

Morphological and phenological characterization of Moroccan *Opuntia* cactus varieties (Karama, Ghalia, Belara, Marjana, Cherratia, Angad, and Melk Zhar) resistant to the cactus cochineal *Dactylopius opuntiae* (Cockerell)

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Abstract. In recent years, Opuntia cactus cultivation has been seriously threatened by the appearance of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) in Morocco, and four species with eight cactus pear varieties have been identified by research as resistant to this cochineal, these varieties are already registered in the official catalog of species and varieties in Morocco. A morphological and phenological characterization using twenty morphological attributes of these eight varieties: Karama, Ghalia, Belara, Marjana, Cherratia, Angad, and Melk Zhar were performed. The principal component analysis allowed distinguishing five groups according to the studied characteristics. Aakria with the smallest bright red fruit and very small cladodes (15.8/7.7 cm), Belara with the highest number of cladodes per plant (101.2), large cladodes (36.4/25.8 cm) and very sweet large vellow-green fruit, Marjana with very thick cladodes (2.3 cm) and large and very sweet fruit. The group of varieties (Melk Zhar, Angad and Cherratia) is characterized by wide and thorny cladodes, the longest thorns and fruits of large size (202.5-276.7 g). The group containing the varieties (Karama and Ghalia) is characterized by large cladodes (33.4-36.0/16.4-25.6 cm) and medium size fruits (98.5-115.6 g). Also, the periods of vegetative and floral budding, and flowering were the longest for Aakria and the shortest for Marjana. The period of fruit development was the longest for Aakria and the shortest for Marjana. In conclusion, these morphological and phenological variations among the eight cactus pear varieties identified as resistant to *D. opuntiae* in Morocco could be the subject of breeding programs.

Keywords: Cactus, morphological attributes, flowering phenology, fruiting phenology, Morocco.

Introduction

The *Opuntia* cactus genus is native to America (Peña-Valdivia *et al.*, 2008). This genus comprises about 300 species (Scheinvar, 1995), of which, twentyfive species have been found in Morocco (Arba *et al.*, 2002a). The most common and economically important *Opuntia* species is *O. ficus-indica* (L.) Mill. (Nefzaoui, 2016). This specie, commonly referred to as prickly pear, is originally from Mexico and diffused in several warm regions of the world by European travelers in the late 15th century (Kiesling, 1998; Griffith, 2004). The cultivation of *Opuntia* cactus pears extended to the hot arid regions of the world due to their resistance to drought, and now *O. ficus-indica* is cultivated in more than 30 countries worldwide stretching from southern Patagonia in Argentina to British Columbia in Canada (Anderson, 2001). It was introduced into North Africa and all along the Mediterranean basin during the 15th century by Christopher Columbus (Anderson, 2001).

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Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC SA) license (https://creativecommons.org/license s/by-nc-sa/4.0/). It is Mexico that comes in first place regarding the surface area occupied by *Opuntia* cactus, estimated at 3 million hectares (ha), of which more than 70,000 ha are cultivated (Inglese, 2010). In Brazil, the surface area occupied by prickly pear cactus is 900,000 ha, of which 600,000 ha are cultivated (Souto Alves *et al.*, 2009; Inglese, 2010). In Italy 3,000 ha of commercial production, 25,000 ha in Tunisia and over 1,000 ha in each of the following countries: Chile, Argentina and South Africa (Arba *et al.*, 2017). In Morocco, the area occupied by cactus pears has evolved remarkably over the last two decades, reaching about 150,000 ha in 2014 before the appearance of the cactus cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) (El Aalaoui *et al.*, 2019; Sbaghi *et al.*, 2019).

Opuntia cactus is an important drought-tolerant crop. It has various beneficial uses, both as food and feed. The fruit is used in human consumption, as a dye, and in cosmetic products (Nefzaoui, 2016). The cladodes are used as animal feed predominantly in the arid and semi-arid zones on degraded lands (Kiesling, 1998; Arba, 2002b; Griffith, 2004).

Characterization of *Opuntia* cactus germplasm is essential to provide information on species diversity, the effect of ecological factor changes and the level of hybridization within the species (Peña-Valdivia *et al.*, 2008; Nefzaoui, 2016). Morphological and molecular data are used to evaluate cactus species and to assess their genetic diversity (Nefzaoui, 2016). Morphological characterization of *Opuntia spp.* is strongly recommended as a first step before attempting an advanced assessment through molecular markers (Hoogendijk and Williams, 2001). A set of morphological attributes (descriptors) can be used to describe *Opuntia* cactus pear plant phenotype. Characteristics of the plant, cladodes (pads), fruits, spines, glochids and seeds can be measured and expressed as numerical values (Nefzaoui, 2016).

Since 2014, the year of the appearance of the *Opuntia* cactus cochineal *D. opuntiae* in Morocco, the *Opuntia* cactus culture is seriously damaged by the attacks of this harmful pest. Thousands of hectares of cactus are totally destroyed causing enormous ecological and economic losses (El Aalaoui *et al.*, 2019). The research carried out in Morocco as a part of the emergency plan for control *D. opuntiae* allowed to identify eight *Opuntia* cactus resistant varieties that showed a very high degree of resistance against this pest (*D. opuntiae*) (Sbaghi *et al.*, 2019). These resistant varieties were recently registered in the Official Catalogue of *Opuntia* cactus in Morocco (Sbaghi *et al.*, 2019). They are also multiplied and transplanted in different regions of Morocco with very different climatic conditions (Sbaghi *et al.*, 2019). In this context, the objective of this research is to characterize the morphological and phenological behavior of the eight cactus varieties that could be the subject of *Opuntia* cactus breeding programs. This characterization enables the selection of desirable traits, targeted crossbreeding, development of disease-resistant cultivars, crop improvement, and conservation of genetic resources, all of which contribute to the advancement and sustainability of cactus breeding efforts.

Material and Methods

Experimental design

The study was carried out at the platform of *Opuntia* cactus resistant to *D. opuntiae* in Tassaout Experimental Station of INRA-Morocco located in northeast of Marrakech (318490 N, 78160 W, 465) on a loamy clay soil. The platform contains only the eight varieties of *Opuntia* cactus identified as resistant to the *Opuntia* cactus cochineal (*D. opuntiae*) by INRA (National Institute of Agricultural Research) (Sbaghi *et al.*, 2019; El Aalaoui and Sbaghi, 2021) (Table 1, Figure 1). Installation of the

platform was carried out using *Opuntia* cactus seedlings. For each of the eight varieties, cladodes were harvested in March 2018 at the Experimental Station of ORMVAD-Zemamra, Doukkala, Morocco (32°37'48" N, 8°42'0" W), then were cut into six pieces of about 140 cm², each piece always having at least two bud areoles in one of the bases and allowed to air dry for 25 days. After 25 days of drying at a temperature between 17 and 28°C, they were soaked for 5 min in 2.5 g/L auxin (IBA). The cuttings were planted in normal polarity in a cylindrical black plastic container, with an average length of 24.6 cm and an average width of 14.1 cm, filled with a mixture of fine sand (2/3 w/w) and peat (1/3 w/w) and placed at an ambient temperature of 28/17°C (day/night) and allowed to grow. The cuttings were transferred to Tassaout Experimental Station for planting. The *Opuntia* cactus plants were distributed in the platform plots (30 ha) with a planting density of 1110 plants/ha, equivalent to a spacing of 3 x 1.5 m. The plants of each variety were planted in separate rows. The plants are grown under intensive fertilization and irrigation with a frequency of three irrigations per year, concentrated mainly between the months of June and October. Mechanical weeding is carried out periodically.

This experimental site is located in the semi-arid ecological zone. Accumulated rainfall values vary between 144 and 388 mm/year. Spring and summer seasons are normally characterized by severe drought stress associated with high temperatures. The average temperature varies from -0.5°C (Dec-Jan) to 40.0 °C (Jul-Aug).

Table 1. List of Opuntia cactus varieties resistant to Dactylopius opuntiae registered in the official	
catalogue of cactus pear in Morocco.	

Species	Varieties	Origin
O. engelmannii Salm-Dyck, 1850	Marjana	Dchira- Inezgane - Morocco
	Karama	Dchira - Inezgane - Morocco
	Ghalia	Dchira - Inezgane - Morocco
<i>O. fícus-indica</i> (L.) Miller, 1768	Belara	Dchira - Inezgane - Morocco
O. robusta (Haw.) Haw., 1812	Angad	Oujda - Morocco
	Melk Zhar	Irradiation O. robusta - Morocco
	Cherratia	Bouznika-Morocco
O. dillenii (Ker Gawl.) Haw., 1819	Aakria	Bouknadel - Morocco



Figure 1. *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco, A) Marjana, B) Belara, C) Karama, D) Ghalia, E) Angad, F) Cherratia, G) Melk Zhar, H) Aakria.

Morphological characterization

Ten plants of each *Opuntia* cactus variety were randomly selected, and 20 morphological attributes were evaluated including whole plant, cladodes, fruits, and seeds morphological parameters using the "Descriptor for Cactus Pear" produced by the FAO-ICARDA Cactusnet (Chessa and Nieddu, 1997). Each of the plant studied was 4 years old and was clearly healthy and vigorous.

Morphological parameters recorded for the whole plant and the cladode: Hp: Height of the plant (cm); Dp: Diameter of the plant in the East-West direction (cm); Nc: Number of cladodes per plant; Lc: Length of one-year-old cladodes (cm); Ic: width of one-year-old cladodes (cm); CSI: Cladode shape index measured by the ratio (Lc/Ic); Ec: Thickness of one-year-old cladodes (cm); Na/10 cm²: Number of areoles/10 cm²; Da: Distance between areoles (cm); LepI: Length of the longest spine (cm).

The characteristics of fruits and seeds recorded: Pf: weight of fruit (g); Lf: Fruit length (cm); Df: Fruit diameter (cm); IFf: Fruit shape index = ratio (Lf/Df); Ee: Bark thickness (mm); Pe: Weight of peel (g); TC: Flesh content (including seeds) in (%) calculated by: [(Pf-Pe)/Pf]x100; Br: Brix measured by a hand refractometer (Model DR-A1, Atago, Japan); Pg: Seed weight per fruit (g); Ng/f: Number of seeds per fruit.

Flowering and fruiting phenology

For each variety, we followed a randomly selected sample of ten four-year-old plants. The method described by Barbara (2007) was chosen to determine the period of the flowering and fruiting phases. Flowering and fruiting parameters recorded: EBvf: Emission of vegetative and floral buds; Fbf: Formation of flower buds; F: Flowering time; MF: Maturation of fruits; PDF: The period of fruit development.

Statistical analysis

A factorial analysis of variance (ANOVA) was performed for each parameter measured, and the means are compared with Student-Newman-Keuls (SNK) test on the least significant difference (LSD) at p= 0.05 using SPSS 18.0 software. To assess the degree of similarity among the analyzed morphological attributes of the eight Opuntia cactus varieties studied and to understand the relationships among them, the data were subjected to Principal Component Analysis (PCA) (James and McCulloch, 1990). For phenotypic classification of the tested Opuntia cactus varieties, a Hierarchical Cluster Analysis (HCA) was used to measure the degree of phenotypic similarity among the Opuntia cactus varieties using SPSS var. 18 software (James and McCulloch, 1990).

Results

Morphological parameters of the whole plant

Plant height is significantly varied among the tested *Opuntia* cactus varieties and ranges from 151.4 cm for Belara to 91.6 cm for Melk Zhar. Almost, the same trend was observed for the plant diameter. The maximum diameter values were recorded for the varieties Karama, Ghalia, and Belara (177.6, 171.0, and 160.6 cm respectively), while the minimum diameter value was recorded for the variety Melk Zhar (98 cm). With the exception of Melk Zhar variety, all the tested varieties had plant height and diameter values exceeding 100 cm. The average number of cladodes per plant is 58.7 and varies between 14.2 for Melk Zhar and 101.2 for Belara (Table 2). The lowest coefficient of variation values (CVs) was observed with plant shape parameters with CV values varying between 4.5 to 28.1 % with an average value 23.0 % for plant height and 23.2 % for plant diameter.

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Varieties	Нр	Dp	Nc
Karama	148.2±8.8 ab (5.9)*	177.6±32.0 a (18.0)	88.6±13.6 b (15.3)
Ghalia	133.8±6.0 bc (4.5)	171.0±12.2 a (7.1)	60.6±7.0 c (11.6)
Belara	151.4±12.3 a (8.1)	160.6±6.4 ab (4.0)	101.2±45.6 ab (45.1)
Marjana	131.2±17.2 c (13.1)	137.4±26.4 bc (19.2)	40.2±7.5 cd (18.7)
Cherratia	122.6±9.0 cd (7.3)	122.2±23.0 cd (18.9)	22.4±2.4 de (10.7)
Angad	113.8±11.1 d (9.8)	118.8±15.5 cd (13.0)	20.2±1.7 de (8.4)
Aakria	72.5±8.9 e (12.3)	140.5±7.2 bc (5.1)	121.8±3.7 a (3.0)
Melk Zhar	91.6±9.7 f (10.6)	98.0±27.4 d (28.0)	14.2±3.3 e (23.2)
Total average	120.6±27.7 (23.0)	140.8±32.7 (23.2)	58.7±42.0 (71.6)
Statistical	<i>F</i> =63.3, df =7,	<i>F</i> =17.5, df=7,	<i>F</i> =56.62, df=7,
analysis	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001

Table 2. Morphological parameters of the whole plant of the *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco (Hp: Height of the plant (cm); Dp: Diameter of the plant in the East-West direction (cm); Nc: Number of cladodes per plant) (Mean ±SD).

^{*}Coefficient of variation. Means within a column followed by the same letters are not significantly different according to LSD test at α = 0.05.

Morphological parameters of the cladode

The morphological parameters of the cladode are significantly varied among the tested *Opuntia* cactus varieties. The average length of the cladodes was 35.7 cm and varies between 46.9 cm for Cherratia and 15.8 cm for Aakria. The average width of the cladodes was 29.5 cm and varies between 26.1 cm for Cherratia and 7.7 cm for Aakria. The average thickness of the cladodes was 2.1 cm and varies between 2.7 cm for Cherratia and 1.3 cm for Aakria. The highest number of areolas per 10 cm² was recorded for Karama (21.4), followed by Ghalia (17.2) and Belara (11.2), and the lowest numbers were recorded for Cherratia (4.8), Angad (3.4), Melk Zhar (3.6) and Aakria (3.0) (Table 3). The CV of the morphological parameters of the cladodes is acceptable and varies between 5.2% and 28.6%. The cladode morphological attributes appear to be more accurate to measure as confirmed by the relatively lower coefficients of variation (Table 3). The lowest CVs are observed for cladode shape parameters with CV values ranging from 5.2 to 18.4% with an average value of 25.5% for length and 40.3% for width (Table 3).

The characteristics of fruits

The average weights of fresh fruit varied significantly from 45.9 g for Aakria to 276.7 g for Cherratia. Fruit length varies significantly from 5.8 cm for Karama to 9.4 cm for Belara. The fruit diameter varied significantly from 4.9 cm for Ghalia to 7.9 cm for Cherratia. Bark thickness varied significantly from 0.4 mm for Aakria to 1.6 mm for Ghalia. Bark weight varied significantly from 24.8 g for Aakria to 181.2 g for Cherratia. The flesh content varied significantly from 29.3% for Melk Zhar to 48.0% for Marjana. Highly significant differences were recorded among the tested *Opuntia* cactus varieties regarding brix degree. The lowest average value of 6.4 °Bx was recorded for Aakria vs. the highest values of 14.5 and 14.6 °Bx for Marjana and Belara, respectively (Table 4). The CVs of the morphological parameters of the fruits are acceptable and vary between 0% and 38.5%. The lowest CVs are observed for the fruit shape parameters with CV values varying between 0 and 8.6% with an average value of 15.5% for fruit length and 16.4% for fruit diameter (Table 4).

Table 3. Morphological parameters of the cladode of the *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco [Lc: Length of one-year-old cladode (cm); Ic: width of one-year-old cladode (cm); CSI: Cladode shape index measured by the ratio (Lc/Ic); Ec: Thickness of one-year-old cladode (cm); Na/10 cm²: Number of areoles/10 cm²; Da: Distance between areoles (cm); Lepl: Length of the longest spine (cm)] (Mean ±SD).

Varieties	Lc	lc	CSI	Ec	Na/10 cm ²	Da	Lepl
Karama	36.0 ±2.5 cd (6.9)*	25.6±1.4 c (5.5)	1.4±0.1 bc (25.0)	2.0±0.2 c (10.0)	21.4±3.8 a (17.8)	3.7±1.0 c (27.0)	2.3±0.3 d (13.0)
Ghalia	33.4±2.5 d (7.5)	16.4±1.8 c (11.0)	1.3±0.2 c (15.4)	2.5±0.5 ab (20.0)	17.2±0.8 b (4.6)	3.5±0.5 c (14.3)	3.5±0.5 bc (14.3)
Belara	36.4±2.7 bcd (7.4)	25.8±1.4 c (5.4)	1.4±0.1 bc (7.1)	1.4±0.4 d (28.6)	11.2±1.2 c (10.7)	3.7±0.3 c (8.1)	0.6±0.1 e (16.7)
Marjana	38.6±2.0 bc (5.2)	24.2±1.5 c (6.2)	1.6±0.1 b (6.3)	2.3±0.4 bc (17.4)	5.8±1.2 d (20.7)	3.6±0.5 c (13.9)	0.6±0.1 e (16.7)
Cherratia	46.9±5.9 a (12.6)	46.1±4.1 a (8.9)	1.0±0.1 d (10.0)	2.7±0.3 ab (11.1)	4.8±0.4 de (8.3)	7.0±0.4 a (5.7)	3.8±0.3 ab (7.9)
Angad	37.0±4.1 bcd (11.1)	41.4±5.4 b (13.0)	0.9±0.1 d (11.1)	2.8±0.6 a (21.4)	3.4±0.5 e (14.7)	5.0±0.7 b (14.0)	4.0±0.0 a-
Aakria	15.8±2.9 e (18.4)	7.7±0.5 d (6.5)	2.1±0.3 a (14.3)	1.3±0.0 d-	3.0±0.0 e-	4.0±0.6 c (15.0)	3.1±0.1 c (3.2)
Melk Zhar	41.1±4.4 b (10.7)	39.0±5.0 b (12.8)	1.1±0.1 d (9.0)	2.0±0.2 c (10.0)	3.6±0.5 e (13.9)	5.6±0.4 b (7.1)	3.9±0.4 a (10.3)
Total average	35.7±9.1 (25.5)	29.5±11.9 (40.3)	1.3±0.4 (30.8)	2,1±0.6 (28.6)	8.8±6.8(77.3)	4.5±1.3 (28.9)	2.7±1.4 (51.9)
Statistical	<i>F</i> =63.2, df=7,	<i>F</i> =146.1, df=7,	<i>F</i> =57.3, df=7,	<i>F</i> =23.5, df=7,	<i>F</i> =212.2, df=7,	<i>F</i> =42.2, df=7,	<i>F</i> =254.8, df=7,
analysis	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001

*Coefficient of variation. Means within a column followed by the same letters are not significantly different according to LSD test at α = 0.05.

Table 4. The characteristics of fruits of the *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco (Pf: weight of fruit (g); Lf: Fruit length (cm); Df: Fruit diameter (cm); IFf: Fruit shape index = ratio (Lf/Df); Ee: Bark thickness (mm); Pe: Weight of peel (g); TC: Flesh content (including seeds) in (%) calculated by: [(Pf-Pe)/Pf]x100; Br: Brix) (Mean ±SD).

Varieties	Pf	Lf	Df	IFf	Ee	Pe	ТС	Br
Karama	115.6±0.5 f (0.4)*	5.8±0.5 d (8.6)	5.5±0.3 e (5.5)	1.1±0.1 d (9.1)	0.9±0.0 cde (0.0)	76.6±0.1 e (0.1)	33.8±0.3 c (0.8)	13.4±0.2 b (1.5)
Ghalia	98.5±0.4 g (0.4)	5.9±0.4 d (6.8)	4.9±0.0 f (0.0)	1.2±0.1 c (8.3)	1.6±0.1 a (6.3)	65.7±0.1 f (15.2)	33.4±0.3 cd (0.9)	13.4±0.2 b (1.5)
Belara	183.4±0.5 d (0.3)	9.4±0.3 a (3.2)	5.9±0.1 d (1.7)	1.6±0.1 a (6.3)	0.9±0.0 de (0.0)	98.4±0.1 d (0.1)	46.4±0.2 a (0.4)	14.5±0.2 a (1.4)
Marjana	134.5±0.7 e (0.5)	7.2±0.3 bc (4.2)	5.5±0.3 e (5.5)	1.3±0.1 b (7.7)	0.8±0.1 e (12.5)	69.9±0.0 ef-	48.0±0.3 a (0.6)	14.6±0.1 a (0.7)
Cherratia	276.7±0.5 a (0.2)	7.2±0.4 b (5.6)	7.9±0.1 a (1.3)	0.9±0.1 e (11.1)	1.3±0.1 abc (7.7)	181.2±0.1 a (0.1)	34.5±0.1 c (0.3)	12.1±0.3 c (2.3)
Angad	220.5±0.6 b (0.3)	7.4±0.3 b (4.1)	7.2±0.4 b (5.6)	1.0±0.1 d (10.0)	1.3±0.5 ab (38.5)	129.3±0.1 c (0.1)	41.4±0.2 b (0.5)	12.1±0.3 c (2.5)
Aakria	45.9±0.4 h (0.9)	6.7±0.2 c (3.0)	5.3±0.2 e (3.8)	1.3±0.1 bc (7.7)	0.4±0.1 f (25.0)	24.8±0.1 g (0.4)	45.9±0.5 a (1.1)	6.4±0.2 d (3.1)
Melk Zhar	202.5±0.7 c (0.3)	7.2±0.4 b (5.6)	6.6±0.2 c (3.0)	1.1±0.1 d (9.1)	1.2±0.4 bcd (33.3)	143.1±17.2 b (12.0)	29.3±8.6 d (29.4)	12.2±0.4 c (3.3)
Total average	159.7±70.3 (44.0)	7.1±1.1 (15.5)	6.1±1.0 (16.4)	1.2±0.2 (16.7)	1.1±0.4 (36.4)	98.6±47.5 (48.2)	39.1±7.4 (18.9)	12.3±2.5 (20.3)
Statistical	F=180870.6, df=7,	F=92.9, df=7,	F=200.3, df=7,	F=77.3, df=7,	F=21.9, df=7,	F=679.7, df=7,	F=55.3, df=7,	F=993.3, df=7,
analysis	P< 0.0001	P< 0.0001	P< 0.0001	P< 0.0001	P< 0.0001	P< 0.0001	P< 0.0001	P< 0.0001

*Coefficient of variation. Means within a column followed by the same letters are not significantly different according to LSD test at α = 0.05.

The characteristics of seeds

Seed weight per fruit varied significantly from 0.8 g for Aakria to 8.1 g for Cherratia. Number of seeds per fruit varied significantly from 77.1 for Aakria to 570.1 for Belara. The CVs of the seed characteristics are acceptable and vary between 0.1 % and 29.3 % (Table 5). The lowest CVs are observed for the number of seeds per fruit with CV values ranging from 0.1 to 0.3%. The seed characteristics seem to be more accurate to measure as confirmed by the relatively lower coefficients of variation (0.03-29.3%) (Table 5).

Table 5. The characteristics of seeds of the Opuntia cactus varieties resistant to Dac	tylopius
opuntiae in Morocco (Pg: Seed weight per fruit (g); Ng/f: Number of seeds per fruit) (Mean	±SD).

Varieties	Pg	Ng/f
Karama	4.2 ±0.1 d (2.4)*	286.4±0.7 e (0.2)
Ghalia	1.5±0.1 e (6.7)	159.2±0.4 g (0.3)
Belara	4.1±0.2 d (4.9)	570.1±0.3 a (0.1)
Marjana	4.1±1.2 d (29.3)	277.1±0.1 f (0.03)
Cherratia	8.1±0.1 a (1.2)	526.2±0.3 b (0.1)
Angad	6.3±0.1 c (1.6)	520.1±0.2 c (0.04)
Aakria	0.8±0.1 f (12.5)	77.1±0.1 h (0.1)
Melk Zhar	7.4±0.1 b (1.4)	398.1±0.2 d (0.1)
Total average	4.6±2.5 (54.3)	351.8±171.0 (48.6)
Statistical analysis	<i>F</i> =5000.5, df=, <i>P</i> <0.0001	<i>F</i> =2803677.5, df=7, <i>P</i> <0.0001

Coefficient of variation. Means within a column followed by the same letters are not significantly different according to LSD test at α = 0.05.

Principal component analysis (PCA) of morphological parameters Data

The resulting correlation matrix and the results of KMO and Bartlett test are presented in Tables 6 and 7, respectively. Plant height (Hp) was significantly correlated to plant diameter (Dp) and number of cladodes per plant (Nc). One-year cladode length (Lc) was significantly correlated to cladode width (Ic) and number of areoles (Na). Cladode thickness (Ec) was positively correlated to bark thickness (Ee). The distance between areoles (Da) was significantly correlated to the length of the longest spine (Lepl), fruit weight (Pf), fruit diameter (Df) and peel weight (Pe). Peel weight was highly significantly correlated to seeds weight (Pg), and number of seeds per fruit (Ng). The sampling accuracy measured by Kaiser-Meyer-Olkin test that gives an overall view of the quality of inter-item correlations is 0.69. This value shows that the inter-item correlation is acceptable.

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Variables	Нр	Dp	Nc	Lc	lc	CSI	Ec	Na	Da	Lepl	Pf	Lf	Df	IFf	Ee	Ре	тс	Br	Pg	Ng
Нр	1																			
Dp	0.43	1																		
Nc	0.04	0.56	1																	
Lc	0.43	-0.19	-0.65	1																
lc	0.16	-0.36	-0.77	0.84	1															
CSI	-0.19	0.27	0.69	-0.61	-0.88	1														
Ec	0.21	-0.18	-0.68	0.49	0.60	-0.55	1													
Na	0.66	0.62	0.33	0.02	-0.20	0.04	-0.06	1												
Da	-0.26	-0.47	-0.54	0.51	0.70	-0.55	0.38	-0.46	1											
Lepl	-0.50	-0.30	-0.39	0.02	0.42	-0.43	0.34	-0.24	0.54	1										
Pf	0.16	-0.45	-0.67	0.78	0.90	-0.77	0.44	-0.34	0.72	0.25	1									
Lf	0.15	-0.14	0.05	0.19	0.16	-0.11	-0.21	-0.36	0.12	-0.41	0.44	1								
Df	-0.13	-0.54	-0.60	0.56	0.80	-0.65	0.38	-0.51	0.81	0.44	0.90	0.32	1							
IFf	0.25	0.34	0.54	-0.29	-0.52	0.45	-0.49	0.10	-0.55	-0.73	-0.36	0.62	-0.55	1						
Ee	0.23	-0.09	-0.50	0.43	0.53	-0.62	0.54	0.17	0.22	0.39	0.40	-0.10	0.29	-0.32	1					
Pe	0.08	-0.47	-0.70	0.78	0.92	-0.78	0.44	-0.30	0.78	0.39	0.97	0.29	0.89	-0.48	0.44	1				
тс	0.02	0.10	0.37	-0.38	-0.47	0.49	-0.26	-0.27	-0.37	-0.59	-0.26	0.42	-0.22	0.57	-0.47	-0.48	1			
Br	0.78	0.20	-0.33	0.68	0.41	-0.44	0.31	0.44	-0.14	-0.45	0.37	0.23	0.01	0.18	0.39	0.30	-0.09	1		
Pg	0.05	-0.53	-0.72	0.77	0.88	-0.73	0.40	-0.35	0.73	0.30	0.93	0.28	0.87	-0.49	0.31	0.94	-0.37	0.31	1	
Ng	0.33	-0.28	-0.42	0.67	0.73	-0.65	0.24	-0.21	0.48	-0.04	0.90	0.68	0.76	-0.04	0.27	0.81	-0.04	0.47	0.79	1

Table 6. Correlation matrix (Pearson (n)) of the morphological parameters for the *Opuntia* cactus pear varieties resistant to *Dactylopius opuntiae* in Morocco.

Values in bold are different from 0 with a significance level alpha=0.05; Dp: Diameter of the plant in the East-West direction (cm); Nc: Number of cladodes per plant; Lc: Length of one-year-old cladodes (cm); Ic: width of one-year-old cladodes (cm); CSI: Cladode shape index measured by the ratio (Lc/lc); Ec: Thickness of one-year-old cladodes (cm); Na/10 cm² : Number of areoles/10 cm²; Da: Distance between areoles (cm); Lepl: Length of the longest spine (cm) ; Pf: weight of fruit (g); Lf: Fruit length (cm); Df: Fruit diameter (cm); IFf: Fruit shape index = ratio (Lf/Df); Ee: Bark thickness (mm); Pe: Weight of peel (g); TC: Flesh content (including seeds) (%); Br: Brix ; Pg: Seed weight per fruit (g); Ng/f: Number of seeds per fruit.

Table 7. KMO and Bartlett's Test.

Kaiser-Meyer-Olkin Measure of Sampling	0.69	
Bartlett's Test of Sphericity	Approx. Chi-Square	3066.39
	df	190
	<i>p</i> -value	< 0.0001

Our data shows that 85.2 % of the total variance is explained by the first four factors/components (Table 8). The first three factors explain 80.1 % of the total variance (Table 8).

Table 8. Eigenvalues (PCA of the	Opuntia cactus varieties	s resistant to Dactylopius opuntiae in
Morocco).		

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Eigenvalue	9.5	3.6	2.9	1.0	0.8	0.6	0.4	0.3	0.3	0.2	0.1	0.1	0.1
Variability(%)	47.3	18.1	147	5.1	3.8	3.2	1.9	1.6	1.3	0.9	0.6	0.5	0.4
Cumulative (%)	47.3	65.4	80.1	85.2	89.0	92.2	94.1	95.7	97.0	97.8	98.4	99.0	99.3

The first factor is represented mainly by fruit weight (Pf), fruit length (Lf), and number of seeds per fruit (Ng). The second factor is represented mainly by length of the longest spine (Lepl), Bark thickness (Ee), and cladode thickness (Ec) (Figure 2).

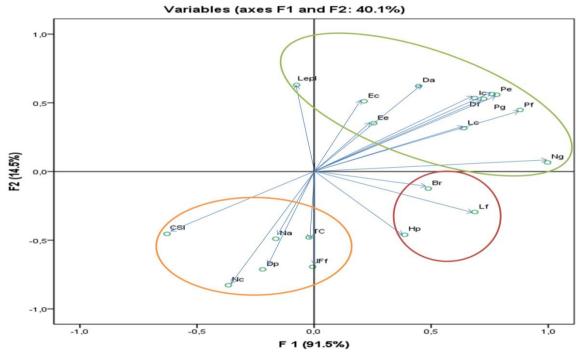


Figure 2. Projection of morphological parameters on F1 and F2 axes.

The projection of the *Opuntia* cactus varieties on principal axes F1 and F2 shows that they are distributed to the five following groups: group 1: Melk Zhar, Cherratia, and Angad; group 2: Ghalia and Karama; group 3: Belara; group 4: Marjana; group 5: Aakria (Figure 3).

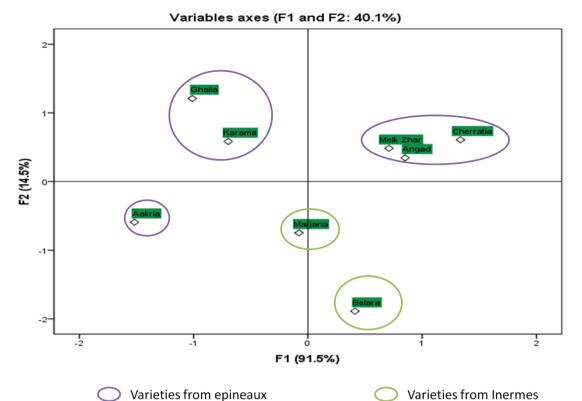


Figure 3. Projection of the *Opuntia* cactus pear varieties resistant to *Dactylopius opuntiae* in Morocco on F1 and F2 axes.

Agglomerative hierarchical clustering (AHC)

To highlight the relationships among the studied *Opuntia* cactus varieties, a dendrogram was constructed based on the proximity matrix using the Euclidian distance (Figure 4). The dendrogram resulting from this matrix distributes the *Opuntia* cactus pear varieties tested to 4 clusters: Cluster 1: Karama, Marjana, and Ghalia; Cluster 2: Aakria; Cluster 3: Chrratia, Angad, and Melk Zhar; Cluster 4: Belara. This distribution is almost concordance with the results obtained with Pearson principal component analysis (Figure 3).

Flowering and fruiting phenology

The emission of vegetative and floral buds and flowering of the eight cactus pear varieties tested were important from February to May, when temperatures started to increase, and the day length became much longer (Table 9). During the studied crop year (2020-2021), the vegetative and floral bud emission phase was the longest for Aakria (103 days) and the shortest for Marjana (87 days). The flowering phase was also the longest for Aakria (91 days) and the shortest for Marjana (85 days). The fruit ripening period was slightly the longest for Belara (86 days) and the shortest for Marjana (79 days). The period of fruit development varied also among the varieties. It was the longest for Aakria (190 days) and the shortest for Marjana (143 days) (Table 9).

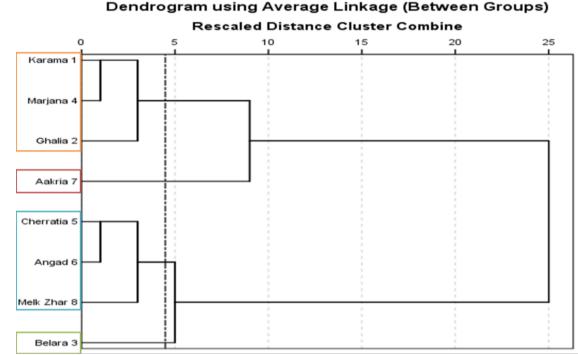


Figure 4. Dendrogram of the *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco, based on Euclidian distance (class 1 in orange, class 2 in red, class 3 in blue and class 4 in green).

Table 9. Duration of the phenological phases of flowering and fruiting of the *Opuntia* cactus varieties resistant to *Dactylopius opuntiae* in Morocco (EBvf: Emission of vegetative and floral buds; Fbf: Formation of flower buds; F: Flowering time; MF: Maturation of fruits; PDF: The period of fruit development).

Varieties	EBvf	Fbf	F	MF	PDF
Karama	90 days ±3:	89 days ±4:	87 days ±5:	81 days ±5:	146 days ±6:
	Feb 18 - May19	Feb 26-May 26	Mar 4- Jun 1	August 1- Sept 20	Feb 26 – Jul 25
Ghalia	90 days ±3:	89 days ±4:	87 days ±5:	81 days ±5:	146 days ±6:
	Feb 18-May 19	Feb 26-May 26	Mar 1-Jun 1	Aug 1-Sep 20	Feb 26-Jul 25
Belara	92 days ±5:	92 days ±4:	90 days ±5:	86 days ±5:	156 days ±4:
	Feb 15-May 18	Feb 25-May 26	Mar 1-Jun 1	Jul 26-Sep 20	Feb 15-Jul 24
Marjana	87 days ±3:	87 days ±4:	85 days ±5:	79 days ±5:	143 days ±6:
	Feb 20-May 18	Feb 28-May 26	Mar 6-Jun 1	Aug 1-Sep 17	Feb 28-Jul 24
Cherratia	90 days ±4:	89 days ±4:	88 days ±5:	81 days ±6:	146 days ±6:
	Feb 28-May 29	Mar 5-June 4	Mar 14-Jun 12	Aug 11-Sep 30	Mar 5-Jul 15
Angad	90 days ±4:	89 days ±4:	88 days ±5:	81 days ±6:	146 days ±6:
	Feb 28-May 29	Mar 5-Jun 4	Mar 14-Jun 12	Aug 11-Sep 30	Mar 5-Jul 15
Aakria	103 days ±5:	104 days ±4:	91 days ±4:	82 days ±5:	190 days ±3:
	Jan 20-May 7	Feb 01-May 15	Mar 20-Jun 20	Jun 20-Sep 10	Feb 01-Aug 10
Melk Zhar	89 days ±4:	88 days ±3:	87 days ±6:	79 days ±6:	146 days ±6:
	Feb 29-May 29	Mar 7-Apr 4	Mar 15-Jun 12	Aug 13-Sep 30	Mar 7-Jul 18

Discussion

The measurements of the morphological parameters of plant and cladode allow calculating the volume of the vegetative system, and considered as an indicator of the global growth of the plant and can be related to the evolution of the cladode surface and to the components of the yield. The ANOVA analysis indicates that the difference among the Moroccan *Opuntia* cactus varieties resistants to *Dactylopius opuntiae* tested was significant for all the morphological parameters measured. The plant height, and diameter, and number of cladodes per plant values were significantly the highest for Belara and the lowest for Melk Zhar. The total average number of

cladodes per plant was 58.7, and exceeded 20 cladodes for all the varieties tested except for Melk Zhar. The length, width and thickness of the one-year cladode, the distance between areoles and the length of the longest spine were significantly higher for Cherratia than for the other varieties. The number of areolae was significantly the highest for Karama, and the lowest for Cherratia, Angad, Melk Zhar, and Aakria. The lowest CVs are observed for plant (4.5-28.1%) and cladode (5.2-18.4%), and fruit (0 and 8.6%) shape. These values are higher than those reported for Mexican accessions by Cervantes Herrera *et al.* (2006) with CV values of 2.7%, 2.6% and 11% for cladode length, width and thickness, respectively, and lower than those reported for some Mediterranean accessions by Arba (2006) with CV values of 5%, 7.7% and 40%, for cladode length, width and thickness, respectively. The plant, cladode and fruit shape parameters appeared to be more important to be measured for accurate morphological characterization. Erre and Chessa (2013) reported that among 38 descriptors studied, only the cladode and spines shape are useful to discriminate Italian pear accessions.

Like the morphological parameters of plant and cladode, the morphological and biochemical (°Brix) characteristics of fruit show a high variability among the studied Opuntia cactus varieties. Aakria, Ghalia, and Karama presented small fruit sizes (45.9-115.6 g). Cherratia, Angad, Melk Zhar, Belara, and Marjana have fruits with large sizes (134.5-276.7 g) that exceed 120 g destined for export (Inglese et al., 1995a). The fruit weight values recorded for the resistant Opuntia cactus varieties studied in this work are relatively higher than the values of El Finti et al. (2013) who found weights between 80.6 g and 106.5 g for 13 cultivars from different regions of Morocco; very higher than the values found by El Kharrassi et al. (2016), who found fruit weights ranging from 53 g to 103 g after evaluation of 30 accessions from different regions in Morocco. Mulas et al. (2006) who worked on 20 accessions of wild Opuntia in Morocco also found values ranging from 41.7 g to 92.2 g. The weight and size of cactus fruits are highly influenced by environmental conditions and cultivation methods. De Wit (2010) found that fruit weights for the same cultivars varied from 71 g to 150 g depending on environmental conditions. Opuntia cactus fruit weight and size is influenced by genotype, seed number (Barbera et al., 1994), water availability, ripening time (Barbera et al., 1994; Nerd et al., 1991), time of bud emergence, cladode fruit load (Inglese et al., 1995b) and environment (Karababa et al., 2004; De Wit, 2010).

The bark thickness and weight were significantly the highest for Cherratia (1.3 mm and 181.2 g, respectively) and the lowest for Aakria (0.4 mm and 24.8 g, respectively). Much higher values of bark thickness were found by Mulas *et al.* (2006) for 20 cultivars in Morocco ranging from 1.8 mm to 4.7 mm. Also, for the 43 accessions evaluated in Lebanon, the bark thickness of the fruit varied from 2.2 mm to 5.8 mm (Chalak *et al.*, 2014). In Lebanon, of the 43 accessions evaluated, most accessions showed moderate bark weight ranging from 35 to 55 g (Chalak *et al.*, 2014). Flesh content varied significantly from 29.3% for Melk Zhar to 48% for Marjana. Mulas *et al.* (2006) found percentages between 29.1% and 44.4% for 20 accessions in Morocco.

The Brix degree, indicating the sugar content, is among the most important characteristics for the choice of varieties for human consumption. Very highly significant differences were recorded among the varieties tested. Aakria showed the lowest average value of 6.4°Bx against the highest value of 14.5°Bx for Belara and Marjana. The other varieties have high sugar content (> 12°Bx). The Brix values obtained in this study are more or less similar or slightly higher than those obtained for *Opuntia* cactus grown in other countries (Felker *et al.*, 2005; Yahia and Mondragón-Jacobo, 2011; Potgietera and D'Aquino, 2018). These minimal differences may be due to genotypic and

environmental effects. Indeed, in a test of the effect of environment on 12 varieties of *Opuntia* in South Africa, De Wit (2010) found variable Brix values for the same genotypes depending on the environment. The Brix values of the varieties tested in the present study are higher than those obtained by El Kharrassi *et al.* (2016) with values ranging from 6.2 to 12.6°Bx, and Harrak *et al.* (2021) with values ranging from 9.3 to 13.6°Bx for Moroccan accessions. With the exception of Aakria, all the resistant varieties tested showed optimal Brix values at harvest (12-15°Bx) (Nerd *et al.*, 1991; Schirra *et al.*, 2002).

The weight of seeds per fruit varies from 0.8 for Aakria to 8.1 for Cherratia and thus represents 1.7 and 2.9% of the total fruit weight respectively. Our results are relatively close to those obtained in Sicily with 2 to 4% seeds (Barbera *et al.*, 1994) and those of Harrak et al. (2021) with 2 to 4% seeds obtained for 36 accessions from Morocco. Also, the number of seeds per fruit varied significantly from 77.1 for Aakria to 570.1 for Belara. Harrak *et al.* (2021) found values ranging from 105 to 269 for 36 Morocain accessions. El Finti *et al.* (2013) found values ranging from 178 to 298. The numbers vary from 171 to 324 for 43 accessions cultivated in Lebanon (Chalak *et al.*, 2014). Our results show that with the exception of Aakria and Ghalia, all other varieties have a high number of seeds (277.1-570.1) indicating that they can be used for oil extraction from the seeds because of the high number of seeds in the fruits. We also noted that late fruits have fewer seeds per unit basis weight compared to seasonal fruits.

The positive and significant correlation between plant height and diameter and the number of cladodes, and between cladode length and width and the number of areoles and between bark weight and seed weight and the number of seeds per fruit seems to be logical. Indeed, an increase in plant height and diameter may increase the number of cladodes and an increase in cladode area may increase the number of areoles, also an increase in bark weight indicates an increase in fruit size and consequently seed weight and number. The significant correlation between cladode thickness and bark thickness and between distance between areoles and length of longest spine, fruit weight, and fruit diameter and peel weight are more difficult to explain.

Our results show that 80.1% of the total variance is explained by the first three factors/components. This result is very satisfactory compared to those of Peña-Valdivia *et al.* (2008) who reported that the first three PCs explained 46% of the total variability. Bendhifi *et al.* (2013) reported that for Tunisian accessions, 93.5% of the total variance is explained by the first three principal components using ten morphological traits. In addition, our results showed that the first factor is represented mainly by fruit weight, fruit length and number of seeds, while the second factor is represented mainly by the length of the longest spine, bark thickness and cladode thickness. This information is very valuable to revise the type of morphological parameters that are appropriate for an improved and more efficient morphological characterization (Nefzaoui, 2016). Erre and Chessa (2013) reported that none of the plant characteristics are useful for discriminating *Opuntia* accessions, except cladode parameters. On the other hand, Peña Valdivia *et al.* (2008) reported that parameter "absence or presence of spines" is a good criterion to discriminate cactus pear accessions.

The PCA analysis showed five main groups of varieties that are distinguished by different characters. It is remarkable the isolation of Aakria which has the smallest bright red fruit and cladodes, Belara with very important number of cladodes per plant and yellow green fruit of big size and Marjana which has very thick cladodes and fruits of big size and very sweet. The group

of varieties (Melk Zhar, Angad and Cherratia) is characterized by large and thorny cladodes and fruits of large size. The group containing the vareties (Karama and Ghalia) is characterized by fruit of medium size. The PCA classification of 36 Opuntia accessions based on morphological and chemical characters in Morocco revealed a high diversity and allowed to distinguish five different groups of accessions (Harrak et al., 2021). Numerical classification revealed a high diversity among 29 Opuntia accessions based on fruit weight and shape in Mexico (Cervantes Herrera et al., 2006). In the same country (Mexico), PCA and cluster analysis were applied to 29 accessions of prickly pear cactus; the analysis identified genotypes with a higher skin proportion and effectively differentiated the accessions into five distinct groups (Valdez-Cepeda et al., 2003). These findings offer valuable insights for the selection of varieties and enhance our understanding of genetic diversity within the studied population. In Lebanon, PCA performed on 43 Opuntia accessions showed high variability, but it did not separate the groups (Chalak et al., 2014). In Algeria, PCA and Hierarchical Cluster Analysis were conducted on a set of five Opuntia species using 49 descriptors. The analysis revealed distinct differences between the species and identified eight key descriptors crucial for their classification; these findings have important implications for the selection and conservation of Opuntia species in Algeria (Hadjkouider et al., 2017).

The hierarchical classification of *Opuntia* cactus varieties resistant to *D. opuntiae* in Morocco using euclidean distance as a statistical dissimilarity index differentiated five different groups. The clustering of the *Opuntia* cactus pear varieties in the dendrogram and the consistency with the results of the principal component analysis suggests a possible indication of phylogenetic closeness. These results imply that the varieties within each cluster may share common ancestry or genetic similarities that influence their morphological and phenological traits. However, it is essential to conduct further analyses, such as molecular studies or genetic markers, to confirm and establish the phylogenetic relationships among these varieties accurately. The dissimilarity dendrogram separates 36 Moroccan accessions into five groups at a Euclidean distance of 21.1 (Harrak *et al.*, 2021). In Lebanon, the dendrogram constructed on 23 attributes according to Euclidean distance separated accessions into six subgroups (Chalak et al. 2014).

Also, our study showed that during the studied crop year (2020-2021), vegetative and floral bud emission and flowering of the tested cactus pear varieties were important from February to May, when temperatures started to increase and the day length became much longer. The periods of vegetative and floral bud emission, floral bud formation, and flowering were the longest for Aakria and the shortest for Marjana. The duration of the budding phase exceeded what has been reported by other studies by 21-60 days in different countries worldwide (Barbara, 2007; Nerd and Mizrahi, 2010; Arba, 2017). In the central zone of Morocco, the flowering period of two varieties in the Khouribga region (the thornless 'Mles' of O. ficus-indica and the spiny 'Draibina' of O. megacantha) was about 50 days and extended from April 9 to May 29 (Arba et al., 2015). The fruit ripening period was the longest for Belara and the shortest for Melk Zhar and Marjana. A study by Arba et al. (2017) in Agadir, Morocco showed that the fruit ripening period was slightly longer for 'Aissa' (79 days) and shorter for 'Achefri' (63 days). The fruit development period was 146 days for Karama, Ghalia, Cherratia, Angad, and Melk Zhar, 156 days for Belara, slightly shorter for Marjana (143 days), and much longer for Aakria (190 days). Arba et al. (2015), recorded that the PDF of 'Moussa lasted 152 days in 2011, and 188 days in 2012 in Morocco. However, Barbara (2007) reported that the varieties with early flower bud emission have a longer PDF, which is consistent with what we reported in the present study for the variety Aakria. The same author reported that for two seasons, the PDF of the majority of varieties in South Africa is ranged from 120-162 days. The PDF of O. ficus-indica is 96 days in Brazil (Segantini et al., 2010) and about 122 days in Italy (Nerd and Mizrahi, 2010).

Conclusion

This work allowed highlighting a significant variability among the eight Opuntia cactus pear varieties identified as resistant to D. opuntiae in Morocco by using different morphological parameters. Most of the morphological traits studied are significantly different among the varieties. Plant height, plant diameter, cladode length, cladode width, fruit length, fruit diameter, seed number and seed weight seem to be more reliable to be used for morphological characterization of the tested varieties as the lowest CVs were observed for these parameters. In general, the PCA and AHC analysis allowed distinguishing five groups according to the studied characteristics. Aakria which has the smallest bright red fruit and very small cladodes. Belara with a very important number of cladodes per plant, large cladodes and yellow-green fruits of big size and very sweet, Marjana which has very thick cladodes and fruits of big size and very sweet. The group of varieties (Melk Zhar, Angad and Cherratia) is characterized by wide and thorny cladodes, the longest thorns and fruits of large size. The group containing the varieties (Karama and Ghalia) is characterized by large size cladodes and medium size fruits. Also, for all the tested varieties, the vegetative and floral budding and flowering typically occur in the spring, between February and May, and may be slightly earlier or later depending on the variety. The periods of vegetative and floral bud emission, floral bud formation and flowering were the longest for Aakria and the shortest for Marjana. The period of fruit development varied among the varieties and was the longest for Aakria and the shortest for Marjana.

The effectiveness of morphological characterization may be hampered by high levels of hybridization within the species and by the many environmental factors that can affect the macromorphological classification of plants. However, genetic characterization of these *D. opuntiae* resistant varieties based on molecular traits is very important to identify both genetically diverse and agronomically preferable varieties for cactus pear improvement. Also, further studies on the effects of climatic conditions on flowering and fruiting phases of the tested varieties are needed.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Conceptualization, Mohamed EL AALAOUI; methodology, Mohamed EL AALAOUI; software, Mohamed EL AALAOUI; validation, Mohamed EL AALAOUI. and Mohamed SBAGHI; formal analysis, Mohamed EL AALAOUI; investigation, Mohamed SBAGHI; resources, Mohamed SBAGHI; data curation, Mohamed EL AALAOUI; writing-original draft preparation, Mohamed EL AALAOUI; writing-review and editing, Mohamed EL AALAOUI; visualization, Mohamed EL AALAOUI; supervision, Mohamed SBAGHI; project administration, INRA Morocco.

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