Morphometric Analysis of 21 Pitahaya (Hylocereus undatus) Genotypes*

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ABSTRACT

Morphological variation was studied in 21 pitahaya genotypes using multivariate analysis. Genotypes were classified by the color of the skin and pulp of the fruit into white, red, magenta, and yellow in order to determine whether other characteristics would also help discriminate among them. The results of principal-component analysis based on 47 features showed three groups of pitahayas, while canonical discriminant analysis analyzing only 28 features selected by the principal-component analysis gave four groups. In both analyses, the magenta and red pitahaya types were assigned to different groups, while yellow and white types, although classified into different groups, were more similar. The differentiation between groups was mainly related to stem features, such as the size of the concave angle, height of the angle vertex, undulation height, and spine number and length. The number and length of internal perianth segments were the most important flower features. Variation in stem features corresponded to variation in *H. undatus*. The four groups were thus considered to belong to this species.

Keywords: Cactaceae, cacti, canonical discriminant analysis, edible fruit, pitahayas, principal-component analysis, stem variation.

INTRODUCTION

The genus *Hylocereus* (Berger) Britton et Rose is widely distributed in the intertropical region of America (Britton and Rose, 1920; Bravo-Hollis, 1978; López, 1996; Rodríguez, 2000) and the genus is well known for its edible fruits all over the world (Tel-Zur *et al.*, 2005). In the circumscription of the genus we follow Anderson (2001) who accepted 18 species. However, Bauer (2003) gave a synopsis of the tribe Hylocereeae in which he recognized *Hylocereus* with 19 species, four of these species were previously classified in the section *Salmdyckia* of *Selenicereus*. Published molecular data suggest that *Hylocereus* and *Selenicereus* are sister taxa, closely related to *Weberocereus*, *Disocactus*, and *Acanthocereus* (Niffeller, 2002; Arias *et al.*, 2005). *Hylocereus* and *Selenicereus* close relationship is supported by their intergeneric hybrid produced in cultivation (Ter-Zur *et al.*, 2004, 2005).

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The three species of *Hylocereus* found in Mexico are *H. undatus, H. purpusii*, and *H. ocamponis*. Bravo-Hollis (1978) distinguished them by variations in their stem color, number and length of spines per areole, and characteristics of outer perianth segments. Natural populations of these three species in the tropical forests of Mexico have been a food source for inhabitants of the regions where they have grown since before the arrival of the Spaniards to the New World (Rodríguez, 2000). The forms of pitahaya, which differ in the colors of their fruits, are known locally as white, magenta, red, and yellow types. In studies of pitahaya, these types are either considered to be different species (Cálix, 1996; Castillo *et al.*, 1996) or variations of *H. undatus* (Mizrahi *et al.*, 1997; Castillo *et al.*, 1999, 2005). The controversy in identifying pitahaya materials is partly due to the custom of regarding fruit color as the sole criterion for defining species, a practice which lacks a firm theoretical taxonomic basis. In addition, various studies with domesticated cacti species have shown that variation in fruit traits, including color, are related to this process of domestication (Arnaud *et al.*, 1997; Casas *et al.*, 1999; Luna-Morales and Aguirre, 2001; Cruz and Casas, 2002; Arellano and Casas, 2003; Otero-Arnaiz *et al.*, 2003; Carmona and Casas, 2005).

In plant populations with divergent morphological patterns, joint analysis of plant characteristics serves as a way to understand the limits of population variation and to phenetically identify similar groups by the similarities between their characteristics (Rhodes *et al.*, 1970; Orozco, 1991). Principal component analysis (PCA), canonical discriminant analysis (CDA), and cluster analysis (CA) jointly analyze different characteristics and identify which characteristics are most important for distinguishing populations (González-Andrés and Ortiz, 1996; Nieto-Angel *et al.*, 1997; Casas *et al.*, 1999; Kephart *et al.*, 1999; Levin, 1999).

In this study, 47 stem, flower, and fruit characteristics were examined in 21 pitahaya genotypes. They were divided by fruit color into magenta, red, yellow, and white using PCA, CDA and CA, with the main purpose being to compare variation among pitahayas and to identify the most important traits for group formation.

MATERIALS AND METHODS

Twenty-one pitahaya genotypes were selected and grouped by their fruit characteristics into four types: white (red skin, white flesh), yellow (yellow skin, white flesh), magenta (red skin, magenta flesh) and red or fine (red skin and flesh). Six white pitahaya genotypes from private family orchards in Xochitlan, Puebla (WP1 and WP2) were studied, three from land adjoining uncultivated lands in Cárdel, Veracruz (WV21, WV22, and WV23), and one from a plantation (WHA) in Halacho, Yucatan. There were seven yellow pitahaya genotypes (Y6, Y7, Y11, Y18, Y28, Y29, Y39), of which two (Y6, Y7) were from the state of Quintana Roo, and the other five from Yucatan (Sucila Y11, Libre Unión Y18, Chapab Y28, Y29, Hecelchakan Y39). These genotypes are all in the Germplasm Bank at the University of Chapingo Botanical Garden in the Yucatan Peninsula. Five magenta genotypes (M1, M2, M3, M4, M5) from private family orchards in Xochitlán, Puebla were studied, as were three red pitahaya genotypes (R1, R2, R3) from private family orchards in Tehuacan, Puebla. Each genotype was represented by five individuals taken from the same site. White, red, and magenta genotype specimens were all more than ten years old, while the plants representing the yellow genotypes were less than five years old.

A total of 47 vegetative and reproductive morphological characteristics were recorded for each genotype (Table 1). Ten of these were stem characteristics, 23 flower and 14 fruit. Stem measurements were taken from ten young branches per individual. Stem lengths were measured using measuring tape, while rib characteristics, such as depth, height at the angle vertex, and undulation height, were measured with a vernier (Scala), as were areole diameter, distance between areoles, and spine length. The concave angle of the opening and the undulation area were obtained from drawings of the rib contours (Figure 1a),

captured using a scanner and processed using the Image Tool program (Wilcox *et al.*, 1996). All rib characteristics were measured in the middle section of selected branches.

Flower characteristics were evaluated from samples of three to eight flowers per individual (Figure 1b), and fruit characteristics from 5 to 15 mature fruits sampled per individual. Maturity was determined by change in pericarp color from green to red or yellow. Peel thickness and Brix values were determined by cutting a cross-section through the middle of the fruit and taking six readings per fruit. Thickness was measured using a vernier (Scala) and Brix by a manual refractometer (American Optical). Seed weight was obtained from a sample of 500 seeds per fruit. Seed area, perimeter, length, width and roundness index were determined from a sample of 20 seeds per fruit. Measurements were made from scanned images of the seeds and processed using the Image Tool program (Wilcox *et al.*, 1996). The formula for calculating roundness index (RI) provided by the package is $RI = (4 \times \pi \times area)/perimeter^2$.

In order to homogenize the variance and meet the necessary multivariate analysis assumptions, mathematical transformations were applied to characteristics evaluated on different scales (Sneath and Sokal, 1973; Hair *et al.*, 1995). A square-root transformation was applied to undulation height and area; areole diameter; spine length; width of internal, intermediate, and external perianth segments; pericarp segment width; nectar chamber width; ovary width; style diameter; fruit bract width; skin thickness; weight of 500 seeds; and maximum projected area, perimeter, length, width, and roundness index of the seed. Characteristics transformed by taking logarithms to base 10 were stem branch length, height at the angle vertex, rib depth, angle opening, areole separation, spine number, flower length, number and length of internal, intermediate and external perianth segments, number and length of stamens, style length, number and length of stigma lobules, fruit weight, length and width, number and length of fruit bracts, and Brix value.

The transformed values were used as inputs to the principal-component analysis (PCA) procedure. The analysis was carried out on the correlation matrix, as the variables had different scales. The variables most important for describing population variation were selected based on maximum values of the eigenvectors and on the correlation coefficient (>0.6) of each variable in the first three principal components. The correlation coefficient was calculated as $CC = (\lambda)^{\frac{1}{2}} X EV$, where λ is the eigenvalue and EV is the value of the eigenvectors of each principal component. Dispersion of the 21 genotypes was graphed for the first two principal components. A canonical discriminant analysis (CDA) was used to test the hypothesis that pitahaya genotypes are separated into different groups on the basis of the selected characteristics by PCA. The variables most important in defining groups were identified using the values of the standardized simple matrix. The statistical differences between groups were measured using Mahalanobis distances. The groups were represented graphically using the first and second discriminant functions. Standard errors of the means were also compared by a Tukey test (p>0.05) for selected characteristics in the first and second discriminant functions. All statistical analyses were performed with SAS software (SAS Institute, 1989). To verify the groups using diagnostic characteristics selected by the CDA, the 21 pitahaya clones were reclassified by the UPGMA method. Before grouping, the data matrix was standardized and the similarity matrix calculated using the distance coefficient. The analysis was carried out using the NTSYS software (Rohlf, 1998).

RESULTS

The PCA using 47 characters for 21 pitahaya genotypes explained 60% of the variation in the first three principal components. The first component explained 28% of the variation. The most important stem characteristics were vertex angle height, undulation height, undulation area, size of the angle, areole distance, areole diameter, number of spines per areole, and spine length. The most important flower

characteristics were nectar chamber length and width, and stigma lobule length, and in the fruit, weight, length, width, number of bracts, and pericarp thickness. Component 2 contributed 21% of the variation, and the most important stem characteristic was rib depth. In the flower, the most important characteristics were number of perianth inner and intermediate segments; perianth inner segment length; pericarp bracts number, length, and width; and ovary length. In the fruit, bract width was the most important. Component 3 explained 11% of the remaining variation; the most important characteristics were ovary width in the flower and seed roundness index in the fruit (Table 2). The first component clearly separated the magenta pitahaya from the others, while the second component was most suitable for separating the red pitahaya from the white and yellow types. White and yellow pitahayas were classified as a single group (Figure 2).

Using CDA on the 28 characters selected by PCA did separate the 21 pitahaya genotypes into four groups (Figure 3). The first three discriminant functions explained 100% of the variation among pitahaya genotypes. The first discriminant function contributed (Eigenvalue 62.62) 63% of the variance, the second 32% (Eigenvalue 31.91) and the remaining 5% (Eigenvalue 5.08) was accounted for by the third discriminant function. The characteristics in the first discriminant function that contributed to classifying the pitahayas into four groups were angle size and the number of spines per areole. In the second discriminant function, the characteristics were vertex angle height, undulation height, spine length, and number of perianth inner segments. The only important characteristic in the third discriminant function was perianth inner segments length (Table 3, Figure 1b). Selected characteristics from the first two discriminant functions are compared in Figures 4 and 5, where it can be seen that the genotypes of the magenta type are statistically different in angle size, varying from 88 to 107 degrees, while in the other genotypes, the angle ranges from 137 to 165 degrees (Figure 4a). Angle vertex heights are similar in red and magenta types (1 to 1.5 cm) and significantly lower in yellow and white pitahayas (2 to 2.7 cm) (Figure 4b). Undulation height was significantly greater in magenta genotypes (0.8 to 1.4 cm), clearly distinguishing them. Other types had undulation height below 0.6 cm, without statistically significant differences between genotypes (Figure 4c). The number of spines per areole helped distinguish the magenta and red types from the yellow and white types. Red and magenta types generally had fewer than 3 spines, while yellow and white types had 3 to 5. The magenta type differed from the red in having only one spine per areole (Figure 5a). The magenta genotypes could also be distinguished by their spine lengths. The longest spines were observed in magenta types, although the WV21 and WV23 white types also had spines as long (Figure 5b). The number of inner perianth segments was a distinctive characteristic of red genotypes, which had 5 to 10 segments more than other pitahayas (Figure 5c). The remaining types did not differ significantly between each other.

The separation into four groups by the CDA was strongly significant (Wilks' λ , P<0.0001, n=105). The analysis also showed that the squared Mahalanobis distances between groups were highly significant (P<0.0001); 100% of the observations were classified correctly (Table 4).

The phenogram obtained shows a separation between the magenta genotypes and the others at a dissimilarity level of 1.76 (Figure 6). The genotypes of the second group were divided into two groups, with a dissimilarity level of 1.53. One of these included the red type and the other the yellow and white types, which in turn, diverged with a dissimilarity level of 1.02. Except for genotypes of the white group, the level of similarity of genotypes within each group was high (≈ 0.50). The white genotypes consisted of two subgroups; one from Veracruz, and the other from Puebla and Yucatán (Figure 6).

DISCUSSION

For the identification of *Hylocereus* species recorded in Mexico, it has been claimed that stem characteristics are the most important, followed by certain flower characteristics (Bravo-Hollis, 1978; Scheinvar, 1985). The findings of the present multivariate analysis of 21 pitahaya genotypes were

consistent with these results; stem characteristics were more important than flower characteristics for separation into groups of *H. undatus* (Table 3).

Six characteristics were identified by the CDA as the important characteristics for separation into four groups. Two of these (height at vertex and spine number) distinguished the magenta and red groups from the yellow and white groups, and the magenta group was differentiated from the remaining groups by angle size and undulation height. The red group was distinguished from the others by the number of perianth segments. The characteristic that varied most among groups was spine length.

The fact that genotypes can be sorted into their respective groups in spite of different procedures and data handling (white and yellow) suggests that the differences between groups do have a genetic basis. When mankind domesticates plants, characteristics of interest to humans (Casas *et al.*, 1997; Mapes *et al.*, 1997; Casas *et al.*, 1999), such as fruit size, color and shape, are favored. This is true of hot peppers (Pickersgill *et al.*, 1979), pitayas (Pimienta-Barrios and Nobel, 1994; Casas *et al.*, 1997) and *Leucaena esculenta* (Casas and Caballero. 1996). Genetic variation created during the domestication process is generally different from that found in wild plants (Casas *et al.*, 1999). This may explain why the red, magenta, and yellow pitahaya phenotypes grown in private orchards are not found in the wild. Another result of domestication is the reduction of within-group variability, which has contributed to the separation between four groups of pitahayas clearly differentiated by color. In this study, it was confirmed that they are differentiated on the basis of mainly vegetative characteristics. The white group, corresponding perhaps to the typical color of the species, incorporates more variability than any of the other genotypes and may be useful in future breeding programs.

It would be difficult to recognize the four groups as different species on the basis of morphological stem differences. Similar conclusions were reached by Ramírez (1999), who analyzed the morphology of white, red, and magenta genotypes, and Grimaldo *et al.* (2001), who carried out chromosome comparisons of the four groups. The variation found in the four pitahaya groups corresponds to that reported by Bravo-Hollis (1978), for *H. undatus*, especially with respect to the number of spines per areole and their length, which together with the green color of the stems, set this species apart. Other characteristics peculiar to the species are the red skin and white flesh of its fruits. Recently, Cálix de Dios (2005) proposed a subspecies, *H. undatus* subsp. *luteocarpus* based mainly on the yellow fruit color and the presence of hairs in the areoles. With the exception of the presence of hairs in the areoles. With the recognized as variations of *H. undatus* related to the process of domestication. To support the recognition of more than one subspecies needs to wait further evidence.

In Mexico, although *H. purpusii* and *H. ocamponis* have red skin and flesh, this is not a determining characteristic for identification of these species. Bravo-Hollis (1978) points to the bluish, glaucous stems, and areoles having three to six short spines as distinguishing characteristics of *H. purpusii*, while *H. ocamponis* can be recognized by its stems of the same color, but areoles of five to eight spines that measure 5 to 12 mm. These characteristics are clearly different than those recorded in the red and magenta groups, genotypes which, in both species, share the same fruit color. The results suggest that variations in fruit color are part of *H. undatus*, as stated by Mizrahi et al. (1997), describing the flesh of *H. undatus* as varying from white to red. Fruit color variation is also considered part of the species *Stenocereus stellatus* (Pimienta-Barrios and Nobel, 1994; Arnaud *et al.*, 1997; Casas *et al.*, 1999).

In conclusion, PCA, CDA, and CA confirmed the separation of pitahaya into magenta, red, yellow, and white groups. The first three had a similar high level of morphological similarity (0.50) between genotypes, while the white group was more variable, showing characteristics typical of *H. undatus*. The variability of this group represents greater capacity to change in response to its environment, demonstrating different phenotypes, which are selected by man as suggested for yellow, red and magenta

pitahayas. Further work might involve molecular comparisons of the four groups identified in the present study, to confirm that the variations in fruits do correspond to the *H. undatus* species. Such studies might include *H. purpusii* and *H. ocamponis* as reference species.

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Number	Character	Number	Character
1	Branch length (cm)	25	Nectar chamber width (cm)
2	Vertex angle height (cm)	26	Ovary length (cm)
3	Rib depth (cm)	27	Ovary width (cm)
4	Undulation height (cm)	28	Stamens number
5	Undulation area (cm ²)	29	Stamens length (cm)
6	Angle size (degrees)	30	Style length (cm)
7	Distance between areoles (cm)	31	Style diameter (cm)
8	Areola diameter (cm)	32	Number of lobules per stigma
9	Number of spines per areole	33	Lobules length (cm)
10	Spines length (cm)	34	Fruit weight (g)
11	Flower length (cm)	35	Fruit length (cm)
12	Number of perianth inner segments	36	Fruit width (cm)
13	Perianth inner segments length (cm)	37	Number of bracts per fruit
14	Perianth inner segments width (cm)	38	Fruit bracts length (cm)
15	Number of perianth intermediate segments	39	Fruit bracts width (cm)
16	Perianth intermediate segments length	40	Brix degrees
17	Perianth intermediate segments width (cm)	41	Pericarpel thickness (cm)
18	Number of perianth outer segments	42	Weight of 500 seeds (g)
19	Perianth outer segments length (cm)	43	Maximum projected seed area (mm ²)
20	Perianth outer segments width (cm)	44	Seed perimeter (cm)
21	Number of bracts in the pericarpel	45	Seed length (cm)
22	Pericarpel bract length (cm)	46	Seed width (cm)
23	Pericarpel bract width (cm)	47	Seed roundness index
24	Nectar chamber length (cm)		

Table 1. Morphometric characters of Hylocereus undatus	evaluated.
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		Principal Components							
Chanastan	1		2		3				
Character	EV	СС	EV	CC	EV	СС			
1	0.142	0.521	-0.175	-0.549	0.169	0.384			
2	0.177	0.650*	0.206	0.646	0.091	0.206			
3	0.050	0.183	0.211	0.662*	0.212	0.482			
4	-0.234	-0.859*	-0.058	-0.182	0.140	0.318			
5	-0.232	-0.852*	-0.047	-0.147	0.117	0.266			
6	0.231	0.848*	0.061	0.191	-0.048	-0.109			
7	-0.167	-0.613*	-0.165	-0.518	-0.032	-0.072			
8	0.182	0.668*	0.189	0.593	0.071	0.161			
9	0.221	0.811*	0.178	0.559	0.018	0.040			
10	-0.204	-0.749*	0.059	0.185	0.105	0.238			
11	0.191	0.701*	0.018	0.056	0.100	0.227			
12	0.053	0.194	-0.280	-0.879*	-0.106	-0.241			
13	0.079	0.290	0.231	0.725*	0.078	0.177			
14	0.056	0.205	-0.082	-0.257	-0.095	-0.216			
15	0.080	0.293	-0.267	-0.838*	0.004	0.009			
16	-0.037	-0.135	0.185	0.581	0.193	0.438			
17	-0.065	-0.238	-0.060	-0.188	-0.049	-0.111			
18	0.132	0.484	-0.267	-0.838*	-0.072	-0.163			
19	-0.116	-0.426	0.093	0.292	-0.212	-0.482			
20	-0.096	-0.352	0.053	0.175	-0.199	-0.452			
21	0.109	0.400	-0.233	-0.731*	-0.071	-0.161			

 Table 2. Eigenvector (EV) and correlation coefficient (CC) for the 47 variables in the first three principal components analyzed in 21 genotypes of pitahaya (*Hylocereus undatus*).

	Principal Components						
	1		2	2		3	
Character	EV	СС	EV	CC	EV	СС	
22	-0.085	-0.312	0.207	0.650*	-0.024	-0.054	
23	-0.024	-0.088	0.227	0.712*	-0.204	-0.463	
24	0.117	0.429	-0.046	-0.144	0.203	0.461	
25	-0.167	-0.613*	-0.019	-0.059	-0.112	-0.254	
26	0.049	0.179	-0.260	-0.816*	0.170	0.386	
27	-0.027	-0.099	0.010	0.031	-0.272	-0.618*	
28	0.127	0.466	-0.083	-0.260	-0.249	-0.256	
29	-0.072	-0.264	-0.076	-0.238	0.127	0.288	
30	0.155	0.569	0.100	0.314	0.040	0.090	
31	-0.080	-0.293	0.114	0.358	-0.169	-0.384	
32	0.147	0.539	-0.139	-0.436	0.079	0.179	
33	0.230	0.844*	-0.055	-0.172	-0.058	-0.131	
34	0.263	0.966*	0.026	0.081	0.018	0.040	
35	0.244	0.896*	-0.081	-0.254	0.015	0.034	
36	0.250	0.918*	0.041	0.128	0.072	0.163	
37	0.170	0.624*	-0.179	-0.562	-0.087	-0.197	
38	0.063	0.231	0.106	0.333	-0.168	-0.382	
39	0.073	0.268	0.294	0.923*	0.008	0.018	
40	-0.129	-0.473	-0.035	-0.109	-0.141	-0.320	
41	0.197	0.723*	0.124	0.389	-0.064	-0.145	
42	-0.040	-0.146	0.035	0.109	0.035	0.079	
43	0.006	0.022	0.018	0.056	0.173	0.393	
44	-0.075	-0.275	-0.038	-0.119	0.162	0.368	

			Principal C	Components		
- Character	1		2		3	
	EV	CC	EV	CC	EV	CC
45	-0.095	-0.348	0.009	0.028	0.255	0.579
46	-0.160	-0.587	-0.001	-0.003	0.231	0.525
47	-0.094	-0.345	0.065	0.204	-0.329	-0.748*

*Selected characters

Discriminant function				Disc	riminant fun	ction	
Characters	1	2	3	Characters	1	2	3
2	0.637	1.237*	-0.873	21	0.103	-0.640	-0.398
3	-0.471	-0.657	0.741	22	-0.062	0.309	0.262
4	0.541	1.049*	-0.419	23	0.497	-0.436	-0.868
5	-0.078	0.438	-0.742	25	-0.257	0.699	-0.192
6	2.436*	-1.075	-1.290	26	0.064	-0.487	0.488
7	-0.338	-0.162	0.200	27	-0.067	0.150	-0.191
8	0.144	-0.737	-0.737	33	0.489	-0.535	-0.007
9	4.990*	2.344	1.783	34	-0.231	-0.087	0.900
10	0.927	1.496*	1.397	35	0.533	-0.453	-0.600
11	-0.206	0.120	-0.163	36	-0.376	0.648	0.566
12	0.930	-1.003*	0.058	37	0.193	-0.596	0.390
13	0.105	0.268	1.112*	39	0.629	-0.529	-0.243
15	0.070	-0.664	0.656	41	0.124	0.012	0.012
18	0.206	-0.885	0.652	47	0.132	0.241	0.007

Table 3. Twenty-eight morphometric characters selected by PCA used in canonical discriminant analysis and their partial contribution to the functions expressed by standardized coefficients of discriminant functions.

*Selected characters

Fruit type	Yellow	White	Red	Magenta
Yellow	0			
White	60.35 (25.5)	0		
Red	238.33 (65.4)	404.79 (105.9)	0	
Magenta	327.22 (124.8)	368.99 (131.6)	327.20 (80.2)	0

Table 4. Mahalanobis distance for square distance among pitahayas (Hylocereus undatus	;)
fruit types and F values in parenthesis with P<0.0001 in all comparisons.	



Figure 1. Diagram showing some characters evaluated in *Hylocereus undatus*. a) stem segment. b) flower.



Figure 2. Principal-component analysis based on 47 morphometric characteristics of 21 genotypes of pitahaya (*Hylocereus undatus*). Each genotype is represented by 5 repetitions.
● = magenta, □ = white, ▲ = yellow, ◆ = red



Figure 3. Ordination of 21 genotypes of pitahaya (*Hylocereus undatus*) in the four fruit types using two canonical discriminant analysis functions. \bullet = magenta, \Box = white, \blacktriangle = yellow, \blacklozenge = red







Figure 5. Mean (μm) and standard deviation of the morphometric characteristics:
a) number of spines per areole, b) spines length, c) number of inner perianth segments of the 21 genotypes of pitahaya (*Hylocereus undatus*).



Distance coefficient

Figure 6. UPGMA phenogram showing clustering of 21 genotypes of pitahaya (*Hylocereus undatus*) based on 28 characteristics selected by PCA (Table 2).