

# Topography and nanosculpture of petals' surfaces of short-lived flowers of the wild species *Cistus creticus*, *Cistus salviifolius*, *Eruca sativa* and *Sinapis arvensis*

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**ABSTRACT.** The adaxial and the abaxial petal surface of short-lived flowers of the successively blossoming species *Sinapis arvensis*, *Eruca sativa*, *Cistus creticus* and *Cistus salviifolius* were examined using light microscopy, scanning electron microscopy and atomic force microscopy. The topography of petals revealed a submicron relief that is expected to influence the visual appearance and the wettability of floral tissues. Adaxial, papillate epidermal cells of petals and mesophylls consistent of loosely arranged cells and large intercellular spaces produce conditions of coordinated light trapping areas, affecting the light use efficiency and the likelihood of changing optical properties of the tissues. Visualization of the petals' epidermises using an atomic force microscope revealed a microrelief that increases the cell surface area of the epidermal cells and this may be a well adapted mechanism to a short floral span. Distinct striations on the petal surfaces of *Sinapis arvensis* and *Eruca sativa* may strengthen the delicate tissues and influence the adhesive contacts, during a three-day floral span. Smooth petal surfaces of ephemeral flowers of *Cistus creticus* and *Cistus salviifolius* may show strong reflections. High resolution imaging shows that roughening of the adaxial surface of petals is higher than that of the abaxial surface, in all the above mentioned species. Traits of micromorphology of the epidermal surface of short-lived petals may be particularly important for the performance of flowers of wild species grown under ambient conditions.

**Keywords:** Adaxial; Abaxial; Flower; Folding; Microsulpture; Petal; Repellent; Surface; Wild species.

## INTRODUCTION

Epidermal cells and cuticular surface of petals are related to the capture of incident light and serve as interfaces between the tissues and their environment (Pfündel et al., 2006; Bhushan, 2009; Koch and Barthlott, 2009). The multifunctional cuticle has attracted the attention of several plant disciplines (e.g. taxonomy, morphology, physiology, biochemistry and evolution), due to a wide range of traits reasonably constant for each species (Olowokudejo, 1993; Barthlott et al., 1998; Mill and Stark Schilling, 2009).

It has been argued that the relief, frequently observed on the surfaces of petals minimizes water loss across the epidermis, it protects the tissues (against physical, chemical and biological attack) and influences their optical properties (Gorton and Vogelmann, 1996; Whitney and Glover, 2007; Zhang et al., 2008; Whitney et al., 2009). Also, it reduces the absorbance of ultraviolet radiation that reaches the cells and it forms favourable sculptures for in-

sect pollinators to walk on petals (Kevan and Lane, 1985; Petanidou and Lambron, 2005; Kerstiens, 2006; Jacobs et al., 2007). The cuticular boundary layer combines many aspects attributed to smart materials (Benítez et al., 2004; Derdej and Koch, 2007) and the way it has evolved seems to be well suited to playing many different roles at a time (Kerstiens, 1996). Hence, major processes contributing to the subtleties of floral life span are related to hydrophobic properties of the petal surfaces (Wagner et al., 2005; Feng et al., 2008; Whitney et al., 2011). The cuticle –consisting of cutin, polysaccharide micro fibrils and waxes– responds to both intrinsic and extrinsic factors in the course of tissues' development (Martens, 1936; Lolle and Pruitt, 1999) and our best understanding comes from cultivated plant species grown under controlled conditions (Li-Beisson et al., 2009).

The aim of this study was to identify structural and functional features of short-lived flower petals of wild species, blossoming in the field, early in the spring. Epidermal cells of petals can influence a diverse set of properties and the life span of floral tissues (Nieto Feliner and Aedo, 1995; Davies and Turner, 2004). We studied the abaxial and the adaxial petal surface of four successively flower-

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ing species, consistent floristic elements of the Mediterranean landscape. Light microscope, scanning electron microscope (SEM) and atomic force microscope (AFM) were used to study the topography of the adaxial and the abaxial petal surface. Scientific work has demonstrated the suitability of SEM and AFM, for observations of structural traits in leaves (Mechaber et al., 1996; Koch et al., 2004; Solga et al., 2007; Agrawal et al., 2009) and petals (Kay et al., 1981; Gale and Owens, 1983; Kaplan, 2008; Whitney et al., 2009; Argiropoulos and Rhizopoulou, 2012). High-resolution imaging using AFM reveals hierarchical micropapillae and striated nanosculptures that increase the size of surface area of petal epidermises, and influence optical and adhesive properties of the delicate tissues (Miller et al., 2011; Rands et al., 2011; Argiropoulos and Rhizopoulou, 2012; Chimona et al., 2012). To the best of our knowledge, structural and functional properties of the petals' surfaces of the examined species (cited alphabetically) *Cistus creticus*, *Cistus salviifolius*, *Eruca sativa*, and *Sinapis arvensis*, using high resolution imaging at the nanometer scale, which may greatly expand our understanding about the microsculpture of the delicate tissues, have not been hitherto reported.

## MATERIALS AND METHODS

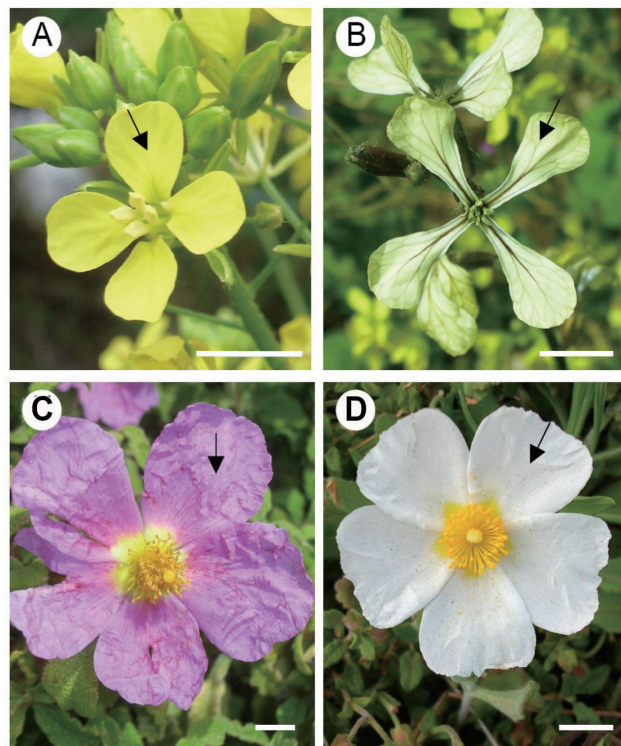
### Plant material

The study was carried out at the Campus of the University of Athens in Greece (38° 57' N, 23° 48' E, altitude 250 m). Expanded, turgid flowers were harvested from four plant species that grow in an open field and are presented here according to the succession of their flowering period: A) *Sinapis arvensis* L., Cruciferae (Figure 1A). B) *Eruca sativa* (Miller) Thell., Cruciferae (Figure 1B). C) *Cistus creticus* L. (*C. incanus* subsp. *creticus*), Cistaceae (Figure 1C). D) *Cistus salviifolius* L., Cistaceae (Figure 1D). Flowering was observed on a regular basis, every day during the blossoming period, of the above mentioned species. Flowers of *S. arvensis* and *E. sativa* exhibit a three-day life span, while those of *C. creticus* and *C. salviifolius* are ephemeral, by exhibiting one-day floral span. Sampling was made at the end of March 2009 and 2010. The above mentioned species begin to bloom in the end of February, when some appearance of spring is seen; their flowering period coincides with a monthly precipitation that varies from 70 mm (February) to 45 mm (March), while the average monthly temperature varies between 10°C and 15°C, respectively.

### Microscopy

The study was carried out in developed petal regions (Figure 1). Samples from the petal blade were carefully cut in square pieces (4 mm<sup>2</sup>) and fixed in 3% glutaraldehyde in Na-phosphate buffer at pH 7, at room temperature, for 2 h. Plant material was washed three times by immersion in buffer for 30 min each time; then, it was post fixed in 1% OsO<sub>4</sub> in the same buffer at 4°C and dehydrated in acetone

solutions. Dehydrated tissues were embedded in SPURR (Serva) resin. Semi-thin sections of resin-embedded tissue (LKB Ultratome III microtome) were stained in Toluidine Blue '0', in 1% borax solution, photographed and digitally recorded using a Zeiss Axioplan light microscope (Carl Zeiss Inc., Thornwood, N.Y.) equipped with a digital camera (Zeiss AxioCam MRc5). Dehydrated samples were dried at the critical point in a Bal-tec CPD-030 dryer, mounted with double adhesive tape on stubs, sputter coated with 20 nm gold in a Bal-tec SCP-050. The adaxial and abaxial epidermises of petals were viewed using the scanning electron microscope JEOL JSM 840 (JEOL Ltd, Tokyo, Japan). Also, the adaxial and the abaxial petal areas (25 μm<sup>2</sup>) were imaged by using a tap mapping atomic force microscope (Multimode SPM; Veeco, Santa Barbara, CA, USA). Several parameters were analysed and processed, using the software package Nanoscope III (Veeco, USA), in order to detect detailed information for the surfaces of petals. The quantitative measurements include surface roughness (Ra) of the tissues, horizontal and vertical distances that represented the height of a step between nanofolds, and the length between the markers that represented the surface distance. The surface area ratio (Sr), representing the density of microfolding, was the percentage of the three-dimensional surface, compared to the projected flat surface area, on the threshold plane. Angles (°) between a straight line connecting the cursors and the horizontal surface were measured on both petal surfaces



**Figure 1.** Flowers of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D); sampling regions of adaxial, fully expanded petal tissues are indicated by arrows. Scale bars: 1 cm.

and varied among the species and between adaxial and abaxial epidermis, i.e. being  $47^\circ$  and  $46^\circ$  respectively for *S. arvensis*,  $56^\circ$  and  $51^\circ$  respectively for *E. sativa*,  $10^\circ$  and  $5^\circ$  respectively for *C. creticus* and  $48^\circ$  and  $40^\circ$  respectively for *C. salviifolius*. Traits, obtained from nine different samples, are given in the representative micrographs (Figures 5, 6) and Table 2. Mean values in Tables 1, 2 are followed by standard errors ( $\pm$  S.E.).

### Statistical analysis

One-way ANOVA was used to analyse differences in traits of petals among species. Then, the data were analysed by Duncan's new multiple range test and the significant difference was defined at  $P \leq 0.05$ . Statistical analysis has been realized with the SPSS statistical program.

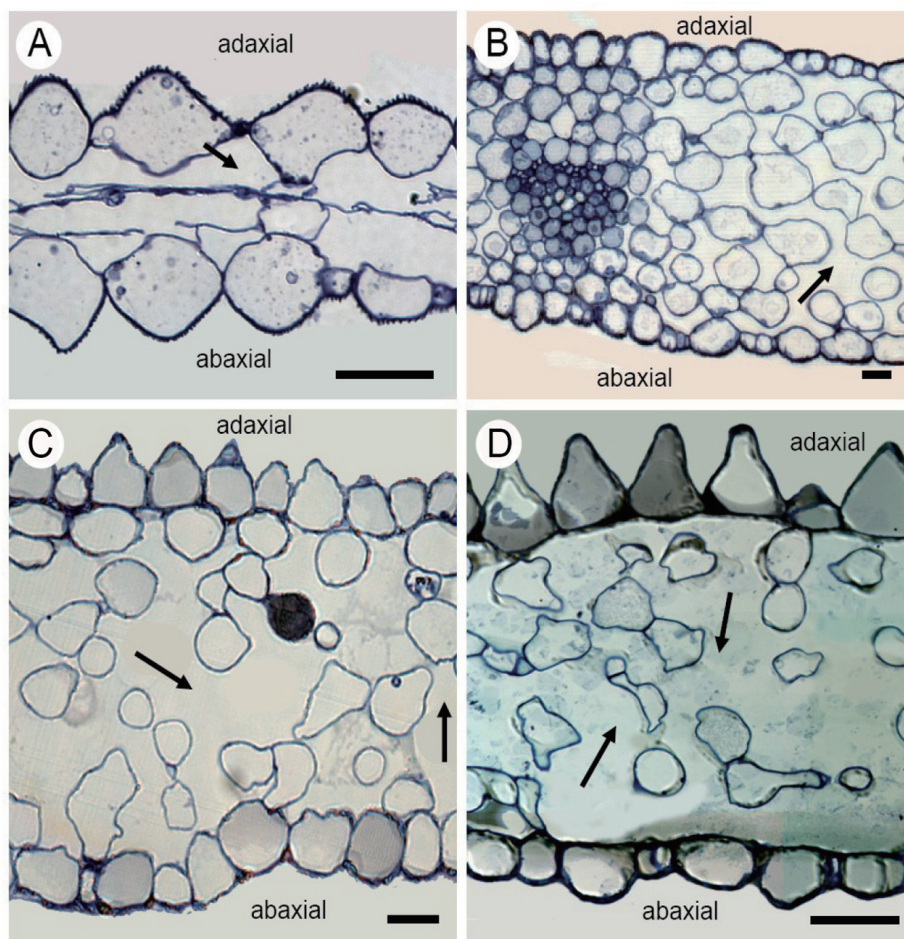
### RESULTS

Flower petals of *S. arvensis* (Figure 1A) possess a narrow mesophyll (Table 1) with a loosely arranged parenchyma between two epidermises (Figure 2A); the thickness of each of the adaxial and the abaxial epidermis is the same order of magnitude with that of the mesophyll.

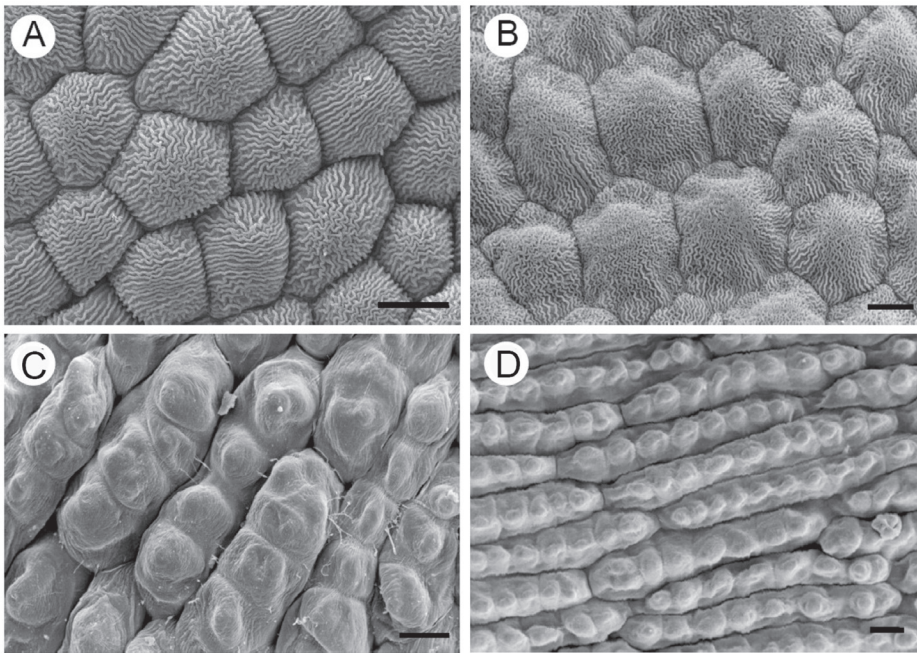
Petals of *E. sativa* (Figure 1B), *C. creticus* (Figure 1C) and *C. salviifolius* (Figure 1D) possess a large mesophyll, with loosely arranged cells and wide intercellular spaces (Figures 2B, 2C, 2D, respectively); the mesophyll thickness is three (*C. salviifolius*), four (*C. creticus*) and five (*E. sativa*) folds thicker, than that of either the adaxial or the

**Table 1.** Mean values of thickness of the mesophyll, the adaxial and the abaxial epidermis of petals of flowers of four successively blossoming species. Each value is the mean of nine measurements  $\pm$  S.E. Means followed by the same letters are not statistically different at  $P=0.05$ .

Species	Petals		
	Thickness		
	Mesophyll ( $\mu\text{m}$ )	Adaxial epidermis ( $\mu\text{m}$ )	Abaxial epidermis ( $\mu\text{m}$ )
<i>S. arvensis</i>	$15.50 \pm 0.45^a$	$19.26 \pm 0.28^a$	$15.75 \pm 0.51^a$
<i>E. sativa</i>	$152.50 \pm 6.50^b$	$25.50 \pm 0.45^a$	$19.40 \pm 0.30^a$
<i>C. creticus</i>	$113.50 \pm 9.50^c$	$21.50 \pm 0.35^a$	$20.50 \pm 0.55^a$
<i>C. salviifolius</i>	$64.00 \pm 4.00^d$	$21.20 \pm 0.20^a$	$11.25 \pm 0.18^c$



**Figure 2.** Transverse sections through the expanded region of petals of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D); intercellular spaces are indicated by arrows. Scale bars: 20  $\mu\text{m}$ .



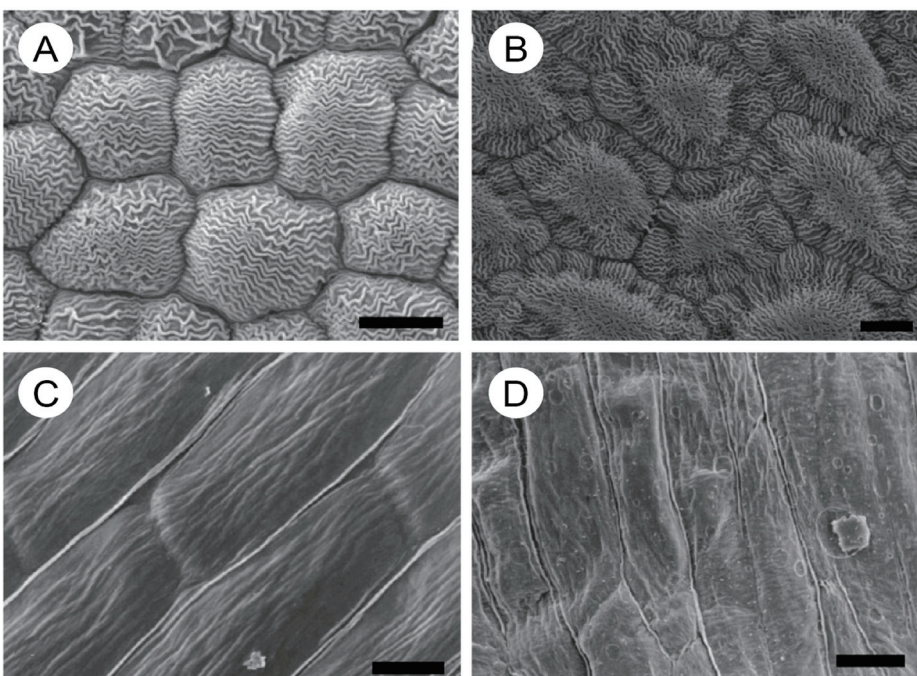
**Figure 3.** Scanning electron micrographs of adaxial, epidermal cells of petals of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D). Scale bars: 10  $\mu$ m.

abaxial epidermis of each of the above mentioned species (Table 1). The adaxial epidermis of the petals is composed of conical-papillate (Figures 2A, 2C, 2D) and lenticular (Figure 2B) cells, while the abaxial epidermis is composed of papillate (Figure 2A), rectangular (Figure 2B) and lenticular cells (Figures 2C, 2D). Adaxial and abaxial papillate cells of *S. arvensis* bear a row of papillate projections in the inner face (Figure 2A). Adaxial papillate cells of *C. creticus* and *C. salviifolius* exhibit relatively flat, lenticular projections on the inner face, facing the mesophyll (Figures 2C, 2D).

Adaxial (Figure 3A) and abaxial (Figure 4A) epidermal

cells of *S. arvensis* are covered by wavy striations. Also, polygonal, convexly shaped cells on the adaxial (Figure 3B) and the abaxial (Figure 4B) epidermises of petals of *E. sativa* are covered by densely packed, wavy striations. Cell wall tortuosities were detected on the basal area of the adaxial (Figure 3B) and the abaxial (Figure 4B) epidermal cells of petals of *E. sativa*.

The adaxial surfaces of petals of *C. creticus* and *C. salviifolius* show certain peculiarities in the shape of epidermal cells. Thus, adaxial epidermal cells of petals of *C. creticus* are elongated and four micro-papillae are arranged in a single row per cell (Figure 3C). Adaxial epidermal



**Figure 4.** Scanning electron micrographs of abaxial, epidermal cells of petals of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D). Scale bars: 10  $\mu$ m.

cells of petals of *C. salviifolius* are extremely elongated and seven to nine micro-papillae are arranged in a single row per cell (Figure 3D). Abaxial epidermal cells of *C. creticus* and *C. salviifolius* possess a flat and smooth surface (Figures 4C, 4D).

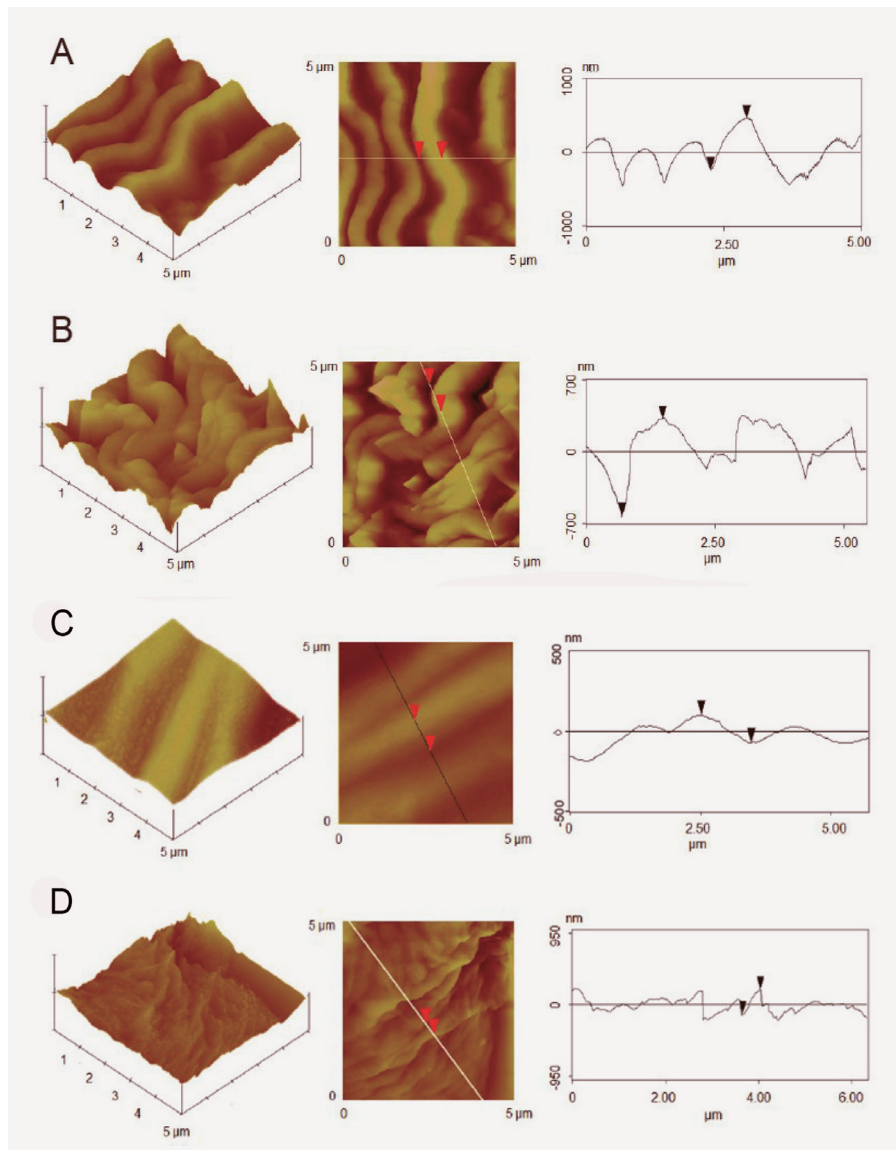
Petal surfaces of the plant species mentioned above possess a different nanosculpture, as indicated by projections in the shape of peaks and cavities which vary in height, density and arrangement (Figures 5, 6; Table 2). The adaxial (Figure 5A) and the abaxial (Figure 6A) surfaces of flower petals *S. arvensis* exhibit the highest roughness (Table 2), among the examined species. The adaxial and the abaxial surfaces of *E. sativa* (Figures 5B, 6B) possess a smaller roughness (Table 2), when compared to roughness of *S. arvensis*. Also, the density of forms on epidermal cells with striated surfaces (represented by values of surface area ratio) differs between the petals' surfaces of the above mentioned species (Table 2).

Micromorphology of the adaxial epidermal cells of ephemeral petals of *C. creticus* and *C. salviifolius* (Fig-

ures 3C, 3D) differs from that of the abaxial epidermal cells (Figures 4C, 4D); in *C. creticus*, the abaxial vertical distance is ten-fold smaller than the abaxial horizontal distance (Table 2). Vertical and horizontal distances on both the adaxial and the abaxial surfaces of the white petals of *C. salviifolius* (Figures 5D, 6D) indicate a quite parallel arrangement of folds (Table 2).

## DISCUSSION

The most prominent feature of the microsculpturing of petal's surface is the epidermal cell shape (Barthlott, 1981). Papillate, epidermal cells of the adaxial surface may absorb light over a greater part of petal surface (Pfundel et al., 2006; Glover, 2007). A papillae shape is expected to reduce reflectance and increase the proportion of incident light that enters the epidermal cells, enhancing light absorption by floral pigments and produce maximum brilliance (Noda et al., 1994; Lee, 2007). In the loosely arranged mesophyll of petals of the examined species



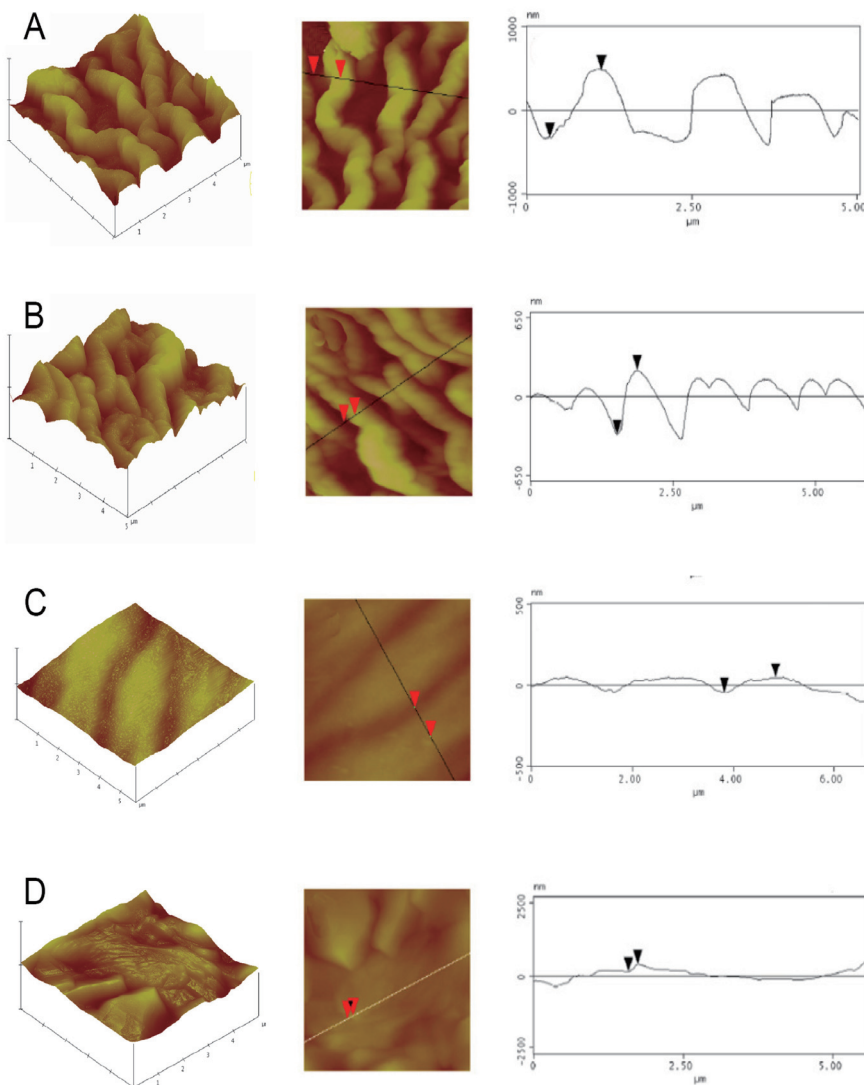
**Figure 5.** Atomic force micrographs of adaxial petal surface of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D): three dimensional profile (left), integrated line of measured points on plane profile (middle) and profile view of the line section (right).

(Figures 2B, 2C, 2D) numerous light reflecting interfaces may increase light scattering within the petal (Kay et al., 1981; Weston and Ryke, 1999; Rhizopoulou et al., 2006). Convexly shaped adaxial and abaxial epidermal cells refract light into focused areas and in combination with cell interiors that exhibit higher refractive indices than air, may act as condensing lenses, sustaining light intensities within petals via intercellular reflectance (Vogelmann, 1993; Lee, 2007).

The papillate inner face of the epidermal cell of *S. arvensis* will act as a light-trap both for light reflected from the mesophyll and for light transmitted from below, via the narrow mesophyll (Figure 2A). Papillate epidermal cells of petals of *S. arvensis* and *C. creticus* absorb light over the greater part of their exposed surface, acting as a light-trap for incident light and, in conjunction with the reflective mesophyll, light is guided through the pigments contained in the epidermal cells and returns to the exterior by a combination of external reflection and refraction (Kay et al., 1981).

It appears that papillate epidermal cells compensate for an increase in the cell surface area, by increasing the area of anticlinal undulations, as in *S. arvensis* and *E. sativa*, modifying the outline of the cells and contributing to the capture of light and wettability of petals (Barthlott, 1981; Gale and Owens, 1983; Whitney et al., 2011).

The longitudinally elongated, epidermal multiple-papillate cells of petals of *C. creticus* and *C. salviifolius* (Figures 3C, 3D) seem to be a characteristic of Cistaceae, which may aid the rapid petal expansion of flowers of *Cistus* species (Barthlott, 1981; Kay et al., 1981; Guzmán and Vargas, 2005). Papillate cells of the adaxial epidermises of *C. creticus* and *C. salviifolius* will absorb incident light (Figures 2C, 2D, 3C, 3D), while flat cells of the abaxial epidermises of *Cistus* species (Figures 2C, 2D, 4C, 4D) may reflect light waves at different angles. Also, surface microsculpture of epidermal cells (Figures 5C, 5D, 6C, 6D) affects the firmness of adhesive contact with droplets of water; thus, smooth surfaces of epidermal cells of *C. creticus* and *C. salviifolius* decline water retention on the



**Figure 6.** Atomic force micrographs of abaxial petal surface of *S. arvensis* (A), *E. sativa* (B), *C. creticus* (C) and *C. salviifolius* (D): three dimensional profile (left), integrated line of measured points on plane profile (middle) and profile view of the line section (right).

**Table 2.** Estimates of roughness on adaxial and abaxial petal surfaces of four successively blossoming species using atomic force microscopy; also, horizontal, vertical and surface distances representing dimensions between nanofolds, and surface area ratio representing density of nanofolds, are given. Mean values (nine samples  $\pm$  S.E.) followed by the same letters are not statistically different at  $P=0.05$ .

Species	Adaxial petal surface				
	Roughness (Ra) (nm)	Vertical distance (nm)	Horizontal distance (nm)	Surface distance (nm)	Surface area ratio (Sr)
<i>S. arvensis</i>	232 $\pm$ 2 <sup>a</sup>	705 $\pm$ 4 <sup>g</sup>	654 $\pm$ 3 <sup>g</sup>	1082 $\pm$ 5 <sup>k</sup>	2.44 $\pm$ 0.04 <sup>n</sup>
<i>E. sativa</i>	163 $\pm$ 3 <sup>b</sup>	944 $\pm$ 3 <sup>h</sup>	791 $\pm$ 2 <sup>g</sup>	1572 $\pm$ 9 <sup>l</sup>	2.16 $\pm$ 0.06 <sup>n</sup>
<i>C. creticus</i>	52 $\pm$ 4 <sup>c</sup>	171 $\pm$ 3 <sup>b</sup>	955 $\pm$ 6 <sup>h</sup>	980 $\pm$ 2 <sup>h</sup>	1.02 $\pm$ 0.02 <sup>o</sup>
<i>C. salviifolius</i>	92 $\pm$ 3 <sup>d</sup>	328 $\pm$ 1 <sup>i</sup>	381 $\pm$ 3 <sup>i</sup>	542 $\pm$ 4 <sup>j</sup>	1.58 $\pm$ 0.03 <sup>p</sup>
Species	Abaxial petal surface				
	Roughness (Ra) (nm)	Vertical distance (nm)	Horizontal distance (nm)	Surface distance (nm)	Surface area ratio (Sr)
<i>S. arvensis</i>	216 $\pm$ 3 <sup>a</sup>	806 $\pm$ 5 <sup>g</sup>	781 $\pm$ 3 <sup>g</sup>	1301 $\pm$ 3 <sup>l</sup>	2.06 $\pm$ 0.05 <sup>n</sup>
<i>E. sativa</i>	127 $\pm$ 3 <sup>e</sup>	527 $\pm$ 4 <sup>j</sup>	353 $\pm$ 1 <sup>i</sup>	699 $\pm$ 3 <sup>m</sup>	1.43 $\pm$ 0.06 <sup>p</sup>
<i>C. creticus</i>	29 $\pm$ 2 <sup>f</sup>	91 $\pm$ 2 <sup>d</sup>	1018 $\pm$ 7 <sup>k</sup>	1030 $\pm$ 6 <sup>k</sup>	1.01 $\pm$ 0.02 <sup>o</sup>
<i>C. salviifolius</i>	72 $\pm$ 6 <sup>c,d</sup>	156 $\pm$ 2 <sup>b</sup>	158 $\pm$ 3 <sup>b</sup>	343 $\pm$ 5 <sup>i</sup>	1.47 $\pm$ 0.04 <sup>p</sup>

delicate petals, during their short life-span (Argiropoulos and Rhizopoulou, 2012).

Striations densely arrayed towards the apex of the adaxial and the abaxial surfaces of *S. arvensis* (Figures 3A, 4A) and *E. sativa* (Figures 3B, 4B) support the delicate tissues with water-repellent properties (Wagner et al., 2003; Koch and Barthlott, 2009) and some extra strength (Gale and Owens, 1983) during their three-day life span. Similar wavy striations exist on the petal surfaces of *Asphodelus ramosus* (Rhizopoulou et al., 2008), which blossoms during the same period in Eastern Mediterranean. In some cases, striated patterns of petals (Figure 3B) may exhibit an illusive, vibratile movement, by possessing a figural intensity, which may help flower discrimination and attraction of pollinators (Dafni et al., 1997). This may be advantageous for *E. sativa*, because the striated, lenticular petal surfaces show weak reflectance (Kay et al., 1981).

Imaging of the relief using atomic force microscopy revealed detailed surface patterns, which may have a great influence on their attributes as interfaces (Glover, 2007; Zhang et al., 2008; Bhushan, 2009). It appears that microfolding, i.e. patterns of ridges and striations, increases cell surface area of the short lived petals and this may be particularly important for their performance in the field. A sculpturally increased surface area of light absorbing papillae cells of adaxial epidermises increases the energy exchange of petals with the surrounding environment and supports the warming of flowers at low ambient temperatures, in the early spring (Barthlott, 1981; McKee and Richards, 1998; Rands and Whitney, 2008).

The adaxial and the abaxial relief of *C. creticus* possess a horizontal distance that is 8-10 folds higher than the vertical distance. In *C. creticus*, vertical distances between folds are comparable to the sub-wavelength regime, i.e. being approximately 170 nm, and thus effective in reflecting radiation of shorter rather than longer wavelengths; if the distance between folds (grating period) is smaller than

the visible wavelength spectrum, then refraction, scattering and polarisation of light of wavelengths greater than the grating period may occur (Gröning, 2005). Traits of petals of *C. creticus* viewed with AFM appear to be capable of producing some structural colour effects (Glover and Whitney, 2010; Feng et al., 2010; Lee et al., 2011).

Adaxial and abaxial wrinkled petal surfaces of *C. creticus* are covered by a smooth relief (Figures 5C, 6C), which coincides with small roughness, low vertical distances and elevated horizontal distances (Table 2) that enhance the cell surface area of adaxial and abaxial petal epidermises, of the short lived, fuchsia flowers. Hence, the nanostructure of petals of *C. creticus* increases the reflectability of the ephemeral, wrinkled, fuchsia tissues (Figure 1C). It seems likely that ephemeral and delicate petals of *C. creticus* blowing in the wind are well adapted to the ambient conditions.

The surface area ratios, which represent the density of forms on the abaxial epidermis, as in *C. salviifolius* and *E. sativa*, are not statistically different (mean values: 1.47 and 1.43 respectively); however, the abaxial surface of *E. sativa* exhibits a significantly higher roughness than that of *C. salviifolius* (mean values: 126 nm and 73 nm respectively). Features of petal surfaces at the nanoscale level indicate adaptation to the environment mostly combined with non wetted tissues. Microsculpturing increases in size the area of the epidermal cells, which aids optical properties and assists the water status of petals (Herminghaus, 2000; Gröning, 2005; Argiropoulos and Rhizopoulou, 2012; Chimona et al., 2012). Conical-papillate cells have a significant impact on how water is retained on the petal surface (Whitney et al., 2011; Argiropoulos and Rhizopoulou, 2012). Adaxial petal surfaces with papillate cells and wavy striations, as in *S. arvensis*, might have developed to profit from sunshine and to not be harmed when exposed to unfavourable environmental conditions, during the early spring flowering period. Abaxial, flat epidermal surfaces of

petals of the examined species seem to be less susceptible to wet conditions. Connected to the reduced wettability appears to be a minimal ability of adhesion of pathogens and dust particles, which may be washed off by rain droplets. Also, surface microsculpture may be a tactile cue for insects to approach sites of rewards, while at the same time the delicate, ephemeral petals should remain unaffected (Bargel et al., 2006; Kutschera, 2008; Harder, 2009; Stelzer et al., 2010).

The topography of the adaxial and the abaxial petal surfaces of the examined species reveal traits linked to an astonishing performance of ephemeral corollas and functionality of boundary layers, which significantly influence the physical properties of petal tissues. In this context, the use of AFM improves the accuracy of “vision” related with biological structures and has greatly increased our knowledge about the function of floral tissues (Bhushan and Nosonovsky, 2010). Nanoridges, playing an important role in optical properties and adhesive contacts, differ among the examined species and between their adaxial and abaxial petal surfaces; roughness (Ra) and density of folds (Sr) of adaxial petal surfaces of the examined species were statistically different at  $P < 0.001$ , while Ra and Sr of the abaxial surfaces at  $P < 0.05$ . Previous studies have described flower petals possessing striations on both the adaxial and the abaxial epidermises and exhibiting a three-day life span, as in *Allium* species, *Ornithogalum umbellatum* and *Trifolium repens* (Kay et al., 1981; Petanidou et al., 1995). While, petals lacking striations expand on ephemeral and short-lived corollas, as in *Erodium cicutarium*, *Hypericum perforatum* and *Silene alba* (Kay et al., 1981; Petanidou et al., 1995). However, striations of petal surfaces and floral life span have never been hitherto correlated. It is noteworthy that texture of petals of *S. arvensis* and *E. sativa* with a greater life span exhibit higher surface roughness (Ra) and density of nanofolds (Sr), while the opposite holds true for ephemeral petals of *C. creticus* and *C. salviifolius*; Ra and Sr of petal surfaces of the examined species are positively correlated with flowers’ life span ( $r^2=0.73$  and  $r^2=0.58$ , respectively).

Adaxial surface distances of the examined petals -which are exposed to the ambient environmental conditions and seen by potential pollinators as they approach flowers-, differ from the abaxial surface distances. In addition, smooth surfaces composed of folding larger than the wavelength of the incident light, as in *S. arvensis*, reflect the light radiation, while, surfaces composed by gratings smaller than the wavelength spectrum, as in *C. creticus*, are more effective in reflecting radiation of shorter rather than longer wavelengths (Table 2). Different features of petal surfaces at the nanoscale level may be species specific and related with their lifespan and adaptations to climatic conditions, mostly combined with light absorption and wettability of tissues grown in the field. It is worth mentioning that submicron patterns of petals’ surfaces, mostly from roses, have already been transferred to biomimetic materials, throughout a rapidly growing and enormously promising field of research (Fratzl, 2007; Feng et al., 2010; Koch et

al., 2009; Stratakis et al., 2009; Qian et al., 2011; Zhang et al., 2012). Further investigation will be required to test these hypotheses in wild species.

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## 野生種 *Cistus creticus*, *Cistus salviifolius*, *Eruca sativa* 和 *Sinapis arvensis* 之短命花之花瓣表面的立體構造及聚合物微泡

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連續開花之野生種 *Sinapis arvensis*, *Eruca sativa*, *Cistus creticus* 和 *Cistus salviifolius* 的短命花之花瓣的向軸面及離軸面分別以光學顯微鏡，電子顯微鏡和原子力顯微鏡檢視。花瓣之立體構造顯示一種次微米之緩解結構，由此可預期其可視外形和花之組織的可溼性。花瓣和葉肉之向軸，乳突的表皮細胞。含鬆散地安排之細胞及大的細胞間隙；由此產生了共軛捕捉光的區域之環境，因此影像日光使用效率以及改變組織之光學性質的可能性。使用原子力顯微鏡檢視花瓣之表皮顯示出一種次微米緩解結構；此結構增加表皮細胞之細胞表面面積，此特徵很可能是短命花之演化適應機制。*Sinapis arvensis* 和 *Eruca sativa* 花瓣表面之不同分層帶可能加強組織之巧妙性和影響黏密的接觸，這些都發生在短命花之三天開發期。*Cistus creticus* 及 *Cistus salviifolius* 之短暫的花之光滑花瓣表面可能顯示強之反射作用。高解析圖像顯示在上述所有花種花瓣向軸面之粗糙化現象都比離軸面高。知命花瓣表面之微細構造之特性可能對生長在自然環境下之野生種的花的表現特別重要。

**關鍵詞：**向軸的；離軸的；花；摺；聚合物微泡；花瓣；反撥的；表面；野生種。