

Morpho-functional adaptations in *Cakile maritima* Scop. subsp. *maritima*: comparison of two different morphological types

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Abstract — Along the Italian maritime foredunes there are two different morphotypes of *Cakile maritima* Scop. subsp. *maritima* differing in leaf shape: one type is characterized by entire lamina, while the other one shows pinnatifid leaves. Therefore, this study was performed to analyze leaf morpho-functional characters of both *C. maritima* morphotypes in order to highlight the peculiar adaptations to the ecological conditions of sand dune environments and to compare the two different morphological types.

The morpho-functional traits analyzed are leaf roll, general morphology of epidermis, vesicular cells, salt crystals, stomatal apparatus, stomatal density (SD), hydathodes, mesophyll, idioblasts, specific leaf area (SLA) and leaf dry matter content (LDMC). The most interesting anatomical adaptations to sand dune habitats are thick epidermal cuticle; high stomatal density; epidermal vesicular cells involved in water storage, sunlight reflexion and together with hydathodes in salt elimination; high development of palisade and water storage parenchyma; bundle sheath typical of C_4 leaf anatomy. Differences in several leaf characters between the two morphotypes seem to suggest that pinnatifid morphotype is more resistant to stress and environmental disturbance than the entire type. The analysis of *C. maritima* distribution in two different coastal traits affected by progradation or erosional processes confirmed this hypothesis.

Key words: *Cakile maritima* Scop. subsp. *maritima*, leaf anatomy, leaf dry matter content (LDMC), morpho-functional adaptations, psammophyte, specific leaf area (SLA), stomatal density (SD).

INTRODUCTION

Sea rocket, *Cakile maritima* Scop. (Brassicaceae), is a halophyte widely distributed in sandy coasts throughout the world (CLAUSING *et al.* 2000; KADEREIT *et al.* 2005). This species is confined to maritime strandlines, where the plant may play a role in trapping blown sand, thus initiating foredune formation and dune succession, especially on prograding coasts.

C. maritima is naturally salt-tolerant plant that displays potential for economical (oilseed), nutrient food and therapeutic (chemotherapeutic drugs) utilization (ZARROUK *et al.* 2003; CASAL

and CASAL 2004; KSOURI *et al.* 2007). Like other Brassicaceae, the high lipid content of seeds may be useful for biofuels production (EL BASSAM 1998; BALDONI and GIARDINI 2001; PAPINI *et al.* 2010).

In Europe, *Cakile maritima* is divisible into at least four subspecies: ssp. *integrifolia* (Hornem.) Hyl. ex Greuter & Burdet (= ssp. *maritima*) distributed along the coasts of west and north-west of Europe from Portugal to Norway; ssp. *baltica* (Jordan ex Rouy & Fouc.) Hylander ex P.W. Ball, of the Baltic and south-east Norway; ssp. *aegyptiaca* (Willd.) Nyman, distributed along the Mediterranean; and ssp. *euxina* (Pobed.) E.I. Nyárády, of the Black Sea (BALL 1964; DAVY *et al.* 2006). Characters of the fruit appear to be the most reliable for distinguishing the subspecies. Leaf shape frequently shows marked plasticity within individual plants and phenotypic variation between populations. There is a morpho-

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logical cline from types with entire leaves in the north of Europe, to types with pinnatifid leaves in the south and east of Europe (BALL 1964; DAVY *et al.* 2006).

In Italy, all the plants belonging to *Cakile maritima* were referred to the ssp. *maritima* (CONTI *et al.* 2005). Evidence from plants grown in a common environment has confirmed the presence of two different morphotypes with different leaf shape (entire or pinnatifid lamina).

Although extensive research has been conducted on *Cakile maritima* focusing the attention on ecophysiological mechanisms at cellular and molecular level (PAKEMAN and LEE 1991a-b; DEBEZ *et al.* 2004; AMOR *et al.* 2006; KSOURI *et al.* 2007; GRATANI *et al.* 2009), very few studies have analyzed the morphology and anatomy of this plant (WRIGHT 1927; BALL 1964; DAVY *et al.* 2006). Moreover, *Cakile maritima* exists as often ephemeral populations that occupy sensitive or fragile habitats in dunes and beaches; where the most important environmental stresses are salt spray, low nutrient and water availability, high temperatures, substrate instability and sandblasting (ROZEMA *et al.* 1985; HESP 1991; MAUN 1997).

In this paper, we focused our attention on the two different leaf morphotypes of *Cakile maritima* analyzing leaf morphological traits such as leaf roll, general morphology of epidermis, stomatal apparatus, stomatal density, mesophyll features, hydathodes, crystals, idioblasts and functional traits such as specific leaf area and leaf dry matter content. Our main objective was to verify the existence of any differences between the two morphotypes focusing the attention on the morpho-anatomical adaptations of the plant to the environmental conditions of coastal sand dune systems. An analysis of distribution of the two morphotypes was conducted in two differ-

ent sites characterized by different processes of progradation/erosion of the shoreline to test which type was more affected by the dynamical processes of the coastline.

MATERIALS AND METHODS

Study site - The Migliarino-San Rossore-Massaciuccoli Regional Park is 240 km² wide and is located at the seaside near Pisa in the northern part of Tuscany (Italy). The Park (latitude, 43.6-43.9° N; longitude, 10.2-10.5° E; altitude, 5 m a.s.l.) consists of a coastal forest of about 40 km², a sandy shore and inland marshes (Fig. 1). This area is characterized by a C2 type of climate, Mediterranean sub-humid (THORNTHWAITE 1948; RAPETTI 2003), with an annual average temperature > 15°C. Average temperature is 8.3°C in the coldest month (January) and 23.4°C during the warmest month (July). Annual rainfall average is 800 mm, 37.9% of which falls in autumn, 26.1% in winter, 22.4% in the spring and only 13.6% during the summer. In October and November average rainfall is 120-130 mm. July is the driest month with an average rainfall of 20 mm.

Material - Samples of two morphotypes of *Cakile maritima* Scop. subsp. *maritima* with different leaf shape (entire or pinnatifid lamina) were collected from coastal sand dunes of Migliarino-San Rossore-Massaciuccoli Regional Park between April and July 2009. *Exsiccata* are in PI.

Light microscopy (LM) - In order to study leaf anatomy, sections of leaves were cut at 3 µm with a Leica 2055 microtome after fixing the fresh material in FAA (10% formaldehyde, 5% acetic acid and 45% ethanol; SASS 1958) and embedding it in LR White acrylic resin (SIGMA). The

TABLE 1 — Morpho-anatomical traits of the two different morphotypes of *Cakile maritima*

	Pinnatifid Leaf (PL)	Entire Leaf (EL)
Leaf roll	dorsi-ventral	absent
Epidermis	thick-walled thick cuticle	thick-walled thick cuticle
Vesicular cells	large and very turned outward	small and slightly turned outward
Salt crystals	present	present
Stomata	abaxial & adaxial	abaxial & adaxial
Starch	present	present
Essential oils in stomata	abundant	few
Hydathodes	present	present
Water-storage parenchyma	present	present
Idioblasts	present	present

sections underwent the following histochemical tests: Toluidine Blue (TBO) as a generic stain and PAS for non-cellulosic polysaccharides (O'BRIEN and McCULLY 1981).

Epidermal strippings of both adaxial and abaxial surfaces of fresh leaves were made to determine stomatal density (SD), which was expressed as the number of stomata per square millimeter leaf area (RADOGLU and JARVIS 1990). For each morphotype of *Cakile*, 20 leaves randomly collected from 10 individuals were analyzed. Counts were made on both surfaces of the leaves for a total of 40 counts per morphotype. For calculating mean and standard error, the average stomatal density for each individual plant was one statistical observation.

In order to analyze epidermal cell features, some strippings underwent the following histochemical tests: Sudan Black B in saturated solution of ethanol 70% (BAYLISS and ADAMS 1972) to detect the presence of lipids; Nadi Reagent (DAVID and CARDE 1964) for essential oils and oleoresins; Lugol solution (potassium iodide) for starch (GAHAN 1984). For image acquisition a Leica DC 300F camera was used.

Scanning electron microscopy (SEM) - Leaves of both morphotypes were fixed in glutaraldehyde (2% in phosphate buffer solution at pH 7.4), dehydrated in a gradient of an alcohol and acetone mixture, critical point dried, mounted with a double adhesive tape on stubs, and sputter-coated with gold. Samples were examined at 10 KV with a JEOL SSM-5410 scanning electron microscope.

Specific leaf area (SLA) and Leaf dry matter content (LDMC) - Specific leaf area is the one-

sided area of a fresh leaf divided by its oven-dry mass, expressed in mm^2/mg . Leaf dry matter content is the oven-dry mass of a leaf divided by its water-saturated fresh mass, expressed in mg/g . For each morphotype we collected 20 leaves from 10 different fully illuminated individuals. Sample storing and processing followed the handbook of CORNELISSEN *et al.* (2003). Leaf projected area was acquired with a CanoScan LiDE 90 (Canon) and determined by CompuEye, Leaf & Symptom Area software (<http://www.ehabsoft.com/CompuEye/LeafSArea/>). For calculating mean and standard error, the average SLA and LDMC for each individual plant was one statistical observation.

Analysis of distribution - Distribution of the two morphotypes of *Cakile maritima* (entire or pinnatifid) was analyzed by a stratified random design in two different sites within the Migliarino-San Rossore-Massaciuccoli Regional Park: Gombo, which is characterized by a strong process of erosion, and Marina di Vecchiano, whose littoral is prograding (Fig. 1). Each sampling station, whose length was 1 km, was divided into ten parcels of 100 m. We selected randomly 5 parcels in each station. In each parcel we randomly placed 5 plots of 2 x 2 m, where percent cover of each morphotype present was recorded, for a total of 50 plots. These analyses were conducted between April and July 2009.

Statistical analysis - Differences in stomatal density (SD), specific leaf area (SLA), leaf dry matter content (LDMC) and distribution of the two morphotypes of *Cakile maritima* were determined by one-way analysis of variance (ANOVA). Bartlett's test was applied to the data to

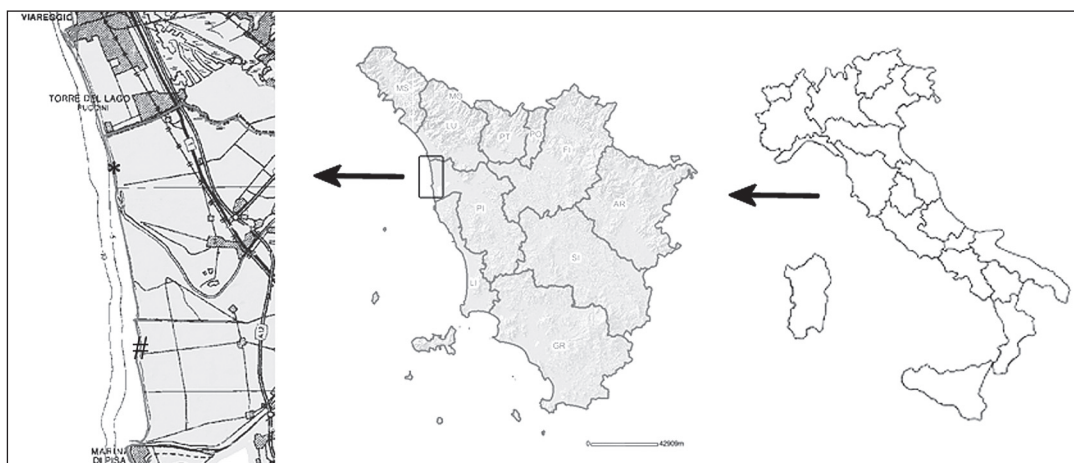


Fig. 1 — Position of Migliarino, San Rossore, Massaciuccoli Regional Park along the seaside of Tuscany. In the detailed map sites affected by progradation (*) and erosion (#) are reported.

verify the homogeneity of variances. When the variances were not homogeneous, we compared the data using the nonparametric test of Kruskal-Wallis. All statistical tests were performed using the software R 2.8.0 (copyright © 2008 The R Foundation for Statistical Computing; <http://www.r-project.org/>).

RESULTS

Leaf anatomy - Analyses of leaf morphology and anatomy were made on the two different morphotypes of *Cakile maritima*: type with entire leaves (EL) and type with pinnatifid leaves (PL) (Fig. 2). The morpho-anatomical characters as leaf roll, general morphology of epidermis, vesicular cells, salt crystals, stomatal apparatus, hydathodes, mesophyll and idioblasts were analyzed focusing the attention on the adaptations to ecological conditions of coastal sand dune systems (Tab. 1).

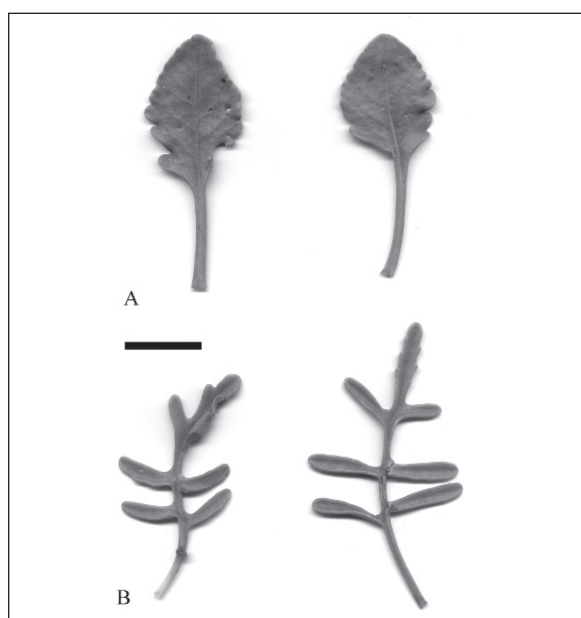


Fig. 2 — Foliar morphology of the two different morphotypes of *Cakile maritima*. - A: entire leaves. - B: pinnatifid leaves (bar = 1 cm).

Plants with entire leaves showed a flat lamina, on the other hand plants with pinnatifid leaves had a dorsi-ventral rolled lamina.

Leaves of both morphotypes were succulent and showed uniseriate epidermis. Epidermal cells were not regular in size, thick-walled with the presence of a thick layer of cuticle that stained with TBO and PAS (Fig. 3A-B). Within the epidermis of both surfaces there were large vesicular cells dispersed among epidermal cells and arranged in groups to delimit fields of stomatal areas (Fig. 3B). Vesicular cells were bigger about 4-5 times than other epidermal cells and considerably projected outwards. This character was more evident in PL than in EL (Fig. 4A). Moreover, vesicular cells were characterized by the presence of salt crystals localized in wall pores especially on the upper and lower sides (Fig. 3B-C).

Stomata occurred on both adaxial and abaxial surfaces of the leaves of both morphotypes (amphistomatic leaves). They were localized within areas of epidermis that appeared depressed because surrounded by the prominent vesicular cells (Fig. 4A). Stomata were formed by two kidney-shaped guard cells that had ledges of wall material on the upper and lower sides and several chloroplasts in the cytoplasm (Fig. 3E, 4B1). They were typically Cruciferous, with three subsidiary cells of which one was smaller than the other two. Moreover, stomata showed wide sub-stomatal chambers (Fig. 3D). Histochemical tests revealed the presence of primary starch granules and inclusions of essential oils in the guard cells of stomata. Essential oils were more abundant in pinnatifid morphotype than in entire one (Fig. 5A-D).

Both morphotypes showed several hydathodes along leaf margins. Each hydathode consisted of many stomatal apertures and a large sub-stomatal chamber which was in contact with the epithem, a little mass of thin-walled cells located between the epidermis and the bundle of tracheids (Fig. 3F, 4B2).

Regarding mesophyll, both entire and pinnatifid leaves showed a subepidermal layer of

TABLE 2 — Mean value and standard error (SE) of stomatal density (SD) of the two different morphotypes of *Cakile maritima* (PL = pinnatifid leaf, EL = entire leaf)

	PL (mean value per mm ² ± SE)	EL (mean value per mm ² ± SE)
Adaxial surface	76.7 ± 8.1	62.3 ± 2.9
Abaxial surface	72.5 ± 8.3	58.8 ± 2.9

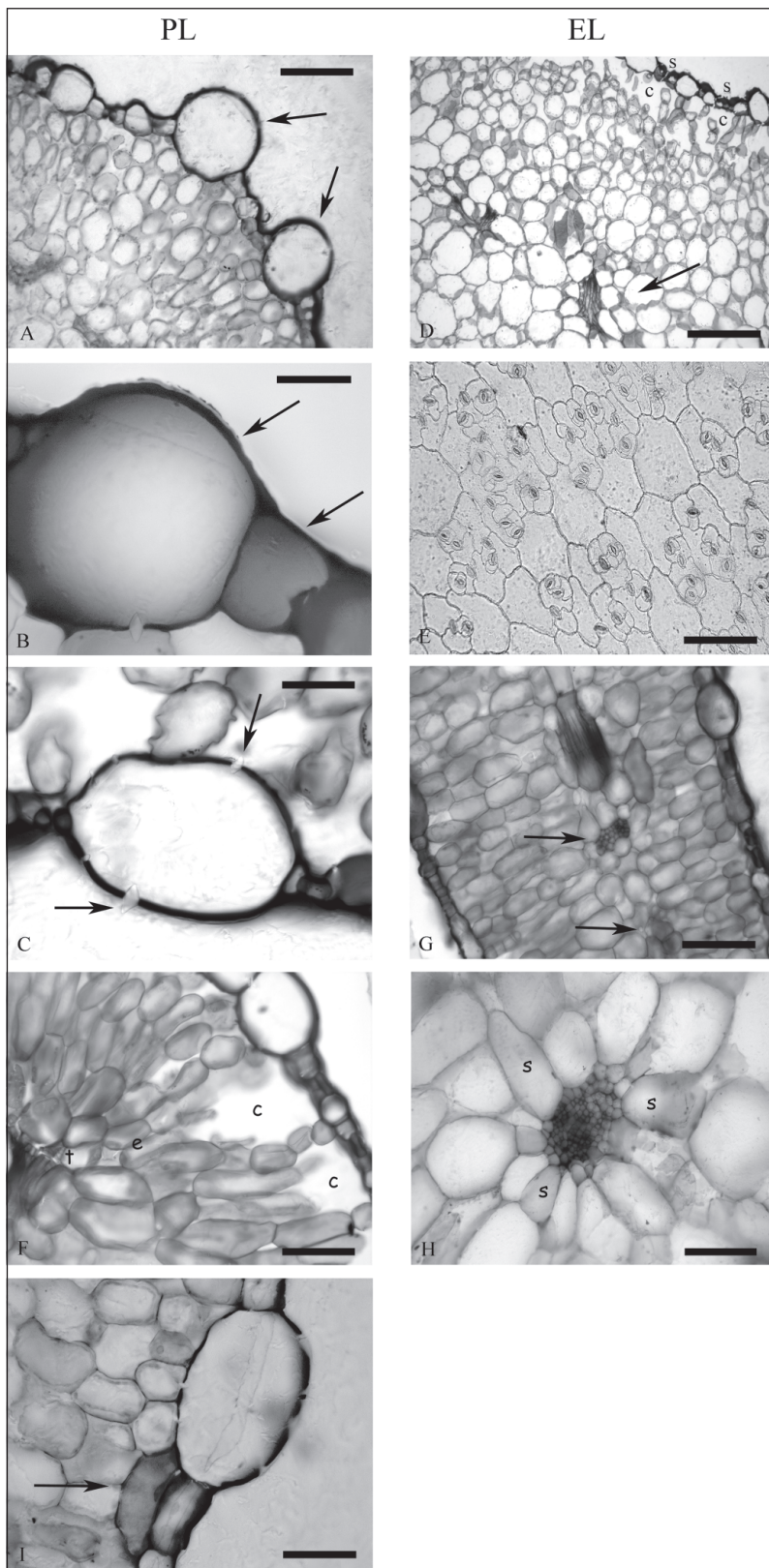


Fig. 3 — Foliar anatomy of *Cakile maritima* (PL = morphotype with pinnatifid leaves; EL = morphological type with entire leaves). (A) leaf of pinnatifid morphotype showing uniseriate epidermis and large prominent vesicular cells (arrows) (TBO) (bar = 38.5 μ m). (B) particular of epidermis where both a vesicular cell and other epidermal cells show a thick outer wall and a thick cuticle (arrows) that stained intensely with PAS-TBO (bar = 9.6 μ m). (C) vesicular cell of a pinnatifid leaf characterized by the presence of salt crystals (arrows) localized in wall pores on the upper and lower sides (PAS – TBO) (bar = 19.2 μ m). (D) water-storage parenchyma in entire leaf, with intercellular spaces, located in the middle layers of the mesophyll close to the central vein (arrow). Two stomata (s) with substomatal chambers (c) are evident (TBO) (bar = 65.2 μ m). (E) epidermal stripping of leaf abaxial surface of an entire morphotype showing stomata with three subsidiary cells one of which is smaller than the other two (bar: 38.5 μ m). (F) hydathode on pinnatifid leaf consisting of stomata and large sub-stomatal chambers (c) in contact with a little epithem (e), a mass of thin-walled parenchymal cells located between the epidermis and the bundle of tracheids (t) (PAS- TBO) (bar = 23 μ m). (G) mesophyll of an entire leaf showing palisade parenchyma on both sides and parallel arrangement of some vascular bundles (arrows) immersed in the water-storage parenchyma (TBO) (bar = 42 μ m). (H) particular of a vascular bundle surrounded by a bundle sheath (s) (TBO) (bar = 14 μ m). (I) idioblast (arrow) localized in a sub-epidermal position close to a vesicular cell (TBO) (bar = 13 μ m).

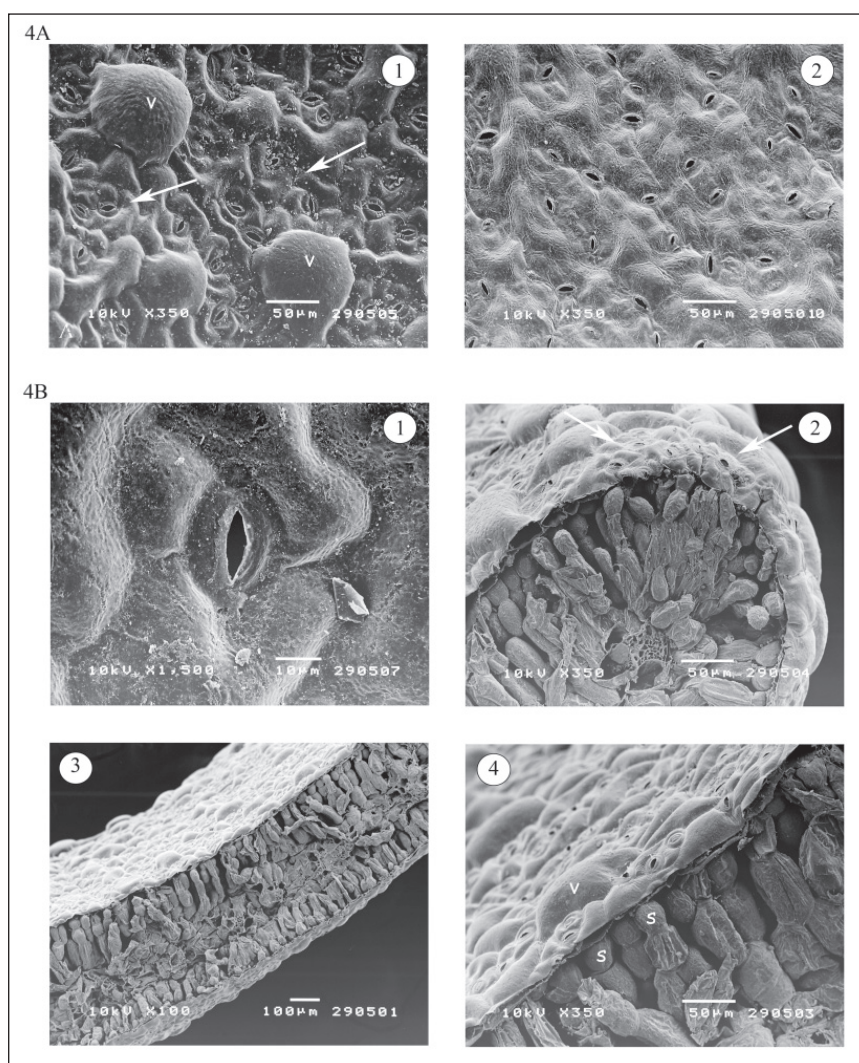


Fig. 4 — Scanning electron microscopy images of the leaves of *Cakile maritima*. — (4A): comparison of epidermal characters between the two different morphotypes. 1: dorsal epidermis of pinnatifid leaf showing stomatal areas (arrows) that appear depressed because surrounded by prominent vesicular cells (v). 2: dorsal epidermis of entire leaf showing the vesicular cells less prominent than pinnatifid leaves. — (4B): morpho-anatomical characters shared by the two different morphological types. 1: particular of stomata on the dorsal epiderm of a pinnatifid leaf: two kidney-shaped guard cells are evident. 2: hydatode with many stomatal apertures (arrows). 3: mesophyll of a pinnatifid leaf with a subepidermal layer of small cells, tightly adherent to vesicular cells and an underlying layer of palisade parenchyma on both sides. 4: particular of the subepidermal small cells (s) tightly adherent to the vesicular cells (v).

small cells, tightly adherent to vesicular cells and an underlying layer of palisade parenchyma on both leaf sides (Fig. 3G, 4B3-4). A typical spongy parenchyma was absent, while it was present a water-storage parenchyma made of living cells particularly large in size and with a thin wall. It was not observed a clear passage from the palisade tissue to the aquifer tissue, but a gradient of increasing size cells from the external to the internal side of mesophyll, associated with a gradual reduction in the number of chloroplasts per

cell. The water-storage parenchyma, located in the middle layers of the mesophyll, was close to the central vein and showed intercellular spaces (Fig. 3G). In addition, in the middle part of the mesophyll there were several vascular bundles surrounded by a bundle sheath (Fig. 3H).

Salt crystals were also observed in some mesophyll cells, especially close to the vascular bundles. Similarly to epidermis, they were localized in wall pores; but they were smaller than those present in vesicular epidermal cells.

Moreover, the mesophyll of both morphotypes was characterized by the presence of idioblasts. They were specialized large cells, usually thick-walled and without chlorophyll, with inclusions intensely staining with TBO. Idioblasts were generally associated with the vascular bundles or localized in a sub-epidermal position, close to the vesicular cells (Fig. 3I).

Stomatal density (SD), Specific leaf area (SLA) and Leaf dry matter content (LDMC) - Measurements of the stomatal density (SD) of both adaxial and abaxial surfaces of *Cakile maritima* leaves of both morphotypes (pinnatifid and entire leaves) are shown in Table 2.

The morphotype with pinnatifid leaves (PL) showed an average number of stomata of 76.7 on the upper (adaxial) surface of the leaves, and 72.5 on the lower (abaxial) surface of the leaves (Tab. 2). Statistical analysis showed no significant differences between the SD values of the two pinnatifid leaf surfaces ($F_{1,18}=0.13$, $P=0.72$).

The morphotype with entire leaves (EL) had a stomatal density of 62.3 on the upper (adaxial) surface, and 58.8 for the lower (abaxial) surface (Tab. 2). Similarly to PL morphotype, statistical analysis showed no significant differences between the SD values of the two entire leaf surfaces ($F_{1,18}=0.75$, $P=0.39$).

Comparing the adaxial and abaxial surfaces of the leaves of both morphotypes, we did not find any significant differences between the SD values ($F_{1,18}=2.82$, $P=0.11$ for upper surfaces; $F_{1,18}=2.47$, $P=0.13$ for lower surfaces; Tab. 2).

Finally, the mean value of stomatal density for each morphological type was calculated as the mean of the abaxial and adaxial values ($SD=74.6\pm 8.0$ for PL and $SD=72.5\pm 8.3$ for EL). Obviously, no significant differences between the SD values of the two different morphotypes were recorded ($F_{1,18}=2.73$, $P=0.12$; Tab. 2).

Moreover, PL morphotype showed a value of SLA of 16.3 mm²/mg and 42.2 mg/g for LDMC, while EL morphotype had a value of SLA of 24.1 mm²/mg and 38.8 mg/g for LDMC (Tab. 3). ANOVA detected significant differences in SLA between the two different morphotypes

of *Cakile maritima* ($F_{1,18}=8.82$, $P=0.008$), but there were no significant differences in LDMC ($F_{1,18}=0.37$, $P=0.55$).

Distribution of the two morphotypes - Percentage cover of both morphotypes of *Cakile maritima* in the two different sites (Gombo and Marina di Vecchiano) are shown in Table 4.

The morphotype with pinnatifid leaves (PL) showed an average cover of 7.6% at Gombo, and 0.63% at Marina di Vecchiano. On the other hand, the morphotype with entire leaves (EL) had an average cover of 3.2% at Gombo, and 0.93% at Marina di Vecchiano (Tab. 4).

Comparing the distribution of both morphotypes, we did not find any significant differences between the percentage cover within each site ($P=0.27$ at Gombo; $P=0.42$ at Marina di Vecchiano). While PL morphotype showed a significant difference between percentage cover values at Gombo and Marina di Vecchiano ($P=0.02$), the EL morphotype had no significant differences ($P=0.35$). Obviously, a significant difference was present between the total percentage cover of *Cakile maritima* between the two sampling stations ($P=0.02$; Tab. 4)

DISCUSSION

The most interesting leaf morpho-anatomical adaptations of *Cakile maritima* to the ecological conditions of coastal sand dune systems seem to be leaf shape, leaf roll, general morphology of epidermis, vesicular cells, salt crystals, stomatal apparatus, hydathodes, mesophyll and idioblasts.

Plants with entire leaves showed a flat lamina; while pinnatifid leaves were dorsio-ventral rolled, a morphology that occurs rarely in plants. The dorsio-ventral rolling of the adaxial surface of the leaf has also been seen in *Otanthus maritimus* (L.) Hoffmanns. & Link (CICCARELLI *et al.* 2009), another psammophyte that lives on foredunes. This unusual feature of the leaf surface could be interpreted as an adaptation to reduce transpiration and water loss creating a sort of stomatal crypt.

Regarding the general morphology of the epi-

TABLE 3 — Mean value and standard error (SE) of specific leaf area (SLA) and leaf dry matter content (LDMC) of the two different morphotypes of *Cakile maritima* (PL = pinnatifid leaf, EL = entire leaf)

	PL (mean value ± SE)	EL (mean value ± SE)
SLA (mm ² /mg)	16.3 ± 1.3	24.1 ± 2.3
LDMC (mg/g)	42.2 ± 4.1	38.8 ± 3.7

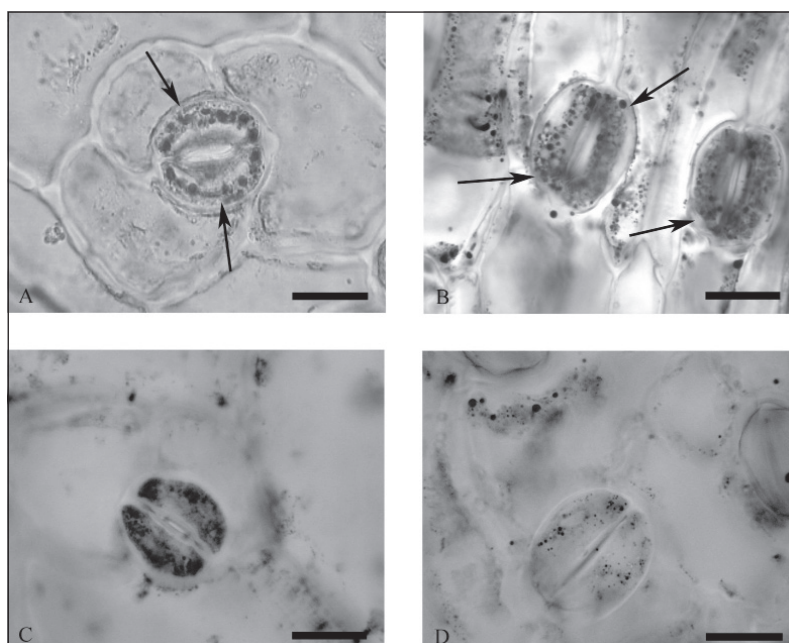


Fig. 5 — Histochemical tests on epidermal stripping of *Cakile maritima* leaves. (A) Lugol solution revealed the presence of primary starch granules (arrows) in the guard cells of stoma of a pinnatifid leaf (bar = 6.5 μm). (B) guard cells of stomata in pinnatifid leaf showing lipid inclusions (arrows, bar = 6.5 μm). (C-D) inclusions of essential oils in the guard cells of stomata, essential oils are more abundant in pinnatifid morphotype (C) than in entire one (D) (bar = 2.9 μm).

dermis of *C. maritima*, we found similar characters showed for other psammophytes present in the same habitats (CICCARELLI *et al.* 2009): one layer of thick-walled epidermal cells, large in size and with a thick cuticle. Obviously, these features are linked to the reduction of transpiration and water loss, even if the typical xerophyte leaf traits are small flat epidermal cells organized in several layers (METCALFE and CHALK 1983). Moreover, the epidermis of both leaf surfaces in both morphological types of *C. maritima* was characterized by the presence of large vesicular cells considerably projected outwards that were arranged in group delimiting fields of stomatal areas. These cells are similar to the epidermal bladder cells (EBCs) found in *Mesembryanthemum crystallinum* L. (AGAIRE *et al.* 2007); they may play a protective role for the stomata from excessive transpiration reflecting the incident sunlight. As the EBCs the vesicular cells may represent an important site of accumulation of organic and inorganic compounds such as flavonoids, betacyanine, chlorine and sodium (AGAIRE *et al.* 2007). In addition, the epidermal vesicular cells of *Cakile* appear to be directly involved in the elimination of salts, as suggested by the presence of salt crystals localized in wall pores especially on the upper and lower sides.

Comparing the two different morphotypes, we found that pinnatifid leaves had epidermal vesicular cells larger and more turned outward than entire leaves. Therefore, it is possible to assume that the benefits brought by the presence of these large cells (partial reflection of light, protection from solar radiation, reserve of water, organic and inorganic compounds, and removal of salts) are more consistent in the morphotype PL than in the morphotype EL.

Both morphotypes of *C. maritima* showed amphistomatic leaves. The presence of stomata on both leaf adaxial and abaxial surfaces of several psammophytes could be interpreted as an adaptation to sand burial, which is a frequent event in maritime sand dune ecosystems (CICCARELLI *et al.* 2009). Moreover, it has been demonstrated that coastal dune species have a high instantaneous water use efficiency (WUE, amount of CO_2 fixed per unit of water lost) as a result of high assimilation rates rather than low transpiration rates (RIPLEY and PAMMENTER 2004).

Histochemical tests revealed the presence of primary starch granules and inclusions of essential oils in the guard cells of stomata. The concentration of starch in guard cells may be related with the closure of stomata leading to a reduction in transpiration (WILLIAM *et al.* 1979); while it is

well known that some terpenoids (the main constituents of essential oils) have a protective function against thermal stress avoiding the denaturation of membranes exposed to high temperature (HARREWIJN *et al.* 2002). Pinnatifid morphotype showed a more abundant presence of essential oils in the guard cells than the entire type.

Along the margins of *C. maritima* leaves we found several hydathodes, which are structures secreting solutions of mineral salts brought to the surface by the terminal tracheids of the veins. Hydathodes of *C. maritima* are similar to those found in *Otanthus maritimus* leaves (CICCARELLI *et al.* 2009): they consisted of many stomatal apertures and an epithem formed of only a few cells, architecture slightly different from the typical hydathodes. It seems that hydathodes prevent an excessive accumulation of minerals within the plant body of the psammophytes.

Leaves of *C. maritima* can be classified as dorsiventral ones, the palisade parenchyma was present on both sides of the leaf and vascular bundles were parallel. *Cakile* showed vascular bundles surrounded by a bundle sheath resembling “Kranz anatomy” characteristic of C4 photosynthetic pathway. This bundle features have also been found in *Calystegia soldanella*, *Euphorbia paralias* and *Otanthus maritimus* (CICCARELLI *et al.* 2009), suggesting an hypothetical changing between C3 and C4 pathways in response to environmental drought, as it was reported by ELHAAK (1999) who studied the behaviour of water-stressed plants.

Another morpho-anatomical adaptation to drought was water-storage parenchyma found in the leaf mesophyll of *C. maritima*. This tissue was made of cells that had a large vacuole with watery or somewhat mucilaginous content. It has been suggested that the ability of mucilage to hydrate may offer a mechanism for plants to resist drought (CLARKE *et al.* 1979). More recently, CLIFFORD and collaborators (2002) hypothesized that the main role of mucilages and glucans, together with foliar starch, may be as significant sources of monosaccharide sugars contributing

to osmotic adjustment in the short-term, and to remobilization of solutes to other sink organs as drought intensified.

In some cells of the mesophyll of *C. maritima*, mainly around the vascular bundles, salt crystals were observed. Similarly to epidermis, they were localized in wall pores; but they were smaller than those present in vesicular epidermal cells. These crystals are unique feature of *C. maritima* and appears to be correlated with the ability of the plants to withstand salt stress. Further investigations are necessary to better understand the scientific value of the presence of these crystals.

Finally, the mesophyll of both morphotypes was also characterized by the presence of idioblasts. Idioblasts are specialized cells that contain myrosin, the enzyme responsible for hydrolysis of glucosinolates. Sixteen different glucosinolates has been identified in *C. maritima*, whose role is a chemical defense against pathogens and predators (DAVY *et al.* 2006).

Statistical analyses of the stomatal density of both adaxial and abaxial surfaces of *C. maritima* leaves of both morphotypes (pinnatifid and entire leaves) did not show any significance differences (Tab. 2). These results are not in accordance with DAVY and collaborators (2006) who found more stomata on the abaxial (96 mm²) than on the adaxial surface (64 mm²) of *C. maritima* leaves collected in British Isles. Plants are able to use both short-term control of stomatal aperture and long-term control of stomatal development and morphology to adapt to changes in the environment. Crucially, it is the prevailing environmental conditions that drive these long-term changes in development (CASSON and HETHERINGTON 2010). It has been demonstrated that increased light quantity positively influences changes in stomatal numbers (SCHOCH *et al.* 1980). Moreover, some reports indicate that growth at elevated temperatures results in reduced stomatal densities and stomatal index (BEERLING and CHALONER 1993; LUOMALA *et al.* 2005). These findings could explain the differences among *C. maritima* from Italy and plants

TABLE 4 — Mean value and standard error (SE) of percentage cover of the two different morphological types of *Cakile maritima* at Gombo and Marina di Vecchiano (PL = pinnatifid leaf, EL = entire leaf)

	PL (mean value ± SE)	EL (mean value ± SE)	PL + EL (mean value ± SE)
Gombo	7.6 ± 3.3	3.2 ± 2.1	10.8 ± 5.0
Marina di Vecchiano	0.63 ± 0.2	0.93 ± 0.3	1.6 ± 0.3

from UK and justify the lower stomatal density values for the Italian specimens that live in environment characterized by higher irradiances and temperature.

Interestingly, ANOVA detected significant differences in SLA between the two different morphotypes of *C. maritima*: PL morphotype, in fact, showed a value of SLA ($16.3 \pm 1.3 \text{ mm}^2/\text{mg}$) lower than EL morphotype ($24.1 \pm 2.3 \text{ mm}^2/\text{mg}$). Our results are comparable with those obtained by GRATANI *et al.* (2009), who studied functional and physiological characters of several psammophytes. From a morphological point of view, they analyzed LMA (leaf mass per unit leaf area calculated as the ratio of leaf dry mass and one-sided leaf area, that is the inverse of SLA) and SI (succulence index calculated as the ratio of the difference between dry mass and fresh mass to the leaf surface area). These authors showed that *Cakile maritima* collected along the Latium coast had the highest SI ($96.4 \pm 0.9 \text{ mg}/\text{cm}^2$) and a low LMA ($8.4 \pm 0.4 \text{ mg}/\text{cm}^2$). Species with low LMA (i.e. high SLA) tend to have a higher photosynthetic capacity per unit leaf mass, resulting from a larger light-capture area per leaf mass (Wright *et al.* 2004), and a shorter diffusion path from stomata to chloroplasts (PARKHUST 1994). Moreover, species in resource-rich environments tend to have larger SLA than those in environments with resource stress. Lower values of SLA, in fact, tend to correspond with relatively high investments in leaf "defences" (particularly structural ones) and long leaf lifespan (CORNELISSEN *et al.* 2003).

Comparing the two different morphological types of *C. maritima* from a morphological, anatomical and functional point of view, it seems that PL morphotype should be more resistant to stress and environmental disturbance, strategy also confirmed by distributional data (Tab. 4). Pinnatifid plants, in fact, are more abundant in the trait of the littoral that is subjected to erosional processes and is, of course, more stressed.

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