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RESEARCH ARTICLE

# Phenotypic and genomic characterization of vine cactus collection (Cactaceae)

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**Abstract** *Hylocereus* (Berger) Britton et Rose, *Selenicereus* (Berger) Britton et Rose and *Epiphyllum* Haw. species have commercial potential as exotic fruit crops in semi-arid and arid lands. The high genetic variability among these species offers an opportunity for commercial cultivation. Toward this end we investigated genomic and morphological characteristics including: nuclear DNA content (2C-values), stomatal length and density, potential yield and reproductive parameters in 64 *Hylocereus*, *Selenicereus* and *Epiphyllum* accessions. Nuclear DNA content ranged from 3.21 pg for *S. grandiflorus* (L.) Britton et Rose spp. *grandiflorus* to 8.77 pg for *H. megalanthus* (Vaup.) Bauer. All species were diploid except the tetraploids *H. megalanthus* and *S. vagans* (Bgek.) Britton et Rose. Stomatal length and density, fruit weight, potential yields, number of viable seeds per fruit and fruit maturation times were highly variable among accessions. No significant correlations were found between stomatal length, density, and nuclear DNA content, nor between fruit weight and seed number. The high genetic variability found between the accessions here provides further support for the excellent prospects of conserving and domesticating these exotic species.

**Keywords** *Epiphyllum* · Flow cytometry · Fruit traits · *Hylocereus* · Polyploidy · *Selenicereus* · Stomata length and density

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## Introduction

Vine cacti are night blooming epiphytes, endemic to the Americas, and belong to Cactaceae, subfamily Cactoideae, tribe Hylocereeae (Britton et Rose) Buxbaum (Barthlott and Hunt 1993). According to the New Cactus Lexicon (Hunt 2006), the genera *Hylocereus* (Berger) Britton et Rose, *Selenicereus* (Berger) Britton et Rose and *Epiphyllum* Haw. comprise 14, 12 and 12 species, respectively. Cacti are exceptionally tolerant to extreme drought having Crassulacean acid metabolism (CAM), a significant benefit for dryland agriculture. Increased interest in vine cactus species has emerged due to their economic potential as exotic fruit crops (Mizrahi and Nerd 1999; Hammer 2001). The edible fruits, known as Pitahaya or Dragon fruit, are currently being marketed worldwide. *Hylocereus* species are characterized by

triangular stems, bearing large edible fruits with broad scales and various peel and flesh colours (Lichtenzveig et al. 2000). *Selenicereus* species have multi-ribbed stems with short spines and bear fruit with a spiny peel (Barthlott and Hunt 1993). *Hylocereus megalanthus* (Vaup) Bauer [=*S. megalanthus* (Schum. ex Vaupel) Moran], resembles *Hylocereus* in its vegetative appearance, but bears tasty medium sized yellow fruit with a spiny peel (Weiss et al. 1994). *Epiphyllum* species have flat, leaf-like spineless stems that are initially four-ribbed, generally becoming two-ribbed upon maturation and bearing small red–purple good tasting fruit (Barthlott and Hunt 1993).

In Cactaceae the base chromosome number is $x = 11$. *Hylocereus undatus*, *H. monacanthus*, *H. ocamponis*, *H. costaricensis* and *S. grandiflorus*, all $2n = 22$, are diploids (Beard 1937; Spencer 1955; Ross 1981; Lichtenzveig et al. 2000; Tel-Zur et al. 2001, 2004; http://mobot.mobot.org/W3T/Search/ipcn.html) while *H. megalanthus*, at $2n = 44$, is tetraploid (Lichtenzveig et al. 2000). Determining ploidy level by chromosome count is technically challenging in many plant groups, thus flow cytometry is used as reliable and rapid estimate of DNA content in cases where chromosome counts have been reported for closely related species (Hcini et al. 2006; Segura et al. 2007). Stomatal features including length and density often can be used as a fast and inexpensive screening technique to assess ploidy. Statistically significant differences in stomatal length and/or density are often strongly correlated with ploidy level (Beaulieu et al. 2008; Inceer and Hayirlioglu-Ayaz 2010).

A collection that includes 20 taxonomically identified species of the genera *Hylocereus*, *Selenicereus* and *Epiphyllum* (Table 1) was established in 1984 at Ben-Gurion University of the Negev (BGU), Israel (Mizrahi and Nerd 1999; Tel-Zur et al. 2004; http://www.bgu.ac.il/life/Faculty/Mizrahi/Gene.html) for developing potential crops for sustainable agriculture in semi-arid and arid lands. The objective of this study was to investigate morphological, reproductive and genomic variation among these vine cacti accessions. The specific research objectives included: determination of nuclear DNA content as an estimate of ploidy level; testing the relation between ploidy level and stomatal length or density; evaluation of potential yield (flowers per plant); and assessment of reproductive parameters such as fruit weight, number of viable seeds per fruit and minimum days to ripening.

## Materials and methods

### Plant material and study site

Vine cactus species in the genera *Hylocereus*, *Selenicereus* and *Epiphyllum* were characterized morphologically and their genome sizes were measured. Plants with unknown taxonomical identification were designated by genus name followed by "sp". A total of 44 *Hylocereus* accessions were studied: *H. costaricensis* (3), *H. monacanthus* (9), *H. undatus* (13), *H. ocamponis* (2), *H. triangularis* (1), *H. bronxensis* (1), *H. escuintlensis* (1), *H. guatemalensis* (1), *"H. zabinski"* (1), and *H. megalanthus* (9), and three morphologically diverse samples (*H.* sp.). A total of 14 *Selenicereus* accessions were studied: *S. grandiflorus* (4), *S. atropilosus* (1), *S. hamatus* (1), *S. inermis* (2), *S. pteranthus* (2), *S. spinulosus* (1), *"S. triangularis"* (1), *S. vagans* (1), and *S.* sp. (1). *Strophocactus testudo* and *Disocactus flagelliformis* originally identified as *Selenicereus testudo* and *Selenicereus innesii*, respectively were also studied. A total of four *Epiphyllum* accessions were studied: *E. oxypetalum* (1) and three morphologically diverse samples that likely represent different species (*E.* sp.).

Plants were grown in a greenhouse located on the Bergmann Campus in Beer-Sheva (31°15′N and 34°48′E). Data were collected from 2004–2010. Plant husbandry was managed as described by Lichtenzveig et al. (2000).

### Flow cytometric analysis and ploidy estimation

Approximately 300 mg of fresh tissue from young stems of each accession was sampled to prepare nuclear suspensions (Doležel et al. 1989; Cisneros and Tel-Zur 2010). Estimation of nuclear DNA content was determined as described by Cisneros and Tel-Zur (2010). $G_0/G_1$ peak positions of diploid *H. monacanthus* and tetraploid *H. megalanthus* were compared, using *Pisum sativum* L. as an internal standard with a reference genome size 9.09 pg of DNA. Each accession was analyzed at least three times to verify reproducibility.

**Table 1** Accession number, country of collection, stomata length and density, mean nuclear DNA (2C) content and estimation of ploidy level according to flow cytometric analysis

| Species | Accession number | Source (country) | Nuclear DNA content pg/ 2C ± SD | Ploidy reported[a] | Ploidy estimated | Stomata | |
|---|---|---|---|---|---|---|---|
| | | | | | | Length (μm) ± SE | Density ($mm^2$) ± SE |
| *Hylocereus costaricensis* (Web.) Britton et Rose | 89-023 | Costa Rica | 4.28 ± 0.08 | 2x | 2x | 49.7 ± 1.0 | 20.3 ± 0.4 |
| | 73-12-32 | Nicaragua | 4.14 ± 0.10 | NR | 2x | 59.4 ± 1.3 | 17.3 ± 1.0 |
| | 99-854 | USA | 4.02 ± 0.10 | NR | 2x | 49.8 ± 0.7 | 16.2 ± 0.6 |
| *H. monacanthus* (Lem.) Britton et Rose | 88-029[b] | USA | 4.05 ± 0.28 | NR | 2x | 58.0 ± 0.6 | 12.7 ± 0.5 |
| | 74-12-08[b] | Surinam | 4.28 ± 0.19 | NR | 2x | 70.0 ± 1.9 | 13.2 ± 0.4 |
| | 72-04-18[b] | Dominican Republic | 4.28 ± 0.13 | NR | 2x | 64.5 ± 1.2 | 18.1 ± 0.9 |
| | 89-028[c] | Nicaragua | 3.89 ± 0.13 | 2x | 2x | 52.3 ± 0.6 | 10.3 ± 0.5 |
| | 89-027[c] | Nicaragua | 3.84 ± 0.22 | 2x | 2x | 50.4 ± 0.8 | 12.1 ± 0.4 |
| | 97-401[c] | Ecuador | 3.97 ± 0.07 | NR | 2x | 49.3 ± 1.0 | 10.3 ± 0.4 |
| | 97-403[c] | Panama | 3.86 ± 0.17 | NR | 2x | 39.9 ± 0.9 | 10.1 ± 0.5 |
| | 97-404[c] | Nicaragua | 3.81 ± 0.24 | NR | 2x | 59.0 ± 1.3 | 12.0 ± 0.5 |
| | 99-858[d] | USA | ND | NR | ND | 60.6 ± 0.5 | 13.6 ± 0.6 |
| *H. undatus* (Haw.) Britton et Rose | 70-02-09 | Mexico | 4.40 ± 0.26 | NR | 2x | 61.7 ± 0.5 | 11.5 ± 0.4 |
| | 88-027 | Colombia | 4.10 ± 0.28 | 2x | 2x | 60.9 ± 0.7 | 9.7 ± 0.4 |
| | 98-334 | Mexico | 3.69 ± 0.17 | NR | 2x | 50.3 ± 1.7 | 7.7 ± 0.5 |
| | 87-601 | Israel | 3.63 ± 0.23 | NR | 2x | 56.8 ± 1.2 | 7.6 ± 0.2 |
| | 89-026 | Mexico | 3.76 ± 0.13 | NR | 2x | 56.7 ± 1.4 | 5.7 ± 0.3 |
| | 92-053 | Israel | 4.35 ± 0.24 | NR | 2x | 61.6 ± 1.5 | 6.7 ± 0.3 |
| | 94-007 | Vietnam | 3.84 ± 0.29 | NR | 2x | 58.2 ± 1.2 | 9.1 ± 0.3 |
| | 95-004 | Mexico | 4.23 ± 0.16 | NR | 2x | 64.2 ± 1.2 | 5.7 ± 0.4 |
| | 70-02-02 | Mexico | 4.20 ± 0.31 | NR | 2x | 69.0 ± 1.3 | 6.7 ± 0.4 |
| | 70-02-07 | Mexico | 3.99 ± 0.21 | NR | 2x | 69.9 ± 1.5 | 6.2 ± 0.3 |
| | 72-06-06 | Australia | 3.82 ± 0.18 | NR | 2x | 59.2 ± 1.2 | 9.4 ± 0.6 |
| | 89-024 | Virgin Island | 3.86 ± 0.12 | NR | 2x | 60.2 ± 1.4 | 8.0 ± 0.6 |
| | 98-338 | Mexico | 4.09 ± 0.30 | NR | 2x | 55.0 ± 0.8 | 7.1 ± 0.5 |
| *H. ocamponis* (S-D.) Britton et Rose | 89-025[e] | Mexico | 3.90 ± 0.23 | 2x | 2x | 86.0 ± 1.5 | 16.7 ± 0.9 |
| | 94-031 | Italy | 3.81 ± 0.21 | 2x | 2x | 90.6 ± 4.0 | 8.7 ± 0.3 |
| *H. triangularis* (L.) Britton et Rose | 71-05-03 | Dominican Republic | 3.73 ± 0.21 | NR | 2x | 66.2 ± 0.6 | 11.8 ± 0.3 |
| *H. bronxensis* Britton et Rose[f] | 99-856 | USA | 3.98 ± 0.27 | NR | 2x | 70.6 ± 1.0 | 14.6 ± 0.5 |
| *H. escuintlensis* Kimnach | 99-853 | Guatemala | 3.55 ± 0.13 | NR | 2x | 55.7 ± 0.5 | 17.5 ± 0.9 |

**Table 1** continued

| Species | Accession number | Source (country) | Nuclear DNA content pg/ 2C ± SD | Ploidy reported[a] | Ploidy estimated | Stomata | |
|---|---|---|---|---|---|---|---|
| | | | | | | Length (μm) ± SE | Density ($mm^2$) ± SE |
| *H. guatemalensis* (Eich.) Britton et Rose | 70-11-07 | USA | 4.12 ± 0.25 | NR | 2x | 86.6 ± 1.8 | 11.3 ± 0.7 |
| *"H. zabinski"*[g] | 99-855 | USA | 3.92 ± 0.08 | NR | 2x | 66.3 ± 0.8 | 16.4 ± 1.3 |
| *H. megalanthus* (Vaup.) Ralf Bauer | 70-11-05 | USA (Hawaii) | 8.77 ± 0.29 | NR | 4x | 80.8 ± 0.5 | 4.8 ± 0.3 |
| | 88-023 | Ecuador | 8.59 ± 0.21 | NR | 4x | 71.6 ± 1.5 | 4.9 ± 0.3 |
| | 88-054 | Colombia | 8.64 ± 0.23 | NR | 4x | 74.8 ± 1.6 | 3.3 ± 0.2 |
| | 93-003D | Ecuador | 8.73 ± 0.29 | NR | 4x | 74.8 ± 1.3 | 3.3 ± 0.2 |
| | 90-002 | Colombia | 8.57 ± 0.04 | 4x | 4x | 76.0 ± 0.7 | 4.3 ± 0.5 |
| | 90-001 | Colombia | 8.57 ± 0.30 | 4x | 4x | 75.5 ± 0.6 | 4.7 ± 0.4 |
| | 90-003 | Colombia | 8.70 ± 0.21 | 4x | 4x | 67.2 ± 1.1 | 4.3 ± 0.2 |
| | 96-667 | Ecuador | 8.45 ± 0.31 | 4x | 4x | 70.6 ± 1.3 | 4.6 ± 0.4 |
| | 96-676 | Ecuador | 8.60 ± 0.22 | NR | 4x | 75.7 ± 1.4 | 4.4 ± 0.4 |
| *Hylocereus* sp. | 73-03-41 | Israel | 3.90 ± 0.18 | NR | 2x | 65.9 ± 1.1 | 6.8 ± 1.1 |
| | 98-337 | Mexico | 3.92 ± 0.11 | NR | 2x | 70.4 ± 1.2 | 11.1 ± 0.5 |
| | 70-02-08 | Mexico | 4.37 ± 0.23 | NR | 2x | 65.3 ± 0.7 | 12.6 ± 0.6 |
| *Selenicereus grandiflorus* (L.) Britton et Rose ssp. *grandiflorus* | 92-080T | Israel | 3.76 ± 0.29 | NR | 2x | 48.9 ± 1.3 | 14.6 ± 0.5 |
| | 94-032F | Italy | 3.21 ± 0.12 | 2x | 2x | 54.8 ± 0.5 | 8.8 ± 0.3 |
| | 98-321[h] | USA | 3.37 ± 0.14 | NR | 2x | 50.6 ± 0.4 | 9.3 ± 0.5 |
| *S. grandiflorus* (S-D.) Ralf Bauer ssp. *donkelaarii* | 98-322[i] | USA | 3.61 ± 0.23 | NR | 2x | 51.3 ± 0.4 | 20.1 ± 0.8 |
| *S. atropilosus* Kimnach | 98-320 | USA | 4.22 ± 0.02 | NR | 2x | 50.3 ± 0.5 | 11.0 ± 0.6 |
| *S. hamatus* (Pf.) Britton et Rose | 98-323 | USA | 3.66 ± 0.11 | NR | 2x | 49.0 ± 0.4 | 12.2 ± 0.6 |
| *S. inermis* (Pf.) Britton et Rose | 98-328[j] | USA | 3.52 ± 0.20 | NR | 2x | 59.4 ± 0.9 | 14.8 ± 1.1 |
| | 98-329[k] | USA | 3.62 ± 0.25 | NR | 2x | 60.9 ± 0.8 | 10.8 ± 0.6 |
| *S. pteranthus* (Dietr.) Britton et Rose | 98-325[l] | USA | 3.51 ± 0.10 | NR | 2x | 52.6 ± 0.4 | 17.9 ± 1.2 |
| | 98-326 | USA | 3.53 ± 0.19 | NR | 2x | 49.6 ± 0.4 | 14.6 ± 0.6 |
| *S. spinulosus* (DC.) Britton et Rose | 98-331 | USA | 3.49 ± 0.28 | NR | 2x | 62.9 ± 0.9 | 15.5 ± 1.1 |
| *"S. triangularis"*[m] | 96-002 | Mexico | 3.64 ± 0.29 | NR | 2x | 59.1 ± 0.7 | 20.0 ± 0.9 |
| *S. vagans* (Bgek.) Britton et Rose | 98-332 | USA | 6.85 ± 0.30 | NR | 4x | 65.5 ± 0.7 | 13.8 ± 0.8 |
| *Selenicereus* sp. | 94-032T | Italy | 3.45 ± 0.21 | NR | 2x | 54.6 ± 0.5 | 12.9 ± 0.8 |
| *Strophocactus testudo* (Zucc.) Ralf Bauer[n] | 98-327 | USA | 3.39 ± 0.19 | NR | 2x | 70.9 ± 0.9 | 13.7 ± 0.6 |
| *Disocactus flagelliformis* (L.) Bthl X?[o] | 98-341 | UK | 3.65 ± 0.09 | NR | 2x | 61.0 ± 0.6 | 10.9 ± 0.4 |
| *Epiphyllum oxypetalum* (DC.) Haw. | T-18 | Israel | 4.04 ± 0.07 | NR | 2x | 53.7 ± 1.1 | 12.7 ± 0.6 |

**Table 1** continued

| Species | Accession number | Source (country) | Nuclear DNA content pg/ 2C ± SD | Ploidy reported[a] | Ploidy estimated | Stomata | |
|---|---|---|---|---|---|---|---|
| | | | | | | Length (μm) ± SE | Density ($mm^2$) ± SE |
| *Epiphyllum* sp. | T-36 | Israel | 4.15 ± 0.06 | NR | 2x | 65.8 ± 0.7 | 9.9 ± 0.4 |
| | T-123 | Israel | 4.10 ± 0.06 | NR | 2x | 57.9 ± 0.6 | 14.6 ± 1.2 |
| | T-24 | Israel | 4.04 ± 0.15 | NR | 2x | 69.9 ± 0.7 | 11.9 ± 0.6 |

[a] Beard (1937), Spencer (1955), Ross (1981), Lichtenzveig et al. (2000), Tel-Zur et al. (2001, 2004)

[b] Originally obtained as *H. lemairei* (Hk.) Britton et Rose

[c] Originally obtained as *H. polyrhizus* (Web.) Britton et Rose

[d] Originally obtained as *H. venezuelensis* Britton et Rose

[e] Originally obtained as *H. purpusii* (Wngt.) Britton et Rose

[f] Name not included in *New Cactus Lexicon*, but found in international plant names index (http://www.ipni.org/ipni/plantnamesearchpage.do)

[g] Unknown name, individual morphologically similar to *H. undatus*

[h] Originally obtained as *S. coniflorus* (Wngt.) Britton et Rose

[i] Originally obtained as *S. donkelaarii* (S-D.) Bail

[j] Originally obtained as *S. rubineus* Kimn

[k] Originally obtained as *S. wercklei* (Web.) Britton et Rose

[l] Originally obtained as *S. macdonaldiae* (Hk.) Britton et Rose

[m] Unknown name, individual morphologically similar to *S. grandiflorus*

[n] Originally obtained as *S. testudo* (Zucc.) Buxb.

[o] Originally obtained as *S. innesii* Kimn., but now known to be a garden hybrid (Hunt 2006)

*NR* Not reported, *ND* Not determined

Previously reported ploidy levels (Table 1) were used as a basis for comparison and as an additional corroboration of ploidy determined in this study.

### Stomatal measurements

Thin strips of epidermis from three fully expanded stems were mounted on a microscope slide in a drop of tap water. Using an Axioimagera1 LED microscope (Zeiss) and Axiocam HRC camera (Zeiss), the number of stomata per field was determined in 10–15 fields. The same samples were used for measuring the length of 25–75 stomata. Measurements were made using the AxioVision 4.6 program (Zeiss).

### Phenological traits

Four quantitative characters (number of flowers per plant, fruit weight, viable number of seeds per fruit and minimum ripening time) were recorded. Fruit were harvested at maturity (when peel colour was full) during 3–4 consecutive years (Nerd and Mizrahi 1998; Nerd et al. 1999). Maturation period was defined as the time elapsing from anthesis till full maturation. The total number of viable (black-coated) seeds per fruit was determined.

### Statistical analysis

To determine the extent to which genome size, determined by flow cytometric analysis, and stomatal size and density are associated, we carried out correlation analyses for all accessions in all three genera studied using Microsoft Excel software. The Pearson correlation coefficient was calculated and its significance at $P < 0.05$ was determined from the table of critical values for Pearson correlation. Correlation between fruit weight and the number of seeds per fruit was also analysed using the same statistical methods.

## Results and discussion

### Flow cytometric analysis and ploidy estimation

The 2C-DNA amount in vine cactus species ranged from 3.21 pg for *S. grandiflorus* ssp. *grandiflorus* to 8.77 for *H. megalanthus* (Table 1). The mean 2C values of the *Hylocereus* (34 accessions, excluding *H. megalanthus*), *Selenicereus* (13 accessions, excluding *S. vagans*) and *Epiphyllum* (four accessions) samples analyzed in this work were 3.99, 3.58 and 4.08 pg, respectively. The mean 2C values for the tetraploid *H. megalanthus* (nine accessions) was 8.62 pg and for *S. vagans* the value obtained was 6.85 pg. The thirteen *H. undatus* accessions had the greatest variation in 2C values, about 21%; i.e. 3.63 pg for accession 87–601 and 4.4 pg for accession 70-02-09. Such variation would be correlated with eco-geographic variables (Murray 2005) or due to expansion or contraction of repeated sequences (including both transposable elements and ribosomal RNA genes), rather than of protein-coding genes (Moscone et al. 2003). Variation among accessions of other species was smaller (2.3–12.5%).

Nuclear genome sizes reported in other cacti are comparable to those found for vine cacti in this study. 2C-values in *Opuntia* (Tourn.) Mill. ranged from 4.17 pg for *O. incarnadilla* Griffiths to 6.53 pg for *O. heliabravoana* Scheinvar (Segura el al. 2007); 3.20 pg for *Mammillaria san-angelensis* Sanchez-Mejorada (Palomino et al. 1999); 4.88 pg for the hexaploid *Consolea* sp. Lem. (Negron-Ortiz 2007), 3.8 pg for *Rebutia albiflora* Ritter and Buin (Zonneveld et al. 2005); 3.05 pg for *Escobaria bella* Britton and Rose and 3.35 pg for *Cleistocactus smaragidifolius* (Weber) Speg. (Bennett and Leitch 2005).

The base number for Cactaceae is x = 11, with many species being $2n = 2x = 22$, but with $2n = 4x = 44$ and $2n = 8x = 88$ being common (Beard 1937; Banerji and Sen 1955; Spencer 1955; Pinkava and McLeod 1971; Ross 1981; Pinkava et al. 1973; Lichtenzveig et al. 2000; Briones et al. 2004; Baker 2006; Negron-Ortiz 2007; Segura et al. 2007). Polyploidy appears to constitute an ongoing process of cyclical polyploidization and diploidization that may play a major role in the evolutionary diversification of plants and animals (Comai 2005). In Cactaceae, diploid, tetraploid and hexaploid were reported in *Echinocereus* Engelm. (Baker 2006). Hexaploid and octoploid species were observed in *Consolea* (Negron-Ortiz 2007). In *Opuntia*, diploid, tetraploid, hexaploid and octoploid species were described (Pinkava and McLeod 1971; Segura et al. 2007). Genome sizes indicated that the species and accessions studied are diploid except the tetraploid *H. megalanthus* (chromosome counts were previously

reported by Lichtenzveig et al. 2000) and *S. vagans* (which is probably also tetraploid). Nine tetraploid *H. megalanthus* accessions studied here were collected from different countries and areas. Previous reports based on cytological data and crossability relations suggest allopolyploid origin, as a result of natural hybridization between two closely related diploid species (Lichtenzveig et al. 2000; Tel-Zur et al. 2004). However, molecular systematic studies suggest that it is taxonomically autopolyploid or perhaps narrowly allopolyploid (N. Tel-Zur, B. Schneider, A. Cisneros, O. Plume, S. Straub and J.J. Doyle, unpublished data).

## Stomatal measurements

A wide range of stomatal lengths (39.9–90.6 μm) was observed (Table 1). Length varied in the diploid *Hylocereus* species from 39.9 μm for *H. monacanthus* (97-403) to 90.6 μm for *H. ocamponis* (94-031). Length was highly variable between accessions of a single species, e.g. *H. undatus*, 50.3 μm for accession 98-334 and 69.9 μm for accession 70-02-07. In *Selenicerus* species, values ranged from 48.9 μm for the diploid *S. grandiflorus* ssp. *grandiflorus* (92-080T) to 65.5 μm for *S. vagans* (98-332). In the diploid *Epiphyllum* species, length varied from 53.7 μm for *E. oxypetalum* (T-18)–69.9 μm for *Epiphyllum* sp. (T-24).

Stomatal density ranged from 3.3–20.3 stomata/mm$^2$ (Table 1). Values ranged from 3.3–20.3 stomata/mm$^2$ in *Hylocereus* species, *H. megalanthus* (89-054 and 93-003D) and *H. costaricensis* (89-023), respectively. Similar to stomatal length, density varied widely among accessions belonging to a single species, e.g., densities in *H. undatus* accessions ranged from 5.7 (89-026 and 95-004)–11.5 (70-02-09) stomata/mm$^2$. Values for *Selenicereus* species varied from 8.8–20.1 stomata/mm$^2$ for *S. grandiflorus* ssp. *grandiflorus* (94-032F) and *S. grandiflorus* ssp. *donkelaarii* (98-322), respectively. Densities in the *Epiphyllum* accessions ranged from 9.9–14.6 stomata/mm$^2$.

Stomatal development and distribution are regulated by genetic, hormonal and environmental factors. Length and/or density have been used as morphological markers for assessing ploidy in several species (Bingham 1968; Beck et al. 2003; Inceer and Hayirlioglu-Ayaz 2010). Stomata size varies according to habitat (Hodgson et al. 2010) and was positively correlated with altitude in *Tripleurospermum* species (Inceer and Hayirlioglu-Ayaz 2010). Vine cactus species occur naturally in a wide range of geographical and environmental conditions (Barthlott and Hunt 1993). The variation in stomata length and density observed in this study could be a result of the different environmental conditions existing in their native area and may be related to adaptive features.

## Phenological traits

Morphological and agronomical traits of the vine cacti under study are summarized in Table 2. Number of flowers/plant/year was evaluated in 54 vine cacti accessions. Among the species studied, a wide range was observed, from 3.7 for *Epiphyllum* sp. (T-36) to 246.7 for *Disocactus flagelliformis* (98-341), a garden hybrid originally obtained as *Selenicereus innesii*. Among *Hylocereus* species, the lowest number of flowers/plant/year was observed in *H. ocamponis* accession 94-031 (4.0) and the highest in *H. monacanthus* accession 97-404 (55.3). Information is lacking for several species, however a range of 15.0–246.7 flowers/plant/year was observed for *"S. triangularis"* and *Disocactus flagelliformis* (98-341), respectively. The range in *Epiphyllum* accessions was 3.7 to 13.3 flowers/plant/year for T-36 and T-123, respectively.

A wide range of fruit weight was found; the lightest mean fruit weight (10 g) in *Epiphyllum* sp. (T-123) and the highest (474 g) in *H. undatus* (70-02-07). Among *Hylocereus* species, the lowest fruit weight (77 g) was recorded in *H. megalanthus* (90-001) and the highest (474 g) in *H. undatus* (70-02-07). For *Selenicereus* species, weight ranged from 30 to 195 g for *S. atropilosus* and *S. grandiflorus* spp. *grandiflorus* (94-032F), respectively. *Epiphyllum* species yielded very light fruits, from 10 g for *Epiphyllum* sp. (T-123) to 37 g for *Epiphyllum* sp. (T-36). Fruit weight was not recorded for three accessions; two non-flowering plants (*S. hamatus* and *S. inermis* (98-328) and one that flowered but had no fruit set, *Disocactus flagelliformis* (98-341).

Potential yield per plant was calculated on the basis of the number of flowers per year and mean fruit weight. Two accessions demonstrated high yield potential, *H. undatus* (89-024) and *H. monacanthus* (89-028) with 15.4 and 14.8 kg/plant/year, respectively.

**Table 2** Accession characterization: flowers per plant, self-pollination success and fruit weight, number of viable seeds and minimum number of days until ripening

| Species | Accession number | Flowers/plant/year ± SE | Fruit weight (g) ± SE[a] | Potential yield/plant (kg)[b] | Number of viable seeds/fruit ± SE | Min. days to ripening |
|---|---|---|---|---|---|---|
| *H. costaricensis* | 89-023 | 25.5 ± 2.6 | 270 ± 8 | 6.9 | 3,366 ± 643 | 30 |
| | 73-12-32 | 20.8 ± 3.7 | 450 ± 27 | 9.4 | 4,673 ± 384 | 30 |
| | 99-854 | 27.0 ± 1.1 | 286 ± 10 | 7.7 | 3,830 ± 623 | 30 |
| *H. monacanthus* | 88-029 | 23.3 ± 1.9 | 280 ± 11 | 6.5 | 3,561 ± 475 | 34 |
| | 74-12-08 | 10.0 ± 4.5 | 278 ± 16 | 2.8 | ND | 36 |
| | 72-04-18 | 23.3 ± 6.5 | 129 ± 8 | 3.0 | 1,863 ± 301 | 33 |
| | 89-028 | 39.1 ± 4.0 | 379 ± 11 | 14.8 | 4,533 ± 375 | 30 |
| | 89-027 | 22.3 ± 5.9 | 239 ± 12 | 5.3 | 4,227 ± 467 | 31 |
| | 97-401 | 15.0 ± 1.4 | 232 ± 17 | 3.5 | 4,445 ± 1,055 | 31 |
| | 97-403 | 38.8 ± 6.9 | 187 ± 6 | 7.2 | 3,411 ± 431 | 30 |
| | 97-404 | 55.3 ± 7.8 | 394 ± 11 | 21.8 | 3,932 ± 257 | 31 |
| | 99-856 | 12.5 ± 3.2 | 126 ± 16 | 1.6 | 5,635 ± 403 | 37 |
| *H. undatus* | 70-02-09 | 31.3 ± 0.9 | 387 ± 15 | 12.1 | 6,037 ± 222 | 30 |
| | 88-027 | 26.7 ± 3.7 | 293 ± 12 | 7.9 | 3,542 ± 359 | 32 |
| | 98-334 | 31.0 ± 6.3 | 439 ± 18 | 13.6 | 5,980 ± 481 | 36 |
| | 87-601 | 24.2 ± 2.8 | 436 ± 17 | 10.5 | 6,644 ± 689 | 30 |
| | 89-026 | 34.8 ± 2.7 | 147 ± 7 | 5.1 | 907 ± 112 | 32 |
| | 92-053 | 15.0 ± 3.2 | 403 ± 16 | 6.0 | 7,225 ± 597 | 29 |
| | 94-007 | 12.3 ± 1.5 | 279 ± 28 | 2.9 | 5,911 ± 554 | 29 |
| | 95-004 | 20.4 ± 2.4 | 343 ± 16 | 7.0 | 3,397 ± 252 | 33 |
| | 70-02-02 | 31.3 ± 3.1 | 350 ± 11 | 10.9 | 5,768 ± 586 | 29 |
| | 70-02-07 | 26.8 ± 3.1 | 474 ± 19 | 12.7 | 3,348 ± 1,095 | 29 |
| | 72-06-06 | 12.0 ± 0.7 | 356 ± 15 | 4.2 | 4,129 ± 555 | 29 |
| | 89-024 | 42.0 ± 5.7 | 372 ± 11 | 15.6 | 5,693 ± 1,015 | 28 |
| | 98-338 | 19.2 ± 3.1 | 436 ± 16 | 8.4 | 5,445 ± 262 | 29 |
| *H. ocamponis* | 89-025 | 8.5 ± 1.5 | 214 ± 14 | 1.8 | 3,111 ± 851 | 32 |
| | 94-031 | 4.0 ± 2.0 | 370 ± 18 | 1.5 | ND | 35 |
| *H. triangularis* | 71-05-03 | 8.5 ± 0.5 | 269 ± 29 | 2.3 | 7,417 ± 1,478 | 41 |
| *H. bronxensis* | 99-856 | 30.3 ± 3.3 | 152 ± 7 | 4.6 | 2,792 ± 508 | 31 |
| *H. escuintlensis* | 99-853 | 11.0 ± 1.4 | 160 ± 17.4 | 1.8 | 4,223 ± 239 | 38 |
| *H. guatemalensis* | 70-11-07 | 5.0 ± 0.0 | 295 ± 24 | 1.5 | 6,087 ± 1,186 | 30 |
| *"H. zabinski"* | 99-855 | 25.3 ± 4.6 | 345 ± 24 | 8.7 | 3,332 ± 308 | 30 |
| *H. megalanthus* | 70-11-05 | 7.8 ± 1.5 | 109 ± 16 | 0.9 | 275 ± 232 | 90–160 |
| | 88-023 | 10.7 ± 3.2 | 86 ± 38 | 0.9 | ND | 90–160 |
| | 88-054 | 20.5 ± 1.7 | 96 ± 5 | 1.9 | ND | 90–160 |
| | 93-003D | 21.3 ± 5.5 | 102 ± 19 | 2.2 | 297 ± 28 | 90–160 |
| | 90-002 | 17.0 ± 2.3 | 99 ± 9 | 1.7 | 270 ± 118 | 90–160 |
| | 90-001 | 16.0 ± 3.7 | 77 ± 16 | 1.2 | ND | 90–160 |
| | 90-003 | 16.3 ± 3.7 | 267 ± 17 | 4.3 | ND | 90–160 |
| | 96-667 | 9.3 ± 3.3 | 82 ± 19 | 0.8 | ND | 90–160 |
| | 96-676 | 10.0 ± 3.2 | 111 ± 13 | 1.1 | ND | 90–160 |

**Table 2** continued

| Species | Accession number | Flowers/plant/year ± SE | Fruit weight (g) ± SE[a] | Potential yield/plant (kg)[b] | Number of viable seeds/fruit ± SE | Min. days to ripening |
|---|---|---|---|---|---|---|
| *Hylocereus* sp. | 73-03-41 | 19.8 ± 1.9 | 301 ± 19.5 | 5.9 | 1,517 ± 143 | 39 |
| | 98-337 | 14.3 ± 1.8 | 359 ± 22 | 5.1 | 6,690 ± 933 | 35 |
| | 70-02-08 | 31.0 ± 1.8 | 424 ± 26 | 13.1 | 4,258 ± 1,027 | 28 |
| *Selenicereus grandiflorus* ssp. *grandiflorus* | 92-080T | ND | 170 ± 14 | ND | 2,358 ± 157 | 84 |
| | 94-032F | 60.7 ± 5.8 | 195 ± 15 | 11.8 | 2,665 ± 229 | 60 |
| | 98-321 | 84.0 ± 24.2 | 154 ± 11 | 12.9 | 3,039 ± 332 | 69 |
| *S. grandiflorus* ssp. *donkelaarii* | 98-322 | 23.0 ± 3.5 | 113 ± 11 | 2.6 | 1,828 ± 189 | 78 |
| *S. atropilosus* | 98-320 | ND | 30 ± 3 | ND | 416 ± 32 | 72 |
| *S. hamatus* | 98-323 | ND | ND | ND | ND | ND |
| *S. inermis* | 98-328 | ND | ND | ND | ND | ND |
| | 98-329 | ND | 43 ± 3 | ND | 597 ± 73 | 92 |
| *S. pteranthus* | 98-325 | 66.0 ± 17.9 | 159 ± 14 | 10.5 | 1,513 ± 349 | 86 |
| | 98-326 | ND | 74 ± 5 | ND | 1,858 ± 195 | 59 |
| *S. spinulosus* | 98-331 | ND | 55 ± 8 | ND | 853 ± 166 | 88 |
| *"S. triangularis"* | 96-002 | 15.0 ± 5.2 | 149 ± 8 | 2.2 | 2,203 ± 221 | 58 |
| *S. vagans* | 98-332 | ND | 31 ± 7 | ND | 471 ± 364 | 94 |
| *Selenicereus* sp. | 94-032T | ND | 57 ± 5 | ND | ND | 79 |
| *Strophocactus testudo* | 98-327 | ND | 17 ± 1 | ND | 65 ± 16 | 73 |
| *Disocactus flagelliformis* | 98-341 | 246.7 ± 43.3 | ND | ND | ND | ND |
| *Epiphyllum oxypetalum* | T-18 | 8.7 ± 2.3 | 35 ± 10 | 0.3 | 0 | 37 |
| *Epiphyllum* sp. | T-36 | 3.7 ± 1.2 | 37 ± 9 | 0.1 | ND | 108 |
| | T-123 | 13.3 ± 7.3 | 10 ± 1 | 0.1 | ND | 108 |
| | T-24 | 6.9 ± 1.0 | 36 ± 3 | 0.2 | 1,106 ± 193 | 115 |

[a] *Fruit weight Hylocereus* species: intra- and interspecific crosses; *H. megalanthus*: intraspecific crosses

*Selenicereus* species: interspecific crosses, except for *S. grandiflorus* ssp. *grandiflorus* (92-080T), *S. pteranthus* (98-325), *S. vagans* (98-332) (intergeneric crosses)

*Epiphyllum* species: interspecific crosses, except for *E. oxypetalun* (intergeneric crosses)

[b] Potential yield per plant was calculated as a number of flowers/year × mean fruit weight

*ND* Not determined

The number of viable seeds/fruit was very high in all 50 accessions studied, and was not correlated with fruit weight. The highest number of viable seeds was observed in *Hylocereus* species, 7,225 and 7,417 in *H. undatus* (92-053) and *H. triangularis*, respectively. Low number of viable seeds/fruit was recorded in *S. atropilosus* and *Strophocactus testudo*; 416 and 65 seeds/fruit, respectively. No viable seeds were observed in *E. oxypetalum*.

The minimum number of days until ripening was determined. Fruits of *Hylocereus* species matured in 28–41 days from anthesis (*Hylocereus* sp. accession 70-02-08 and *H. triangularis*, respectively). Flowers of *H. megalanthus* blooming in early autumn matured in 90 days while those that bloomed later (November and December) matured in 160 days. Fruit maturation time in *Selenicereus* species was prolonged, ranging from 58 days (*"S. triangularis"*) to 94 days (*S. vagans*). Maturation time in *Epiphyllum* species varied from 37 days (*E. oxypetalum*) to 115 days *Epiphyllum* sp. (T-24).

High levels of variation for the agronomical traits examined were observed even among accessions of a single species, e.g., *H. undatus*. Potential yield/plant calculated on the basis of flowers per year and mean fruit weight is a very important agricultural trait and

**Table 3** Correlations between stomata measurements and nuclear DNA (2C) content and fruit weight and seed number

| Variables | All species* |
|---|---|
| Stomata length versus stomata density (d$f = 56$) | $y = -0.1522x + 20.486$ |
| | $R^2 = 0.1134$ |
| Stomata length versus nuclear DNA content (d$f = 56$) | $y = 2.69x + 19.102$ |
| | $R^2 = 0.2673$ |
| Stomata density versus nuclear DNA content (d$f = 56$) | $y = -1.3076 + 17.352$ |
| | $R^2 = 0.305$ |
| Fruit weight versus seed number (d$f = 11$) | $y = 12.038x + 499.36$ |
| | $R^2 = 0.6008$ |

* All correlations were not significant at the $P < 0.05$ level of probability

is a target in breeding programs. Number of seeds per fruit was relatively high in most of the accessions with high variability. The seeds are very small and soft, and their presence or absence does not affect fruit quality. Ripening is relatively fast, about a month for most *Hylocereus* species and between 2–3 months for *Selenicereus* and *Epiphyllum* species. Ripening was prolonged in the tetraploid *H. megalanthus*, developing more slowly at lower temperatures. Ripening time is genotype-specific with considerable variability among *H. megalanthus* accessions (Dag and Mizrahi 2005).

Phenotypic and genomic characterization of the vine cactus core collection studied in this work indicated a high level of variability for most of the traits studied. Although the heritability of these traits has yet to be studied, and some are likely to have a substantial environmental component, the levels of variation reported here constitute a strong suggestion that these accessions have high potential for breeding programs as exotic fruit crops intrinsically adapted to dry areas.

## Statistical analysis

Several previous reports have identified a positive relationship between nuclear DNA content (genome size) and cell size (Darlington 1965; Bennett 1972; Beaulieu et al. 2008). Beaulieu et al. (2008) reported a significant negative relationship between genome size and stomata density in 101 species of angiosperms. In the work reported here four correlations were calculated: stomata length versus stomata density, stomata length versus nuclear DNA content, stomata density versus nuclear DNA content and fruit weight versus seed number (Table 3). No correlations were significant at the $P < 0.05$ level, thus in vine cacti ploidy level could not be estimated using stomata measurements. Likewise, no statistically significant correlations between fruit weight and seed number were found among species and genera.

One of the most important goals of maintaining core collections is to conserve genetic diversity of crop species and their wild relatives, thus supporting breeding programs as well as basic research. The vine cactus species in the core collection characterized in this work have diverse geographical origins, and this is reflected in the wide range of morphological variation observed. Several of the traits described here are significant for the development and improvement of vine cacti as crop species.

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