# Flowering and fruiting phenology, and physico-chemical characteristics of 2-year-old plants of six species of *Opuntia* from eight regions of Morocco

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## **SUMMARY**

We have described the flowering and fruiting phenology of 14 accessions of six *Opuntia* species grown in Morocco: *O. ficus-indica*, *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha*, and *O. stricta*. We also determined the physicochemical characteristics of their cladodes. For each accession, we used four-to-20 plants. Two systems were used to measure phenology: the extended Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH)-scale and the National Phenology Monitoring System. Five principal stages were described: the development of vegetatively-propagated organs (vegetative buds), the development of flower buds, flowering, fruit development, and fruit maturity. The number of vegetative buds varied significantly among the 14 accessions, while only four accessions produced flower buds. After reaching their final size, fruit lengths varied from 3.40 – 6.40 cm, while fruit diameters varied from 1.93 – 3.90 cm. The average cladode number differed significantly among genotypes and varied from 1.75 – 7.75. There were also significant differences in cladode length (13.75 – 30.63 cm), width (6.25 – 17.33 cm), thickness (0.65 – 1.38 cm), fresh weight (FW; 67.50 – 766.00 g), and dry weight (DW; 8.75 – 67.15 g) between species. Water content and ash content, as well as total protein and total sugar concentrations, showed significant differences and were within the ranges of 86.67 – 92.04% (w/w), 12.97 – 22.08 g 100 g<sup>-1</sup> DW, 4.64 – 11.56 g 100 g<sup>-1</sup> DW, and 3.22 – 12.51 g 100 g<sup>-1</sup> DW, respectively. Our results will help in the development of agronomic management practices and improve the characterisation, valorisation, and use of cactus pear species grown in Morocco.

Cactus pear (*Opuntia* spp.), a member of the family Cactaceae, is an economically and ecologically important tree native to arid and semi-arid regions of Mexico (Abdel-Hameed *et al.*, 2014). It is now distributed in temperate, sub-tropical, and cold regions of many parts of the World such as Central and South America, South Africa, and Mediterranean countries (Feugang *et al.*, 2006; Gurrieri *et al.*, 2000).

Cactus pear is cultivated for fresh fruit consumption (Abdel-Hameed *et al.*, 2014; Yahia and Mondragon-Jacobo, 2011), as a forage crop (Costa *et al.*, 2012), for pharmaceutical and cosmetic purposes, and as a food additive (Piga, 2004). In addition, cactus pear has beneficial effects on human health due to its anti-cancer, anti-viral, anti-diabetic, and anti-oxidant properties (Feugang *et al.*, 2006; Abdel-Hameed *et al.*, 2014). In Morocco, the area of *Opuntia* cultivation is approx. 120,000 ha (Arba, 2009), with *O. ficus-indica* being the main species (El-Mostafa *et al.*, 2014).

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The cultivation of cactus pear in various ecosystems and under different climatic conditions may affect its phenological development. In fact, environmental factors such as photoperiod and temperature affect the production of fruit and flowers in most plant species (Hamann, 2004; Munguía-Rosas and Sosa, 2010). In addition, both geographic origin and species could affect phenology (Vitasse et al., 2009). Differences in the patterns of flowering and fruiting are probably due to interactions between many factors (Fenner, 1998). Studying and understanding plant phenology is crucial to understanding species interactions, community functions, and diversity (Fenner, 1998). Furthermore, phenology is the main element contributing to the ecology of communities and ecosystems, and during evolution and adaptation (Forrest and Miller-Rushing, 2010). Despite the importance of phenological studies, there have been few studies on the phenology of cactus pear species. Most of the species concerned were from Latin America (Lenzi and Orth, 2012; Pimienta-Barrios et al., 2000; Sánchez et al., 1991). Therefore, it was of interest to

understand and determine the phenological stages of cactus pears growing under Moroccan conditions.

In addition to the limited data available regarding the phenology of cactus pear, there is also a lack of data on the physico-chemical characteristics of cladodes in species growing in Morocco. Indeed, most studies have been performed on species grown in Mexico (Hernández-Urbiola et al., 2011; Ramírez-Moreno et al., 2013; Rodríguez-Garcia et al., 2007). Cactus pear cladodes could be used for human consumption, as food supplements, or as forage, due to their nutrient composition (Feugang et al., 2006). However, nutrient concentrations depend on cultivation site, climate, and genotype (Felker et al., 2005; Sáenz, 1995). Thus, differences in ash content, total protein and total carbohydrate concentrations were observed in O. ficusindica genotypes in Mexico (Ramírez-Moreno et al., 2013), Tunisia (Ayadi et al., 2009), and India (Shilpa et al., 2012). Determination of the physico-chemical characteristics of cladodes of Opuntia species grown in Morocco would assist in the characterisation, valorisation and future use of these species.

The aims of this investigation were: (i) to study flowering and fruiting phenology in six recently-planted *Opuntia* species: *O. ficus-indica*, *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha*, and *O. stricta*, collected from eight different regions of Morocco; and (ii) to determine the physico-chemical characteristics of the cladodes of these species.

#### MATERIALS AND METHODS

Plant material

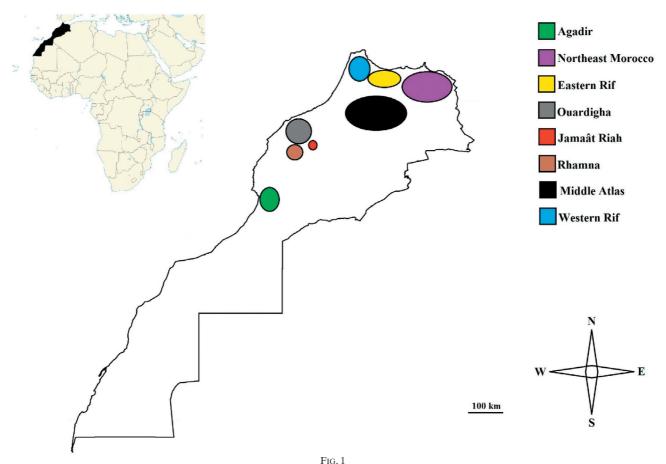
Six Opuntia species were investigated: *O. ficus-indica*, *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha*, and *O. stricta*. The species were identified by comparing our field observations with two taxonomic sources (Boujghagh, 2011; Britton and Rose, 1919).

For each species, we used one-to-eight accessions (total n=14 accessions). For *O. ficus-indica* (n=8 accessions) and for *O. leucotricha* (n=2 accessions), each accession came from a different region. There was one accession of each of the other four species.

Four-to-20 plants of each accession of these species were collected from different regions in Morocco (Figure 1), planted in June 2011 and grown under the same conditions at 'Ain Zagh' (32° 95' N; 7° 62' W; 400 m asl), part of the National Institute of Agronomic Research of Settat, INRA, Settat, Morocco. At the start of the experiments, each plant consisted of one cladode.

## Phenological observations

Based on the extended Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH)-scale (Hack *et al.*, 1992) and The National Phenology Monitoring System (Thomas *et al.*, 2010), we observed the following five growth stages: the development of vegetatively-propagated organs (Stage 4); the development of flower buds (Stage 5); flowering (Stage 6); the development of fruit (Stage 7); and maturity of



Map of Morocco showing the eight collection sites for the 14 accessions of the six species of *Opuntia* used in this study.

fruit (Stage 8). In addition, we counted the number of vegetative buds and flower buds, and measured fruit lengths and diameters after each plant had reached its final size. Phenological observations were performed between April – August 2013 on the four-to-20 plants of each accession.

# Physico-chemical analysis

The mean number of cladodes in each accession of each species, as well as traits such as cladode length, cladode width, and cladode thickness were measured. Again, we used the four-to-20 plants of each accession. These data were recorded in April 2013.

We measured cladode fresh weights (FW) and cladode dry weights (DW) after drying to constant weight in an oven (XU980, France-Etuves, Chelles, France) at 55°C for 10 h. Subsequently, the water contents of cladodes were calculated using the formula:

Water content [% (w/w)] = (FW - DW) / FW

To measure ash contents [in g  $100 \, \mathrm{g}^{-1} \, \mathrm{DW}$ ], 2 g of dried cladode tissue from each plant (n = 4 – 20) of each accession (n = 14) was placed in a platinum crucible (58 mm in diameter and 53 mm in height) and incinerated in an electric muffle furnace (P-Selecta Horn; JP Selecta, Barcelona, Spain) at  $550^{\circ}\mathrm{C}$  for 4 h. Total protein concentrations were determined by calculating the total nitrogen concentration according to the Kjeldahl method (Kjeldahl, 1883) then multiplying by 6.25. Total sugar concentrations were determined by the phenol-sulphuric acid method of Dubois *et al.* (1956), using glucose as a standard.

For all analyses, we used two cladodes per plant and four-to-20 plants of each accession. These analyses were carried out between September – October 2013.

## Statistical analysis

All experiments were laid out and measured in a completely randomised design. Data were subjected to analysis of variance and means were separated by the Student Newman- Keuls (SNK) test at  $P \le 0.05$ . Prior to analysis, percentage data were arcsine transformed. All statistical analyses were performed using SPSS Version 16.0 for Windows (IBM-SPSS Inc., Chicago, IL, USA).

#### **RESULTS**

Phenological observations

Phenological observations were made according to the extended BBCH scale (Hack *et al.*, 1992) or The National Phenology Monitoring System (Thomas *et al.*, 2010).

Stage 4: Development of vegetatively-propagated organs: Vegetative buds appeared during the first week of May in all species except *O. robusta*. The mean number of vegetative buds per accession varied from 0.25 in *O. leucotricha* from The Middle Atlas, to 12.35 in *O. ficusindica* from Agadir (Table I). The following five phases of Stage 4 were observed: vegetative buds began to develop (Figure 2A); cladodes reached 30% of their final size (Figure 2B); cladodes reached 50% of their final size; cladodes reached their final size.

Stage 5: Flower buds: Flower buds started to appear during the first week of May (Figure 2C). However, they were observed in only four accessions of two species: O. ficus-indica from Jamâat Riah, O. ficus-indica from The Middle Atlas, O. ficus-indica from Agadir, and O. dillenii from Ouardigha. The average number of flower buds per accession varied significantly within and between species and ranged from 0.05 in O. ficus-indica from Jamâat Riah, to 13.75 in O. dillenii from Ouardigha (Table I).

Stage 6: Flowering: The flowering period started in the second week of May in four accessions of two species: O. ficus-indica from Jamâat Riah (Figure 2D), O. ficus-indica from The Middle Atlas, O. ficus-indica from Agadir, and O. dillenii from Ouardigha. The following five phases of flowering (Stage 6) were observed: first flowers open; beginning of flowering (10% of flowers open); full flowering (50% of flowers open); flowering finishing (majority of petals fallen); end of flowering (fruit set visible).

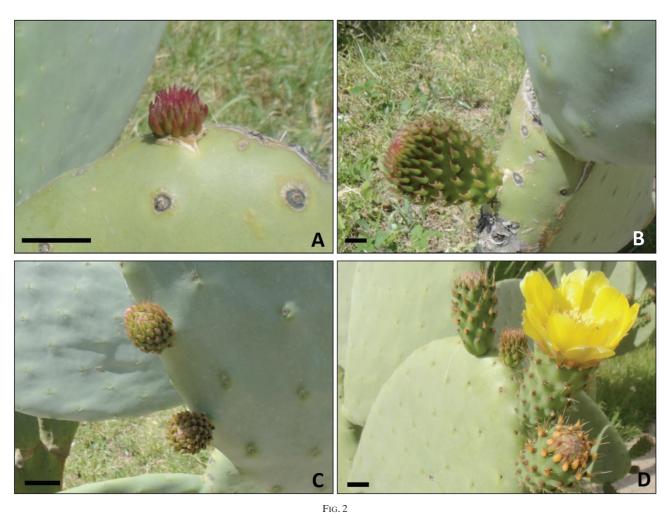
Stage 7: Development of fruit: The formation of fruit started in the fourth week of May in O. ficus-indica from Jamâat Riah, O. ficus-indica from The Middle Atlas, O. ficus-indica from Agadir, and O. dillenii from Ouardigha. Fruit growth continued for 4 weeks until all fruit reached their final size. Fruit lengths and diameters were

Table I

Vegetative bud and flower bud production, and fruit lengths and diameters in six species of Opuntia collected from up to eight different regions of Morocco

Species	Accession/ Geographic origin <sup>‡</sup>	No. of plants sampled	No. of vegetative buds	No. of flower buds	No. of plants that produced fruit	Fruit length after reaching the final size (cm)	Fruit diameter after reaching the final size (cm)
O. ficus-indica	Jamaât Riah	20	$7.40 \pm 5.60 \text{ ab}^{\dagger}$	$0.05 \pm 0.22$ a	1	$6.40 \pm 0.0$	$3.90 \pm 0.00$
•	Rhamna	20	$10.65 \pm 8.64$ ab	_	_	_	-
	Ouardigha	20	$4.20 \pm 3.09 \text{ ab}$	_	_	_	-
	Middle Atlas	16	$2.69 \pm 2.50 \text{ ab}$	$0.56 \pm 1.36 a$	3	$4.90 \pm 0.20$	$3.00 \pm 0.10$
	Northeast Morocco	8	$0.63 \pm 1.06 \text{ a}$	_	_	_	_
	Eastern Rif	16	$1.44 \pm 1.55$ a	-	-	_	_
	Western Rif	8	$6.88 \pm 7.02 \text{ ab}$	_	_	_	-
	Agadir	20	$12.35 \pm 11.72 \text{ b}$	$1.10 \pm 2.25 a$	5	$4.98 \pm 0.43$	$2.84 \pm 0.19$
O. aequatorialis	Rhamna	4	$10.00 \pm 2.58$ ab	-	-	_	_
O. stricta	Rhamna	4	$5.50 \pm 3.79 \text{ ab}$	_	_	_	_
O. dillenii	Ouardigha	4	$10.75 \pm 2.22$ ab	$13.75 \pm 13.87 \text{ b}$	3	$3.40 \pm 0.17$	$1.93 \pm 0.05$
O. robusta	Northeast Morocco	4	_	_	_	_	_
O. leucotricha	Middle Atlas	4	$0.25 \pm 0.50$ a	_	_	_	_
	Western Rif	8	$1.38 \pm 2.07 \text{ a}$	_	_	_	-

<sup>&</sup>lt;sup>†</sup>All values are means  $\pm$  standard deviation (n = 4 – 20 plants of each accession). Mean values followed by the same lower-case letters in each column for each accession are not significantly different (P > 0.05) by the Student-Newman-Keuls test. <sup>‡</sup>See Figure 1.



Examples of the phenological growth stages of *Opuntia ficus-indica*. Panels A, B, Stage 4 development of vegetatively-propagated organs. Panel A, vegetative bud begins to develop. Panel B, cladode at 30% of its final size. Panel C, Stage 5 flower bud. Panel D, Stage 6 flowering. Scale bars = 2 cm.

recorded after reaching the final size. Our observations indicated that fruit dimensions depended on the species studied (Table I).

Stage 8: Maturity of fruit: After reaching their final size, fruit started maturity colouration and all *O. ficus-indica* accessions that produced fruit exhibited yellow-orange-skinned fruit at full maturity, while *O. dillenii* produced purple-red-skinned fruit. The following three fruit maturity phases of Stage 8 were observed: beginning of ripening and fruit colouration; advanced ripening and fruit colouration; fully-ripe fruit showing fully-ripe colour.

#### Physico-chemical analysis

Mean values for the different characteristics measured among the 14 accessions (n = 4-20 plants per accession) of the six species are presented in Table II. The average number of cladodes per plant ranged from 1.75 in *O. ficus-indica* (from Ouardigha) to 7.75 in *O. dillenii* (from Ouardigha). Cladode lengths varied from 13.75 – 30.63 cm, while cladode widths and cladode thicknesses varied from 6.25-17.33 cm and from 0.65-1.38 cm, respectively (Table II). *O. aequatorialis* from Rhamna had the greatest cladode length and was significantly different ( $P \le 0.05$ ) from all other accessions, while the

greatest cladode width and thickness were observed in *O. robusta* from Northeast Morocco. *O. dillenii* had the highest number of cladodes per plant. However, its cladodes had, on average, smaller dimensions compared to the other five species.

Average cladode FWs ranged from 67.50-766.00 g. The greatest cladode FW was observed in *O. ficus-indica* from Agadir, while the lowest FW was observed in *O. dillenii* from Ouardigha. Significant differences ( $P \le 0.05$ ) were observed among and within species (Table II), except for the two accessions of *O. leucotricha*, which showed no significant difference, even though they were collected from two different regions, The Middle Atlas and Western Rif.

Cladode DWs varied from 8.75 – 67.15 g, with no significant difference between the two *O. leucotricha* accessions. Water contents varied slightly among the different accessions and ranged from 86.67 – 92.04% (w/w). Ash contents varied from 12.97 – 22.08 g 100 g<sup>-1</sup> DW, with significant differences within and among species. The highest ash content was observed in *O. ficus-indica* from The Middle Atlas, while the lowest value was in *O. ficus-indica* from Rhamna.

Total protein concentrations were between 4.64 g 100 g<sup>-1</sup> DW in *O. robusta* (from Northeast Morocco) and 11.56 g 100 g<sup>-1</sup> DW in *O. leucotricha* (from Western Rif),

Physico-chemical characteristics of cladodes on six species of Opuntia collected from up to eight different regions of Morocco

		No. of	No. of Average number						Water	Ash	Total	Total
Species	Accession/ plants Geographic origin* sampled	plants sampled	of cladodes per plant	Cladode length (cm)	Cladode width(cm)	Cladode thickness (cm)	Fresh weight (g)	Dry weight (g)	content [% (w/w)]	$\begin{array}{c} \text{content} \\ \text{(g 100 g}^{-1}  \text{DW)} \end{array}$	content proteins sugars $(g 100 g^{-1} DW) (g 100 g^{-1} DW) (g 100 g^{-1} DW)$	$\underset{(g\ 100\ g^{-1}\ DW)}{\text{sugars}}$
O. ficus-indica	Jamaât Riah	20	$2.60 \pm 1.14 \text{ a}^{\dagger}$	$23.07 \pm 3.52 \text{ bc}$	$12.45 \pm 1.88 \text{ bc}$	$0.86 \pm 0.21 \text{ ab}$	$686.56 \pm 154.43$ cd	$54.83 \pm 10.15$ bc	$91.90 \pm 0.86 \text{ b}$	$18.24 \pm 3.38  \text{def}$	$6.65 \pm 1.47 \text{ bcd}$	$5.91 \pm 1.24 \text{ ab}$
	Rhamna	20	$3.05 \pm 2.26$ a	$25.01 \pm 2.91 c$	$12.41 \pm 1.75$ bc	$0.87 \pm 0.33$ ab	$708.30 \pm 275.83$ cd	$49.75 \pm 18.75$ bc	$92.00 \pm 4.75 \text{ b}$	$12.97 \pm 1.38 a$	$7.48 \pm 1.43$ cde	$8.95 \pm 3.27 c$
	Ouardigha	20	$1.75 \pm 0.79 \text{ a}$	$20.33 \pm 3.57 \text{ bc}$	$10.65 \pm 1.81 \text{ bc}$	$0.80 \pm 0.24 \text{ ab}$	$389.50 \pm 145.90$ bc	$33.89 \pm 10.84 \text{ b}$	$91.15 \pm 1.14$ ab	$16.08 \pm 0.75 \text{ bcd}$	$7.76 \pm 1.35$ cde	$6.45 \pm 1.57 \text{ bc}$
	Middle Atlas	16	$2.13 \pm 1.20 \text{ a}$	$20.15 \pm 6.12 \text{ bc}$	$12.05 \pm 1.32$ bc	$0.75 \pm 0.13$ ab	$551.25 \pm 218.97$ bcd	$52.25 \pm 15.51$ bc	$89.74 \pm 3.07$ ab	$22.08 \pm 0.79 \mathrm{g}$	$7.97 \pm 1.09  de$	$5.21 \pm 1.38 \text{ ab}$
	Northeast Morocco	∞	$2.00 \pm 0.76$ a	$23.54 \pm 1.11 \text{ bc}$	$11.41 \pm 0.73$ bc	$0.71 \pm 0.14$ ab	$286.00 \pm 43.14 \mathrm{b}$	$33.14 \pm 6.18 \mathrm{b}$	$88.46 \pm 0.71 \text{ ab}$	$20.61 \pm 4.04  \mathrm{fg}$	$5.56 \pm 0.36 \text{ ab}$	$6.88 \pm 0.91 \text{ bc}$
	Eastern Rif	16	$1.94 \pm 1.61 a$	$23.87 \pm 4.89 \text{ bc}$	$12.94 \pm 1.64$ bc	$0.92 \pm 0.19 \text{ ab}$	$583.38 \pm 150.45$ bcd	$56.60 \pm 15.22$ bc	$90.08 \pm 2.01$ ab	$19.19 \pm 3.97 \text{ defg}$	$7.71 \pm 1.21$ cde	$5.63 \pm 2.05 \text{ ab}$
	Western Rif	∞	$3.13 \pm 0.64 a$	$22.05 \pm 1.85 \text{ bc}$	$11.91 \pm 0.79 \text{ bc}$	$0.89 \pm 0.17$ ab	$453.75 \pm 152.55$ bcd	$36.88 \pm 10.60 \text{ b}$	$90.65 \pm 5.99 \text{ ab}$	$16.10 \pm 1.12$ bcd	$8.93 \pm 1.15$ ef	$4.80 \pm 0.31 \text{ ab}$
	Agadir	20	$3.35 \pm 1.50 \text{ a}$	$25.76 \pm 4.55 c$	$13.43 \pm 1.99 c$	$0.79 \pm 0.27$ ab	$766.00 \pm 305.57 \text{ d}$	$66.49 \pm 24.94 c$	$91.06 \pm 2.41$ ab	$19.75 \pm 1.74 \text{ efg}$	$6.11 \pm 0.80 \text{ abcd}$	$8.62 \pm 2.62 c$
O. aequatorialis	Rhamna	4	$3.25 \pm 0.50 \text{ a}$	$30.63 \pm 2.95 \mathrm{d}$	$14.03 \pm 9.99 c$	$1.08 \pm 0.39 \text{ b}$	$655.10 \pm 151.46$ cd	$57.77 \pm 18.22$ bc	$91.27 \pm 0.76$ ab	$18.73 \pm 0.13  \text{def}$	$9.10 \pm 0.24$ ef	$3.22 \pm 0.18 a$
O. stricta	Rhamna	4	$5.75 \pm 0.96 \text{ b}$	$20.73 \pm 3.26 \text{ bc}$	$9.60 \pm 1.82 \text{ b}$	$0.78 \pm 0.28$ ab	$484.50 \pm 0.71 \text{ bcd}$	$38.56 \pm 10.27 \text{ b}$	$92.04 \pm 2.13 \text{ b}$	$16.48 \pm 0.12$ bcd	$10.15 \pm 0.25 \mathrm{f}$	$8.70 \pm 0.21 c$
O. dillenii	Ouardigha	4	$7.75 \pm 3.50 \text{ c}$	$13.75 \pm 1.84 a$	$6.25 \pm 0.86 \text{ a}$	$0.65 \pm 0.04 a$	$67.50 \pm 24.75 a$	$8.75 \pm 1.92 a$	$86.67 \pm 2.05 \text{ a}$	$14.17 \pm 0.07 \text{ abc}$	$7.53 \pm 0.49$ cde	$5.40 \pm 0.22 \text{ ab}$
O. robusta	Northeast Morocco	4	$2.75 \pm 0.96 \text{ a}$	$17.90 \pm 3.59 \text{ ab}$	$17.33 \pm 3.45 d$	$1.38 \pm 0.05 c$	$580.00 \pm 56.57 \text{ bcd}$	$67.15 \pm 0.81 c$	$88.37 \pm 0.99 \text{ ab}$	$16.95 \pm 0.23$ cde	$4.64 \pm 0.13 a$	$4.75 \pm 0.07$ ab
O. leucotricha	Middle Atlas	4	$4.00 \pm 0.82 a$	$24.75 \pm 3.10 \mathrm{c}$	$14.38 \pm 0.25 c$	$1.13 \pm 0.31 \text{ b}$	$530.00 \pm 21.21$ bcd	$51.01 \pm 1.36 \mathrm{bc}$	$90.37 \pm 0.13$ ab	$13.69 \pm 0.10$ ab	$5.78 \pm 0.25 \text{ abc}$	$12.51 \pm 0.23 d$
	Western Rif	∞	$3.63 \pm 1.19 a$	$25.63 \pm 4.46 \mathrm{c}$	$13.54 \pm 1.69 c$	$0.85 \pm 0.22$ ab	$531.25 \pm 269.95$ bcd	$56.95 \pm 28.69 \text{ bc}$	$89.19 \pm 0.74$ ab	$20.74 \pm 0.18  \mathrm{fg}$	$11.56 \pm 3.25 \mathrm{g}$	$5.29 \pm 2.03 \text{ ab}$
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different (P > 0.05) by significantly not are each accession column for each .⊟ letters lower-case same the þ 20 plants of each accession). Mean values followed E) Values are means ± standard deviation ( Student-Nowman-Keuls test. \*See Figure 1. and significant differences ( $P \le 0.05$ ) were revealed among and within species. Total sugar concentrations ranged from 3.22-12.51 g 100 g<sup>-1</sup> DW. The highest total sugar concentration was observed in *O. leucotricha* from The Middle Atlas, while the lowest concentration was in *O. aequatorialis* from Rhamna. Again, statistical analysis showed significant differences within and among species (Table II).

Regarding geographic sites of origin, we observed that accessions from different species, but with the same geographic origin, or *vice versa*, might show significant differences in the parameters studied. Interestingly, there were significant differences between the two accessions of *O. leucotricha* in terms of ash contents, total protein concentrations, and total sugar concentrations. In fact, *O. leucotricha* plants from The Middle Atlas had significantly higher concentrations of total sugars than those from Western Rif, whereas the opposite was observed in terms of ash contents and total protein concentrations (Table II), which might be used as indices to distinguish cultivars.

#### **DISCUSSION**

Phenological observations

Studies on the flowering and fruiting phenology of *Opuntia* species are scarce. Similar to findings for *O. monacantha* from southern Brazil, reported by Lenzi and Orth (2012), our findings showed that the cladodes of *O. ficus-indica* and *O. dillenii* produced vegetative and floral buds. According to our observations, floral development from bud to anthesis occurred between May and June. In *O. ficus-indica* from Mexico, Pimienta-Barrios *et al.* (2000) reported that flower development lasted from early-March to late-April. In a review of the literature, Reyes-Agüero *et al.* (2006) reported that floral development from bud to anthesis required between 21 – 75 d, depending on the species, and could take place at different times, depending on the geographical area.

Our results also showed that the flowering period ranged from the second week of May to early-June. Lenzi and Orth (2012) reported longer periods of flowering in *O. monacantha*, from the end of September to mid-January, but an April-to-May flowering period in *O. diploursina* from Arizona (Stock *et al.*, 2014). According to Reyes-Agüero *et al.* (2006), the flowering period of *Opuntia* species in general began in late-Winter and could last until early-Autumn. Nerd and Mizrahi (2001) indicated that flowering continued for a number of weeks in *O. ficus-indica* and occurred simultaneously with the formation of areoles and young fruit, which was consistent with our observations.

Fruit production was observed between May and June. This agreed with the findings of Reyes-Agüero *et al.* (2006), who reported that fruit were produced between April – November in the northern hemisphere. Our results indicated that fruit growth and maturation depended on species. The fruit of *O. ficus-indica* were larger than those of *O. dillenii*, with a yellow-orange skin colour. The latter were smaller and had a purple-red skin colour. Fruit development started in mid-April and ended in late-August in *O. ficus-indica* from Mexico (Pimienta-Barrios *et al.*, 2000). In *O. diploursina* from Arizona, fruit ripened in late-May to June (Stock *et al.*,

2014). According to Kuti (1992), fruit-ripening periods ranged from 45 – 154 d in *O. ficus-indica*, *O. stricta*, *O. hyptiacantha*, and *O. lindheimeri*. In *O. joconostle* from San Martín de las Pirámides, Mexico, fruit ripening occurred 224 d after anthesis (Sánchez *et al.*, 1991). In *O. monacantha* from southern Brazil, fruit growth and development were reported to last 120 d before the start of maturation, while complete maturation was observed after 240 d (Lenzi and Orth, 2012).

These diverse results indicate that the phenology of *Opuntia* spp. varied considerably among species and with environmental conditions. In fact, studies have revealed that plant phenology depended on many factors such as climatic variables (e.g., temperature, rainfall), growing conditions, species, and geographic site (Carl *et al.*, 2013; Schwendenmann *et al.*, 2010; Woog, 2002). In addition, Reyes-Agüero *et al.* (2006) reported that fruit ripening in *Opuntia* species was asynchronous.

## Physico-chemical analysis

Our results showed that the physico-chemical characteristics of Opuntia spp. from Morocco varied significantly, depending on species and site of origin. Studies on the physico-chemical characteristics of cladodes of Opuntia species are scarce. In fact, most studies have been conducted on the physico-chemical characteristics of O. ficus-indica. Rodríguez-Garcia et al. (2007) reported that the chemical composition of dehydrated cladodes varied with cladode age in O. ficusindica 'Redonda' from Mexico. Thus, ash contents varied from 18.41 - 23.24 g 100 g<sup>-1</sup> DW, while total protein concentration ranged from 11.39 -14.22%. Hernández-Urbiola et al. (2011) also indicated that the physicochemical composition of cladodes varied with cladode age, and that, in O. ficus-indica from Mexico, cladode DWs were 100 - 550 g, ash contents were 17.65 - 24.30 g  $100 \text{ g}^{-1}$  DW, protein concentrations were 5.85 - 8.99 g 100g<sup>-1</sup> DW, and carbohydrate concentrations were 42.94 – 60.77 g 100 g<sup>-1</sup> DW. Ramírez-Moreno et al. (2013) reported 18.38 g and 18.23 g 100 g<sup>-1</sup> DW of ash, 12.90 g and 13.84 g of protein 100 g<sup>-1</sup> DW, and 25.38 g and 18.99 g of total carbohydrate 100 g<sup>-1</sup> DW in the cladodes of Mexican cultivars O. ficus-indica, from Milpa Alta and Atlixco, respectively. In O. ficus-indica f. amyloceae and O. ficus-indica f. inermis from Tunisia, Ayadi et al. (2009) reported 25.65 and 23.30 g 100 g<sup>-1</sup> DW for total ash, 8.74 and 8.88 g 100 g<sup>-1</sup> DW for total protein, and 60.36 and 60.93 g 100 g<sup>-1</sup> DW for total carbohydrates, respectively. In O. ficus-indica from India, ash contents varied from 19  $-23 \text{ g} 100 \text{ g}^{-1} \text{ DW}$ , protein concentrations from 4 - 10 g100 g<sup>-1</sup> DW, and carbohydrate concentrations from 64 – 71 g 100 g-1 DW (Shilpa et al., 2012). In O. ficus-indica from Marrakech, Morocco, the ash content of cladodes was 19.6 g 100 g<sup>-1</sup> DW (Malainine *et al.*, 2003).

All these results indicate that the physico-chemical characteristics of *O. ficus-indica* vary with geographic site. In addition, we observed that the carbohydrate contents of cladodes of *O. ficus-indica* from Morocco were lower than in *O. ficus-indica* from all other countries. This may be due to the environmental conditions in Morocco.

## **CONCLUSIONS**

Our study revealed how the phenological stages of 14 accessions of six species of 2-year-old *Opuntia* plants differed depending on species and site of origin, and that the physico-chemical characteristics of the cladodes of all six species differed significantly. Our results provide important information when planting new orchards of *Opuntia* species and will be beneficial for the valorisation and future use of these species.

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