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Seed shape quantification in *Capparis spinosa* L.: Effect of subspecies and geographic regions

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Abstract:

Seed shape quantification is an important tool in taxonomy and biodiversity studies. Seed images are compared with geometric figures and percent of identity is obtained (J index). Seed images of Arabidopsis and model legumes (Lotus and Medicago) adjust well to a cardioid allowing studies of shape variation in development and germination and mutant phenotyping. In this work we compare seed shape in two subspecies of *Capparis spinosa*: *rupestris* and *spinosa*. *Capparis spinosa* seed images adjust well to a cardioid. J index values are higher in subsp. *spinosa*. Division of the image in quadrants allows the identification of morphological types with different proportions in the subspecies. Seeds of subsp. *rupestris* have more morphological diversity. Among the four quadrants differences between subspecies are found in Q1 and Q2. The comparison between geographic locations for subsp. *rupestris* reveals reduced area and J index partial values in Q3 and Q4 in the seeds obtained in the desert.

Keywords: *Capparis spinosa*, biodiversity; cardioid; shape; morphometry; seeds

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1.Introduction

Shape is an important characteristic of biological objects and nevertheless it is not normally submitted to quantification. Seeds are the products of sexual reproduction in plants and in general are compact structures with geometrical shapes that, in a small volume, contain the genetic information for development and completion of the plant life cycle [1]. The analysis of variation in seed shape can be of great utility in plant taxonomy as well as in the description of mutant genotypes and adaptive changes in response to particular environments. Seed morphology may give useful information in the phenotypic characterization and phylogenetic relationships between varieties and cultivars [2].

Morphological description of seeds is, in general, required in the study of plant biodiversity as well as in other particular aspects, such as for example in genetics for the characterization of mutants and agronomy as a proof of seed quality. Modern methods of scanning and image analysis allow rapid measurements of seed dimensions in a large number of samples obtaining morphological data on general aspects such as circularity index, sphericity or roundness [3, 4, 5]. Nevertheless, more specific quantitative methods for measuring the shape of seeds, consisting in their comparison with particular geometrical figures [6, 7, 8] may be more informative from a biological point of view.

Quantification of seed shape consists in the comparison of seed images with geometric figures and has been applied to seeds of the model plant *Arabidopsis thaliana* [7], model legumes *Lotus japonicus* and *Medicago truncatula* [8], and species from other plant families such as Capparaceae [9], Euphorbiaceae [10], and others [2].

In the model plant *Arabidopsis thaliana* as well as in model legumes *Lotus japonicus* and *Medicago truncatula* the shape of seeds was described and quantified based on their similarity with a cardioid curve [7, 8]. In *Capparis spinosa*, the comparison of seed images with the cardioid curve was helpful to describe differences between two subspecies, *rupestris* and *spinosa* [9]. J index gives the percent of similarity of seed images with a cardioid whereas the comparison of shape in each of the four quadrants of the cardioid may be useful in the description of different seed types. Higher variation in seed shape was found in populations of *Capparis spinosa* subsp. *rupestris* than in subsp. *spinosa* [9]. In addition morphological comparison of seed types revealed differences between subspecies.

This work applies a new semi-automated method for quantification of seed shape (roundness) to seeds of *Capparis spinosa* and insists in the comparison between subspecies *rupestris* and *spinosa* for roundness and J index. Our results confirm and expand previous results of seed morphology for this species. In addition we compare seed morphology in populations of subsp. *rupestris* from different geographic locations.

2.Results

The comparison between subspecies reveals differences in area, J index total and partials. There are no differences in roundness. Area and J index values are higher in subsp. *spinosa*. Among the four quadrants differences between subspecies are found in Q1 and Q2 (Table 1).

The comparison between geographic locations for subsp. *rupestris* reveals differences in area and J index partial values in Q3 and Q4 (Table 2).

2.1. Seed size

According to previous reports the area of seed images is smaller in subsp. *rupestris* (Table 1).

Table 1: Comparison between varieties (standard dev.)

	rupestris	*spinosa*	Signification (P < 0.05)
N	220	40	
Area	6.6 (1.0)	9.4(1.2)	0.000
Roundness	0.8 (0.06)	0.8 (0.04)	0.984
J Index	91.0 (3.29)	92.3 (2.26)	0.021
J Index Q1	87.1 (4.56)	91.2 (3.39)	0.000
J Index Q2	92.3 (5.14)	95.6 (2.51)	0.000
J Index Q3	93.7 (3.04)	94.2 (2.12)	0.377
J Index Q4	82.4 (9.30)	82.2 (7.96)	0.889

For subsp. r*upestris*, seeds of southern regions are smaller (Table 2).

Table 2: Comparison between geographic regions (standard dev.)

	North	South	Signification (P < 0.05)
N	40	180	
Area	7.4 (1.4)	6.4 (0.8)	0.000
Roundness	0.8 (0.1)	0.8 (0.1)	0.125
J Index	91.0 (3.1)	91.1 (3.3)	0.936
J Index Q1	88.2 (4.3)	86.9 (4.6)	0.107

	North	South	Signification (P < 0.05)
J Index Q2	93.7 (5.5)	92.0 (5.0)	0.061
J Index Q3	94.6 (3.4)	93.5 (2.9)	0.036
J Index Q4	86.0 (6.9)	81.6 (9.6)	0.001

2.2. Seed shape

There are no differences in roundness between subspecies (Table 1). Differences between subspecies are found in J index with lower values in subsp. *rupestris* (Table 1) and also in the values of J index in the first and second quadrants (Q1 and Q2; Table 1).

In subspecies *rupestris,* there are no differences in roundness and J index values between geographic locations. Among the four quadrants differences between geographic regions are found in Q3 and Q4 (Table 2).

2.2.1. *Comparison of seed types between subspecies*

Of the six single morphological seed types (A, B, C, D, E and 0), higher percentages of type B and C, denoting alterations in the first and second quadrants (Q1 and Q2), are observed in subspecies *rupestris* (Table 3).

Table 3: Quantities and frequencies of single morphological seed types

	Total 0	Total A	Total B	Total C	Total D	Total E
spinosa	3	3	14	2	1	33
	7.5%	7.5%	35%	5%	2.5%	82.5%
rupestris	10	19	158	54	22	167
	4.5%	8.6%	71.8%	24.5%	10%	75.9%

As a result of the combination of single morphological seed types, a total of sixteen seed types were found in the seeds (Table 4). From these, all were observed in *rupestris* and eight in *spinosa*.

Table 4: Quantities and frequencies of observed morphological seed types

	A	AB	ABE	AE	0	B	BC	BE	BCE	BCDE	BDE	C	CE	CDE	DE	E
Spinosa	2 5%			1 2.5 %	3 7.5 %	2 5%		10 25%	1 2.5 %	1 2.5%						20 50%
Rupestris	5 2.3%	8 3.6 %	2 0.9 %	4 1.8 %	10 4.5 %	20 9.1 %	5 2.3 %	78 35.5 %	28 12.7 %	10 4.5%	7 3.2 %	5 2.3 %	4 1.8 %	2 0.9 %	3 1.4 %	29 13.2 %

2.2.2. *Comparison of seed types between seeds from subspecies rupestris grown in different climatic regions*

Seeds from plants grown in the dessert and arid regions of the south have higher percentages of each of the single morphological types (A, B, C, D and E) but type 0 is more frequent in the north (Table 5). This result indicates that size alterations in the four quadrants are more frequently observed in seeds obtained from plants of subspecies *rupestris* grown in the the desert and southern arid regions.

Table 5: Quantities and frequencies of single morphological seed types from subspecies rupestris grown in different climatic regions

	Total 0	Total A	Total B	Total C	Total D	Total E
North	6 15 %	2 5.0 %	23 57.5 %	7 17.5 %	2 5 %	22 55 %
South	4 2.2 %	17 9.4 %	135 75 %	47 26.1 %	20 11.1 %	145 80.6 %

All the observed combinations (16) were detected in seeds obtained from plants grown in the south and only 10 of them in seeds obtained from plants grown in the north (Table 6).

Table 6: Quantities and frequencies of observed morphological seed types

	A	AB	ABE	AE	0	B	BC	BE	BCE	BCDE	BDE	C	CE	CDE	DE	E
North	2 5%				6 15 %	7 17.5 %	2 5%	12 30%		1 2.5%	1 2.5 %	1 2.5 %	3 7.5 %			5 12.5 %

	A	AB	ABE	AE	0	B	BC	BE	BCE	BCDE	BDE	C	CE	CDE	DE	E
South	3 1.7%	8 4.4%	2 1.1%	4 2.2%	4 2.2%	13 7.2%	3 1.7%	66 36.7%	28 15.6%	9 5%	6 3.3%	4 2.2%	1 0.6%	2 1.1%	3 1.7%	24 13.3%

Representative seeds are shown in Figure 1 where the population of origin is indicated.

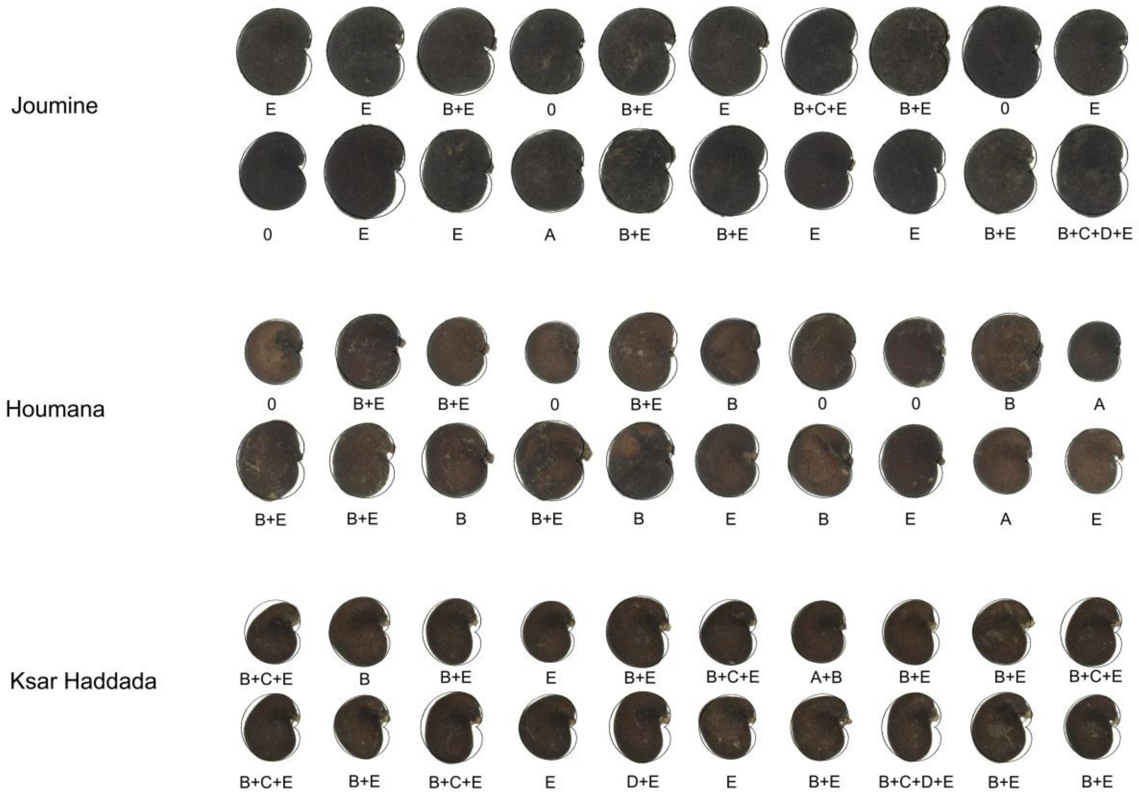


Figure 1. Representative images of Capparis spinosa belonging to two subspecies: spinosa and rupestris. The two rows in the top contain seeds from subspecies spinosa grown in Joumine (see figure 2 for localization in the map). The two rows in the middle contain seeds from subspecies rupestris grown in Houmana and the two rows in the bottom seeds from subspecies rupestris grown in Ksar Haddada, in the desert (south).

3. Discussion

The J index gives the percent of similarity of the seed images with a cardioid curve. It reaches values superior to ninety in a majority of seeds of *Capparis spinosa* analyzed in this study. The values obtained for J index are superior to the values obtained for roundness; thus, J index is a magnitude that describes better the shape of *Capparis* seeds than roundness [2].

The adjustment of *C. spinosa* seeds to a cardioid is accurate and may be a useful tool to analyze and classify seeds from diverse provenances and to study biodiversity in seed shape. Of the six populations under study here, only one (CHE) doesn't contain seeds with values of J index superior to ninety five (type A seeds; not shown). This population is also unique in that both subspecies (*spinosa* and *rupestris*) coexist, ending in a frequent presence of hybrids [11].

In relation with seed size our results confirm and expand those of Saadaoui et al. [9, 11] reporting differences between both subspecies. *C. spinosa* subsp. *spinosa*, restricted to the north, has larger seeds; and *C. spinosa* subsp. *rupestris*, established throughout the Tunisian territory including southern deserts, has smaller seeds. These results support the hypothesis of Fici [12] that *C. spinosa* subsp. *rupestris* represents a primitive type closer to the tropical stock of the group; whereas *C. spinosa* subsp. *spinosa* is a derived form of this. The primitive type corresponding to subsp. *rupestris* would be here closer to the concept of "r" type strategy [13]. In Ecology, "r" type is the strategy based on high rates of reproduction ("r" comes from reproduction); in contrast to the K strategy, based on the quality (i.e. on the production of a reduced number of individuals with highly sophisticated structures in response to specific adaptations). In agreement with this, the comparison between both subspecies indicates that subsp. *rupestris* has characteristics closer to an "r" type strategy: smaller seeds, simpler plant structure (trailing, thornless), larger number of stamens and self-reproduction. In contrast, subsp. *spinosa* may have diverged from the "r" type strategy towards more specialized adaptations: Larger seeds, more complex structure (erect and thorny), reduced number of stamens and cross-reproduction [9, 11, 12].

The diversity between both subspecies (*spinosa* and *rupestris*) is also supported by chemical composition of the seed. Saadaoui et al. observed high diversity among populations with differences between subspecies [14]. The difference concerns both oil content and fatty acid composition. In addition, total content of phenolic compounds and tannins is higher in subsp. *spinosa* [15]. In fact, this variability is manifested at the physiological behavior; caper populations of the arid region showed a higher tolerance to salinity during germination stage [16].

4. Materials and Methods

4.1. Plant Material

Figure 2. Map of Tunisia showing the locations of the populations used as source of seeds. The populations underlined (Chemtou and Joumine) correspond to *C. spinosa* subsp. *spinosa.* All the other populations are from *C. spinosa* subsp. *rupestris*

Seeds were collected in the field in natural populations corresponding to six different locations in Tunisia (Figure 2). The distribution of *C. spinosa* subsp. *spinosa* is restricted to the north, thus seeds of this subspecies are from populations located in two geographical sites in Joumine (JOU) and Chemtou (CHE). *C. spinosa* subsp. *rupestris* seeds were collected from two populations in the north (Houmana, HOU; Ghar el Melh, GEM), and two populations in the south (Chenini Tataouine, CHT; Ksar Hdada, KH).

Seeds from each population were pooled and allowed to dry at room temperature. Seeds from CHE were collected in July, 2003, the rest of populations in July, 2013.

4.2. Image capture

Photographs of longitudinal views of seeds were taken with a digital camera Nikon DS-Fi1 adapted to a Nikon 'SMZ-1500' stereo-microscope.

4.3. Quantitative morphology

Composed images containing the cardioid (model) and each seed were elaborated with the software image Corel PHOTO-PAINT X7. Area values were calculated with Image J (Java

Image Processing Program). In this process, a microscopy photograph of a graph paper was used to convert pixel into mm.

Two magnitudes were used for the quantitative morphological analysis: Roundness and J index.

Roundness is defined by Ferreira and Wayne [17] as:

$$\mathbf{R} = \frac{\mathbf{4\ x\ area}}{\boldsymbol{\pi}\ [\mathbf{Major\ axis}]^{\mathbf{2}}}$$

It was determined automatically with the Image J program.

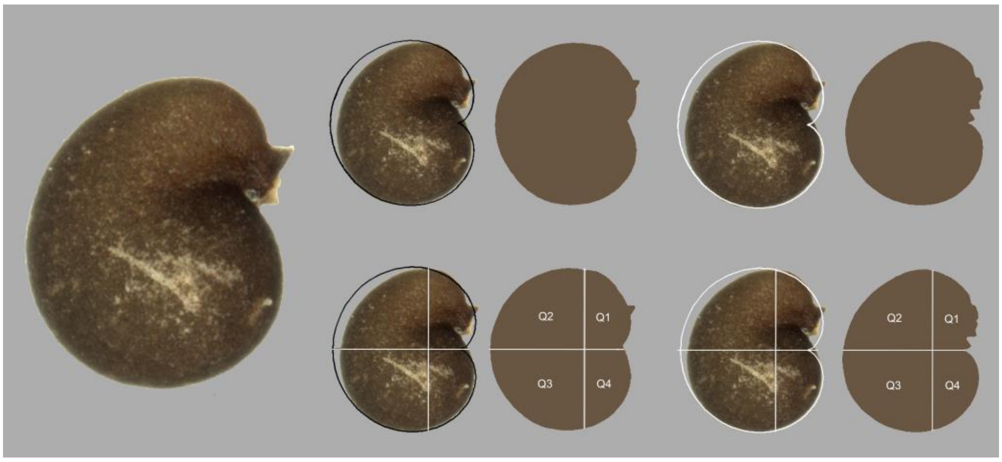
J index reflects the percent similarity of the seed image with a cardioid. The measurement of J index requires a manual procedure done in each seed by superimposing the seed image with the cardioid and calculating the ratio of the areas in two regions: The common region in the cardioid and the seed image (C), and the total area occupied by the ellipse and the seed image (see Figure 3a). The index of adjustment (J) is defined by:

$$J = \frac{\text{area (C)}}{\text{area (C)} + \text{area (D)}} \times 100$$

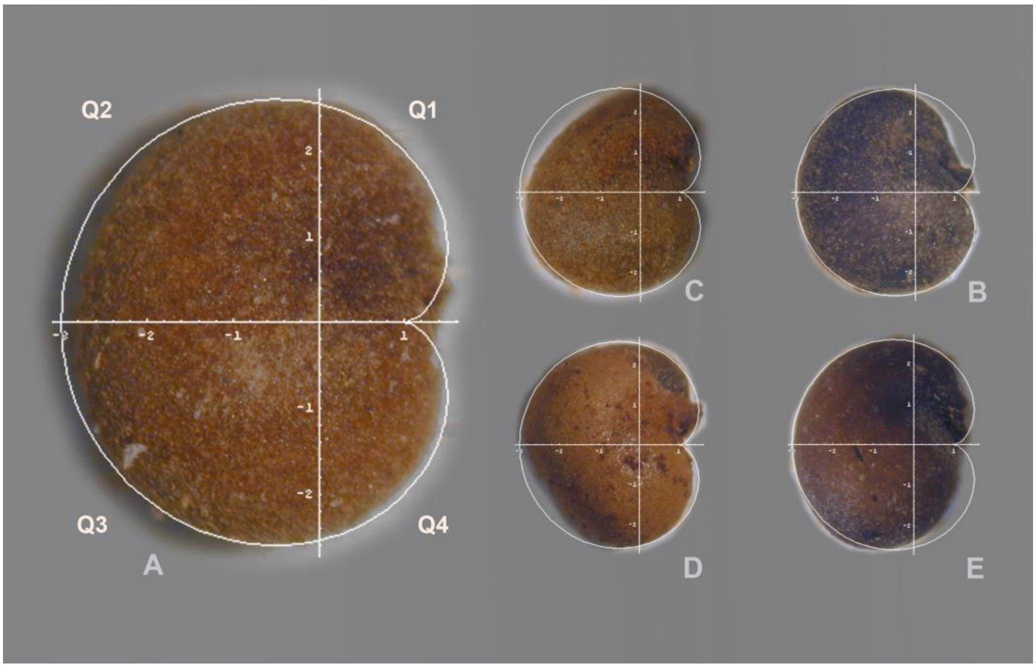
where C represents the common region and D the regions not shared (total area is the sum of shared and non-shared). Note that J ranges between 0 and 100, and decreases when the size of the non-shared region grows. It equals 100 when cardioid and seed image areas coincide, i.e., area (D) is zero. The seeds that have values of J index superior to ninety five have been termed as type A seeds (Figure 3b).

The images of the seeds with the superimposed cardioid curve were divided in four quadrants (Q1 to Q4; Figure 3) and percent of identity with the cardioid was calculated for each quadrant. This gave four additional seed types (B, C, D, E) depending on which quadrant contains a difference with the cardioid (Figure 3b). Types B, C, D and E were defined for seeds whose values of similarity with the cardioid curve were lower than 90 per cent in Q1, Q2, Q3 and Q4 respectively. The five basic seed types (A, B, C, D and E) don't exclude each other. Thus each seed may belong in theory to a total of $2^5 = 32$, non-excluding morphological combinations of the five basic types. In addition, for those seeds not belonging to any of the above types, type "0" (zero) was described, resulting in a total of 33 possible combinations, of which only 16 were observed (Tables 4 and 6).

J Index was calculated for a total of 260 seeds (20 seeds corresponding to one plant per each population; except in the population of KH with 160 seeds corresponding to eight plants).



(a)



(b)

Figure 3. Schematic representation of the method used in the quantification of seed shape. a) Seed image (left) and its comparison with a cardioid with representation of total (left) and shared (right) areas. b) Seed image with the four quadrants (left), and examples of the morphological seed types.

4.4. Statistical analysis

ANOVA was used for the comparison between different groups. Post-hoc analysis was carried out using Scheffé test for samples of heterogeneous size. Statistical analysis was done with software IBM SPSS Statistics.

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