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Seed Shape of Castor Bean (*Ricinus communis* L.) Grown in Different Regions of Tunisia

José Javier Martin Gómez[1], Ezzeddine Saadaoui[2] and Emilio Cervantes[1*]

[1]IRNASA-CSIC, Cordel de Merinas, 40, 37008, Salamanca, Spain.
[2]University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, BP 67, Gabès Manara, 6011, Tunisia.

Authors' contributions

This work was carried out in collaboration between all authors. The three authors worked together in the process of design, writing and discussion of the manuscript. Author ES collected the seeds. Author JJMG did most of the image analysis. Author EC coordinated the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Seed size, shape and colour intensity were compared in castor bean (*Ricinus communis* L.) grown in the field in twelve locations corresponding to four climatic regions in Tunisia.
Study Design: Seeds were obtained from plants grown in the field in twelve locations corresponding to four climatic regions in Tunisia.
Place and Duration of Study: Samples were taken in summer 2014 from plants growing spontaneously.
Methodology: Size is estimated as the area of the seed images. Shape is measured by the roundness and J index that gives the percent of similarity of the figure plane of a seed with an ellipse. Colour is measured as gray intensity in seed images.
Results: Among the parameters indicating seed shape, roundness varied more than J index. Values of J index were superior, thus J index describes better than roundness the shape of *Ricinus communis* seeds. Seed size and colour presented higher variation than magnitudes concerning

**Corresponding author: E-mail: ecervant@usal.es;*

shape indicating that shape is more conserved in *Ricinus communis* seeds, than size or colour.
Conclusion: Differences in size and shape were found among the climatic regions. Reduced size and J index values were observed together with increased values of roundness in seeds obtained from the population grown in the dessert.

Keywords: Ricinus communis; seeds; shape; diversity; Tunisia.

1. INTRODUCTION

Castor bean (*Ricinus communis* L.; Fig. 1) is the unique species of the genus Ricinus in the family Euphorbiaceae. Traditionally used in agriculture, the seeds are a source of lipid reserves, secondary metabolites and fuels in the pharmaceutic and oil industry [1]. The first mention of *Ricinus communis* as a laxative can be traced back to 3500 year-old Egyptian papyrus scrolls [2]. The seeds are poisonous containing 2.8–3% toxic substances such as ricin, a potent inhibitor of protein synthesis, and agglutinin-1 [3,4].

Ricinus communis (2n = 20) is native to north eastern Africa and the Middle East, but it has escaped cultivation to become a naturalized species and a weed under tropical and subtropical climates [5]. The plants grow well in diverse ecosystems ranging from cool temperate (moist to wet) through tropical desert to wet forest life zones. They support high temperatures, but seeds may fail to set above 38℃. The crop tolerates annual precipitation of 2.0 to 42.9 dm, temperatures of 7.0 to 27.8℃ and soil pH of 4.5 to 8.3. Plants require 140–180 days growing season and are readily killed by frost [5,6]. Castor bean is a hardy crop, easy to establish on the field, drought resistant, tolerant to different soil types [1]. It was naturalized in Tunisia, where it exists from the North to the South in spontaneous state occupying marginal soils, urban areas and agricultural land. Castor bean plants grow at a fast rate when situated in full sun and provided with ample fertilizer and water.

Fig. 1. *Ricinus communis;* From top to bottom and left to right: adult plant, floral bud, leaves, flower, inflorescence fruits and seeds

Anastasi et al. [7] registered a seed yield of 7.65 ton/hectare and seed oil content of 45% for a Tunisian genotype cultivated in Italy. Perdomo et al. [8] report oil yields in the range between 40 and 54% in Tunsian genotypes. The major fatty acid, ricinoleic acid, representing between 74 and 86% of the total [9].

Different results concern variation at the morphological and molecular levels in *Ricinus communis*. Plants range from short-lived dwarf annuals to large perennials developing into small trees. The tree and short-internode types are commonly referred to as giant and dwarf castor types respectively [10].

Goodarzi et al. [11] analyzed the diversity of 12 Iranian accessions and showed a low genetic diversity. Other studies show limited molecular diversity [12-14]. The low observed heterozygosity values suggest the predominance of autogamy, with a mixed mating system, being both self- and cross-pollinated by wind [15]. Also, based on polymorphism analysis by ISSR and RAPD markers respectively, Wang et al. [16] and Lakhani et al. [17] suggest high genetic diversity of castor bean germplasm.

Seed morphology may give information useful in the phenotypic characterization and phylogenetic relationships between varieties and cultivars. The seeds are characterized by high variability essentially in colour, size, weight and shape. Colour varies from red, white, grey, faint chocolate to purple and high variation is exhibited by the surface area [18], hundred-seed weight showed a wide range of variation from 11.6 to 59.1 g [19].

Accurate geometric description of seeds requires the comparison with geometric figures that resemble seed images. The approach has been developed in the model plant (*Arabidopsis thaliana*; [20]) as well as in the model legumes, *Lotus japonicus* and *Medicago truncatula* [21]. In *Capparis spinosa* the comparison of seed images with the cardioid curve was helpful to describe differences between two subspecies. Higher variation in seed shape was found in populations of *Capparis spinosa* Subsp. spinosa than in Subsp rupestris [22]. In *Jatropha curcas* a relationship was found between yield and seed size and shape, lower yields being associated with smaller seed size and poorer adjustment of the seed to morphological models [23]. In *Rhus tripartita*, lower values of J index were obtained in plants grown in the dessert regions than for those grown in mediterranean climates [24].

In *R. communis*, high phenotypical variability is observed in the seeds [18], that also represent the interesting part of plant, as oil source. This work presents the variations in size, shape, and colour of seeds in plants of Castor bean grown in thirteen locations in Tunisia. In the analysis of seed shape, the model applied for the geometric description has been an ellipse.

2. MATERIALS AND METHODS

2.1 Plant Material

Mature seeds were obtained in summer 2014 from plants growing spontaneously in 12 locations in Tunisia (Fig. 2; Table 1). For the analysis, 50 seeds per plant were used. The number of plants used per location oscillated between 1 and 8.

2.2 Image Capture

Images of the seeds were obtained with a scanner ScanJet 5300 C (HP).

2.3 Seed Size

The analysis includes area, perimeter and weight of 50 seeds per individual.

2.4 Seed Morphology

Roundness is a measure of the similarity between a plane figure and a circle given by

$$\mathrm{I} = 4\,\frac{\mathrm{area}}{\pi \times A^2}$$

Where A is the length of the major axis. It ranges from zero to one giving the value of 1 for circles and it is a useful magnitude as a first approximation to seed shape. It is preferred here to circularity index [25], because the latter is very sensitive to alterations in the margin of the figure.

J index measures the degree of similarity of a plane figure with an ellipse. An ellipse can be defined as the locus of all points that satisfy the equation $\frac{a^2}{x^2} + \frac{b^2}{y^2} = 1$

Where (x, y) are the coordinates of any point on the ellipse, and (a, b) are the radius on the x and y axes respectively.

Seed images of R. communis were adjusted to an ellipse whose relation between the major and minor axes (a and b) equals 1.43. The ratio was chosen after measuring the ratios between axes in a sample of seeds. J index was defined previously for quantification of Arabidopsis seed shape in comparison to a cardioid [20], and here is used to measure the adjustment of seed shape to the ellipse. To obtain J index, composed images containing an ellipse and the seed (Fig. 3) were elaborated for each seed with the software Corel PaintShop Pro X5. The ellipse was drawn with CorelDRAW X6. Area quantification was done with Image J (Java Image Processing Program).

Quantification of the adjustment was done in each seed as a proportion between the areas in two regions: The common region in the ellipse and the seed image (area C), and the sum of total regions of both images, the seed and the ellipse (Fig. 3). The index of adjustment (J index) is defined by:

$$J = \frac{\text{area}\,(C)}{\text{area}\,(C) + \text{area}(D)} \quad ,$$

Where area (C) represents the common region and area (D) the regions not shared. Note that J index ranges between 0 and 100, and decreases when the size of the non-shared region grows. It equals 100 when ellipse and seed image areas coincide, i.e., area (D) is zero. J Index was calculated for a total of 1426 seeds (Table 4).

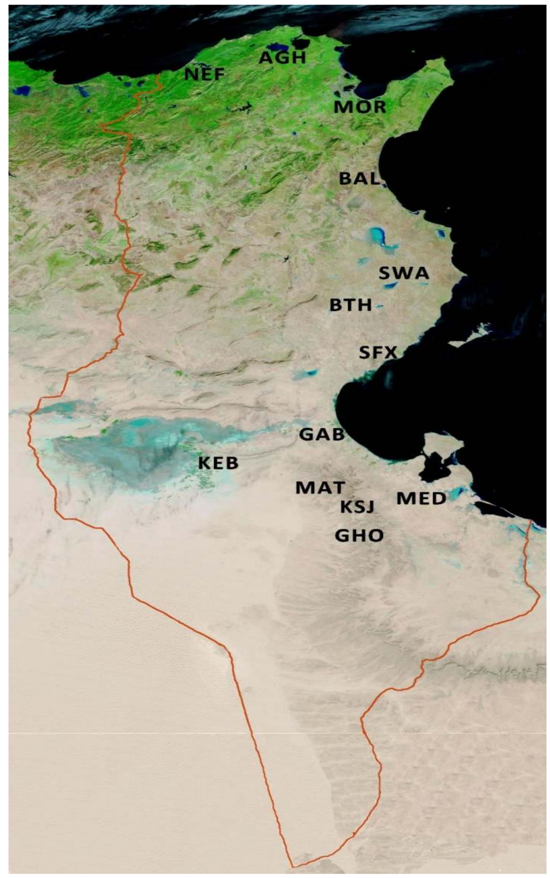

Fig. 2. Map of Tunis showing the localization of the populations used in this study

Table 1. Name and main characteristics of the sites of origin of the populations used in this study

Population	Abbreviation	Latitude (N)	Longitude (E)	Altitude (m)	Bioclimate	Soil
Nefza	NEF	36°58' 36.53"	9°03' 51.01"	34	Humid and	Silt-
Mateur	AGH	37°01' 51.80"	9°52' 51.67"	23	Sub-humid	sandy
Mornag	MOR	36°41' 40.78"	10°18' 21.19"	52	Upper and	
Sidi Bou Ali	BAL	35°57' 05.03"	10°28' 17.92"	20	inferior semi-arid	sandy
Souassi	SWA	35°20' 20.77"	10°32' 24.30"	55	Upper and	
Bouthady	BTH	35°04' 10.43"	10°15' 56.32"	124	inferior arid	
Gabes	GAB	33°52' 23.25"	10°07' 40 04"	46		
Matmata	MAT	33°32' 25.81"	09°57' 95.03"	375		
Ksar Jedid	KSJ	33°18' 03.93"	10°17' 37.84"	208		
Mednine	MED	33°21' 40.50"	10°28' 59.44"	100		
Ghomrassen	GHO	33°03' 32.33"	10°19' 35.01"	93		
Kebili	KEB	33°38' 44.94"	8°59' 38.66"	45	Saharan	

Fig. 3. Seeds of *Ricinus communis* showing the method used in the calculation of J index
To obtain J index, composed images containing an ellipse and the seed were elaborated for each seed with the software Corel PaintShop Pro X5. The ellipse was drawn with CorelDRAW X6. Area quantification was done with Image J (Java Image Processing Program)

Quantification of the adjustment was done in each seed as a proportion between the areas in two regions: The common region in the ellipse and the seed image (area C), and the sum of total regions of both images, the seed and the ellipse (Fig. 3). The index of adjustment (J index) is defined by:

$$J = \frac{\text{area (C)}}{\text{area (C)} + \text{area(D)}},$$

Left: Above: Seed; Below: Ellipse used as model.

Right: Above: Ellipse is superimposed to seed image showing total Area: area (C) + area (D); Below: Shared area between seed image and ellipse: area (C) is delimited by a white space.

2.5 Seed Colour

Intensity of grey and RGB values were obtained with CorelDRAW X6. The RGB colour model is one of the most common ways to encode colour in computing and diverse programs may give for each image the relative contribution of each of the three colours (Red, Green and Blue). The corresponding histograms were obtained with Photoshop CS3 (Fig. 4a and 4b).

2.6 Statistical Analysis

Analysis of variance (ANOVA) was applied to the comparison of magnitudes between plants as well as to the comparison between different populations. Magnitudes analyzed are those related with seed size (area, perimeter, weight of 50 seeds) and morphology (Circularity index, J index). The statistical analyses were made with the software IBM SPSS Statistics version 21. In the comparison between populations, ANOVA was done with the populations having five or more plants. Post-hoc analysis was carried out using Tuckey test (samples of similar sizes).

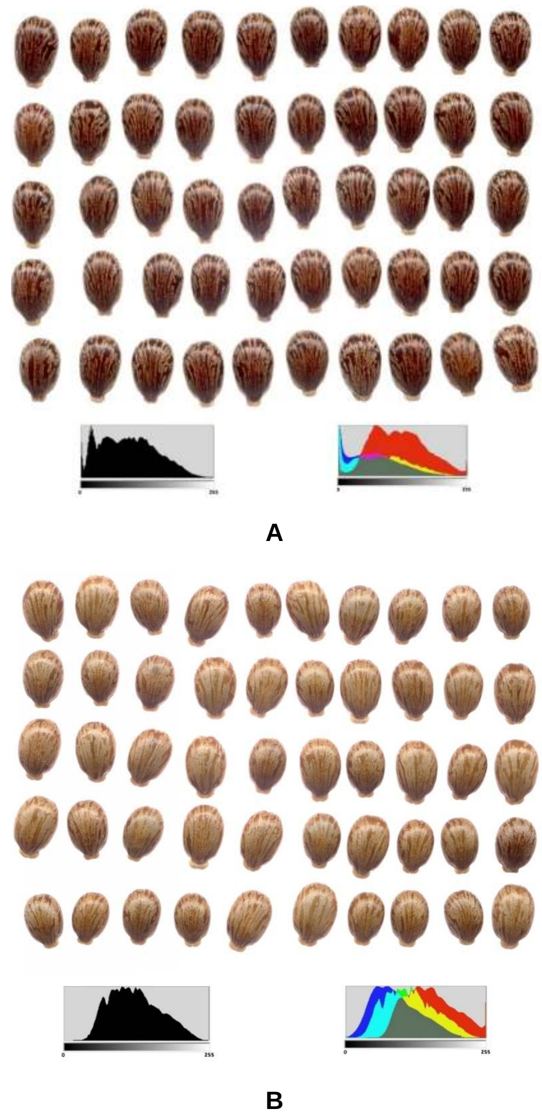

Fig. 4. Seeds of MED (a) and BAL (b) and histograms showing gray and RGB values obtained with photoshop

3. RESULTS AND DISCUSSION

Table 2 presents a summary of the results concerning area of seed images, roundness, J index, and gray values in the upper and lower sides of the seeds. The data are analyzed in detail below. The description follows a similar schema for all magnitudes and includes: 1) A general view of the data distribution in all seeds, 2) A description of the data per plant, 3) A description of the data per populations and 4) The comparison among climatic regions (Table 2).

Table 2 Summary of the results

Table 2A. Summary of area, weight and roundness

Climatic región	Number of seeds	Area (cm^2)	Weight	Roundness
Humid and sub-humid	446	1.04^a	0.49^a	0.65^b
Upper and inferior semi arid	500	1.11^c	0.51^b	0.65^b
Upper and inferior arid	1600	1.14^d	0.54^c	0.64^a
Saharan	300	1.07^b	0.48^a	0.68^c
Total	2846	1.11	0.52	0.65

Table 2B. Summary of J index

Climatic región	Number of seeds	J index
Humid and sub-humid	226	0.87^b
Upper and inferior semi arid	250	0.87^b
Upper and inferior arid	800	0.87^b
Saharan	150	0.85^a
Total	1426	0.87

Table 2C. Summary of gray values in the upper and lower side of the seeds

Climatic región	Number of plants	Value gray (upper)	Value gray (lower)
Upper and inferior arid	32	108^a	95^a
Upper and inferior semi arid	10	112^a	100^a
Humid and sub-humid	9	112^a	101^a
Saharan	6	128^b	127^b
Total	57	111	100

3.1 Seed Size (Area of Seed Image)

Seed size was estimated as the area corresponding to the seed images. The mean area in all seeds is of 1.12 cm2 and the values oscillate between 0.73 and 1.60 cm2 (Table 3).

Per populations (Table 3), the minimum area values correspond to BAL (0.95), AGH and BTH (1.00) and maxima to KSJ (1.37), SWA (1.27) and GHO (1.25). Differences were found between populations with BTH and AGH having lower area values and SWA and GHO higher (Table 3).

The comparison among climatic regions reveals four groups with lower area values in the humid and sub-humid regions, followed by the dessert, the upper and inferior semi arid, and finally larger seeds in the upper and inferior arid region (Table 2A).

3.2 Roundness

A summary of the results for roundness is presented in Tables 2 and 4. Mean roundness in all seeds is 0.65. The values oscillate between 0.53 and 0.85 (Table 4).

Table 3. Area values (cm^2) per images of seeds in 12 populations

Population	Number of seeds	Mean	Standard deviation	Min	Max
NEF	196	1.10^d	0.093	0.89	1.34
AGH	250	1.00^b	0.082	0.78	1.20
MOR	350	1.19^e	0.140	0.87	1.60
BAL	150	0.95^a	0.089	0.73	1.14
SWA	300	1.27^f	0.107	1.00	1.56
BTH	350	1.00^b	0.108	0.74	1.26
GAB	300	$1.04^{b,c}$	0.088	0.84	1.36
MAT	50	$1.07^{c,d}$	0.073	0.92	1.26
KSJ	50	1.37^g	0.095	1.10	1.53
MED	300	1.18^e	0.074	1.00	1.43
GHO	250	1.25^f	0.077	1.04	1.51
KEB	300	$1.07^{c,d}$	0.099	0.85	1.30
Total	2846	1.12	0.145	0.73	1.60

Per populations, the minimum roundness corresponds to MAT (0.62) and maximum to GHO and KEB (0.68; Table 4).

Differences were found between climatic regions with highest values in the Saharan region, lowest in the upper and inferior arid region and intermediate values in the other two regions (Table 2A).

3.3 J Index

A summary of the results for J index is presented in Tables 2 and 5. The mean value of J index in all seeds is of 0.87 and the values oscillate between 0.77 and 0.93 (Table 5).

Differences were found between populations with KEB and GHO having the lowest J index values (0.85) and AGH, BTH and BAL, the highest (0.88; Table 5; Fig. 5).

J index is smaller in the Sahara than in the other three climatic regions (Table 2B).

3.4 Gray Values

A summary of the results for gray values is presented in Tables 2, and 6. Table 6 contains data of gray values for the upper side (6a) and the lower side (6b) of the seeds. Higher gray values correspond to less coloured seeds.

Mean gray value in the upper side is 112 (Table 6a) and the values oscillate between 79.4 and 135.7.

Mean gray value in the lower side is 101.4 (Table 6b) and the values oscillate between 72.9 and 135.9.

In the upper side of seeds, the mean values in populations (Table 6a) oscillate between 94.9 (MED, 10) and 134.1 (BAL, 4).

In the lower side of seeds, the mean values in populations (Table 6b) oscillate between 72.9 (MOR) and 135.9 (KEB).

Table 4. Roundness per images of seeds in 12 populations

Population	Number of seeds	Mean	Standard deviation	Min	Max
NEF	196	$0.64^{b,c}$	0.02456	0.57	0.70
AGH	250	0.66^{e}	0.02684	0.59	0.85
MOR	350	$0.66^{d,e}$	0.02870	0.56	0.75
BAL	150	$0.64^{b,c,d}$	0.01931	0.61	0.70
SWA	300	$0.63^{a, b}$	0.02944	0.56	0.73
BTH	350	0.63^{a}	0.02297	0.53	0.71
GAB	300	$0.64^{b,c}$	0.02354	0.54	0.69
MAT	50	0.62^{a}	0.03124	0.56	0.72
KSJ	50	$0.65^{b,c,d}$	0.02187	0.60	0.68
MED	300	$0.65^{c,d}$	0.01762	0.60	0.69
GHO	250	0.68^{f}	0.02546	0.60	0.77
KEB	300	0.68^{f}	0.02887	0.60	0.77
Total	2846	0.65	0.03091	0.53	0.85

Table 5. J index per images of seeds in 13 populations

Population	Number of seeds	Mean	Standard deviation	Min	Max
NEF	100	$0.86^{a,b,c}$	.0205	.81	.90
AGH	126	$0.88^{d,e}$	.0170	.83	.91
MOR	175	$0.87^{b,c,d,e}$	.0197	.81	.91
BAL	75	0.88^{e}	.0134	.85	.91
SWA	150	$0.86^{a,b,c}$	.0178	.81	.89
BTH	175	$0.88^{d,e}$	.0199	.78	.93
GAB	150	$0.87^{c,d,e}$	.0178	.82	.90
MAT	25	$0.86^{a,b,c}$	.0213	.82	.91
KSJ	25	$0.86^{a,b,c}$	.0290	.77	.92
MED	150	$0.87^{a,b,c,d}$	.0154	.82	.90
GHO	125	$0.85^{a,b}$	.0234	.77	.90
KEB	150	0.85^{a}	.0269	.78	.91
Total	1426	0.87	.0219	.77	.93

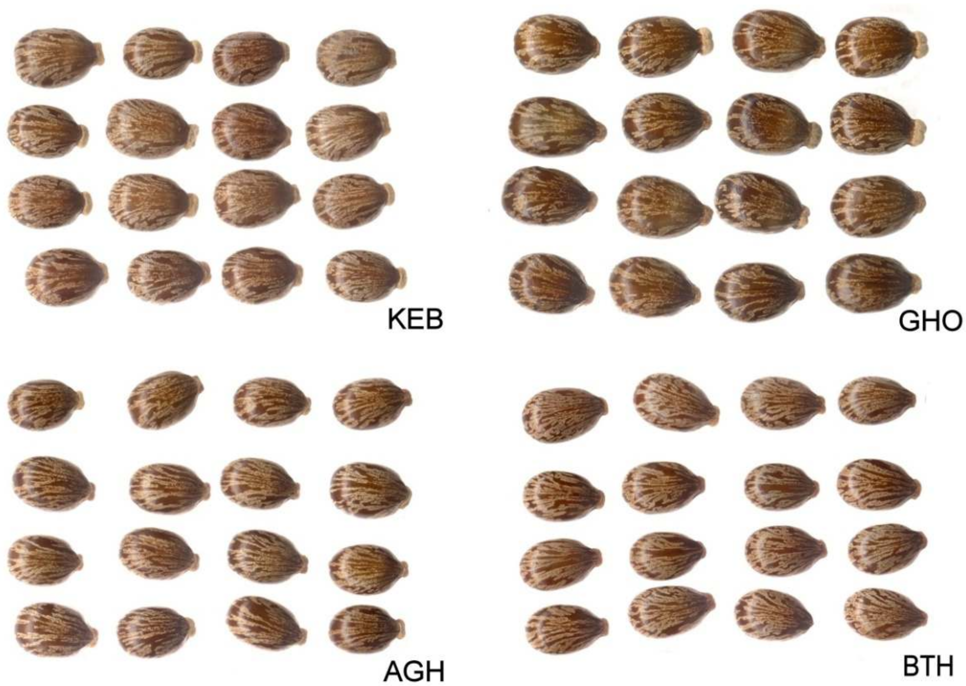

Fig. 5. Seeds of KEB, GHO, AGH and BTH. KEB has the lowest J index values and AGH and BTH, highest. GHO have larger seeds of higher circularity index and lower J index, whereas plants in AGH and BTH have smaller seeds

Table 6a. Mean grey values for seeds corresponding to plants in 10 populations (upper side)

Population	Number of plants	Mean	Standard deviation	Min	Max
NEF	4	110.8[a,b,c]	2.199	108.3	113.2
AGH	5	113.1[b,c]	3.454	107.7	116.5
MOR	7	102.6[a,b]	11.158	79.4	114.0
BAL	3	134.1[d]	1.980	131.9	135.7
SWA	6	106.9[a,b]	9.860	88.8	115.9
BTH	7	117.4[b,c,d]	4.808	107.1	121.4
GAB	6	110.8[a,b,c]	5.675	102.3	116.5
MED	6	94.9[a]	7.423	80.1	100.8
GHO	5	113.1[b,c]	2.726	109.2	116.9
KEB	6	128.4[c,d]	4.930	121.5	135.5
Total	55	112.0	12.080	79.4	135.7

Table 6b. Mean grey values for seeds corresponding to plants in 10 populations (lower side)

Population	Number of plants	Mean	Standard deviation	Min	Max
NEF	4	100.2[a,b]	2.769	98.4	104.3
AGH	5	103.1[a,b]	4.598	96.4	107.9
MOR	7	93.9[a]	10.827	72.9	104.9
BAL	3	114.5[b,c]	4.252	110.1	118.6
SWA	6	94.6[a]	10.795	74.3	104.7
BTH	7	106.7[a,b]	2.626	103.8	111.2
GAB	6	90.8[a]	4.821	84.8	96.1
MED	6	88.7[a]	7.579	74.9	96.8
GHO	5	100.5[a,b]	2.798	96.6	104.0
KEB	6	127.7[c]	5.731	120.8	135.9
Total	55	101.4	13.087	72.9	135.9

Differences were found between populations, both in the upper as in the lower side, with MED having the lowest gray values and BAL and KEB, the highest. Fig. 5 shows seeds of MED (Fig. 5a) and BAL (Fig. 5b).

Differences in gray values were found between climatic regions with highest values in the Saharan region, and lower in the other regions (Table 2C).

3.5 Discussion

Size, shape and colour are important characteristics in the description of seeds [18,19,26]. Our estimation of size is based in the area of seed images. Shape is evaluated using two magnitudes: roundness and J index. For colour, grey and RGB values are obtained with Photoshop from the analysis of 50 seeds. Thus, the analysis of colour does not involve comparison between plants in each population (there is only one data available for each plant). In the other instances (area, roundness and J index), the study involves a comparison between plants in a population, the comparison between populations, and finally between climatic regions.

A large amount of the total variation observed for size and shape is concentrated at the level of a single plant. For example, area values expand a total of 0.87 units and the area values for the seeds of a single plant expand 0.60 units in the case of MOR; 3-4. For the J index, variation total expands 0.16 units, and variation in a single plant reaches 0.10 units in GHO; 11-1.

The shape of *R. communis* seeds adjust better to an ellipse than to a circle. Values obtained are higher for J index than for roundness, thus J index is a magnitude more appropriate to study seed shape variations in this species than roundness.

In general there is no correlation between size and shape, or between roundness and J index. Nevertheless a combination of these magnitudes may give an idea of particular variations between plants or populations. For example, the seeds of Souassi (SWA, 5) and Ghomrassen (GHO; 11) are larger than in the other locations, and seeds in GHO have higher roundness than in SWA. The populations in BTH and AGH had highest J index values, with small seed size; and KEB and GHO, lowest J index values with the larger seed size in the case of GHO. This is in contrast with the results of our recent study in *Jatropha curcas* (Euphorbiaceae), where lower J index values were associated with reduced seed size, lower weight and reduced productivity [23].

In this study, highest J index values correspond with populations having smaller area values (BTH and AGH). The difference with the results obtained with *Jatropha curcas* may be explained because in Jatropha, we were comparing seed yield in culture between cultivars of different provenances, and low yield involves difficult growth conditions with higher proportion of aborted seeds, smaller seeds and alterations in shape, resulting in lower J index values. In contrast, the *Ricinus communis* seeds of this study were obtained from plants growing in the field, where smaller seeds with higher J index may represent special adaptations to particular environments.

Seed colour is a distinctive characteristic of Ricinus seeds, and it may present characteristic variations useful to distinguish between subspecies or varieties [1,27]. Among the populations used in this study, seeds are darker in MED and lighter in KEB and BAL. The analysis of both, the upper and the lower side yielded similar results in all populations.

The comparison of size, shape and colour among climatic regions revealed interesting peculiarities. As expected, seeds grown in the dessert are small, due to lack of water and nutrients. But the seeds grown in the dessert have also lower values of J index and higher values of roundness. In addition, they are darker (have larger grey values) as an adaptation for protection against high irradiation. Studies with other plant species reveal an increased production of total antioxidants, and of total phenolics in the arid conditions of the dessert [28,29].

Among the magnitudes considered, there is large variation in seed size or colour than in those concerning seed shape. Among the latter, J index describes better the shape of the seed, because the seed images resemble more an ellipse than a circle. Consequently there is more variation in the values of circularity index than in those of J index.

4. CONCLUSION

Ricinus communis is adapted to Tunisian climatic conditions; it is present in the spontaneous state in the different regions and bioclimates throughout the country. Castor bean oil has a great interest in international market. Our results show a large variability of seed parameters, including shape, size and colour. Size and colour present higher diversity than shape. Bioclimatic conditions affect all three magnitudes, and plants from the dessert have smaller seeds with reduced values of J index but increased circularity and colour intensity. These parameters are high interest to any work of selection and initiation of castor bean culture in Tunisia. Previous studies on the correlation between these parameters and castor bean oil yield seem promising.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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