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ORIGINAL ARTICLE

# Comparative morphological and anatomical characters of *Cakile arabica* from different habitat in eastern region of Saudi Arabia



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**Abstract** Morphological, anatomical and physiological plasticity was examined for *Cakile arabica* from three different sites at the coastal part of the Arabian Gulf near Ad Dammam city in the eastern region of Saudi Arabia. Morphological investigation showed that the size and number of lobes of the leaves are increased in sites (I) which have high salt stress. Also anatomical investigation using a light microscope showed that the plant is adaptive for salt stress by increasing the thickening of the cuticle or epidermis layer and increase in the area of vascular bundles. Physiological studies showed that plant growing under high salt stress is characterized by increase content of electrical conductivity and increase in chlorophyll a, b, carotenoids and proline content in the plant tissues. This can be explained as an osmotic adjustment mechanism for the investigated species growing under high salinity stress.

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## 1. Introduction

The genus *Cakile* (family Brassicaceae) is an annual succulent halophyte widely distributed in sandy coasts throughout the world; it has seven species, only one species (*Cakile arabica*) recorded in Saudi Arabia (Mandaville, 1990).

Salinity is an environmental stress that limiting plant growth and productivity around the world. This problem is more severe in arid and semi-arid regions (Munns, 2002). Increasing salinity induces specific changes on cell, tissue and organ levels. These changes are physiological, morphological and anatomical (Shannon, 1997; Isla et al., 1998). It has been revealed from many studies that high salinity mostly causes anatomical alterations such as reduction of stomata number, leaf thickness, distance between vascular bundles and epidermis cell number (Shennan et al., 1987; Hwang and Chen, 1995; Kiliç et al., 2007; Çavuşoğlu et al., 2007; Vijayan et al., 2008; Çavuşoğlu et al., 2008). Other structural changes that occurred in salt stressed plants such as inhibition of differentiation, diameter and number of xylem vessels have also been recorded. The pigment system of the plants is very

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sensitive to the salt stress as it has been reported by many workers (Wu et al., 2010; Chookhampaeng, 2011; Ahmad et al., 2012).

Halophytes can grown in saline to extremely saline habitats and have particular characteristics which enable them to evade and/or tolerate salinity by various eco-physiological mechanisms. Anatomical features of different organs by halophytes are considered an adaptive response to habitat ecology of a certain species (Chrispeels and Sadava, 2003; Grigore and Toma, 2007; Ianovici et al., 2010; Ianovici, 2011a,b).

Anatomical features of different plant organs are considered an adaptive response to habitat ecology of a certain species (Grigore and Toma, 2007). Many studies have shown that plants are plastic for numerous ecologically important traits, ranging from morphology, physiology and anatomy, to developmental and reproductive timing, breeding system and offspring developmental patterns (Sultan, 1992; Heschel et al., 2004; Al-Taisan, 2010).

Very little research has been done on the morphological and anatomical plasticity for the species of *Cakile* as (Maun and Payne, 1989; Pakeman and Lee, 1991a,b; Boyd and Barbour, 1993; Daniela et al., 2010; Jianu et al., 2014).

The plasticity of *Cakile arabica* has not been done. The present study examined the different morphological, anatomical and some physiological characters of *C. arabica* from three different habitats.

## 2. Material and methods

The species of *Cakile arabica* Vel. et Bornm, was collected fresh from three different localities in the coastal part of the Arabian Gulf near Ad Dammam city in the eastern region of Saudi Arabi (Table 1). The materials studied were identified according to plant key of (Mandaville, 1990).

Foliar and floral details were examined with the aid of binocular stereo microscope under incident light and photographs. For anatomical investigation each specimen was fixed according to (Nassar and El-Sahhar, 1998) in F.A.A. (formalin – glacial acetic acid – 70% alcohol) with the ratio of 5:5:90 by volume. The stems and leaves (petiole & blade) were hand sectioned at 20–30  $\mu$ m in thickness; the stem taken from 2nd, 4th and 6th internodes. The sections were stained according to Dilcher (1974) in safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) then photographed.

### 2.1. Soil analysis

Soil samples were taken at three random points from each site as a profile (composite samples) at a depth of 0–30 cm close to naturally growing plants, by using a digging tool. Soil samples

were air-dried, and then passed through a 2 mm sieve. Textural analysis was performed using the hydrometer method (Day, 1965). Percentages of soil water content (SWC%) determined by evaluating weight loss after drying at 105 for 24 h (Wilde et al., 1979). The electrical conductivity (EC) of soil–water extract (1:5) expressed as (mmohs/cm) of soil was measured by a conductivity meter (Wilde et al., 1979). The soil acidity (pH) was determined in distilled water at soil solution ratio of 1:5 with a potentiometric glass electrode using KNICK pH meter according to (Al-Busaidi et al., 2005).

### 2.2. Plant analyses

Shoot fresh weight (FW) was measured soon after harvest while dry weight (DW) was determined after placing plant samples in a forced-draft oven at 60 °C for at least 48 h or until a constant weight was achieved. Leaf water content is the difference between FW and DW. Tissue water content percentage of plant shoots was determined as  $TWC(\%) = 100 \times (FW - DW)/DW$  according (AOAC, 2008).

### 2.3. Measurement of chlorophyll content

The following physiochemical measurements were determined in the fresh harvested shoot of the fourth cutting: chlorophyll a & b and carotenoid concentrations were extracted and estimated from known fresh leaves in 80% (v/v) aqueous acetone to suitable concentrations for spectrophotometric measurements using a spectrophotometer (UV-1800) according to the method used by, following the standard method of (Arnon, 1949). Values were expressed in  $\text{mg g}^{-1}$  F. wt.

### 2.4. Measurement of proline content

Free proline accumulation was determined using the method of (Bates et al., 1973). 0.5 gm fresh weight of leaf contents of proline was expressed as  $\mu\text{mol g}^{-1}$  F. wt.

## 3. Results and discussion

### 3.1. Physiological analysis

#### 3.1.1. Soil analysis

Data in (Table 2) indicated that, soil texture was sandy, loamy sand and sandy clay loam at the three sites II, I and III respectively. Soil moisture content (SWC%) ranged from 0.608% at site I to 0.22% at site III. Soil pH varied from moderate alkaline 7.01, at site III to high alkaline (8.99) at site I. Electrical conductivity (E.C.) ranged between 194.60 and 531.66 mmohs/cm.

**Table 1** The site and the date of the *Cakile arabica* collection.

Sites	Area	Habitat ecology	Date
Site (I)	Sehat area (about 70 m from the Gulf shore)	Coastal sands	12/2/2015
Site (II)	Rayan – Dammam (about 18 km to the south west of site I)	Deep sand sheets	12/2/2015
Site (III)	Dammam – Al Khobar road (about 21 km to the south east of site I)	Loose sandy soil	15/2/2015

**Table 2** Some physical and chemical properties of soil supporting *C. arabica* in the three investigated sites.

Characters site	Sand%	Silt%	Clay%	Texture	SWC%	pH	EC (m.mohs/cm)
(I)	88.60	1.40	10.00	Lomy sand	0.6081	8.99	531.66
(II)	93.54	3.38	3.07	Sandy	0.3015	7.56	203.33
(III)	73.03	10.01	16.96	Sandy clay loam	0.2193	7.01	194.60

**Table 3** The variation water content (%), chlorophyll a, b, carotenoid and proline of *C. arabica* in the three investigated sites.

Site	Characters		Chlorophyll		
	Water content	Proline $\mu\text{mol g}^{-1}$ F. wt.	Chl. a $\text{mg g}^{-1}$ F. wt.	Chl. b $\text{mg g}^{-1}$ F. wt.	Carot. $\text{mg g}^{-1}$ F. wt.
(I)	$5.11 \pm 0.03^*$	$60.223 \pm 0.15^{**}$	$2.837 \pm 0.05^*$	$1.223 \pm 0.15$	$4.170 \pm 0.10$
(II)	$3.90 \pm 0.03^*$	$37.25 \pm 0.10^*$	$2.538 \pm 0.07$	$0.953 \pm 0.05^*$	$3.528 \pm 0.06$
(III)	$1.52 \pm 0.09$	$20.800 \pm 0.08^*$	$1.709 \pm 0.10$	$0.499 \pm 0.04$	$2.565 \pm 0.08^*$

Soil salinity could be classified into two orders:

- Moderate salinity at site III.
- High salinity at the others sites.

The salinity varied among them and site I recorded the highest salinity.

### 3.1.2. Plant analysis Table 3

The current study investigated the response of shoot moisture content, pigment system and proline content of *C. arabica* in different sites. Leaf water content of *C. arabica* was not affected by increases in salinity at site I. The result was 5.11%, 3.90% and 1.52% at I, II and, III respectively. Salt stress increases the water content significantly at site I due to the proximity of the site from the sea and the increase in salinity caused increase in leaf succulence. The growth response of *C. arabica* is similar to that observed by Erdei and Kuiper (1979) in *Plantago maritima*. Another characteristic of halophytes is the increase in water content significantly at salt concentrations, which brings about an increase in leaf volume, or succulence (Flowers et al., 1986).

Concerning the photosynthetic pigments of *C. arabica* during the period of investigation in winter, it was found that higher values of chl. a, chl. b and carotenoid were attained at site I, which is more saline than the other two sites. Maximum increase in carotenoid content was observed, 4.170 at site I. It is evident from the results that chlorophyll content of the experimental *C. arabica* was not much affected by the salinity. This might be due to the increased leaf area in this site, which is considered a measure of leaf density or thickness (Hunt, 1982; Cramer et al., 1994). According to Dadkhah (2011) leaves of stressed plants became thicker than no stressed plants, and thicker leaves contain more cells per unit leaf area, both photosynthesis and chlorophyll concentration were expressed per unit leaf area. Similar result (Mane et al., 2010) was observed that the total chlorophyll content of the mature leaves was increased considerably due to increasing concentrations of NaCl up to 200 mM in *Cymbopogon nardus*. Moreover, (Morsy et al., 2008) in their study on the desert plants along Alamain Wadi El-Natron area, it was concluded that chlorophyll a, b and carotenoids were remarkably decreased in summer and increased during winter for all stressed plant species.

**Table 4** The main different morphological characters of *Cakile arabica*.

Characters		Sites		
		Site (I)	Site (II)	Site (III)
Length of root (cm)		8–13.5	10–13	8–17
Length of shoot (cm)		34–45	41–47	37–42
Lower leaf	Length $\times$ width (cm)	11–15 $\times$ 7–9	9.5–13 $\times$ 6.5	11–13 $\times$ 5–10
	No. of lobes	4–7	5–6	4–6
Upper leaf	Length $\times$ width (cm)	5–5.6 $\times$ 4.5–5.5	4–5.5 $\times$ 3–5.5	4–5 $\times$ 3.5–4
	No. of lobes	2–3	2–3	1–2
No. of inflorescence		14–21	8–10	10–23
No. of fruits per branch		15–27	10–20	20–27
Size of fruit (cm)		1.5–1.7 $\times$ 0.3	1.6–2 $\times$ 0.3	1.8–1.9 $\times$ 0.2
Size of seed (mm)		3 $\times$ 0.5	3.5 $\times$ 0.8	3.5 $\times$ 1



**Plate 1** Different vegetative characters of *Cakile arabica*. Fig. 1: from site one; Fig. 2: from site two; Fig. 3: from site three; A = lower leaves; B = upper leaves.

The proline concentration of leaves was significantly higher at Site (1) 60.22% and 37.25%, 20.800% at site II and site III respectively. (Greenway and Munns, 1980) indicated that the adaptive role of proline is related to survival rather than to maintenance of growth. Plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological status (Edreva, 1998). According to the findings of our study, proline content in leaves of *C. arabica* increased with increasing salt concentration. Similar result (Ghoulama et al., 2002) was seen in five sugar beet cultivars, (Vicentea et al., 2004) in *Plantago crassifolia*, (Abositta and Al-Taisan, 1995) for *Limonium axillare*,

(Liu et al., 2006) in *Limonium bicolor* and *Suaeda salsa* and (Al-Taisan, 2010) for *Senecio glaucus*.

### 3.2. 2-Macromorphology Table 4 and Plate 1

The *Cakile arabica* species is an annual, glabrous and succulent herb arising from tap root; stem erect, terete, solid and ascending branched. Leaves alternate and pinnate. Lower leaves oblong-ovate in outline, petiolate. Petiole glabrous and up to 7.5 cm long. Blade 9.5 – 15 cm × 4.5 – 10 cm, pinnately divided almost to base into 4–7 narrowly linear lobes. The upper leaves are pinnate and petiolate. Petiole up to 1.7 cm

**Table 5** The main different anatomical characters of *Cakile arabica*.

Characters		Sites		
		Site (I)	Site (II)	Site (III)
Stem anatomy	Cuticle thickness ( $\mu\text{m}$ )	48.60	62.23	59.20
	Epidermis thickness ( $\mu\text{m}$ )	242.36–390.04	201.87–265.64	483.00
	Cortex thickness ( $\mu\text{m}$ )	968.73	874.32	1218.92
	No. of V.B.	15–16	15–17	
	Area of V.B. ( $\mu\text{m}^2$ )	1440267.84– 2290597.76	570945.76– 875981.92	387374.24– 1005326.08
Petiole anatomy	Cuticle thickness ( $\mu\text{m}$ )	76.84	102.72	70.95
	Epidermis thickness ( $\mu\text{m}$ )	162.80–382.83	261.72–393.01	376.25–402.33
	No. of main V.B.	3 (united)	1	1
	Area of main V.B. ( $\mu\text{m}^2$ )	3735337.76	3306862.24	5868500.00
	No. of subsidiary V.B.	3,3	3,3	3,3
	No. of row of sclerenchyma associated with V.B.	5	4	4
	Area of sclerenchyma ( $\mu\text{m}^2$ )	2419574.08	1504233.28	2182317.28
Blade	Cuticle thickness ( $\mu\text{m}$ )	59.85	44.00	50.17
Rachis	Epidermis thickness ( $\mu\text{m}$ )	236.46–442.85	222.67–403.34	114.40–452.43
	No. of main V.B.	1	2 (united)	1
	Area of main V.B. ( $\mu\text{m}^2$ )	1942679.20	1831514.08	5541025.96
	No. of subsidiary V.B.	5,5	5,4	5,5
Lobe	Cuticle thickness ( $\mu\text{m}$ )	74.88	74.80	53.53
	Epidermis thickness ( $\mu\text{m}$ )	299.88–410.05	122.73	183.01–321.32
	Midrib thickness ( $\mu\text{m}$ )	1433.41	2251.51	2187.80
	Wings thickness ( $\mu\text{m}$ )	928.69	1948.01	1179.21
	Area of main V.B. ( $\mu\text{m}^2$ )	1170834.72	572513.92	967244.99

long. Blade 4–6 cm  $\times$  3–5.5 cm, ovate with 1–3 lateral lobes.

Inflorescence raceme. Sepals hairy, green, ovate oblong in outline with narrow membranous margin. Petals violet, clawed, limb obovate with obtuse apex. Stamen with glabrous, filament and ovate anthers. Ovary is smooth with inconspicuous style and flattened stigma. Silique 1.5–2 cm  $\times$  0.4–0.6 cm, ribbed, glabrous, indehiscent with 2-segmented. The upper segment longer than the lower with pyramidal shape and one seeded. The lower segment is short, cylindrical with two prominent lateral projections basally and one seeded. Beak 2–3 mm long and seedless. Seeds are oblong, 3–3.5 mm  $\times$  0.5–1 mm brown with sub-terminal hillum and smooth surface.

### 3.3. 2-Micromorphology

#### 3.3.1. Stem anatomy

The outline in cross section is terete in *Cakile* from site two and terete with wavy margin in remainder [Table 5](#) and [Plate 2](#). Epidermal cells are tangential in site two and radially mixed with tangentially elongated cells in site one and three, covered with thick, warty cutin. Cortex consists of 3–4 layers of chlorenchyma cells followed by 1–2 layers of parenchyma. Pericycle consists of parenchymatous cells. Vascular cylinder is eustele, composed of 15–16 bundles in *Cakile* from site one, 15–17 in site two and 18–20 in site three, each with well defined patches of phloem and well defined xylem vessels. The medullary rays are wide. Pith is wide and homogenous, consists of thin walled

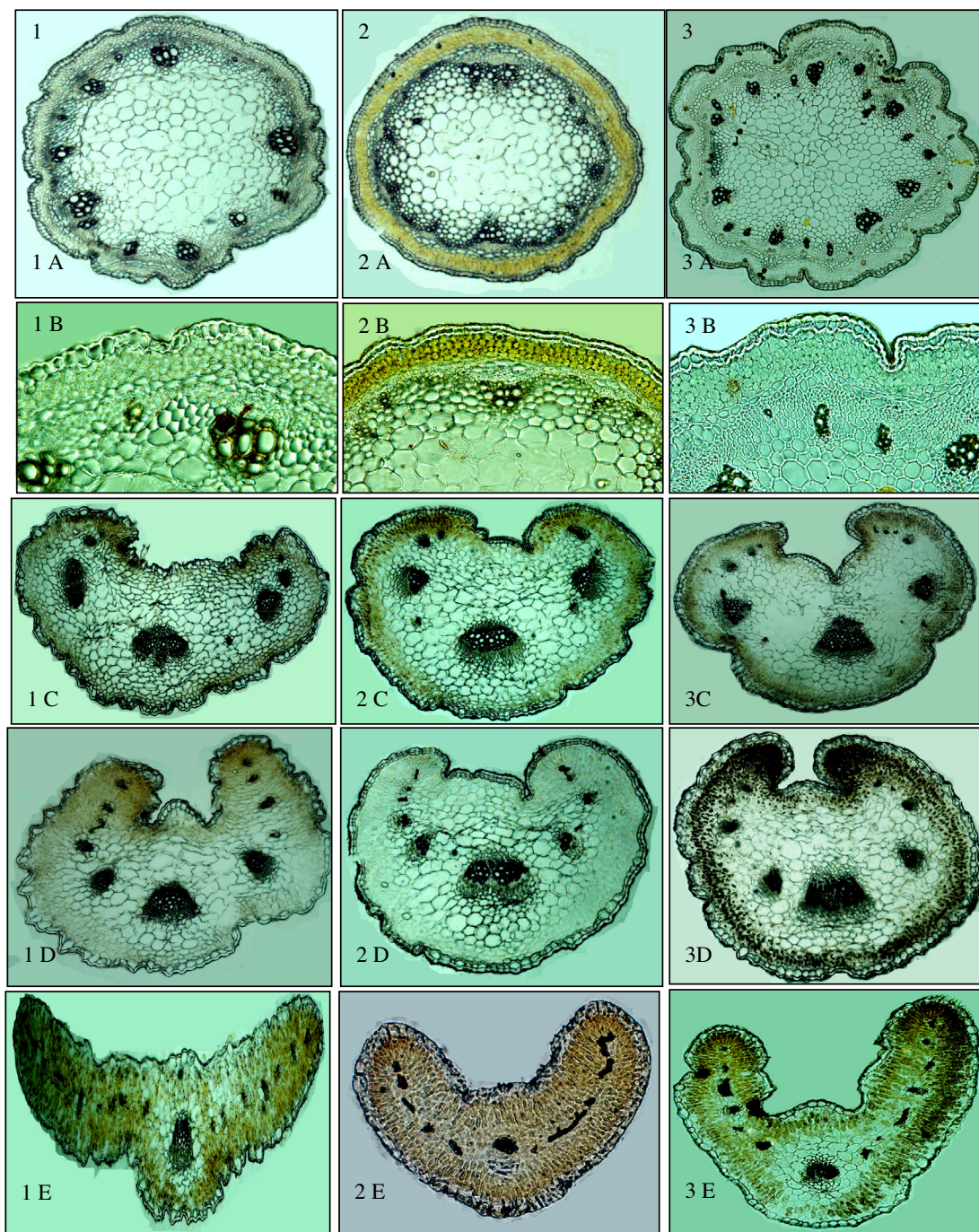
round to polygonal parenchymatous cells. Schizogenous canals are recorded in cortex and pith.

#### 3.3.2. Leaf anatomy

**3.3.2.1. a-Petiole.** The outline in cross section is  $\pm$  crescent with two prominent ridges. Epidermis is composed of radially elongated cells covered with thick and warty cutin. Ground tissue consists of 3–4 layers of chlorenchyma tissue found abaxially and ridges followed by round to irregular thin cell wall parenchyma cells. Vascular system is made up of 10 bundles arranged in crescent form, three (united) main and 7 (3, 4) small, unequal in size in each side for site one and 7 bundles, one main and 6 (3, 3) subsidiary in each side for site two & site three. Each bundle with well defined patches of phloem, wide xylem vessels, surrounded by a bundle sheath of wide parenchyma and associated with fibers (sclerenchyma), the number of rows of sclerenchyma range from 4–5 in each row. Schizogenous canals are present.

#### 3.3.2.2. c-Blade.

**3.3.2.2.1. c.1-Rachis.** The outline in cross section is  $\pm$  crescent with two prominent ridges. Epidermis is composed of radially elongated cells mixed with some tangentially covered with thick and warty cutin. Mesophyll is centric, composed of palisade in the form of outer 3–4 layers of loose cells, followed by parenchyma tissue which is composed of 4–6 layers of large thin-walled round to polygonal. The vascular system is in the form of 11 collateral bundles, one main and 10 (5, 5) subsidiary vascular bundles in site 1



**Plate 2** Anatomical structure of *Cakile arabica* from different habitat. Fig. 1: from site one; Fig. 2: from site two; Fig. 3: from site three; A = outline of stem; B = sector of stem; C = petiole outline; D = rachis of blade; E = lobe of blade.

& site three and two (united) main vascular bundles and 9 (5,4) subsidiary in site two. Schizogenous canal are recorded.

**3.3.2.2.2. c.2-Lobe.** The outline in cross section is duplicate. Epidermal cells are radial mixed with bulliform cells covered with thick and warty cutin and interrupted by Anisocytic semi depressed stomata. Mesophyll is isobilateral, composed of 3–4 layers of cubic cells of palisade tissue continuously adaxial at the midrib region followed by one layer of thin cell wall parenchyma cells. Vascular system is composed of one large main

bundle at midrib region and many small bundles on each side at the wing region. Each bundle is surrounded by a bundle sheath of wide parenchyma and associated with fibers.

#### 4. Conclusion

From all characters of physiology, morphology and anatomy for the *C. arabica* from three different habitats, this study indicates that, the *C. arabica* which grows in site one (high salinity)

is characterized by increase in the leaf size, lobe number, thickening of epidermis, area of vascular bundles, chlorophyll a, b, carotenoid and proline as adaptive characters for salinity stress; this agrees with the view of (Vijayan et al., 2008; Çavuşoğlu et al., 2008; Wu et al., 2010; Chookhampaeng, 2011; Ahmad et al., 2012; Kishor and Sreenivasulu, 2014). This study supports the use of *C. arabica* and these characters are used as an indicator of the salinity of the soil.

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