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Morphological and chemical characteristics of fruits and seeds of two cultivars of *Opuntia ficus-indica* (L.) Miller from northern Morocco

Hanane Bougdaoua[1], Noureddine El Mtili[*1]

[1] Laboratory of Biology and Health, Department of Biology, Faculty of Sciences, Abdelmalek Essaâdi University, PO Box 2121, 93002 Tétouan, Morocco.

*Corresponding Author: elmtili@hotmail.com

Abstract. Fruits of two cultivars of *Opuntia ficus-indica* (the "Hajria" and "Dellahia" cultivars) growing in the Tetouan and Al-Hoceima provinces of northern Morocco were harvested at the same ripening stage. The chemical properties (total lipids, proteins, total sugars, moisture, and dry matter) of the peel and seeds were determined and the morphological characteristics (whole fruit weight, pulp weight, peel weight, and the number and weight of fertilized and aborted seeds) of the fruits and their seeds from both cultivars were compared. Surface-sterilized seeds were separated into six groups according to their length and germinated on Murashige and Skoog basal culture medium. Seed viability, by analyzing the correlation between (1) germination rate and seed length, and (2) germination rate and seed weight was determined. Results showed that the province or cultivar factor had significant effects on the morphological characteristics of fruits and seeds. Fruit and pulp weights were positively correlated with the seed number. In addition, a marked difference in the sugar content of the peel was observed (57.29% in the ''Dellahia'' cultivar compared to 36.66% in the "Hajria" cultivar). The seeds of the "Dellahia" cultivar contained 10.41% fat and 8.12% protein, whereas the seeds of "Hajria" contained 12.27% fat and 8.67% protein. Seed viability was assured from a length of 3.5 mm in both cultivars. The seed germination rate correlated positively with increased length and weight; however, this rate was significantly different between the two cultivars.

Keywords: *aborted, fat, fertilized, germination, protein.*

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Introduction

The genus *Opuntia* is native to Mexico and includes approximately 300 to 400 species and an even greater number of varieties (Odoux and Dominguez-Lopez, 1996; Stintzing and Carle, 2005). This genus was introduced to Morocco at the beginning of the 17th century from Spain (Schielief *et al*., 2000) and currently occupies a significant area of over 120,000 ha (Anonymous, 2007). It is cultivated for its delicious edible fruits, which have a relatively high sugar content and low acidity (Sepúlveda and Sáenz, 1990; Joubert, 1993). The *Opuntia ficus-indica* fruit, also known as prickly pear, is an oval and elongated berry with thick skin; its granular pulp is delicately flavored, and its pads are used as fodder (Arba, 1983; Duru and Turker, 2005; Feugang *et al.*, 2006). Moreover, the fruits contain an equal number of viable and aborted seeds (Lawes *et al*., 1990; Barbera and Inglese, 1993; Weiss *et al*., 1993). This species shows extensive diversity in fruit weight and size (Lamb, 1993; Arba *et al*., 2002). The best Italian cultivars are those that contain a weight of 160 g (very large fruit) or weight between 120 and 160 g (premium fruit), with flesh percentage not less than 55-60%, yellow-orange or red color, total soluble solids (TSS) range from 13 to 15 °Brix, and a high ratio between

empty and normal seeds (Inglese *et al*., 1995a; Rodriguez-Felix, 2002). However, the relationship between fruit size and seed content varies from one variety to another, with variations in the number of seeds ranging from 100 to 300 (Barbera and Inglese, 1993; Barbera *et al*., 1994). Furthermore, the prickly pear forms the basis of products and by-products intended for various industrial, medicinal, pharmaceutical, and cosmetic uses (Barbera, 1995). Although the peel represents a substantial percentage of the fruit, knowledge of its chemical composition remains limited (Anwar and Sallam, 2016).

Recently, prickly pear seeds have garnered much interest compared to other seeds because they contain good quality edible oil (Sawaya and Khan, 1982; Ennouri *et al*., 2005). Piga (2004) noted that the high content of certain chemical constituents could add value to this fruit based on its nutritional and technological functionality.

Furthermore, studies on seed germination have contributed to the conservation of this natural resource (Flores *et al*., 2008). Multiplication by seeds maintains the genetic diversity of populations and species (Rojas-Aréchiga and Vázquez-Yañes, 2000). It, however, remains one of the riskiest stages in the reproduction of cacti because many seeds do not germinate (Bregman and Graven, 1997; Bougdaoua and Mtili, 2020).

The main objectives of this study were: 1) to describe the morphological characteristics of the fruits and seeds of two *O. ficus-indica* cultivars, “Hajria” and ‘‘Dellahia’’, growing in the provinces of Tetouan and Al-Hoceima (northern Morocco); 2) to compare chemical parameters (total lipids, total sugars, protein, moisture, and dry matter) of the seeds and peel from these cultivars, and 3) we also attempted to determine the relationship between (a) viability and seed length and (b) viability and seed weight in the two cultivars.

Material and Methods

Plant material

Fruit samples of two cultivars of *Opuntia ficus-indica* (L.) Miller were collected at the same ripening stage from two different regions in North of Morocco in August 2017. The first cultivar “Hajria”, with Orange-Yellow fruit’’ was collected from the Tetouan region (35°34'49"N, 5°16'52"W; Altitude 14 m), and the second cultivar “Dellahia”, with Green fruit” was harvested from the Al-Hoceima region (34°56'04''N, 4°20'34"W; Altitude 1007 m) (Figure 1). A total of 35 fruits were randomly selected for each cultivar. The thorns of each fruit were removed using a sponge, and the whole fruit was weighed. Next, the fruits were cut lengthwise to separate the peel from the pulp, and their weights were recorded separately. The pulp was then placed in a sieve and washed several times under running water to obtain the seeds, which were drained and dried at room temperature. These seeds were sorted into two groups: fertilized and aborted. The total dry weight and the number of seeds of each group were determined.

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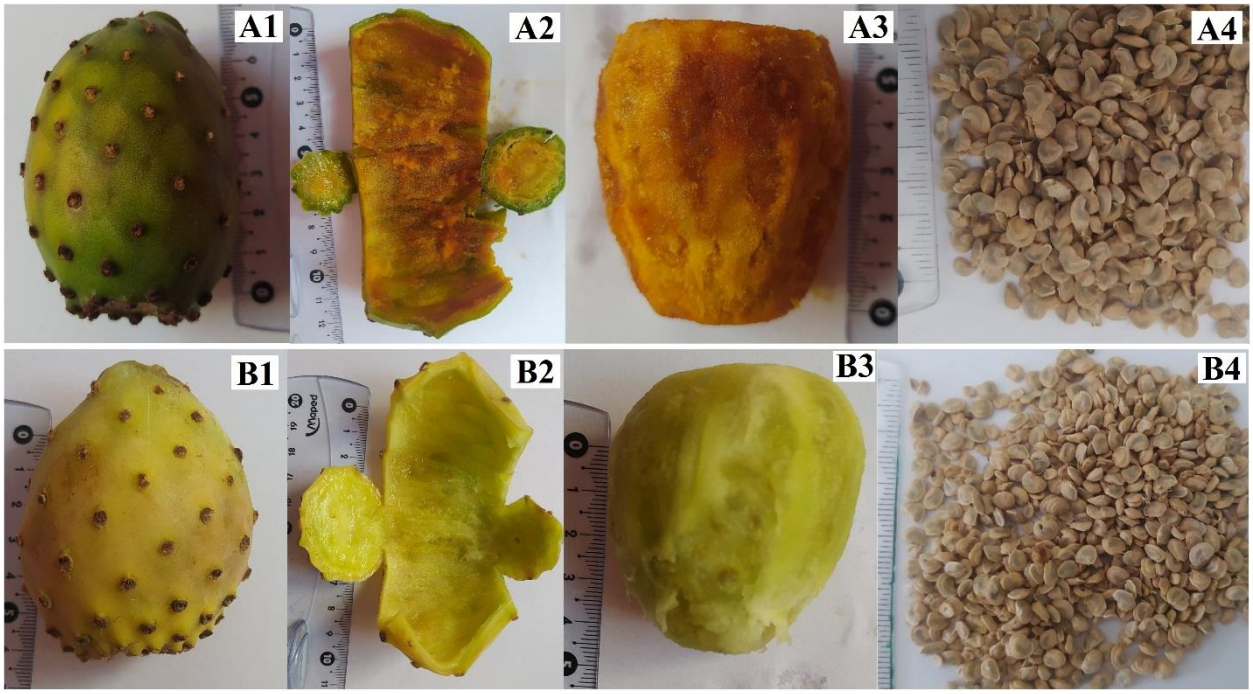

Figure 1. The different parts of the two studied cultivars of *Opuntia ficus-indica* fruits harvested from Tetouan and Al-Hoceima regions. (A1, A2, A3 and A4 represent the fruit, peel, pulp and seeds of the "Hajria" cultivar, respectively; and B1, B2, B3 and B4 represent the fruit, peel, pulp and seeds of the "Dellahia" cultivar, respectively).

Chemical analysis

Fruit peels and seeds were placed in an oven at 50°C until completely dried and ground to a fine powder. Chemical analyses were subsequently performed using the ground peel and seeds from "Hajria" and "Dellahia" cultivars.

Moisture content was determined by drying in an oven at 105°C for 24 hours until a constant weight was obtained (AOAC, 2000).

The dry matter content was calculated according to the method described by AFNOR (1986), using the following formula:

$$Dry\ matter\ rate\ (\%) = 100 - moisture\ (\%)$$

The total lipid content was obtained using the methanol/chloroform method [2:1 (v/v)] according to the extraction procedure described by Bligh and Dyer (1959). First, 1.5 g of sample was added to methanol (10 mL), chloroform (5 mL) and distilled water (4 ml), and the mixture was homogenized for 30 min. Next, chloroform (5 mL) and 1.5% $NaSO_4$ (5 mL) were added to each homogenized sample. The mixture was stirred for 4 min and centrifuged for 2 min at 1,000 rpm. The organic phase was recovered by separation and mixed with 0.5 g of anhydrous $NaSO_4$. Finally, the extract was filtered and evaporated, and the retained lipids were weighed.

The total sugars were determined according to the method described by Dubois *et al*. (1956) using phenol-sulfuric acid followed by alkaline hydrolysis with NaOH (1N).

Proteins were analyzed according to the method described by Bradford (1976).
All chemical analyses were performed with three repetitions.

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Seed germination

Seed viability was determined by germination, and the relationship between viability and seed length was established. A seed was considered germinated when the radicle pierced the seed coat.

The seeds of each cultivar were divided into six groups (n = 40) according to their length using a microscope fitted with graph paper. For each group, the average weight of 25 seeds was determined for the two cultivars (Table 1). Next, the seeds were disinfected by immersion in 0.01% (w/v) mercury chloride for 20 minutes and rinsed three times with sterile distilled water. Their ends were cut using a scalpel to promote germination. The seeds were carefully placed in Petri dishes with MS basal culture medium (Murashige and Skoog, 1962) supplemented with 30 g/L of sucrose and solidified with 7 g/L of agar. The Petri dishes were incubated in a culture chamber at 26°C under a 16-h photoperiod.

Table 1. The length and weight of seeds by groups.

Group	Seed length (mm)	Average weight of 25 seeds from ‘‘Dellahia’’ fruits (g)	Average weight of 25 seeds from “Hajria” fruits (g)
1	2.5–2.9	0.0581	0.0922
2	3.0–3.4	0.1102	0.1111
3	3.5–3.9	0.1820	0.1675
4	4.0–4.4	0.3314	0.3019
5	4.5–4.9	0.3685	0.3782
6	≥5	0.4030	0.3975

Statistical analysis

All statistical analyses were carried out using SPSS. The mean values obtained for the studied variables of different groups were compared at a significance level of 0.05 using a two-sample independent Student's t-test (for equal means) and analysis of variance (ANOVA), followed by the post-hoc Duncan's test. Data obtained from chemical analyses were compared using the Mann-Whitney test and Moses test. A simple linear correlation analysis was used to measure the strength of the relationship between the variables.

Results and discussion

The morphological characteristics of each studied cultivar are presented in Table 2. The fruits of the “Hajria” cultivar collected from the Tetouan site weighed more (80.171 ± 3.243) than those of the ‘‘Dellahia’’ cultivar collected from the Al-Hoceima site (74.102 ± 2.432). Conversely, the pulp of the ‘‘Dellahia’’ cultivar fruits (49.833 ± 1.753) was heavier than that of the “Hajria” cultivar fruits (41.590 ± 2.111). In addition, the peel of “Hajria” cultivar fruits weighed more than that of ‘‘Dellahia’’ cultivar fruits. No significant difference in the total number of seeds per fruit between the two sites was observed, although the fruits of the “Hajria” cultivar contained a higher number of fertilized seeds and were heavier than those of the ‘‘Dellahia’’ cultivar. By contrast, the weight and the number of aborted seeds showed significant variations between the two cultivars. The fruits of the ‘‘Dellahia’’ cultivar were relatively heavy, with many aborted seeds. The “Hajria” cultivar (6.533 ± 0.3467) contained a higher percentage of peel in relation to the pulp than the ‘‘Dellahia’’ cultivar (4.632 ± 0.209).

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Table 2. Comparison of fresh weight, pulp and peel weight, and seed types between the two *Opuntia ficus-indica* cultivars.

Morphological parameters	Origins	Means±SD	*p* values
Fresh weight	‘‘Dellahia’’	74.102±2.432	0.139
	“Hajria”	80.171±3.243	
Pulp weight	‘‘Dellahia’’	49.833±1.753	0.004**
	“Hajria”	41.590±2.111	
Peel weight	‘‘Dellahia’’	24.054±1.000	<0.001***
	“Hajria”	38.807±1.494	
Number of fertilized seeds	‘‘Dellahia’’	104.085±6.837	<0.001***
	“Hajria”	158.257±9.235	
Number of aborted seeds	‘‘Dellahia’’	151.771±7.283	<0.001***
	“Hajria”	99.485±5.834	
Number of feconded seeds	‘‘Dellahia’’	1.543±0.092	<0.001***
	“Hajria”	2.092±0.102	
Weight of aborted seeds	‘‘Dellahia’’	0.739±0.033	<0.001***
	“Hajria”	0.495±0.041	
Total number of seeds	‘‘Dellahia’’	255.857±10.823	0.902
	“Hajria”	257.742±10.749	
Total seed weight (g)	‘‘Dellahia’’	2.282±0.102	0.057
	“Hajria”	2.588±0.120	
% seed weight/pulp weight	‘‘Dellahia’’	4.632±0.209	<0.001***
	“Hajria”	6.533±0.3467	

Correlation coefficients were calculated to determine the relationship among the morphological characteristics of *O. ficus-indica* fruits and seeds (Table 3). Fruit weight was positively correlated with pulp weight, showing a value of 0.939 for the ‘‘Dellahia’’ cultivar and a value of 0.934 for the “Hajria” cultivar. However, the weight of the peel in both cultivars correlated with the whole weight and the weight of the pulp. In both cultivars, the number of fertilized seeds did not correlate with the weight of the fruit, the weight of the pulp, or the weight of the peel. By contrast, better correlations were observed between (1) the number of aborted seeds and whole weight, and (2) the number of aborted seeds and peel weight in the “Hajria” cultivar (0.637 and 0.538, respectively) compared with the ‘‘Dellahia’’ cultivar (0.326 and 0.412, respectively). Similarly, a better correlation was observed between total seed weight and the weight of fertilized seeds in the two cultivars (0.753 for ‘‘Dellahia’’ and 0.790 for “Hajria”) than between total seed weight and the weight of aborted seeds (0.282 for ‘‘Dellahia’’ and 0.488 for “Hajria”).

The total number of seeds was also correlated with the number of fertilized seeds and aborted seeds. The weight of aborted seeds positively correlated with the whole weight and pulp weight in the “Hajria” cultivar, compared with the ‘‘Dellahia’’ cultivar.

The percentage of pulp, peel, and seeds in the fruit is presented in Table 4. No significant difference in the percentage of seeds between the ‘‘Dellahia’’ cultivar (3.1260 ± 0.139) and the “Hajria” cultivar (3.2860 ± 0.144) was observed. However, the percentage of peel was higher in “Hajria” fruits (48.8154 ± 1.753) than in ‘‘Dellahia’’ fruits. By contrast, the percentage of pulp was higher in the ‘‘Dellahia’’ cultivar (64.4242 ± 0.8177) than in the “Hajria” cultivar (47.8986 ± 0.97249).

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Table 3. Correlation matrix for the properties of fruits and seeds of the two cultivars (“Hajria” and ‘‘Dellahia’’).

Correlation matrix	Cultivars	Whole weight (g)	Pulp weight (g)	Skin weight (g)	Number of fertilized seeds	Number of aborted seeds	Total number of seeds	Weight of fertilized seeds (g)	Weight of aborted seeds (g)
Whole weight (g)	‘‘Dellahia’’	**0.939****	**0.792****	0.251	0.326	0.378	0.356	0.210	0.395
	“Hajria”	0.934**	0.865**	0.246	0.637*	0.557*	0.333	0.453*	0.613*
Pulp weight (g)	‘‘Dellahia’’		*0.537**	0.382	*0.412**	*0.519**	*0.433**	0.297	*0.466**
	“Hajria”		0.641*	0.158	0.538*	0.427*	0.227	0.440*	0.505*
Skin weight (g)	‘‘Dellahia’’			-0.063	0.091	0.022	0.110	0.020	0.156
	“Hajria”			0.307	0.653*	0.618*	0.438*	0.405*	0.657*
Number of fertilized seeds	‘‘Dellahia’’				0.174	0.**749****	0.**812****	0.303	0.**771****
	“Hajria”				-0.035	0.840**	0.642*	-0.306	0.435*
Number of aborted seeds	‘‘Dellahia’’					0.**783****	0.259	0.*486**	0.296
	“Hajria”					0.513*	0.232	0.779**	0.593*
Total number of seeds	‘‘Dellahia’’						*0.687**	*0.518**	*0.686**
	“Hajria”						0.678*	0.160	0.696*
Weight of fertilized seeds (g)	‘‘Dellahia’’							*0.402**	0.**753****
	“Hajria”							0.080	0.790*
Weight of aborted seeds (g)	‘‘Dellahia’’								0.282
	“Hajria”								0.488*

Table 4. Weight of *Opuntia ficus-indica* fruits from the two cultivars and the percentages of different components of the fruit.

Properties	‘‘Dellahia’’	“Hajria”
Fruit weight (g)	74.102±2.432	80.171±3.243
Pulp (%)	64.4242±0.818	47.8986±0.973
Peel (%)	32.4497±0.860	48.8154±1.753
Seeds (%)	3.1260±0.139	3.2860± 0.145

The chemical parameters of the seed and peel from the two cultivars are presented in Table 5. No significant difference was observed in the moisture content, dry matter, and total sugars of the seeds between the two cultivars. However, the non-parametric tests revealed a small significant difference in the lipid contents between the ‘‘Dellahia’’ (10.41 ± 0.964) and “Hajria” (12.27 ± 0.865) cultivars. The protein level was 8.12 ± 0.176 and 8.67 ± 0.459 for the ‘‘Dellahia’’ and “Hajria” cultivars, respectively. A significant difference in the total sugar content of the peel between the two cultivars (57.29 ± 12.100 in the ‘‘Dellahia’’ cultivar and 36.66 ± 6.095 in the “Hajria” cultivar) was noted.

The germination percentage of seeds, sorted according to their length and weight, is presented in Figure 2. Seeds measuring less than 3.5 mm failed to germinate in both cultivars. Germination occurred in seeds ≥3.5mm long and weighing more than 0.1102 g (for the ‘‘Dellahia’’ cultivar) and 0.111 g (for the “Hajria” cultivar), at a rate that did not exceed 10% and 15% in the “Hajria” and ‘‘Dellahia’’ cultivars, respectively. In particular, significant germination occurred in group 6 (seeds measuring ≥5mm) at 57% and 55% for the ‘‘Dellahia’’ and “Hajria” cultivars, respectively. This germination percentage also increased with increasing seed weight and length in both cultivars.

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Table 5. Chemical characteristics of *Opuntia ficus-indica* seeds and peel (% w/w of dry matter) from the "Hajria" and ''Dellahia'' cultivars.

Structure	Parameters	Cultivar	N	Means± SD	Mann-Whitney test	Moses test	*t*-test (p<0.05)
Seeds	moisture	''Dellahia''	3	13.75±2.069	0.275	0.8	0.539
		"Hajria"	3	12.54±2.344			
	Dry matter	''Dellahia''	3	86.25±2.069	0.275	0.8	0.539
		"Hajria"	3	87.46±2.344			
	Total lipids	''Dellahia''	3	10.41±0.964	0.05*	<0.001***	0.068
		"Hajria"	3	12.27±0.865			
	Total sugars	''Dellahia''	3	7.04±0.973	0.127	0.5	0.122
		"Hajria"	3	5.74±0.610			
	Proteins	''Dellahia''	3	8.12±0.176	0.05*	<0.001***	0.125
		"Hajria"	3	8.67±0.459			
Peel	moisture	''Dellahia''	3	82.60±0.876	0.275	0.5	0.192
		"Hajria"	3	86.47±4.187			
	Dry matter	''Dellahia''	3	17.40±0.870	0.275	0.5	0.192
		"Hajria"	3	13.52±4.187			
	Total lipids	''Dellahia''	3	4.49±1.480	0.659	0.8	0.862
		"Hajria"	3	4.74±1.850			
	Total sugars	''Dellahia''	3	57.29±12.100	0.05*	<0.001***	0.058
		"Hajria"	3	36.66±6.095			
	Proteins	''Dellahia''	3	5.26±0.543	0.127	0.5	0,175
		"Hajria"	3	4.49±0.540			

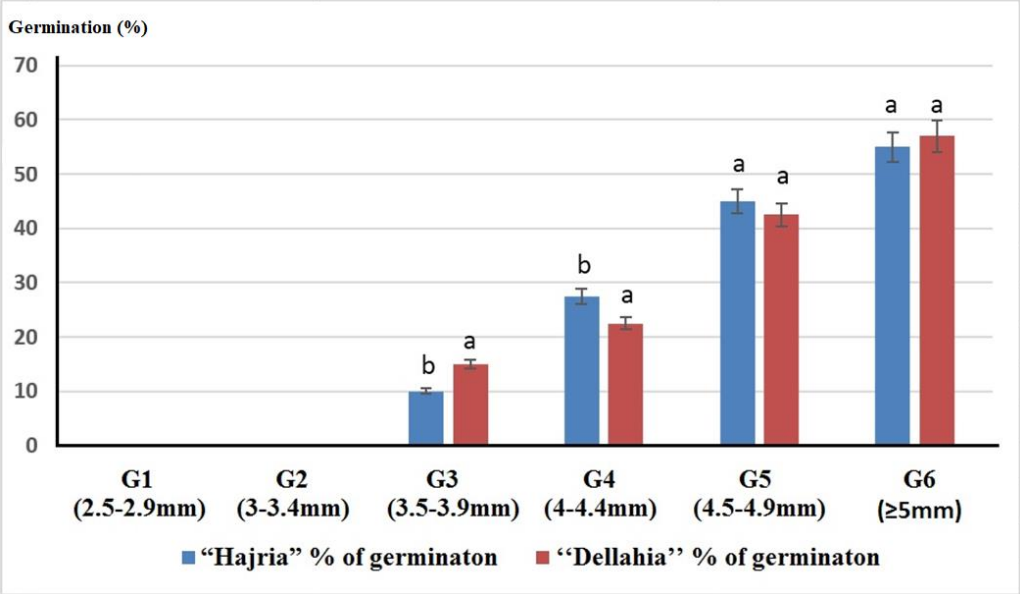

Figure 2. Seed germination rate in the two *Opuntia ficus-indica* cultivars. Seeds from each cultivar were separated into six groups according to their size.

The results obtained in this study show a difference in whole fruit weight between the two cultivars, possibly due to the environmental conditions of the province, consistent with previous studies. Barbera *et al*. (1994) detected no difference in the total number of seeds and percentage of seeds in the fruits between the two tested sites. The values reported here are similar to those reported by Mondragon-Jacobo and Perez-Gonzalez (1996). Pimienta-Barrios (1994) reported lower values in Mexican cultivars, which enhanced the appreciation of *O. ficus-indica* fruits in certain markets (Barbera *et al*., 1994). The fruits of the ''Dellahia'' cultivar are characterized by a higher percentage of pulp than those of the "Hajria" cultivar, which can be explained by the difference in climate between the two sites. Felker *et al.* (2002) reported that higher rainfall during the ripening increases

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the fruit size and the percentage of pulp, this part of fruit increases its value because in the export market pulp percentage should not be less than 55-60% (Inglese *et al.*, 1995b). Moreover, the ‘‘Dellahia’’ fruits are rich in aborted seeds and thus more sought after in the local markets than the “Hajria” fruits, which contain more fertilized seeds.

The difference in seed type may be due to several factors such as flowering date, flower quality, and soil type, as indicated by Lawes *et al*. (1990). However, this relationship is unclear, as fruit weight also depends on the number of fruits per cladode (Inglese *et al*., 1995b), water availability, and fruit ripening time (Barbera *et al.*, 1994; Nerd *et al.*, 1991), also the time of bud emergence and environment (Mashope, 2007). Although some varieties naturally produce larger fruits, with no effect on cladode load and time of bud emergence, it is concluded that fruit mass may be genetically controlled according to Karababa *et al.* (2004) and Bekir (2006). In addition, De Wit *et al.* (2010) showed that prickly pear fruit quality was influenced by genotype × environment interactions, this is by the findings of Ochoaa and Leguizamón (2009). Also, the fruit color of the *O. ficus-indica* is an index of its richness in antioxidants which increases its quality (Du Toit *et al.*, 2018) this criterion could complete this study with the involvement of other genotypes.Correlation analyses revealed a relationship between the number of aborted seeds and the weight of fruit pulp in both cultivars, implying that the seeds influence fruit size in *O. ficus-indica* (Barbera *et al*., 1994). The growth of cactus fruits is generally influenced by their seed type and weight.

Chemical analyses showed no significant difference in the lipid content recovered from the peel of the two cultivars, although the values obtained were notable, compared to the findings reported by Anwar and Sallam (2016) 3.66% ± 0.43 and 2.43% ± 0.32 (El Kossori *et al*., 1998). These recovered lipids could be suitable for commercial exploitation as a source of lipids for food use, for the manufacture of soaps, or the production of cosmetics (Ramadan and Mörsel, 2003). Because that peel lipids are rich sources of essential fatty acids and lipid-soluble antioxidants. Conversely, we observed a significant difference in the total sugar content of the peel between the two cultivars. This part of the fruit can therefore be exploited to manufacture other products or used as a carbon source to produce ethanol (Jha *et al*., 2019). Moreover, the protein content in the peel of the two cultivars is close to that reported by Ashraf *et al.* (2011). This increases its nutritional value, which makes us suggest its use in some food. On the other hand, the protein content of the seeds reported in the present study remained lower than those found by Sawaya *et al*. (1983) and El Kossori *et al*. (1998); 16.6% ± 0.40 and 11.8% ± 1.17 respectively. We also noted a higher seed moisture content in *O. ficus-indica* than previously reported in *O. boldinghii* 5.3 ± 0.51 (Sawaya *et al*., 1983) and 7.66 ± 0.06 (García-Pantaleón *et al*., 2009), due to the humid climate in northern Morocco. Higher lipid content was extracted from “Hajria” seeds than from ‘‘Dellahia’’ seeds. This difference is explained by the richness of “Hajria” fruits in fertilized seeds than in aborted seeds. The difference in chemical compositions between these genotypes suggests that the geographical location or environmental conditions of each province affected the characteristics of the fruits and seeds, as demonstrated by Karababa *et al*. (2004).

In the present study, germination only occurred in seeds longer than 3.5 mm. The percentage of germination increased with increasing seed length and weight in both cultivars but in a distinct manner. Thus, seed viability increased with increasing germination rate (TeKrony *et al*., 1980). However, in both cultivars, germination failed to reach 100%, even in seeds longer than 5 mm. This phenomenon can be explained by the known dormancy in *Opuntia* species (Romo-Campos *et al*., 2010; Jurado *et al*., 2006). Here, however, the humid climate of the collection sites may have influenced seed germination, and seed moisture humidity may have played an important role in the germination of these two cultivars compared to other species. By contrast, Jurado and Flores

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(2005) showed that seed dormancy is very common in unpredictable and difficult environments such as those in arid and semi-arid areas. In the present study, we observed a difference in germination percentage and mass among seeds of the same length, in both cultivars. There are, therefore, differences between the seeds based on their provenance, suggesting that dormancy is associated with environmental heterogeneity (Angevine and Chabot, 1979; Morpeth *et al*., 2000). These previous studies showed that several perennial species exhibit a combination of endogenous (morphological and physiological) and exogenous (physical and mechanical) dormancy. Indeed, environmental variations affect plant characteristics such as seed morphology, as well as physiological processes such as seed germination (Luzuriaga *et al*., 2005).

Conclusion

In northern Morocco, *Opuntia ficus-indica* exhibits a marked diversity between the “Hajria” and ‘‘Dellahia’’ cultivars. Differences were noted on a morphological and chemical level, as well as during physiological processes such as germination. The present findings showed that seed germination rate was positively associated with increased length and weight. These results can be used to select the best seeds to constitute a seed bank for this species. In addition, data on the chemical composition of the peel and the seed reinforce the potential of these two fruit by-products in the manufacture of various commercial items, both in the food industry as an additive and in the cosmetic industries. However, further research is needed to arrive at stronger results.

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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