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RESEARCH ARTICLE

Genetic variability, characters association and principal component study for morphological and fodder quality of *Opuntia* and *Nopalea* sp. in India

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Abstract A morphological and quality analysis of 64 global core collections of cactus genotypes was conducted using 30 growth and fodder quality traits. Results indicated a high coefficient of variation for primary cladode perimeter (91.0%), root fresh weight (50.8%), and number of cladodes per plant (47.7%). The length, width, and thickness of the primary cladode ranged between 14.4 to 53.9 cm, 5.0 to 13.7 cm, and 1.5 to 4.3 cm, respectively. Principal component analysis showed a 77.6% variation in the first 10 major components with an eigenvalue > 1.0. The first and second principal components explained 18.5% and 14.8% of the total variation, respectively. The fresh weight of the shoot was positively correlated with the dry weight of the shoot (0.72), dry weight of the root (0.48), root length (0.38), and fresh weight of primary cladodes (0.29). The dendrogram obtained using a Ward analysis confirmed the results of the PCA analysis. The cactus pear accessions were grouped into four major clusters with a further four sub-clusters, containing 6, 26, 17 and 15 genotypes, respectively. Genotypes, CAZRI-Kukma, Clone 1308, Jalpa, Mexico Unknown, Trunzara Bianca Bronte, Copena F1, CAZRI Botanical Garden, Piantra-25 and IPA-90-18 are positioned at a wider angle and are more diverse and useful genotypes for use as parent populations for developing new genotypes in future breeding programs.

Keywords Cactus pear · Correlation · Diversity · Fodder quality

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Introduction

Cactus (prickly pear or green gold) is an important species in the cactaceae family. There are approximately 130 genera and 1500 dicotyledonous

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angiosperm species under *Cactaceae* (Shetty et al. 2012). Within the cactaceae family, *Opuntia* and *Nopalea* genera are the two most valuable to humans (Valdez and Osorio 1997). Cactus is well recognized for its fodder, food and medicinal uses (Nazareno 2017). There are 258 known *Opuntia* species, and only 10 recorded species in the genus *Nopalea* (Bravo-Hollis 1978). These species are found in almost every climatic regions of the world (Feugang et al. 2006). However, they are most abundant in arid and semi-arid regions between 35° N and 35° S (Oldfield 1997). It is cultivated widely in Brazil (600,000 ha), Tunisia (600,000 ha), Mexico (230,000 ha), Morocco (150,000 ha), Algeria (150,000 ha), and South American countries (75,000 ha) (De Waal et al. 2015) to produce forage and fodder for cattle, sheep, and goats (Inglese 2010).

In arid and semi-arid regions, the cactus pear is an important and useful food and forage crop due to its ability to retain food and moisture in its cladodes even under extreme heat and prolonged drought conditions (Bomfim et al. 2013; Kumar et al. 2017). Therefore, it can serve as an excellent food and fodder source for animals in arid and semi-arid regions, particularly in hot months when other green fodder sources are insufficient.

Cactus plants can grow up to 3.5–5 m. The succulent cladodes are the major part and the main source of fodder and food (Sudzuki et al. 1993). In addition to food and fodder value, cactus cladodes are an excellent source of nutrients (betalains), amino acids (taurine), minerals, vitamins, and antioxidants. The young and thin green pads (leaves) can be used cooked as a vegetable, used in salads, pickled, and juiced. Cactus flowers can also be cooked as a vegetable (Villegas y de Gante 1997).

To mitigate fodder scarcity in arid and semi-arid regions in India, thornless cactus accessions were introduced initially to ICAR-Central Arid Zone Research Institute (CAZRI) through the International Centre for Agricultural Research in the Dry Areas (ICARDA) during the 1970s. Various investigations on adaptation and fodder quality were carried out on these limited accessions in Central Western India (Mathur et al. 2009; Pareek et al. 2003). However, field-level adoption was limited and exploitation of the cactus in other dryland regions of the country was poor. Cactus introduction and evaluation were revived with the introduction of more thornless accessions through ICARDA's South Asia and China Regional Program, India. Since the thornless accessions were introduced to many habitats, their adaptability needs to be studied through characterization (Lutatenekwa et al. 2020).

The Kachchh region in Gujarat was chosen for large-scale research and adaptation trials. In this study, 64 core cactus accessions were evaluated to select genotypes for high forage yield. The initial evaluation at the experimental farm of Central Arid Zone Research Institute, Regional Research Station, Bhuj, and on farmer's fields showed an average survival rate of 86.0% (Feugang et al. 2006). This high survival is attributed to low water requirements, high-temperature tolerance and high growth rate (Louhaichi et al. 2015). Additionally, the cactus pear uses crassulacean acid metabolism (CAM) pathways that are four times more effective than either C_4 or C_3 plants in transforming water into biomass (Han and Felker 1997). Of India's 141 million ha of agricultural land, 80 million ha are under dryland farming systems producing 40% of the agricultural commodities and supporting at least 65% of the livestock population (Ravindranath et al. 2011). A recent study indicates that of the 329 total geographical areas of India, about 102 million ha falls under the 'suitable' (29%) and 'most suitable' (3%) category for cactus cultivation, mainly in Rajasthan, Gujarat, Chhattisgarh, Odisha, Telangana, Andhra Pradesh and Karnataka (Acharya et al. 2019). The western and east-central part of India is the most suitable for cactus cultivation.

However, the performance of different cactus accessions varies by region. Therefore, it is essential to identify the best-suited accession for a particular region. In the present study, we focus on the characterization of all 64 accessions in the northwest part of India, i.e. arid Kachchh region and identify the most appropriate accession for high fodder yield, survival, and morphological growth. The aim was to identify potential genotypes for high fodder during the lean period in the arid Kachchh region and other similar regions.

Material and methods

Sixty-four cactus accessions were collected with support from ICARDA's South Asia and China

Regional Program, India. Two accessions did not survive, and two promising local thornless accessions were collected from the Kachchh region of Gujarat and Jodhpur Rajasthan (CAZRI-Kukma, CAZRI Botanical Garden) (Table S1). Of the 62 exotic collections, 27 were of Italian origin, 25 Brazilian, one from the USA, one from Mexico and eight were of unknown origin. The crop was raised and evaluated during 2016–2018 at the experimental farm of ICAR-Central Arid Zone Research Institute, Regional Research Station, Kukma-Bhuj.

The experimental soil was sandy loam (8.8% clay, 7.0% silt), alkaline (pH 8.6), non-saline (electrical conductivity: 0.47 dSm^{-1}) and deficient in organic carbon (0.24%). The region has an arid climate with very low, erratic rainfall with an average of 346 mm (average of data from 1998 to 2015) and an average of 13 rainy days a year. The annual maximum temperature ranged from 39 to 45 °C. In 2016, two rows of one-year-old nursery-raised cladodes were transplanted in an alfa lattice design with two replications. The row length was 5 m with row-to-row spacing of 2 m. Plants were spaced 1 m apart within each row. No fertilizers were applied during the experiment except the initial application of dry farmyard manure of cattle origin at the rate of 1 kg per plant.

Irrigation was provided once a fortnight until the regeneration of new shoots occured. Subsequently, watering was done at one-month intervals in summer and every two months in winter. For each irrigation event, about 2 L of water was applied per plant. Different morphological observations were recorded on the whole plant, primary cladode, and secondary cladodes (Mangalassery et al. 2017). The parameters recorded at the harvest during July 2018 are listed below.

- Cactus survival (%)
- Plant height (PH)
- Number of cladodes per plant (CL/pl)
- Root length (RL)
- Whole plant fresh weight (FW_{wp})
- Whole plant dry weight (DW_{wp})
- Root fresh weight (FW_{rt})
- Root dry weight (DW_{rt})
- Shoot fresh weight (FW_{sh})
- Shoot dry weight (DW_{sh})
- Number of areoles per cladode (AN/cl)

In the laboratory, the following features were recorded:

- Primary cladode length (CL_{pc})
- Secondary cladode length (CL_{sc})
- Primary cladode width (CW_{pc})
- Secondary cladode width (CW_{sc})
- Primary cladode thickness (CT_{pc})
- Secondary cladode thickness (CT_{sc})
- Primary cladode area ($area_{pc}$)
- Number of roots per plant (RN^{-pl})
- Primary cladode perimeter (CP_{pc})
- Secondary cladode perimeter (CP_{sc})
- Primary cladode leaf area (LA_{pc})
- Secondary cladode leaf area (LA_{sc})
- Primary cladode fresh weight (FW_{pc})
- Primary cladode dry weight (DW_{pc})
- Primary cladode length: width ratio ($L{:}\ W_{pc}$)
- Secondary cladode length: width ratio ($L{:}\ W_{sc}$)

Observations were made from the middle ten plants of each accession and the mean of the data was used for statistical analysis. Plant height, root length, shoot length, primary and secondary cladode length, and width were measured using a meter scale. Root length was measured on five randomly uprooted plants of each genotype and averaged. The plant fresh and dry weight and root fresh and dry weight were obtained with a precision scale (accuracy, 0.01 g). For recording the dry weight, cladodes were cut into four pieces and dried in a hot air oven at 72° C until the constant weight was achieved. The moisture content in the cladode was calculated as recommended by Guimarães and Stone (2008) using the following equation.

$$Moisture\,(\%) = \frac{FW(c) - DW(c)}{DW(c)} \times 100$$

where FW(c) is the fresh weight of the cladode and DW(c) is the dry weight.

The cladode area (cm^2) was calculated as described by Nobel and Cortázar (1991) for the cactus plant.

$$Cladode\ area = Length \times Width \times 0.632\,(factor)$$

The length and width were measured on the middle portion of the cladode, while thickness was estimated from the middle, upper, and lower parts of the cladode using a digital Vernier caliper and averaged.

The plant fresh and dry weights and shoot fresh and dry weights were defined as the primary fodder yield contributing traits. Similarly, the length and width of primary and secondary cladodes and the cladode surface area of the primary and secondary cladodes were considered secondary fodder yield contributing traits. The total photosynthetic area was estimated by multiplying the cladode area by the total number of cladodes per plant. To estimate available calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na), the oven-dried samples were ground and sieved. The concentrations of K, Mg and Na were determined using an atomic absorption spectrophotometer (AA500, PG Instruments, UK). The Ca was estimated with EDTA complexometry.

Statistical design

The 64 accessions of cactus pear were planted in a randomized block design with two replications. The obtained data were subjected to multivariate cluster analysis based on Ward's method (Ward 1963) and principal component analysis using R software. The PCA graphics, including Biplot-PCA, Variables-PCA and Individuals-PCA were done using devtools, ggplot2, and factoextra packages in R. Correlation among the traits was calculated using the metan package in R. Basic descriptive statistics and principal component analysis were completed using PAST4 software (Hammer 2001).

Results

Diversity in morphological and fodder traits

The results indicated significant variability among the 64 cactus accessions for most morphological and fodder quality traits as presented in the supplementary Table S2. A high coefficient of variation (CV) was observed for different primary cladode traits, namely length (24.7%), width (20.5%) and thickness (22.9%) (Table 1). The length, width and thickness of the primary cladode varied between 14.4 to 53.9 cm, 5.0 to 13.7 cm, and 1.5 to 4.3 cm, respectively. Similarly, the length, width, and thickness of secondary cladodes ranged from 15.8 to 31.6 cm, 4.2 to 11.1 cm, and 0.6 to 5.8 cm, with a CV value of 14.6%, 16.70% and 44.6%, respectively. The primary cladode length was highest in the genotype CAZRI-Kukma (43.9 cm) followed by Jalpa (41.4 cm), Clone 1308 (34.0 cm), Clone 1287 (31.5 cm), Rossa Roccapalumba (31.4 cm), Blue Motto (30.1 cm), Bianco Macomer (29.4 cm), Clone 1270 (28.2 cm), whereas, the width followed the order Clone 1270 (13.7 cm), Mexico unknown (12.5 cm), Jalpa (12.4 cm), Trunzara Bianca-San Cono (12.4 cm), Rossa Roccapalumba (11.6 cm), Crystallina (12.8 cm), Clone 1287 (11.7 cm), Roso San Cono (11.8 cm); and thickness followed the order Trunzara Gialla Bronte (4.3 cm), Spineless (4.0 cm), Gymnocarpe (3.6 cm), Palma Redonda (3.6 cm), White Roccapalumba (3.6 cm), Palma Grande (3.6 cm), IPA-90-115 (3.6 cm). The length and width ratio of primary and secondary cladode ranged from 1.4 to 4.7 and 1.7 to 5.1 with a coefficient of variation of 21.9 and 17.0%, respectively. Plant height and number of cladodes per plant varied considerably during harvest, with height and number of cladodes per plant ranging from 38.5 to 119.5 cm and 2.5 to 19.0, respectively. The genotype like Giall×Giall (119 cm), Trunzara Gialla San Cono (117.5 cm), Bianca-San Cono (116.5 cm), White Roccapalumba (116.75 cm), Seedless Santa Margherita Belice (117 cm) are grouped under taller genotypes (> 100 cm height), and genotypes like CAZRI Botanical Garden (38.5 cm), Orelha de Elefante Africana (40 cm), CAZRI-Kukma (57 cm), Clone 1270 (52), Piantra-25 (58) are grouped under shorter genotype (< 60 cm).

In general cladodes traits of *Nopalea* sp. (Clone 1308, Palma miuda ou doce, IPA Sertania ou baiana, Orelha de onca) are moderate to low in range compared to accessions of *Opuntia* species. Similarly, fodder yield contributing characters like shoot fresh weight and dry weight ranged from 291.5 to 1534.3 g and 11.4 to 117.5 g with a coefficient of variation of 30% and 40.5%, respectively. The differential fresh and dry shoot weight may be attributed due to genetic characteristics of diverse genotypes of different environment origin and their growth performance in a similar growth environment. The primary cladode fresh weight and dry weight of various accessions studied ranged from 100 to 940 g and 10 to 72 g respectively. Secondary yield contributing traits like leaf area of primary and secondary cladode varied noticeably from 50.2 to 363.9 cm^2 and 55.7 to 198.7 cm^2 with the coefficient of variation value 38.5% and 26.6%, respectively. Mineral analysis of fresh

Table 1 Descriptive statistics for thirty morphological, fruit quality, and yield traits of 64 cactus pear genotypes

Para-meters	Abbreviation	Min	Max	Mean	Std. error	Variance	Stand. dev	Skewness	Kurtosis	Coeff. var
Plant										
Survival rate	Sur (%)	21.0	100.0	72.5	3.1	620.8	24.9	−0.1	−1.5	34.4
Plant height	PH (cm)	38.5	119.5	91.8	2.4	365.3	19.1	−0.9	0.4	20.8
No. of cladodes per plant	Cl/pl	2.5	19.0	6.6	0.4	9.9	3.2	2.2	5.8	47.7
Plant fresh weight	FW_{wp} (g)	300.0	1540.0	858.0	32.1	65,813.3	256.5	0.3	0.6	29.9
Plant dry weight	DW_{wp} (g)	11.9	122.5	48.6	2.4	357.7	18.9	1.3	2.9	38.9
Shoot fresh weight	FW_{sh} (g)	291.5	1534.3	852.6	32.0	65,573.4	256.1	0.3	0.7	30.0
Shoot dry weight	DW_{sh} (g)	11.4	117.5	45.8	2.3	342.9	18.5	1.3	2.8	40.5
Root fresh weight	FW_{rt} (g)	1.6	13.0	5.3	0.3	7.3	2.7	0.8	0.0	50.8
Root dry weight	DW_{rt} (g)	0.5	6.9	2.8	0.2	1.4	1.2	0.5	0.7	42.9
Root length	RL (cm)	11.5	60.0	31.2	1.2	95.1	9.8	0.3	0.1	31.2
No. of roots per plant	RN^{-pl}	5.0	29.0	19.9	0.7	28.6	5.3	−0.4	−0.4	26.9
Calcium	Ca (ppm)	0.0020	0.0080	0.0045	0.000173	0.000001	0.0013	0.6	0.2	30.8
Magnesium	M (ppm)	0.0054	0.0443	0.0017	0.000797	0.000004	0.0064	1.7	4.8	37.4
Potassium	K (%)	4.4	12.3	8.1	0.2	2.6	1.6	−0.2	0.4	19.9
Sodium	Na (%)	0.2	0.6	0.3	0.0	0.0	0.1	0.9	0.2	32.3
Primary cladode										
Fresh weight	FW_{pc} (g)	100.0	940.0	394.5	19.9	25,241.1	158.9	1.0	2.4	40.3
Dry weight	DW_{pc} (g)	10.0	72.6	27.5	1.3	103.4	10.2	1.7	5.4	37.0
Leaf Area	LA_{pc} (cm^2)	50.2	363.9	158.0	7.6	3691.9	60.8	0.9	1.4	38.5
Cladode length	CL_{pc} (cm)	14.4	43.9	21.5	0.8	36.6	6.1	2.0	8.3	24.7
Cladode width	CW_{pc} (cm)	5.0	13.7	9.5	0.2	3.8	2.0	−0.2	−0.5	20.5
L:W ratio	$L:W_{pc}$	1.4	4.7	2.6	0.1	0.3	0.6	0.9	2.0	21.9
Thickness	CT_{pc} (cm)	1.5	4.3	2.7	0.1	0.4	0.6	0.1	−0.4	22.9
Perimeter	CP_{pc} (cm)	1.2	550.4	174.4	19.8	25,193.8	158.7	1.4	0.5	91.0
No. of areoles per cladode	AN/cl	14.4	95.9	32.6	1.4	130.5	11.4	2.6	14.2	35.0
Secondary cladode										
Cladode length	CL_{sc} (cm)	15.8	31.6	23.4	0.4	11.7	3.4	0.1	−0.1	14.6
Cladode width	CW_{sc} (cm)	4.2	11.1	7.5	0.2	1.6	1.3	0.0	0.6	16.7
L: W ratio	L: W_{sc}	1.7	5.1	3.2	0.1	0.3	0.5	0.2	2.5	17.0
Thickness	CT_{sc} (cm)	0.6	5.8	1.6	0.1	0.5	0.7	3.4	18.9	44.6
Perimeter	CP_{sc} (cm)	32.5	334.9	131.6	5.9	2234.6	47.3	1.5	4.7	35.9
Leaf area	LA_{sc} (cm^2)	55.7	198.7	119.9	4.0	1019.8	31.9	0.5	0.0	26.6

cladodes of accessions indicated a high coefficient of variation for magnesium (37.4%), sodium (32.3%), calcium (30.8%), and potassium (19.9%). The minimum and maximum values for these traits ranged between 0.0044 to 0.0054 (%), 0.2 to 0.6 (%), 0.0020 to 0.0080 (%) and 4.4 to 12.3 (%), respectively. The presence of high CV was ascertained in the traits namely, primary cladode perimeter (91.0%), root FW (50.8%), no. of cladode/ plant (47.7%), the thickness of secondary cladode (44.6%), root DW (42.9%) and primary cladode FW (40.3).

Principal component analysis

The first three significant components explained 42.24 per cent of the total variation. Out of 30 morphological and fodder traits assessed, 17 traits were correlated to the first 10 principal components. These 17 traits were found to be most efficient to discriminate the cactus genotypes in our study. The maximum magnitude of variation of 18.50% was reported in the first major component (Table 2). The traits FW_{sh} (0.69) and FW_{wp} (0.70) contributed positively to the first component variance. The FW_{pc} (0.68), CP_{pc} (0.67), and LA_{pc} (0.20) all contributed positively to the second principal component, which accounted for a 14.80 percent of the variance. The positive contributing traits for the variation in the third component (9.0% variation) were the FW_{pc} (0.62) and LA_{pc} (0.25) and the negative contributor was CP_{pc} (−0.73). Additional traits like survival percentage, (PC8), plant height (PC9 & PC10), calcium (PC11), RL_{pc} (PC13) and DW_{pc} (PC14 & PC15) have high PC value in later components with PC values of 0.83, 0.60 & 0.73, 0.98, 0.84, 0.81 and, 0.59 and 0.77, respectively. The use of a biplot between PC1 and PC2 to graphically illustrate accession diversity revealed substantial variability between and within accessions (Fig. 1, Supplementary Figure S1, and Figure S2). Based on numerous morphological and fodder characteristics, PC1 and PC2 dispersed the 64 cactus pear genotypes into groups. These are grouped (closer) based on the certain related characteristics of genotypes. Genotypes like CAZRI-Kukma, Clone 1308, Jalpa, Mexico Unknown, Trunzara Bianca Bronte, Copena F1, CAZRI Botanical Garden, and IPA-90-18 are positioned in the gaps and found to be more diverse and potentiall useful as parent population for developing new genotypes. The genotypes dispersed among the quarters may be less diverse for traits unique to that quarter and more distinct for traits unique to other quarters. It could be the supplement that, all the quarters represent the diversity pattern in the cactus pear germplasm being studied.

Cluster analysis

The dendrogram obtained by Ward analysis confirms the results of the PCA analysis; cactus accessions were grouped into four major clusters with many sub-clusters. The first cluster, which is further divided into two groups, has six genotypes. The second major cluster grouped genotypes into the three distinct sub-clusters with 26 accessions. The third and fourth major clusters are further classified into two sub-clusters with 17 and 15 genotypes, respectively. The dendrogram was made to investigate the distance between the 64 cactus genotypes based on 30 quantitative characteristics. According to the dendrogram produced by cluster analysis using the hierarchical Ward analysis technique (Ward.D2), the cactus pear accessions were divided into four primary clusters (Figs. 2 and 3). The first cluster comprises six genotypes that are further separated into two groups. The first group contains five genotypes (Rosa San Cono, Red San Cono, Crystallina, Clone no. 1270, Jalpa) and the second group contains twenty-six genotypes characterized for higher RN perpl, Mg, CTsc, PH, Ca, LApc, CLsc, CWsc, CPpc, FWrt, RL, Na. The Blue Motto was found to have a maximum root length (60 cm) in the second cluster. The third major cluster grouped genotypes into the three distinct sub-clusters with17 distinct genotypes. The genotypes clustered in this group were greater in FWwp, FWsh, CTpc, DWsh, Ca and medium in LApc, CLsc and CWsc. Maximum Ca content was reported in orelha de onca (80.16 mg) in the third cluster. The fourth cluster comprised 15 genotypes that were further split into four groups. Each of these encompasses 6, 5, 3, and 3 genotypes and was determined to be the most different from the major cluster one genotype. Genotypes grouped under this category were characterized by higher CPsc, L: Wsc, CPpc, CN perpl and CPpc and medium L: Wpc and CTpc. Among the two genera studied, *Napalea* accessions like Palma miuda ou doce, IPA Sertania ou baiana, Orelha de onca were found to be less vigorous in comparison to genotypes belonging to *Opuntia*.

Table 2 Principal component loading of 34 morphological characters in 64 cactus pear accessions

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12	PC 13	PC 14	PC 15
Sur (%)	0.00	0.05	0.00	0.00	0.11	−0.07	0.42	0.83	0.31	−0.09	−0.09	−0.05	−0.08	0.07	0.00
PH (cm)	0.01	−0.01	0.01	−0.04	0.00	−0.23	−0.10	−0.13	0.60	0.73	−0.15	0.05	−0.01	0.01	0.06
CN/pl	0.00	0.00	−0.01	0.00	−0.01	0.03	−0.02	0.00	0.00	0.03	−0.03	0.02	−0.07	−0.05	−0.07
FW_{wp} (g)	0.70	−0.12	−0.04	−0.01	0.00	0.01	−0.01	0.00	0.03	−0.03	0.00	0.02	−0.03	−0.03	0.09
DW_{wp} (g)	0.04	−0.01	−0.02	0.00	0.01	0.01	0.04	0.20	−0.49	0.45	−0.02	−0.05	0.06	−0.01	−0.01
FW_{sh} (g)	0.69	−0.12	−0.04	−0.01	0.00	0.03	0.01	0.00	0.03	−0.02	0.00	0.00	0.02	0.03	−0.08
DW_{sh} (g)	0.04	−0.01	−0.02	0.00	0.01	0.01	0.05	0.21	−0.49	0.45	−0.02	−0.05	0.02	0.02	−0.03
FW_{rt} (g)	0.00	0.00	0.00	0.00	0.00	−0.02	−0.02	0.00	0.00	−0.01	0.00	0.01	−0.05	−0.06	0.17
DW_{rt} (g)	0.00	0.00	0.00	0.00	0.00	−0.01	−0.01	0.00	0.00	0.01	0.00	0.00	0.04	−0.03	0.02
RL (cm)	0.01	−0.01	0.00	−0.01	−0.06	0.00	0.08	−0.03	0.09	−0.02	0.00	−0.51	0.81	0.19	−0.09
RN/pl	0.00	0.00	0.00	0.02	−0.01	0.02	−0.07	−0.04	0.02	0.03	0.04	0.03	−0.22	0.76	−0.54
FW_{pc} (g)	0.16	0.68	0.62	−0.03	0.01	−0.29	−0.16	0.04	−0.07	−0.05	0.01	−0.03	0.01	−0.02	−0.04
DW_{pc} (g)	0.01	0.04	0.04	−0.02	0.00	0.03	0.02	−0.02	−0.07	−0.01	0.02	0.08	0.01	0.59	0.77
LA_{pc} (cm^2)	0.03	0.20	0.25	−0.05	−0.32	0.79	0.29	−0.09	0.12	0.15	−0.07	−0.02	−0.08	−0.03	−0.02
CL_{pc} (cm)	0.00	0.02	0.02	0.00	−0.03	0.08	0.04	0.02	0.03	0.04	−0.01	0.11	0.11	−0.08	0.09
CW_{pc} (cm)	0.00	0.01	0.01	0.00	−0.01	0.02	0.01	−0.01	−0.01	−0.01	0.01	−0.03	−0.03	0.02	−0.03
$L{:}W_{pc}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.02	−0.02	0.02
CT_{pc} (cm)	0.00	0.00	0.00	0.00	0.00	−0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.02	0.01
CP_{pc} (cm)	0.08	0.67	−0.73	0.02	0.00	0.05	−0.01	−0.04	0.02	0.01	0.00	0.01	0.01	0.00	0.00
AN_{pc}	0.00	0.02	0.03	0.01	−0.04	0.01	0.08	0.06	−0.01	0.00	0.07	0.84	0.48	0.05	−0.14
LA_{sc} (cm^2)	0.02	0.05	0.04	0.01	0.26	−0.26	0.80	−0.44	−0.09	0.03	0.04	0.00	−0.08	0.01	−0.04
CL_{sc} (cm)	0.00	0.01	0.01	0.00	0.03	−0.02	0.07	−0.02	0.04	0.02	0.01	0.02	0.07	−0.09	0.10
CW_{sc} (cm)	0.00	0.00	0.00	0.00	0.00	−0.01	0.03	−0.02	−0.01	0.00	0.01	−0.02	0.00	0.01	−0.01
$L{:}W_{sc}$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.01	−0.01	0.01
CT_{sc} (cm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	−0.01	0.01	0.00	0.01	0.00	0.01	0.01	−0.03
CP_{sc} (cm)	0.00	0.04	0.07	−0.06	0.90	0.38	−0.17	0.00	0.06	0.05	0.02	0.00	0.08	0.00	−0.01
Ca (ppm)	0.01	0.00	0.01	0.03	−0.04	0.03	0.01	0.07	0.11	0.13	0.98	−0.06	−0.04	−0.05	0.02
Mg (ppm)	0.02	0.02	0.05	0.99	0.04	0.04	−0.01	−0.01	0.03	0.04	−0.03	−0.01	0.01	0.00	0.02
K (%)	0.00	0.00	0.00	0.00	0.00	−0.01	0.00	0.00	0.01	−0.01	−0.01	−0.03	−0.02	0.04	0.10
Na (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eigenvalue	5.53	4.43	2.70	2.21	1.92	1.46	1.44	1.28	1.21	1.05	0.96	0.90	0.86	0.68	0.60
% variance	18.46	14.78	9.0	7.39	6.41	4.88	4.83	4.29	4.05	3.53	3.22	3.01	2.87	2.29	2.02
Cumulative	18.46	33.24	42.24	49.64	56.05	60.94	65.77	70.06	74.11	77.64	80.86	83.88	86.76	89.06	91.08

Correlation study

An important objective of the study was to consider high forage yield genotype, higher in weight, and cladode yield (Fig. 4). DW_{sh} (0.72^{***}), DW_{rt} (0.48^{***}), CL_{rt} (0.38^{**}), FW_{pc} (0.29^{*}), DW_{pc} (0.22^{*}), and CT_{pc} (0.30^{*}) were all positively linked to FW_{sh}. The DW_{wp} was highly correlated with the DW_{sh} (1.0). The FW_{pc} was strongly correlated to DW_{pc} (0.87^{***}), LApc (0.79^{***}), CL_{pc} and CW_{pc} (0.62^{***} & 0.72^{***}, respectively). Areole number per cladode was positively correlated to CL_{pc}. CL_{pc} was found to be negatively influenced by the number of cladodes per plant (-0.49^{***}) and the RN^{-pl} (-0.35^{**}). It is also worth noting that plant height did not significantly contribute positively to the fresh per dry yield of the whole plant or the fresh per dry yield of the shoot. Therefore, the height of the plant alone should not be used as a selection criterion for high fodder yield. The results of the correlation analysis revealed that most morphological traits of primary and secondary cladodes studied were positively correlated, with the exception of a few traits.

Discussion

Thirty morphological growth and fodder parameters were evaluated. Seventeen showed great potential for differentiating among cactus genotypes (Table 2). We encourage future research to concentrate on these informative traits to save time and resources on cactus germplasm characterization.

The descriptive analysis revealed significant variability among 64 cactus accessions for most morphological and fodder quality traits (Table S1). Some traits (potassium, secondary cladode length, secondary cladode width, and secondary cladode L: W ratio) showed moderate diversity (CV: < 20%). Cladode characteristics have been useful in identifying valuable fodder *Opuntia* species (Peña-Valdivia et al. 2008). Genetic variability has been reported in *Opuntia* (Buxbaum 1958); however, cladode traits are dependent on genotype and environmental factors (Nobel 1988). (Muñoz-Urias et al. 2008) stated that crossbreeding between *Opuntia ficus-indica* and other wild *Opuntia* species led to Mexican cultivated accessions. This could help clarify some morphological similarities between the accessions of Mexican origin included in the current study. The species outcrossing and the persistence of several individuals over generations from large populations before separation could justify this high genetic diversity (Young et al. 1996). Additionally, significant variability among accessions may be due to the high level of phenotypic plasticity, interspecific hybridization, and polyploidy (Wallace and Gibson 2002).

High variability in cladode traits (length, width perimeter, leaf area and primary cladode) was recorded in the present study. This result supports earlier findings (Bendhifi et al. 2013) that used RAPD markers on cacti to discover marked variation between cactus accessions for fodder traits. According to Stuppy (2002) and Peña-Valdivia et al. (2008), the cladode size (length and width), along with the presence of spines and seed morphology should be considered important traits to characterize and discriminate wild and domesticated *Opuntia* spp. As in a previous study (Munoz et al. 1995), larger cladode size can be a ploidy predictor of domesticated cactus accessions. Cladode size is a species-dependent characteristic and wide variability of cladode size occurs between species in the wild (Bravo-Hollis 1978). The morphological descriptors can be seen as a successful instrument in the study of genetic diversity (Adli et al. 2017).

In the present PCA analysis, more than 77% of the total variation is explained by the first 10 principal components. Quantitative fodder traits such as the fresh weight of primary cladodes, main shoot and whole plant, perimeter and area of the leaf (cladode) dominated in the first and second principal components. The multivariate methods for morphological and agronomic descriptions (Reyes-Aguero et al. 2007) and industrial descriptions (Hammouch et al. 2013) were previously used in *Opuntia*. The use of morphological descriptors comprising cladode, flower, and fruit traits yielded a high number of morphotypes and allowed the discrimination of all the studied species. Previously, positive loading for only green matter yield with the highest value of 0.993 in the first principal component and plant height (0.998) in the second principal component was documented in 33 cactus pear PCA analyses (Nadaf et al. 2016).

The PCA biplot revealed that genotypes scattered in quarters could be less diverse for traits belonging to that particular quarter and more distinct from the traits of other quarters. It could

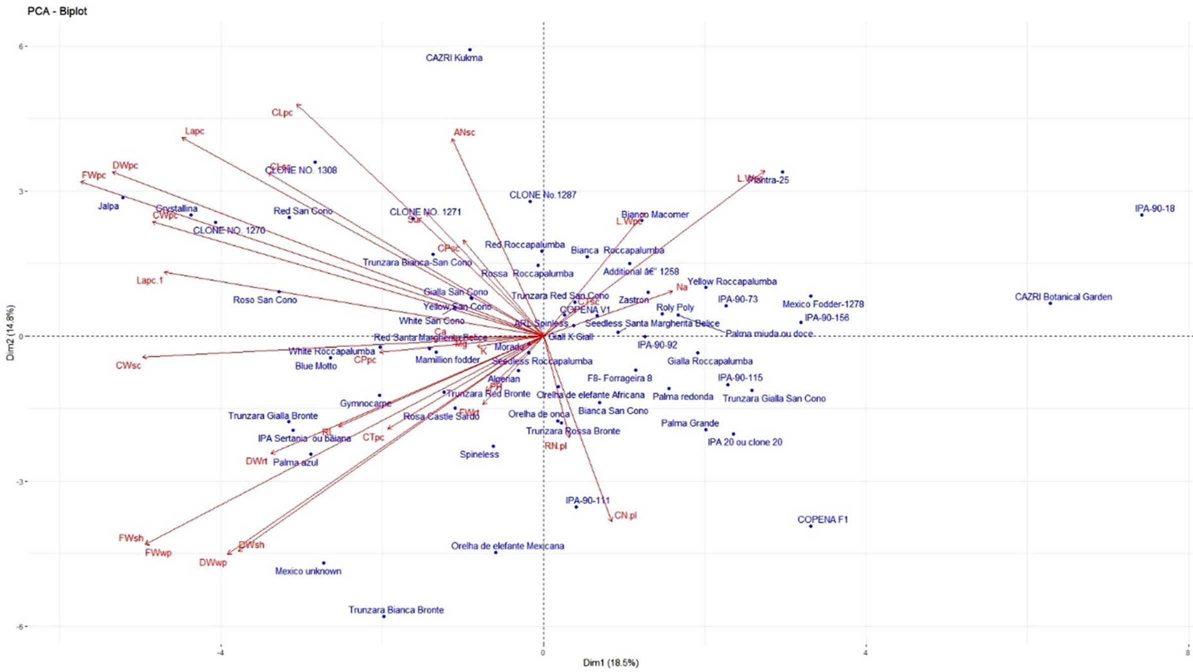

Fig. 1 Biplot of principal components 1 (PC1) and 2 (PC2) showing scatter of 64 cactus pear accessions. *Note* Survival rate: Sur (%); Plant height: PH; No. of cladodes/plant: Cl/pl; Plant fresh weight: FWwp; Plant dry weight: DWwp; Shoot fresh weight: FWsh; Shoot dry weight: DWsh; Root fresh weight: FWrt; Root dry weight: DWrt; Root length: RL; No. of roots/plant: RN-pl; Calcium: Ca; Magnesium: M; Potash: K; Sodium: Na; Primary cladode Fresh weight: FWpc; Primary cladode Dry weight: DWpc; Primary cladode Leaf Area: LApc; Primary cladode length: CLpc; Primary Cladode width: CWpc; Primary cladode L: W ratio: L: Wpc; Primary cladode Thickness: CTpc; Primary cladode Perimeter: CPpc; No. of areole/cladode: AN/cl; Secondary Cladode length: CLsc; Secondary Cladode width: CWsc; Secondary Cladode L: W ratio: L: Wsc; Secondary Cladode Thickness: CTsc; Secondary Cladode Perimeter: CPsc; Secondary Cladode Leaf area: LAsc

supplement all the quarters representing the diversity pattern in the cactus pear germplasm being studied. The variation reported in the present study is in line with the results of earlier research (Mondragón-Jacobo and Pérez-González 2001; Munoz et al. 1995). Previously (Chalak et al. 2014) in PCA analysis of 43 cactus genotypes confirm 45.7% in the first three components. The variation in the first component (21.4%) is mostly due to fruit weight, juice weight, pulp weight, and seed number, while the second component (13.3%) is explained by the peel thickness, plant vigor, habit, and shape. The receptacle diameter and cladode shape led to variation in the third component (10.9%).

However, the distances between the subcluster of the second major cluster are minimal, suggesting they are very similar to common properties, a similar observation to that of Chougui et al. (2016); short distances within subgroups, represent their closeness with mutual properties. Previously, Peña-Valdivia et al. (2008) separated 46 cactus accessions into two groups through multivariate analysis. Accessions fall under groups that differed for the width and length of the cladodes, the width of the areoles, the number of areoles per cladode face, and cladode length. The genotype of the most diverse cluster can be used in a breeding program with accessions belonging to other clusters (Nadaf et al. 2016). The specific grouping of Nopalea accessions was observed in the second main cluster during this investigation. This may be when the accession of different centers of origin is introduced directly to a new place for adaptation (Chalak et al. 2014).

Correlation analysis using metan package in R revealed a weak association between plant height and cladode number, fresh and dry weight and shoot of the plant. However, the fresh weight of the shoot is positively associated with root length, cladode

Fig. 2 Principal component analysis of 64 cactus accessions using 30 morphological and fodder traits

thickness and dry weight of the plant. The leaf area of the primary cladode was strongly positively correlated with Fresh weight (0.79***), Dry weight (0.79***), cladode length (0.86***) and width (0.87***) of the primary cladode. Previously, Cunha et al. (2012) and Guimarães et al. (2018), independent of the cladode order, verified a positive correlation of cladode area and cladode weight in cactus pear. In contrast, Karababa et al. (2004) observed positive correlations between plant height, cladode number, and fresh and dry matter yields. Our results indicate DWsh, DWrt, CLrt, FWpc, DWpc, and CTpc could be used as selection criteria for improving the productivity of forage cactus populations. However, the height of the plant alone should not be used as a selection criterion for high fodder yield. It is beneficial to do indirect selection for traits which are significantly positively correlated, to obtain advances in te traits of commercial importance (Cruz et al. 2012).

Conclusion

The study revealed genetic diversity within and among 64 *Opuntia* and *Nopalea* species in India through morphological descriptors. A significant variation was reported for primary and secondary cladodes traits. *Nopalea* accessions are found to be less productive than *Opuntia* accessions. The accessions: CAZRI-Kukma, Clone 1308, Jalpa, Mexico Unkown, Trunzara Bianca Bronte, Copena F1, CAZRI Botanical Garden, and IPA-90-18 are most distinct from each other, and can be used for cross-breeding programs. The morphological traits as shoot fresh weight, whole plant fresh weight, fresh weight of primary cladodes, primary cladode perimeter and cladode surface area are the most important morphological traits to distinguish the accessions and selection for productivity.

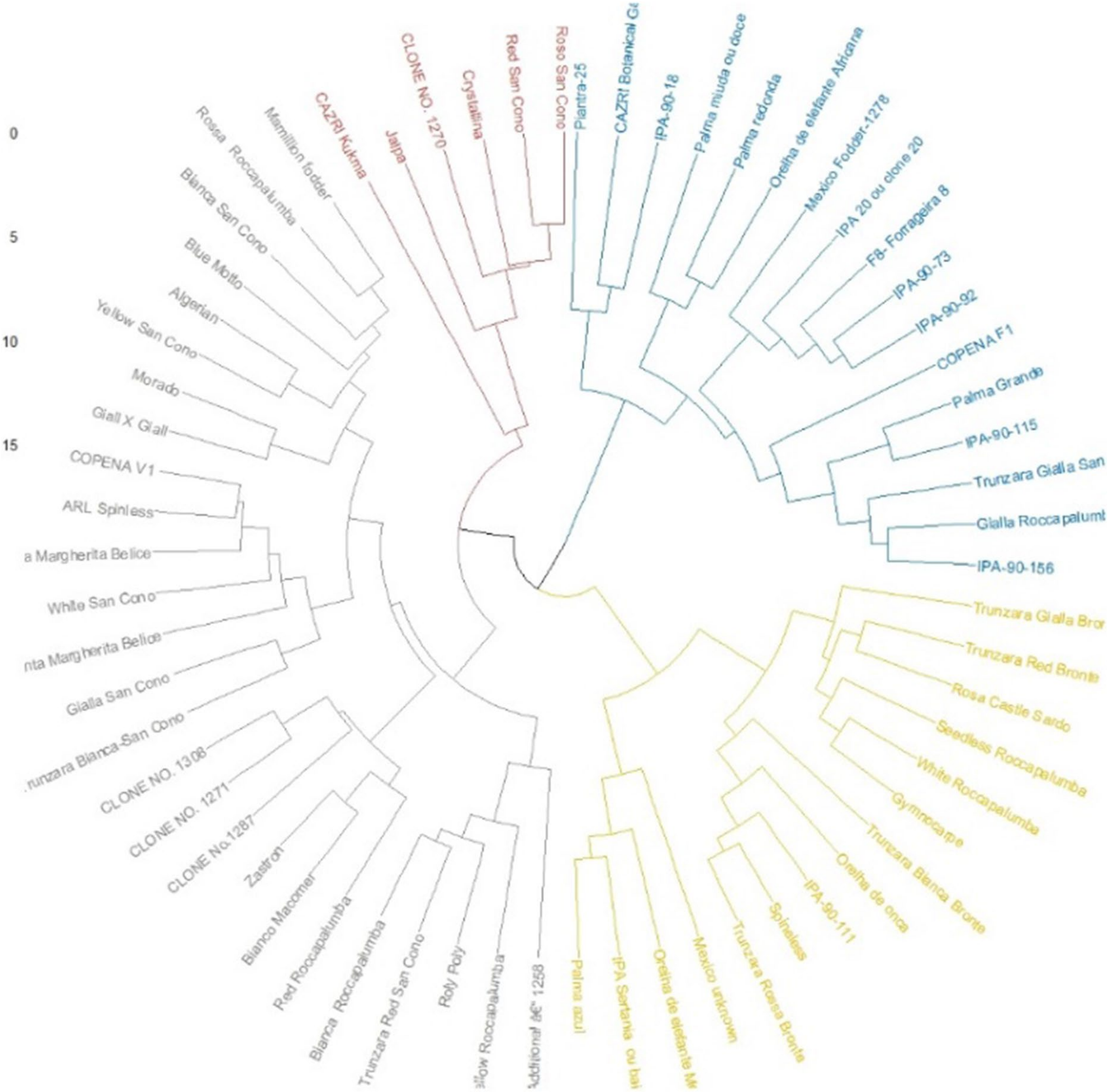

Fig. 3 Dendrogram of 64 cactus pear genotypes obtained by average distance between cluster analyses based on 34 morphological and fodder yield traits

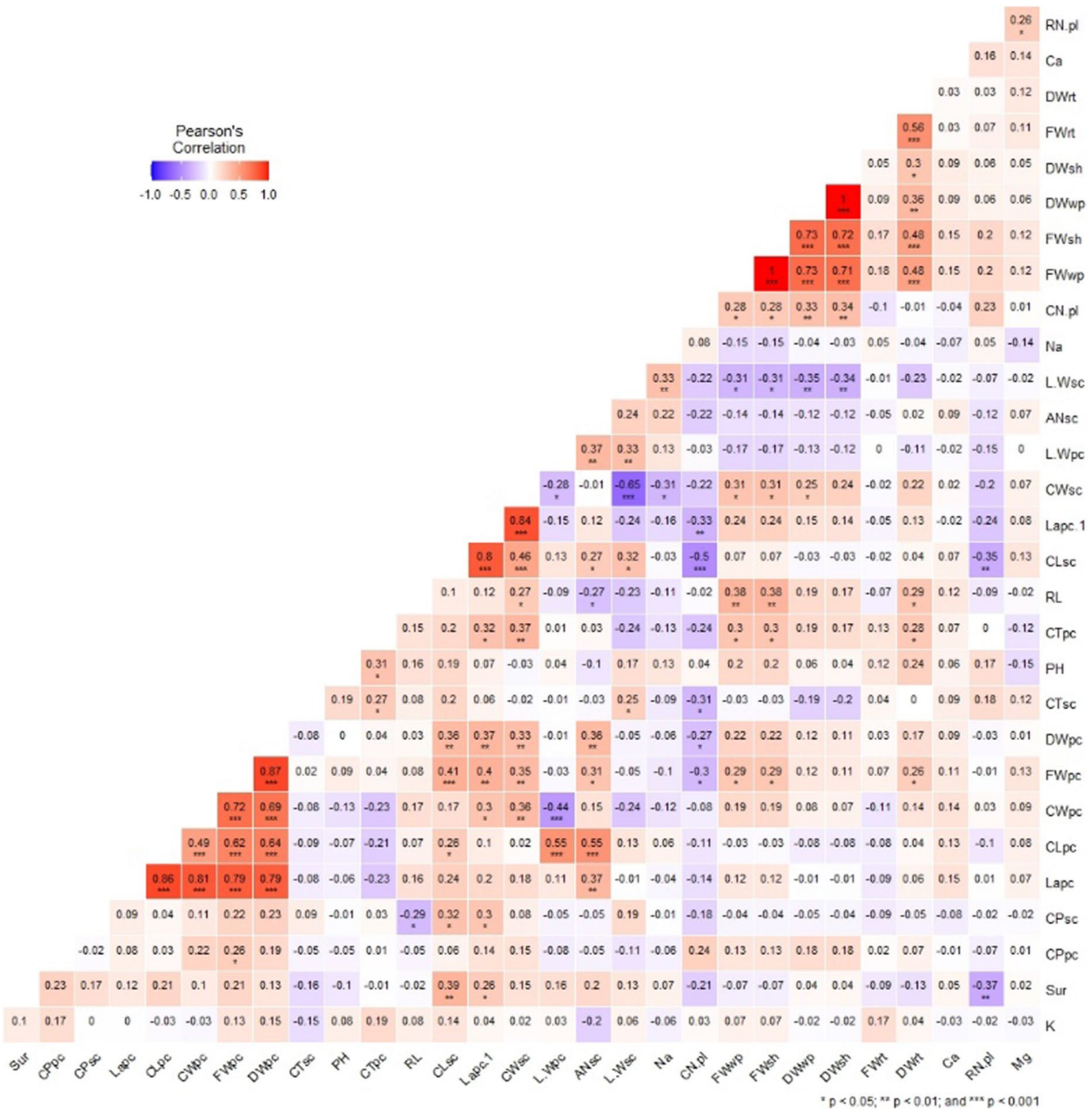

Fig. 4 Correlation among the 34 recorded traits in 64 cactus pear accessions. *Note* Survival rate: Sur (%); Plant height: PH; No. of cladodes/plant: Cl/pl; Plant fresh weight: FWwp; Plant dry weight: DWwp; Shoot fresh weight: FWsh; Shoot dry weight: DWsh; Root fresh weight: FWrt; Root dry weight: DWrt; Root length: RL; No. of roots/plant: RN-pl; Calcium: Ca; Magnesium: M; Potash: K; Sodium: Na; Primary cladode Fresh weight: FWpc; Primary cladode Dry weight: DWpc; Primary cladode Leaf Area: LApc; Primary cladode length: CLpc; Primary Cladode width: CWpc; Primary cladode L: W ratio: L: Wpc; Primary cladode Thickness: CTpc; Primary cladode Perimeter: CPpc; No. of areole/cladode: AN/cl; Secondary Cladode length: CLsc; Secondary Cladode width: CWsc; Secondary Cladode L: W ratio: L: Wsc; Secondary Cladode Thickness: CTsc; Secondary Cladode Perimeter: CPsc; Secondary Cladode Leaf area: LAsc

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Data availability All data generated or analyzed during this study are included in the paper or supplementary information.

Declarations

Conflict of interest The authors declare they have no conflicts of interest.

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