

## INTRASPECIFIC VARIABILITY OF SEED MORPHOLOGY IN *CAPPARIS SPINOSA* L.

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Morphological description of seeds is a required step for analysis of biodiversity in natural populations and may give clues to adaptive strategies in species evolution. A cardioid is the curve described by a point of one circumference rolling around another circumference of equal radius. Models based on adjustment of seed shape with cardioid curves have been described for *Arabidopsis thaliana* and the model legumes *Lotus japonicus* and *Medicago truncatula*. In this work the model is applied to analyze seed morphology in populations of two subspecies of *Capparis spinosa* growing in Tunisia. Adjustment of seed images to cardioid curves, followed by statistical analysis of similarity in the complete images as well as in each of four quadrants, allows an accurate description of seed shape. The results show differences in morphology between subspecies. Seeds of subsp. *rupestris* present higher diversity of shape than seeds of subsp. *spinosa*. This may indicate primitiveness of *C. subsp. rupestris* seeds, associated with nonspecialization. The results are discussed in relation to the ecological strategies of both subspecies in their evolution.

**Key words:** Adaptation, biodiversity, cardioid curve, ecology, morphometry, *Capparis spinosa*.

### INTRODUCTION

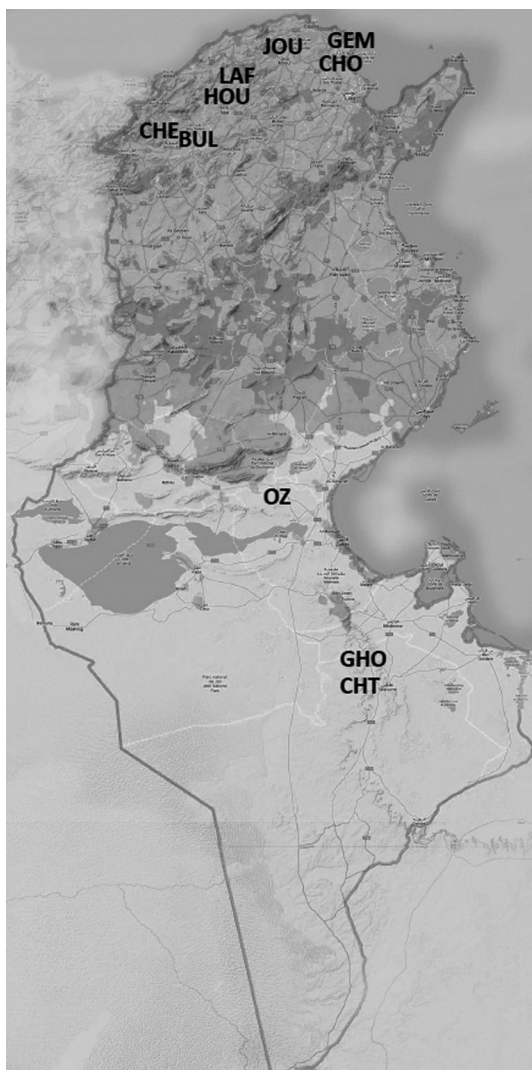
Caper (*Capparis spinosa* L.) is an important crop in Mediterranean countries, where it also grows spontaneously in diverse ecosystems. The name *Capparis* was already used in the 4th century BC by Theophrastus (Sharples, 1994) and in the 1st century AD by Dioscorides.

The Genus *Capparis* corresponds to number DCCIII in *Codex Botanicus Linneanus* (Richter, 2003) and contains more than 250 species (Jacobs, 1965; Willis, 1988; Barbera, 1991). In his *Flora Aegyptiaco-Arabica* published in 1775, Pehr Forsskal described three different species of *Capparis* (*Capparis dahi*, *C. decidua*, *C. inermis*; Hepper and Friis, 1994). In the Mediterranean region and Asia, Zohary (1960) described six species comprising fifteen varieties, of which two species were reported in Tunisia: *C. spinosa* and *C. ovata*. A wider species concept led Jacobs (1965) to include all the species of *Capparis* from the Mediterranean region in a single species, *Capparis spinosa*. In Tunisia, Pottier-Alapetite (1979) described four varieties within this species: var.

*egyptica* (Lamk) Boiss., var. *genuina* Boiss., var. *coriacea* Coss. and var. *rupestris* (S. & Sm.) Viv. Later, Higton and Akeroyd (1991) divided *C. spinosa* into two subspecies: subsp. *spinosa* and subsp. *rupestris* (Sm.) Nyman.

Saadaoui et al. (2011) applied morphometric analysis to fifteen populations of *C. spinosa* from diverse locations covering all the Tunisian territory. The analysis was based on three qualitative traits and eight quantitative parameters. The qualitative traits were creeping or erect plant; type of thorny stipule development (thin, developed, and curved); and the presence or absence of epidermal hairs. The quantitative parameters were blade length, blade width, blade surface, internode length, number of stamens per flower, seed length, seed width and weight of 1000 seeds. The analysis revealed two sub-clusters in accord with the recent botanical subdivision of Higton and Akeroyd (1991), Tutin et al. (1993) and Heywood (1993): *C. spinosa* subsp. *rupestris* is more creeping and smaller, and adapted to a variety of diverse conditions including drought and arid conditions in southern regions, where mean annual precipitation is under 100 mm.

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**Fig. 1.** Map of Tunisia showing the sites of the ten *Capparis spinosa* populations used in this work.

Tlili et al. (2011) collected seeds in diverse Tunisian populations. Their work shows differences in the biochemical composition of the two subspecies. In addition, seeds of *C. subsp. spinosa* were larger than those of *C. subsp. rupestris*.

Analysis of seed size based on scanning technology and image analysis algorithms (Herridge et al., 2011; Tanabata et al., 2012) gives automated measurements of seed dimensions which may be useful in plant taxonomy and evolution studies (Grillo et al., 2010). Other methods for morphology and carpology are based on a combination of image analysis with diverse morphological parameters (Borkowski, 2001; Toma, 2008). Quantitative morphology may be based on comparison with geometric models (Keefe and Davis, 2010). Quantitative morphological analysis applied to *Arabidopsis*, *Lotus* and

*Medicago* revealed the similarity of seeds of these model plants to a cardioid curve (Cervantes et al., 2010, 2012). A geometric model based on similarity to a cardioid curve allowed comparison between varieties and mutants in seeds of these model species, opening the way to quantitative analysis of seed morphology. This approach may be of interest in evolution and developmental studies.

The aim of this study was to apply morphological analysis based on comparison with a cardioid curve to seeds of two wild subspecies of Tunisian populations of *C. spinosa*, in order to determine any relationships between seed shape and other characteristics of the subspecies related to their habits, structure or life cycles.

## MATERIALS AND METHODS

### PLANT MATERIAL

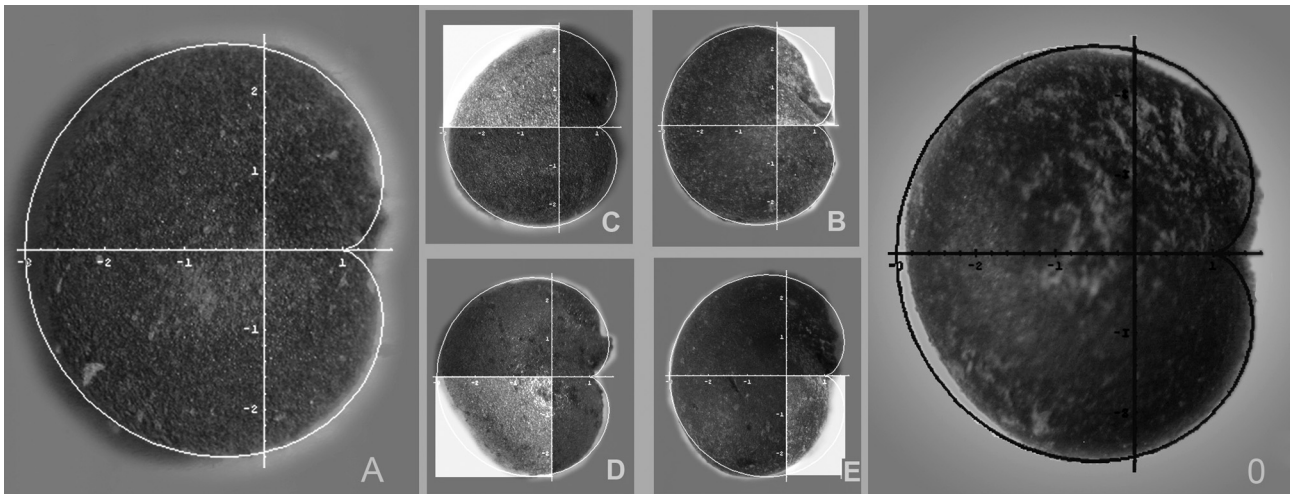
Seeds were collected in the field in July 2003 in natural populations corresponding to ten different locations in Tunisia. The distribution of *C. spinosa* subsp. *spinosa* is restricted to the north. Its seeds for this study are from populations at five sites in northern Tunisia: Chouiqui (CHO), Joumine (JOU), Chemtou (CHE), Bullaregia (BUL) and Lafareg (LAF). Seeds of *C. spinosa* subsp. *rupestris* were collected from two populations in the north (Houmana, HOU; Ghar el Melh, GEM), one in the center (Oued Zayied, OZ), and two in the south (Chenini Tataouine, CHT; Ghomrassen, GHO). The seeds of each population were pooled and allowed to dry at room temperature. Figure 1 shows the locations of the populations and Table 1 describes their ecological characteristics.

### PHOTOGRAPHY AND IMAGE ANALYSIS

Seeds were observed with a Nikon SMZ-2T stereomicroscope. The seeds were placed on a flat surface with the micropyle oriented to the right and photographs of orthogonal views were taken with a Nikon Coolpix 950 digital camera. Composed images containing the cardioid curve (see later) and the seed were made for each seed with Photoshop CS4 (Adobe). Areas were quantified with Image J (Java Image Processing Program). In this process, graph paper was used to convert pixels to  $\mu\text{m}$ . Seed size estimates are based on the seed image area expressed in  $\text{mm}^2$ .

### QUANTITATIVE MORPHOLOGY

The pictures of seeds were superimposed on cardioid images (Fig. 2). A cardioid is the trajectory described by a point of a circle rolling around another



**Fig. 2.** Left: Adjustment of *C. spinosa* seeds to a cardioid curve. The cardioid curve is described by the point of a circle of radius  $r$  turning around another circle of the same radius. Q1, Q2, Q3 and Q4 indicate the four quadrants used in the analysis to describe types B, C, D and E of seeds. Type A applies to seeds whose percentage of similarity to the cardioid curve (J index) exceeds 95. Center: Seeds representative of types B, C, D and E. Types B to E are seeds whose similarity in Q1 to Q4 is below 90 percent (B refers to Q1, C to Q2, D to Q3 and E to Q4). Right: Seed of type 0 is a seed whose J index is below 95 but whose similarity to a cardioid in each quadrant is over 90. Thus it does not belong to type A or any of the other four types.

TABLE 1. Basic ecological characteristics of the studied Tunisian populations of *Capparis spinosa*

Subspecies	Population	Abbreviation	Bioclimatic zone	Latitude (N)	Longitude (E)	Altitude (m)	Soil pH	Total limestone (%)	Soil texture
<i>C. spinosa</i> subsp. <i>rupestris</i>	Ghomrassen	GHO	Arid	33°06'	10°18'	326	8.6	23	Rocky outcrops
	Chenini Tataouine	CHT	Saharan	33°42'	9°23'	211	8.1	45	Rocky outcrops
	Oued Zayied	OZ	Arid	34°08'	9°31'	180	7.6	56	Rocky outcrops
	Houmana	HOU	Sub-humid	36°40'	9°08'	371	7.2	11	Rocky outcrops
	Ghar El Melh	GEM	Sub-humid	37°10'	10°11'	5	7.9	71	Rocky outcrops
<i>C. spinosa</i> subsp. <i>spinosa</i>	Chouiqui	CHO	Semi-arid	36°53'	9°46'	158	7.5	38	Rocky outcrops
	Joumine	JOU	Sub-humid	36°57'	9°31'	106	7.8	38	silty
	Bullaregia	BUL	Sub-humid	36°33'	8°44'	204	8.1	61	silt-sandy
	Chemtou	CHE	Sub-humid	36°29'	8°44'	189	7.7	31	silt-sandy
	Lafareg	LAF	Sub-humid	36°28'	8°33'	171	7.4	45	Silt-sandy

er fixed circle with the same radius (see in Fig. 2A). Quantification of the adjustment was done for each seed by comparing the areas of two regions: the region common to the cardioid and the seed picture, and the region not shared by them (see Fig. 2). The index of adjustment (J index) is defined as:

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

where area (C) represents the common region and area (D) the regions not shared. Note that J ranges between 0 and 100, and decreases when the size of the nonshared region grows. It equals 100 when the cardioid and seed image areas coincide, that is, area D is zero.

The J index was calculated for a total of 100 seeds (10 seeds per population; 50 seeds per sub-species). Seeds having J index values equal or supe-

TABLE 2. Summary of results. Means ( $\pm$ SD) for area, J index and JQ1, JQ2, JQ3 and JQ4 values

<i>Capparis</i> subsp. <i>rupestris</i>						
	CHT	HOU	GEM	OZ	GHO	Mean
Area (mm <sup>2</sup> )	6,1 (0,87)	8,4 (1,74)	6,0 (1,27)	5,9 (0,87)	7,6 (0,94)	6,8 (1,53)
J Index	93,4 (2,17)	92,9 (3,16)	94,0 (2,45)	92,8 (3,61)	91,9 (2,84)	93,0 (2,86)
JQ1	90,8 (2,94)	85,5 (8,36)	91,0 (2,24)	82,9 (13,21)	83,7 (7,86)	86,8 (8,43)
JQ2	94,1 (4,81)	95,1 (1,99)	95,9 (3,31)	96,1 (3,75)	96,3 (1,75)	95,5 (3,28)
JQ3	97,0 (1,89)	96,3 (2,35)	95,3 (2,84)	96,4 (1,77)	92,9 (5,60)	95,6 (3,40)
JQ4	88,9 (8,53)	85,4 (9,99)	89,5 (6,61)	87,1 (9,52)	84,6 (9,42)	87,1 (8,73)
subspecies <i>spinosa</i>						
	CHO	JOU	CHE	BUL	LAF	Mean
Area (mm <sup>2</sup> )	9,1 (1,23)	9,6 (2,80)	9,6 (0,86)	9,6 (1,35)	9,8 (1,56)	9,5 (1,64)
J Index	93,3 (2,98)	91,8 (5,21)	90,8 (2,96)	93,3 (1,69)	93,5 (2,74)	92,5 (3,35)
JQ1	86,1 (4,51)	80,3 (9,45)	71,6 (10,4)	87,1 (6,61)	83,5 (5,65)	81,7 (9,26)
JQ2	96,1 (3,30)	95,2 (4,08)	94,4 (2,58)	96,4 (1,65)	95,5 (3,52)	95,5 (3,09)
JQ3	97,4 (1,15)	95,5 (3,76)	94,5 (3,15)	94,7 (2,73)	96,0 (2,39)	95,6 (2,86)
JQ4	85,0 (11,22)	86,7 (8,51)	92,0 (3,73)	89,4 (6,99)	90,7 (5,24)	88,8 (7,72)

rior to 95 (95 percent identity with the cardioid) were designated Type A (Fig. 2).

The images of the seeds with the superimposed cardioid curve were divided into four quadrants (Q1 to Q4; Fig. 2) and the percentage 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 (Fig. 2). Types B, C, D and E apply to seeds whose values of similarity with the cardioid curve are lower than 90 per cent in Q1, Q2, Q3 and Q4 respectively. The five basic seed types (A, B, C, D and E) are not mutually exclusive. Theoretically each seed may belong to one of a total  $2^5 = 32$  nonexclusive morphological combinations of the five basic types. For seeds not belonging to any of the above types, the additional type 0 (zero) was described (Fig. 2), resulting in a total of 33 possible combinations.

#### STATISTICAL ANALYSIS

ANOVA was used to test the significance of differences between the groups. Post-hoc analysis employed the Scheffé test for samples of heterogeneous size. Tukey's test was used for samples of similar size. Statistical analyses were done with IBM SPSS Statistics.

## RESULTS

### SEED SIZE

Seeds are larger in *C. spinosa* subsp. *spinosa* (mean area 9.5 mm<sup>2</sup>) than in *C. spinosa* subsp. *rupestris*

(6.8 mm<sup>2</sup>). Within *C. subsp. rupestris*, seeds from the Oued Zayied and Ghar el Melh populations were smaller (5.9 and 6.0 mm<sup>2</sup>) than those from Ghomrassen and Houmana (7.6 and 8.4 mm<sup>2</sup>). The *C. subsp. spinosa* populations did not differ in seed size (Tab. 2).

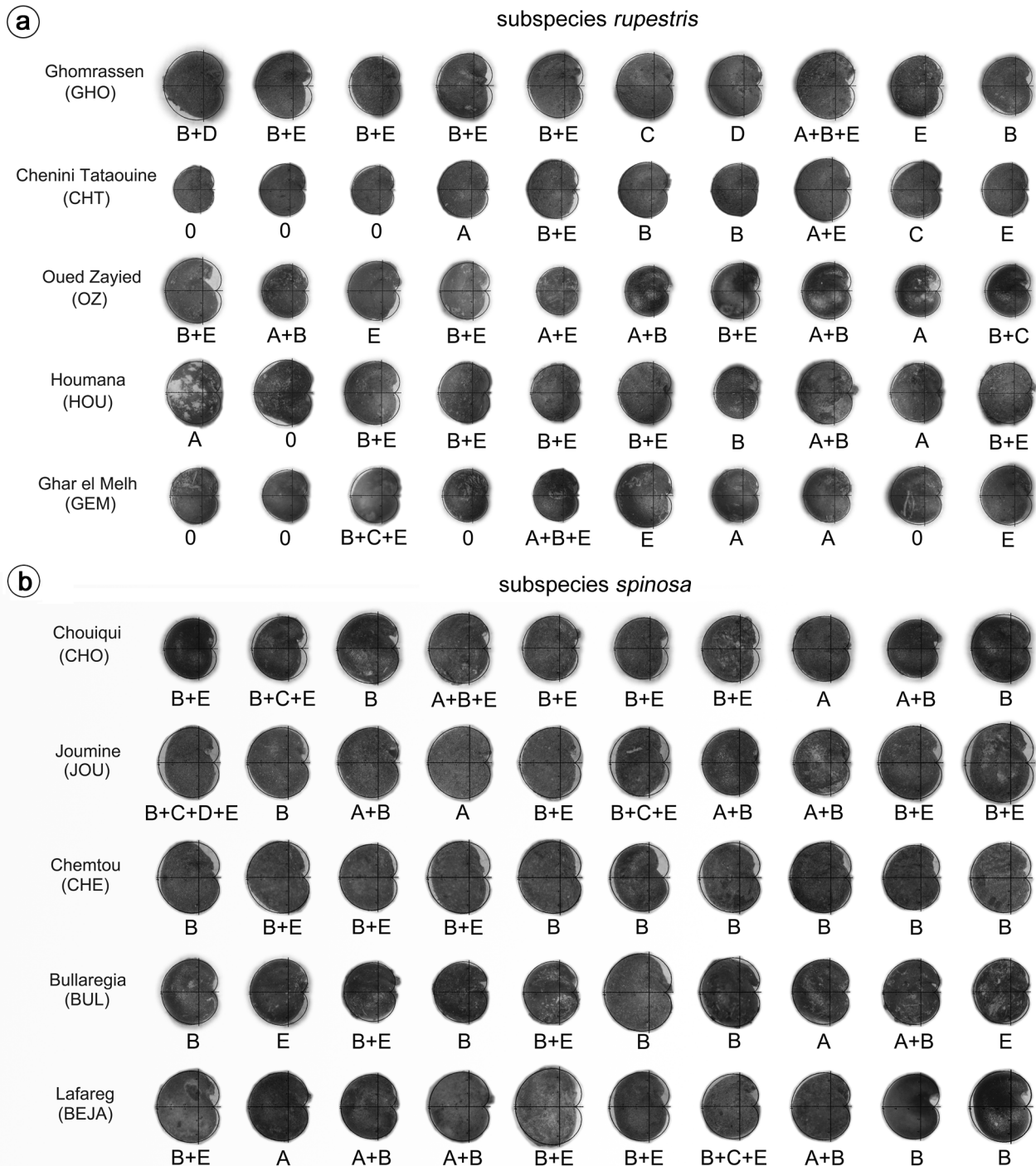
### SEED SHAPE

The J index was calculated for 10 seeds of each population (50 seeds per subspecies, total 100 seeds). The J index gives the degree of similarity of the seed image with a cardioid curve and has a maximum value of 100. In this work the J index ranged between 83.5 and 97.9 (mean 92.8), and did not differ between subspecies or populations (mean 92.5 for *C. subsp. spinosa*, 93.0 for *C. subsp. rupestris*; see Tab. 2). Seeds with a J index over 95 were designated Type A.

Of the 100 seeds analyzed, 28 were type A (Fig. 3, Tab. 3b). The only population not containing type A seeds was Chemtou (*C. spinosa* subsp. *spinosa*). The division of the seed images into four quadrants yielded four data per seed, corresponding to the efficiency of cardioid adjustment in each of the four quadrants (Q1 to Q4; Fig. 2). Morphological analysis was then focused on each of the four sections.

Values JQ1 to JQ4 represent the percentage of similarity of the seed image to the cardioid in the respective quadrant (Fig. 2, Tab. 2). Types B, C, D and E describe seeds with respective JQ1, JQ2, JQ3 and JQ4 values lower than 90.

Similarity with the cardioid curve was lower in the quadrants on the right side of the images above



**Fig. 3.** Seeds of *Capparis spinosa* subsp. *rupestris* (a) and subsp. *spinosa* (b) from five different locations in Tunisia.

and below the micropyle (JQ1 mean 84.2, JQ4 mean 87.9) and more variable (JQ1 SD 9.2, JQ4 SD 8.2) than in the quadrants opposed to the micropyle on the left side of the images (JQ3 mean 95.5, SD 3.2; JQ4 mean 95.6, SD 3.1). Morphological variation concentrated in the half of the seed that con-

tains the micropyle (right side of images); in consequence, more seeds belong to types B and E than to C or D (Figs. 3, 4, Tab. 3B).

A comparison of the JQ1 values reveals differences between subspecies: mean JQ1 was 86.8 for *C. subsp. rupestris* and 81.7 for *C. subsp. spinosa*;

TABLE 3. Distribution of morphotype combinations in the two *C. spinosa* subspecies (A). Total number of seeds per morphological type (A, B, C, D, E). For details see Materials and Methods

A)														
	A (J index > 95)					No A (J index < 95)								
	A	AB	ABE	AE	0	B	BC	BD	BE	BCE	BCDE	C	D	E
<i>rupestris</i>	7	4	2	2	8	4	1	1	13	1	0	1	1	5
<i>spinosa</i>	4	8	1	0	0	16	0	0	15	3	1	0	0	2
Total	11	12	3	2	8	20	1	1	28	4	1	1	1	7

B)						
	Tot. A	Tot. B	Tot. C	Tot. D	Tot. E	Nor B neither E
<i>rupestris</i>	15	26	3	2	23	17
<i>spinosa</i>	13	44	4	1	22	4
Total	28	70	7	3	45	21

TABLE 4. Number of morphotype combinations in the two *Capparis spinosa* subspecies

subspecies <i>rupestris</i>					
CHT	HOU	GEM	OZ	GHO	Mean
7	5	5	6	7	6
subspecies <i>spinosa</i>					
CHO	JOU	CHE	BUL	LAF	Mean
6	6	2	5	5	4.8

in agreement with this result, seeds of type B were more frequent in *C. subsp. spinosa* (Tab. 3B). JQ2, JQ3 and JQ4 did not differ between subspecies. Of the 33 possible combinations of types only 14 were found (Tab. 3A), six of which were found exclusively in *C. subsp. rupestris* (AE, BC, BD, C, D, 0) and only one of which (BCDE) was exclusive to *C. subsp. spinosa*.

Eight seeds in three populations of *C. subsp. rupestris* (CHT, HOU, GEM) could not be assigned to any of the five described seed types and were classified as type 0 (Tab. 3). In all of them the adjustment to a cardioid (J index) was below 95 but the partial similarity to the cardioid curve in each of the four quadrants was above 90. This morphotype was found only in seeds of *C. subsp. rupestris*. Variation of seed shape was higher in the populations of *C. spinosa subsp. rupestris* than in *C. spinosa subsp. spinosa* (Tab. 4). The number of morphotypes ranged from 5 to 7 in the former (mean 6), and from 2 to 6 in the latter (mean 4.8).

ANOVA with JQ1 values revealed population differences, with seeds from Chemtou having smaller values (71.5) than the other populations. The differences in mean JQ1 values between Chemtou and Joumine: (80.3) or Oued Zayied: (82.9) were not significant: Chemtou and Joumine belong to *C. subsp. spinosa*. ANOVA of Q3 revealed differences between

Ghomrassen (92.9) and Chouiqui (97.33). The Ghomrassen population is *C. subsp. rupestris* and the Chouiqui population is *C. subsp. spinosa*. JQ2 and JQ4 did not differ between populations.

All seeds of Chemtou correspond to type B (Fig. 3). This result, together with the lower Q1 values, indicates morphological differences for this population which may deserve further attention.

## DISCUSSION

Our seed size results confirm and expand those of Saadaoui et al. (2011) who, reported differences between subspecies of *Capparis spinosa*. *C. spinosa subsp. spinosa*, restricted to the north, has larger seeds, and *C. spinosa subsp. rupestris*, established throughout the Tunisian territory including the southern deserts, has smaller seeds. In his study of Sicilian populations of this species, Fici (2001) suggested 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. In support of this hypothesis, *C. subsp. rupestris* has several characteristics of a plant with an "r"-type strategy (Begon et al., 2005): small seeds, simple structure (trailing, thornless), larger number of stamens and self-reproduction. *C. subsp. spinosa* may have diverged from the "r" strategy towards more specialized adaptations: larger seeds, more complex structure (erect and thorny), reduced number of stamens and cross-reproduction (Saadaoui, 2012).

Morphological description of seeds is a step required for analysis of biodiversity in natural populations as well as an important aspect of genetic, taxonomic and biodiversity studies. Modern methods of scanning technology and image analysis algorithms (Herridge et al., 2011; Tanabata et al., 2012) allow rapid measurement of seed dimensions in a large

number of samples, but from a biological point of view quantitative methods may be more informative when considering seed shape, that is, their similarity to geometrical figures (Keefe and Davis, 2010).

Recently seed shape was described in the model plant *Arabidopsis* and in the model legumes *Lotus japonicus* and *Medicago truncatula*, based on their similarity to a cardioid curve (Cervantes et al., 2010, 2012). In ecological terms the model plants belong to the "r" strategy (Begon et al., 2005), having short life cycles, production of large amounts of seeds, and simple morphological features. We used a geometric model based on similarity to a cardioid curve to describe *Capparis spinosa* seeds collected from ten different field locations throughout Tunisia. The J index gives the percentage of similarity between images of the seeds and a cardioid curve. It exceeded 95 in 28 of the 100 seeds analyzed. We termed these seeds type A (Figs. 2, 3). Thus the adjustment of *C. spinosa* seeds to a cardioid curve is appropriate and should prove useful for analyzing and classifying seeds from diverse provenances and for studying the variation of seed shape. Only one of the populations (CHE) did not yield seeds of type A. This population is also unique in that the two subspecies, *spinosa* and *rupestris*, coexist, resulting in frequent hybrids (Saadaoui, 2012).

The analysis of quadrants identified those on the micropylar side of the seed (Q1, Q4) as the regions of maximum variation. Those opposite the micropyle (Q2, Q3) showed lower variation (Tab. 2). Independent analysis of similarity in the four quadrants (J index for quadrants, JQ) allowed us to define four additional seed types (B, C, D, E) as those in which similarity of the seed to the cardioid is below 90% in a particular quadrant (see Materials and Methods; Figs. 2, 3, Tab.2). Seeds of types B, C, D and E showed less than 90% similarity to the cardioid in their first (Q1), second (Q2), third (Q3) and fourth (Q4) quadrants respectively. Analysis of Q1 revealed differences between subspecies. Mean JQ1 values were higher in *C. subsp. rupestris* than in *spinosa*.

In *C. subsp. rupestris*, 26 of its 50 seeds were type B, whereas 44 of the 50 *C. subsp. spinosa* seeds were type B. Five basic morphotypes were described (A, B, C, D, E); a seed could belong to any combination of morphotypes, from a total  $25 = 32$  theoretical possibilities. Of these, only 14 combinations were observed (Tab. 4), 6 of which are exclusive to *C. subsp. rupestris* and one exclusive to *C. subsp. spinosa*. Thus, both seed size (see first paragraph of Results) and seed shape are more diverse in *C. subsp. rupestris*. Of particular interest is the combination called 0, for seeds that do not adjust well to a cardioid (J index <95) but do not have major differences from the cardioid in any of the four quadrants (Q1 to Q4 >90). This morphotype is

absent in *C. subsp. spinosa* and is present in three populations of *C. subsp. rupestris* (Chenini Tataouine, Houmana, Ghar el Melh). This morphotype, together with the higher morphological diversity of *C. subsp. rupestris* and the higher presence of type B seeds in *C. subsp. spinosa* (Tab. 3), seems in agreement with Fici's (2001) suggestion of primitiveness in seeds of *C. subsp. rupestris*, possibly associated with the nonspecialization characteristic of an "r"-type strategy.

Our results show differences in morphology between subspecies. The higher JQ1 values in *C. subsp. rupestris* than in *C. subsp. spinosa* support the assertion that *C. spinosa* subsp. *rupestris* has characteristics of an "r"-type strategy: *Arabidopsis* and the model legumes *Lotus japonicus* and *Medicago truncatula* are typical examples of an "r"-type strategy and their seeds bear remarkable similarity to the cardioid model. There also were differences in JQ1 values between populations, with Chemtou having lower values than most of the other populations. The differences in JQ3 may be attributed to populations or to a combination of subspecies and geographical location, as they were found between Ghomrassen (*rupestris*, south) and Chouiqui (*spinosa*, north). Analysis of such population variation requires a larger number of seeds.

The variation of JQ2 and JQ4 is not attributable to subspecies, nor to populations, and may be associated with developmental aspects related with growth in the pods, environmental or harvest/storage conditions of the seed, though a more detailed analysis might reveal population differences.

The two populations in the south, Ghomrassen and Chenini Tataouine, are geographically isolated from the eight other populations by a large salt lake called Chott Jerid. These two populations have specific characteristics: the presence of hairs and a large number of stamens (Saadaoui et al., 2011), but they do not have a characteristic seed shape. This result should be considered together with Ghorbel et al.'s (2001) RAPD molecular analysis which showed that the genetic effect is more responsible for the polymorphism of *C. spinosa* in Tunisia than the geographical effect.

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