indicating high genetic control among these characteristics. For the other characteristics CT, CFM, H_2O, PW, PH, TPA, CAI, FM, DM, DESIR and DIS, the (GV)/(EV) indices were below one, indicating the dominance of the environment on these characteristics (Table 2).

Neder et al. (2013) report that CW, CL, CT, PW, PH, FM and DM are controlled by genetic factors and that NC is controlled by environmental factors. However, as the researchers reported that the genetic correlations among these characteristics were higher than the phenotypic and environmental, but not significant, it may indicate possibly the effect of the environment on the association with the genetic characteristics (Gonçalves et al., 1996).

Paixão (2012) studying variance components and genetic parameters for the variables PH, PW, NC, CW and CL on cactus pear progenies, genera *Opuntia* and *Nopalea*, reported that environmental variance is greater than genotypic variance for these characteristics, indicating that the variability was biased, and that it could be overestimating the genetic variance. In general, the variance caused by the environment is an important source of error, capable of reducing experimental precision (Falconer, 1987; Paixão, 2012). However, those same researchers reported that the estimates of broad-sense heritability coefficients were of high magnitude to PH, CW and CL, demonstrating good genetic control and the possibility of genetic advances (Paixão, 2012).

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 analysis of variance was significant, differences were identified (p ≤ 0.05) by the Scott-Knott's test, and observed the formation of two (CL, CDM, TPA, CAI, CAR and WIL), three (CA and NC) and four (CW and ESC) groups of means (Table 3). The Tocher grouping method gathered the seven varieties into two distinct groups. The group I was represented by genotypes 1, 2, 3, 4, 5 and 6. Group II consists of the genotype 7 (Table 4).

According to the dendrogram obtained by hierarchical clustering method UPGMA, the cactus pear varieties were gathered into three groups, considering the cut of 38% of relative genetic distance, according to the criterion mentioned by Arriel et al. (2006) and Cruz et al. (2012), in which the high-level change points are considered delimiters of the number of genotypes for a certain group. Group I was composed by genotypes 1, 3, 4, 5 and 6; II group by 2; and group III by 7 (Figure 1).

The principal component analysis demonstrated that the use of the first three variables was sufficient to account for almost 85% of the total variation obtained in the seven genotypes (Table 5). Thus, a reasonable description of the genetic diversity of genotypes can be made by these components, since, according to Cruz et

Table 3. Mean of morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Variable	Varieties							C.V
	1	2	3	4	5	6	7	
CW	18.16^{b}	22.33^{a}	11.06^{c}	8.50^{d}	11.10^{c}	13.60^{c}	17.33^{b}	9.99
CL	32.33^{a}	30.50^{a}	21.90^{b}	19.90^{b}	25.00^{b}	28.40^{a}	22.50^{b}	6.67
CT	0.70^{a}	0.60^{a}	0.50^{a}	0.43^{a}	0.36^{a}	0.76^{a}	0.70^{a}	32.92
CA	372.56^{a}	434.90^{a}	153.46^{c}	107.40^{c}	176.76^{c}	248.46^{b}	250.80^{b}	15.32
CFM	268.33^{a}	401.66^{a}	88.33^{a}	59.46^{a}	71.66^{a}	232.23^{a}	188.33^{a}	75.93
CDM	65.06^{a}	81.06^{a}	25.60^{b}	20.20^{b}	21.90^{b}	73.8^{a}	53.00^{a}	41.67
H_2O	309.72^{a}	333.12^{a}	245.20^{a}	197.40^{a}	228.60^{a}	215.71^{a}	257.92^{a}	25.91
NC	24.66^{c}	21.66^{c}	46.83^{b}	79.83^{a}	53.16^{b}	30.83^{c}	12.16^{c}	23.31
PW	114.11^{a}	87.66^{a}	84.00^{a}	90.00^{a}	72.88^{a}	98.89^{a}	65.66^{a}	19.38
PH	80.33^{a}	68.66^{a}	52.33^{a}	61.33^{a}	63.66^{a}	65.33^{a}	60.33^{a}	18.93
TPA	9157.06^{a}	9356.16^{a}	7292.75^{a}	8579.86^{a}	9898.06^{a}	7799.16^{a}	2949.90^{b}	29.52
CAI	1.83^{a}	1.87^{a}	1.45^{a}	1.71^{a}	1.97^{a}	1.55 ^{a}	0.59^{b}	29.52
FM	6680.00^{a}	8356.25^{a}	4279.48^{a}	4753.83^{a}	3776.93^{a}	7180.35^{a}	2164.58^{a}	52.92
DM	1628.35^{a}	1718.91^{a}	1235.51^{a}	1611.43^{a}	1160.95^{a}	2293.86^{a}	611.55^{a}	35.25
DESIR	2.33^{a}	2.66^{a}	2.66^{a}	2.66^{a}	3.00^{a}	2.33^{a}	3.00^{a}	17.68
CAR	1.66^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	91.65
SCA	0.00^{d}	2.00^{a}	0.66^{c}	0.00^{d}	0.00^{d}	1.33^{b}	0.00^{d}	58.33
DIS	1.33^{a}	1.00^{a}	1.66^{a}	1.00^{a}	2.00^{a}	1.33^{a}	1.33^{a}	46.07
WIL	1.66^{b}	1.66^{b}	2.33^{a}	2.33^{a}	3.00^{a}	1.66^{b}	2.00^{b}	18.53

CW Cladode width, (cm) CL cladode length (cm), CT cladode thickness (cm), CA cladode area (cm^2), CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), NC total number of cladodes, PW plant width (cm), PH plant height (cm), TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, FM fresh matter production of the plant (g), DM dry matter production of the plant (g), DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt Index. The varieties followed by the same letter belongs to the same group by Scott-Knott criterium ($P \leq 0.05$).

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

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

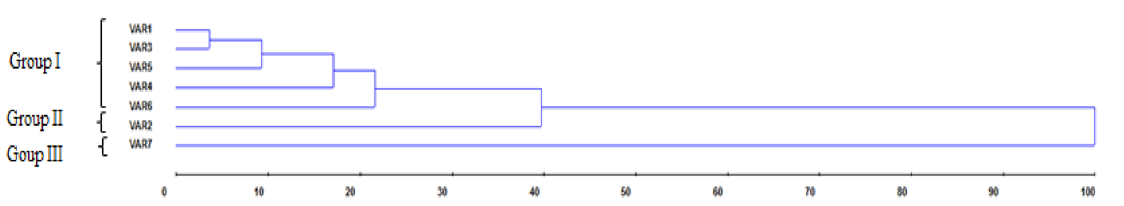

Figure 1. Representative dendrogram of the grouping by UPGMA of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, based on morphological and behavioral characteristics.

Table 5. Estimates of the eigenvalues associated to the principal components and relative importance (eigenvectors) for 19 morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Components	Root (eigenvalue)	Root %	Accumulated %	Relative Importance (eigen vectors)										
				CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI	PW
1	10.05	52.87	52.87	0.30	0.30	0.29	0.23	0.25	0.28	0.29	-0.20	0.07	0.07	0.21
2	4.06	21.38	74.26	-0.11	-0.13	-0.11	-0.22	-0.23	0.07	-0.10	0.34	0.44	0.44	0.29
3	2.03	10.71	84.96	-0.10	-0.07	-0.11	0.11	0.01	0.14	0.04	-0.11	-0.06	-0.06	0.20
4	1.81	9.50	94.46	0.14	-0.01	0.17	-0.32	0.25	0.21	0.25	-0.12	0.28	0.28	-0.26
5	0.82	4.31	98.77	-0.08	0.11	-0.14	0.27	-0.14	0.21	-0.09	-0.29	-0.03	-0.03	0.01
6	0.23	1.23	100.00	-0.02	0.28	-0.10	0.24	-0.15	0.13	-0.10	0.056	-0.001	-0.001	-0.26

Components	Raiz (eigenvalue)	Root %	Accumulated %	Relative Importance (eigenvectors)							
				PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
1	10.05	52.87	52.87	0.23	0.26	0.18	-0.27	-0.16	0.15	-0.21	0.19
2	4.06	21.38	74.26	0.12	0.21	0.30	0.11	0.04	0.11	-0.23	-0.007
3	2.03	10.71	84.96	0.35	-0.21	-0.22	0.02	0.20	0.57	-0.06	-0.52
4	1.81	9.50	94.46	0.12	0.006	-0.24	0.30	0.32	-0.01	0.38	0.06
5	0.82	4.31	98.77	-0.19	0.007	0.20	0.073	0.74	-0.09	-0.19	0.20
6	0.23	1.23	100.00	0.53	-0.07	0.37	0.15	-0.09	-0.34	0.26	-0.25

CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), CT cladode thickness (cm), CW cladode width (cm), CL cladode length (cm), CA cladode area (cm^2), NC total number of cladodes, TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, PW plant width (cm), PH plant height (cm), FM fresh matter production of the plant (g), DM dry matter production of the plant (g), WIL wilt Index, DIS Disease Incidence, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), DESIR Desirability and SCA Infestation by cochineal Scale (*Diaspis echinocacti*).

al. (2012), it is necessary that the first principal components exceed 80% of the accumulated value to account for the variability manifested among individuals, leading the interpretation of the phenomenon with considerable simplification of characters. By analyzing the chart of the score dispersions of the principal component analysis, the formation of four groups was observed. The genotypes were divided into group I (3, 4 and 5), group II (1 and 6), group III (2) and Group IV (7) (Figure 2).

In the analysis of canonical variables, the first two variables explained 96.75% of the total variation among the varieties of cactus pear, providing good reliability of the variability among genotypes in the two-dimensional plane (Table 2). When analyzing the chart of the score dispersion of canonical variables, the formation of three groups was observed. Group I, represented by genotypes 1, 3, 4, 5 and 6, group II by genotype 7 and group III by genotype 2 (Figure 3).

The grouping methods of the genotypes were similar to each other. However, the grouping order of genotypes among groups was different. For example, in UPGMA methods, principal components and canonical variables, the genotype 2 was classified into one group, diverging from the Tocher method that classified this genotype as similar to genotypes 1, 3, 4, 5 and 6. The method of principal components classified genotypes 1 and 6 in different groups, differing from other methods that classified the genotypes 1, 3, 4, 5 and 6 as similar (Figure 1, 2, 3 and Table 4).

The grouping analysis (cluster analysis) identifies groups of similar individuals after estimation of a dissimilarity matrix. There are several grouping methods that differ by type of result and by 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 mainly based on hierarchical and optimization methods. 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

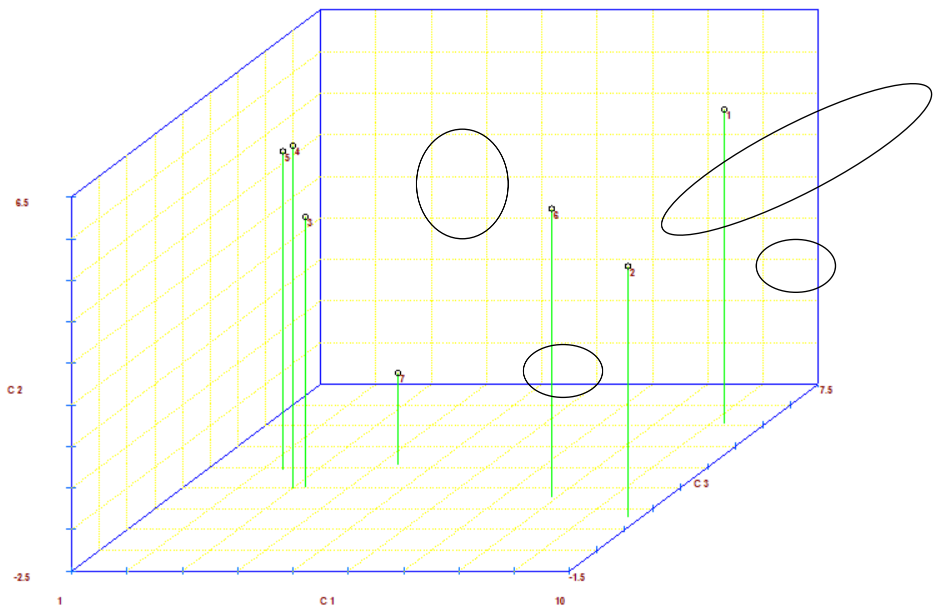

Figure 2. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third principal component (C1, C2, C3), based on six morphological and behavioral characteristics.

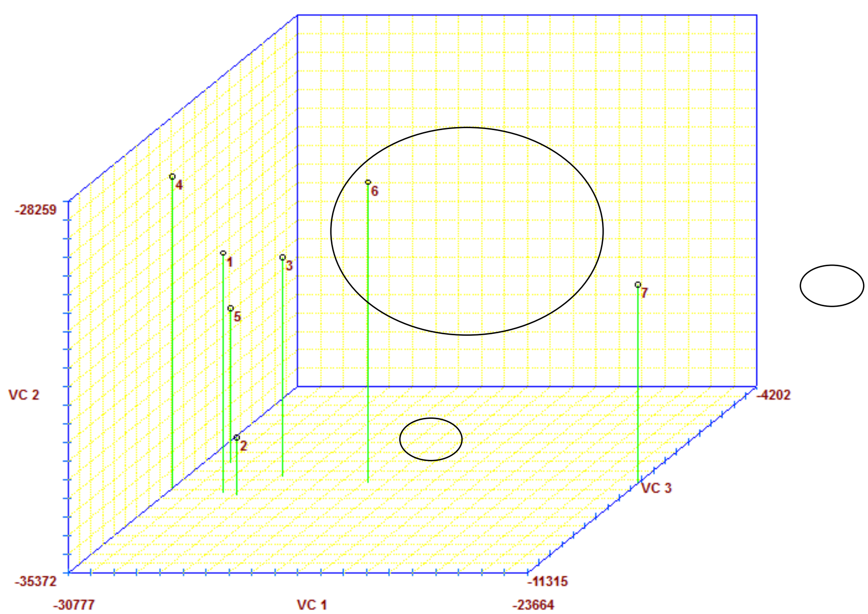

Figure 3. Graphic dispersion of seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, in relation to the first, second and third canonical variable (CV1, CV2 and CV3) based on six morphological and behavioral characteristics

does not take into account the optimal number of groups. In optimization methods, there is the Tocher algorithm, in which the goal is 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 the groups obtaining the optimal number of groups (Cruz et al., 2012).

Table 6. Estimate of the eigenvalues associated to canonical variables and relative importance (eigenvectors) for 19 morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco.

Canonical variable	Root (eigenvalue)	Root %	Accumulated %	Relative importance (eigenvectors)									
				CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI
1	5950441.88635	65.76975	65.76975	0.00005	0.00027	0.0	0.00039	0.00069	-0.0006	-0.00223	-0.00299	0.00043	-0.00009
2	2803191.65355	30.98345	96.75320	-0.00027	0.00009	0.0	0.00084	-0.0022	-0.0002	0.00154	0.00309	-0.0008	0.0
3	172614.96673	1.90789	98.66110	-0.00148	-0.0015	0.0	0.00074	-0.0068	-0.0007	0.00131	0.00603	0.00051	0.00007
4	120244.63357	1.32905	99.9901	0.0006	0.00135	0.0	0.00098	0.00485	0.00399	-0.00211	-0.01036	0.00329	-0.00014
5	886.82921	0.00980	99.99996	0.0099	0.02711	0.0	0.00552	0.00003	0.01874	-0.00932	-0.4237	0.03919	-0.00011
6	3.19831	0.00003	100.0000	0.12444	0.36019	0.00001	-0.0743	0.20859	0.40727	0.02923	-0.2794	0.16108	0.00692

Canonical variable	Root (eigenvalue)	Root %	Accumulated %	Relative Importance (eignevectors)								
				PW	PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
1	5950441.88635	65.76975	65.76975	0.00502	0.00072	0.00303	0.00285	-0.3576	-0.3529	0.58204	0.07891	0.6344
2	2803191.65355	30.98345	96.75320	0.00582	-0.0008	-0.0042	-0.0015	0.31095	0.29452	0.05789	0.88437	0.17614
3	172614.96673	1.90789	98.66110	0.00474	-0.0039	-0.0072	0.00092	0.25413	0.70908	0.11707	-0.4296	0.48388
4	120244.63357	1.32905	99.9901	-0.0099	0.00595	0.01342	-0.0054	0.12349	0.17886	0.79893	-0.4384	-0.5586
5	886.82921	0.00980	99.99996	-0.02294	-0.0354	0.0601	-0.0846	0.82691	-0.4990	0.07197	-0.1570	0.14183
6	3.19831	0.00003	100.0000	-0.05287	0.601	0.38999	-0.1260	-0.0397	0.03136	-0.0169	0.00784	0.00594

CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O cladode water content (g), CT cladode thickness (cm), CW cladode width (cm), CL cladode length (cm), CA cladode area (cm^2), NC total number of cladodes, TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, PW plant width (cm), PH plant height (cm), FM fresh matter production of the plant (g), DM dry matter production of the plant (g), WIL wilt Index, DIS Disease Incidence, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), DESIR Desirability and SCA Infestation by cochineal Scale (*Diaspis echinocacti*).

The techniques of principal component analysis and canonical variables aims to reduce the dimensionality of the variables so that the new combination of resulting uncorrelated linear variables explains the structure of variance and covariance of the set of original variables(Cruz et al., 2012).

The use of multivariate techniques in the detection of genetic diversity requires a certain degree of structure in the data. It is important that different grouping criteria be used and that the consensus structure of most of them be considered as correct, so as to assure that the result is not an artifact of the technique used (Arriel et al, 2006; Viana, 2013).

The formation of two groups of cactus pear by the Tocher method, three by UPGMA methods and canonical variables and four groups by the method of principal components, provides relevant information to the conservation of genetic material as a source for breeding programs. According to Silva et al. (2011), crossbreeding between genotypes from different groups provide superior lines for the improvement of characteristics of interest. Obtaining lineages from commercial varieties is a viable alternative because they represent improved and tested genotypes in various cultivation environments. Considering the hybrids, it is also possible to have a high proportion of fixed different 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 individual performance of the genotype for each characteristic of agronomic and zootechnical interest (Ferreira et al., 2003), besides the possibility and ease to have crossings between individuals of different genera (Paixão, 2012).

In the principal component analysis, the characteristics that contributed most to the total variance of the first component were CFM, CDM, H_2O and CA. In the second component, TPA, CAI and NC stood out; in the third component, CAR and PH (Table 5); in the analysis of canonical variables, the characteristics that contributed most to the total variance in the first variable were SCA and CAR, and in the second variable DESIR and WIL (Table 6). The most important characteristics are those whose weighting coefficients (eigenvectors) are of greater magnitude, in absolute value, in the first principal components or canonical variables (Cruz et al., 2012). Therefore, these would be the most responsive characteristics in the selection process among cactus pear populations.

In the relative contribution analysis of the characteristics for the genetic diversity among the seven varieties of cactus pear by Singh methodology (1981), H_2O contributed with 43.29% and CFM with 39.63% for the variability among genotypes. These two characteristics contributed with 82.92% of the total variability among the materials. The characteristics that contributed least to the divergence were CT, DESIR, SCA and DIS (Table 7). The lower contribution variables are little informative in the characterization of genetic variability, and can be discarded in genetic diversity studies (Rêgo et al., 2011).

Table 7. Relative contribution of 19 morphological and behavioral characteristics for the genetic diversity of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco, through Singh's methodology (1981).

Variable	Relative contribution (%)
CW	2.69
CL	2.86
CT	0.01
CA	0.13
CFM	39.63
CDM	0.89
H_2O	43.29
NC	0.23
PW	0,15
PH	0.23
TPA	2.66
CAI	0.37
FM	5.51
DM	0.83
DESIR	0.02
CAR	0.26
SCA	0.04
DIS	0.05
WIL	0.12

CW Cladode width, (cm) CL cladode length (cm), CT cladode thickness (cm), CA cladode area (cm^2), CFM cladode fresh matter (g), CDM cladode dry matter (g), H_2O water content of cladode (g), NC total number of cladodes, PW plant width (cm), PH plant height (cm), TPA total photosynthetic area of the plant (cm^2), CAI cladode area index, FM fresh matter production of the plant (g), DM dry matter production of the plant (g), DESIR Desirability, CAR Infestation by carmine cochineal (*Dactylopius opuntiae*), SCA infestation by cactus scale (*Diaspis echinocacti*), DIS Incidence of Diseases and WIL wilt Index.

Paixão (2012) and Ferreira et al. (2003) cite as most important characteristics for the divergence in genera *Opuntia* and *Nopalea*: CW, CL, CT, NC and CFM. Although this study present CFM and H_2O as the main characteristics of diversity, they are positively and significantly correlated in the ($p \leq 0.01$) and corelated to CDM, CW, CA, and in the ($p \leq 0.05$) with CL. These results indicate the importance of the morphological characteristics related to cladodes for the study of genetic diversity in cactus pear (Table 8).

The CDM was correlated positively and significantly with H_2O, CA, CT, CW, CL. The CW was positively correlated with CA. The CL was positively correlated with CA, PH and FM. The TPA was positively correlated with CAI. The PW was positively correlated with DESIR. The PH was positively correlated with CAR. FM was positively correlated with DM and SCA. The DM was positively correlated with DESIR. The NC was negatively correlated with CDM, CT, CW and AC. The WIL was negatively correlated with CDM, CT, CFM and H_2O (Table 8).

Most of the morphological characteristics of cladodes evaluated in this study correlated positively and significantly with each other. This is expected because they are characteristics related to agronomic production. Positive correlations among agronomic characteristics of these pests with the plant. An important aspect to consider is the CA of the genotype: the larger the area, the greater the H_2O and more resistant is the plant to water deficit and WIL.

This characteristic is of interest to the breeding program of cactus pear aimed at the selection of genotypes more tolerant to water deficit. In addition, the CA was positively correlated with the CFM and CDM, relevant agronomic characteristics for the production of forage for food and animal nutrition.

A priori, the selection of genotypes with increased production of CDM should be preferred, since variation in water content harms the nutritional calculations. The selection by CFM or CA can be used, since these characteristics are significantly and positively correlated with each other and would not require the determination of the CDM (Neder et al., 2013). According to Cruz et al. (2012), the existence of significant correlations among characteristics indicates the feasibility of indirect selection in order to obtain gains in the characteristic of great importance. The CFM and CA are very important characteristics in terms of technical and economic aspects of a rural property. For being of easy viewing and production measurement in the Brazilian semiarid region, it is used as calculation basis for sizing the number and flow of animals on farms (Amorim, 2011). The genotypes that obtained the highest means for production characteristics, water retention, and resistance to pests and diseases were 2 and 6 (Table 3). Then, crossings that involve these genotypes could generate superior progenies in characteristics of agronomic, zootechnical or physiological interests. (NC, CT, CL, CW, PH, PW, FM and DM) were also reported by Neder et al. (2013), who studied 19 accesses of cactus pear (*Opuntia ficus indica*) at 30 months of age, except for the NC, which was negatively correlated with CT and CW, corroborating the results of our work.

The positive correlations of PH and FM with CAR and SCA, respectively, are given by the greater contact area Thus, the 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, physiological, biochemical and chemical characteristics, and chromosomal variation for the determination of the genetic variability; thereby providing a complementary analysis to studies through morphological and behavioral characteristics. Additional studies must be performed in other locations and in several years.

Table 8. Correlations among morphological and behavioral characteristics of the seven varieties of cactus pear, genera *Opuntia* and *Nopalea*, grown in the semiarid region of Pernambuco

Variable	CFM	CDM	H_2O	CT	CW	CL	CA	NC	TPA	CAI	PW	PH	FM	DM	WIL	DIS	CAR	DESIR	SCA
CFM	1	0.94**	0.99**	0.64Ns	0.93**	0.81*	0.96**	-0.74ns	0.08ns	0.08ns	0.36ns	0.60Ns	0.73ns	0.36ns	-0.81*	-0.51ns	0.28ns	0.39ns	0.71ns
CDM	-	1	0.91**	0.83*	0.84*	0.80*	0.88**	-0.78*	-0.04ns	-0.04ns	0.41ns	0.59Ns	0.69ns	0.45ns	-0.88**	-0.48ns	0.27ns	0.50ns	0.66ns
H_2O	-	-	1	0.59Ns	0.94**	0.80*	0.96**	-0.71ns	0.11ns	0.11ns	0.34ns	0.59Ns	0.72ns	0.33ns	-0.78*	-0.51ns	0.28ns	0.35ns	0.70ns
CT	-	-	-	1	0.59ns	0.54ns	0.58Ns	-0.78*	-0.42ns	-0.42ns	0.39ns	0.41Ns	0.34ns	0.28ns	-0.87**	-0.39ns	0.34ns	0.53ns	0.31ns
CW	-	-	-	-	1	0.72ns	0.96**	-0.85*	-0.09ns	-0.09ns	-0.15ns	0.56Ns	0.47ns	0.02ns	-0.69ns	-0.38ns	0.32ns	0.13ns	0.51ns
CL	-	-	-	-	-	1	0.87**	-0.59ns	0.42ns	0.42ns	0.63ns	0.84*	0.76*	0.50ns	-0.61ns	-0.14ns	0.61ns	0.57ns	0.46ns
CA	-	-	-	-	-	-	1	-0.76*	0.14ns	0.14ns	0.37ns	0.71Ns	0.65ns	0.23ns	-0.71ns	-0.37ns	0.45ns	0.33ns	0.54ns
NC	-	-	-	-	-	-	-	1	0.43ns	0.43ns	-0.01ns	-0.35Ns	-0.18ns	0.12ns	0.63ns	0.08ns	-0.26ns	-0.08ns	-0.34ns
TPA	-	-	-	-	-	-	-	-	1	1.00**	0.51ns	0.41Ns	0.61ns	0.58ns	0.16ns	0.08ns	0.24ns	0.35ns	0.22ns
CAI	-	-	-	-	-	-	-	-	-	1	0.51ns	0.41Ns	0.61ns	0.58ns	0.16ns	0.08ns	0.24ns	0.35ns	0.22ns
PW	-	-	-	-	-	-	-	-	-	-	1	0.68Ns	0.72ns	0.74ns	-0.57ns	-0.34ns	0.72ns	0.94**	0.16ns
PH	-	-	-	-	-	-	-	-	-	-	-	1	0.57ns	0.38ns	-0.50ns	-0.26ns	0.80*	0.51ns	0.04ns
FM	-	-	-	-	-	-	-	-	-	-	-	-	1	0.85*	-0.64ns	-0.47ns	0.27ns	0.74ns	0.75*
DM	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.47ns	-0.36ns	0.13ns	0.83*	0.56ns
WIL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.69ns	-0.37ns	-0.68ns	-0.52ns
DIS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.05ns	-0.38ns	-0.38ns
CAR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.54ns	-0.30ns
DESIR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.32ns
SCA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

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

Conclusion

The varieties studied of cactus pear, genera *Opuntia* and *Nopalea*, present genetic divergence. The uni and multivariate methods used for the divergence differ and gather genotypes in two, three or four groups.

Conflict of interests

The authors have not declared any conflict of interests.

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