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

Wafa’a A. Al-Taisan a,*, Dalia G. Gabr b

a Department of Biology, Faculty of Science, University of Dammam, Saudi Arabia
b Department of Basic Science, Faculty of Education, University of Dammam, Saudi Arabia

Received 23 February 2016; revised 5 April 2016; accepted 14 April 2016
Available online 25 May 2016

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.

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
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 grow 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 off-spring 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 Arabia (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 μm in thickness; the stem taken from 2nd, 4th and 6th internodes. The sections were stained in safranin (1% solution in 50% ethanol) with the ratio 70% alcohol) 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 (mmhos/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 × (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 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 μmol 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 mmhos/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 |
Soil salinity could be classified into two orders:

(a) Moderate salinity at site III.
(b) 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

| Site | Characters     | Chlorophyll | Proline μmol g⁻¹ F. wt. | Water content |
|------|---------------|-------------|-------------------------|--------------|
| (I)  | 5.11 ± 0.03*  | 2.837 ± 0.05* | 60.223 ± 0.15**       |
| (II) | 3.90 ± 0.03*  | 2.538 ± 0.07  | 37.25 ± 0.10*         |
| (III)| 1.52 ± 0.09   | 1.709 ± 0.10  | 20.800 ± 0.08*        |

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-Natrun area, it was concluded that chlorophyll a, b and carotenoids were remarkably decreased in summer and increased during winter for all stressed plant species.
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
long. Blade 4 – 6 cm × 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. Siliqua 1.5 – 2 cm × 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 × 0.5 – 1 mm brown with sub-terminal hilum 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 reminder 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 will 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 ±crescent with two prominent ridges. Epidermis is composed of radially elongated cells covered with thick and warty cutin. Ground tissue is 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 ±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.

| Table 5 | The main different anatomical characters of Cakile arabica. |
|---------|----------------------------------------------------------|
| Characters | Sites (I) | Sites (II) | Sites (III) |
| Stem anatomy | Cuticle thickness (µm) | 48.60 | 62.23 | 59.20 |
| | Epidermis thickness (µm) | 242.36–390.04 | 201.87–265.64 | 483.00 |
| | Cortex thickness (µm) | 968.73 | 874.32 | 1218.92 |
| | No. of V.B. | 15–16 | 15–17 | 15–17 |
| | Area of V.B. (µm²) | 1440267.84–2290597.76 | 570945.76–875981.92 | 387374.24–1005326.08 |
| Petiole anatomy | Cuticle thickness (µm) | 76.84 | 102.72 | 70.95 |
| | Epidermis thickness (µm) | 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. (µm²) | 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 (µm²) | 2419574.08 | 1504233.28 | 2182317.28 |
| Blade | Cuticle thickness (µm) | 59.85 | 44.00 | 50.17 |
| Rachis | Epidermis thickness (µm) | 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. (µm²) | 1942679.20 | 1831514.08 | 5541025.96 |
| | No. of subsidiary V.B. | 5,5 | 5,4 | 5,5 |
| Lobe | Cuticle thickness (µm) | 74.88 | 74.80 | 53.53 |
| | Epidermis thickness (µm) | 299.88–410.05 | 122.73 | 183.01–321.32 |
| | Midrib thickness (µm) | 1433.41 | 2251.51 | 2187.80 |
| | Wings thickness (µm) | 928.69 | 1948.01 | 1179.21 |
| | Area of main V.B. (µm²) | 1170834.72 | 572513.92 | 967244.99 |

W.A. Al-Taisan, D.G. Gabr
& site three and two (united) main vascular bundles and 9 (5:4) subsidiary in site two. Schizogenous canal are recorded.

3.3.2.2. 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, cartenoid and protein as adaptive characters for salinity stress; this agrees with the view of (Vijayan et al., 2008; Çavuşoğlu et al., 2008; Wu et al., 2010; Chookkampaeng, 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.

Acknowledgments

The authors would like to express their sincere appreciation to Damman of University, Saudi Arabia for giving us the opportunity to conduct the practical part of the research in the research units of College of Science research.

References

Ahmad, P., Hakeem, K.R., Kumar, A., Ashraf, M., Akram, N.A., 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (Brassica juncea L.). Afr. J. Biotech. 11, 2694–2703.

AOAC. 2008. Official Methods of Analysis. In: Arnon (Ed.), Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris L., Plant Physiol., 24. Association of Official Analysis Chemists. International Arlington, Virginia, U.S.A., pp. 1–15. 1949.

Abosita, Y.M., Al-Taisan, W.A., 1995. Salinity effect on water relations and proline accumulation in Luminium axillare (Forssk) Kuntze. Desert Inst. Bull. Egypt 47 (1), 285–309.

Al-Busaidi, A., Cookson, P., Yamamoto, T., 2005. Methods of pH determination in calcareous soils: use of electrolytes and suspension effect. Aust. J. Soil Res. 43, 541–545.

Al-Taisan, W.A., 2010. The Relation between phenotypic plasticity of Senecio glaucus L. and some soil factors. Aust. J. Basic Appl. Sci. 4 (6), 1369–1375.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris L. Plant Physiol. 24, 1–15.

Bates, L.S., Waldren, R.P., Teare, J.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.

Boyd, R.S., Barbour, M.G., 1993. Replacement of Cakile edentula by C. maritima in the strand habitat of California. Am. Midlad Nat. 130, 209–228.

Çavuşoğlu, K., Kılıç, S., Kabar, K., 2007. Some morphological and anatomical observations during alleviation of salinity (NaCl) stress on seed germination and seedling growth of barley by polyamines. Acta Physiol. Plant 29, 551–557.

Çavuşoğlu, K., Kılıç, S., Kabar, K., 2008. Effects of some plant growth regulators on leaf anatomy of radish seedlings grown under saline conditions. I. Appl. Biol. Sci. 2, 47–50.

Chookkampaeng, S., 2011. The effect of salt stress on growth, chlorophyll content proline content and antioxidative enzymes of pepper (Capsicum annuum L.) seedling. Eur. J. Sci. Res. 49, 103–109.

Chrispeels, M.J., Sadava, D.E., 2003. Plants, Genes, and Crop Biotechnology. Jones and Bartlett Learning, p. 562.

Cramer, G.R., Alberico, G.J., Schmidt, C., 1994. Leaf expansion limits dry matter accumulation of salt-stressed maize. Aust. J. Plant Physiol. 21, 663–674.

Daddkhah, A., 2011. Effect of salinity on growth and leaf photosynthesis of two sugar beet (Beta vulgaris L.) Cultivars J. Agric. Sci. Technol. 13, 1001–1012.

Daniela, C., Mirko, B., Maria, P.A., Costantina, F.L.M., 2010. Morpho-functional adaptations in Cakile maritima scop, subsp. maritima: comparison of two different morphological types. Caryologia 63 (4), 411–421.
Morsy, A.A., Youssef, A.M., Mosallam, H.A., Hashem, A.M., 2008. Assessment of selected species along Alamein-Wadi El-Natrun desert road, Egypt. J. Appl. Sci. Res. 4 (10), 1276–1284.
Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239–250.
Nassar, M.A., El-Sahhar, K.F., 1998. Botanical Preparation and Microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, p. 219 (in Arabic).
Pakeman, R.J., Lee, J.A., 1991a. The ecology of the strandline annuals Cakile maritima and Salsola kali. 1. Environmental factors affecting plant performance. J. Ecol. 79, 143–153.
Pakeman, R.J., Lee, J.A., 1991b. The ecology of the strandline annuals Cakile maritima and Salsola kali. 2. The role of nitrogen in controlling plant performance. J. Ecol. 79, 155–165.
Shannon, M.C., 1997. Adaptation of plants to salinity. Adv. Agron. 60, 76–119.
Shennan, C., Hunt, R., Macrobbie, E.A.C., 1987. Salt tolerance in Astertripolium L. I. The effect of salinity on growth. Plant Cell Environ. 10, 59–65.
Sultan, S.E., 1992. What has survived of Darwin’s theory? Phenotypic plasticity and the neo-darwinian legacy. Evol. Trends Plants 6, 61–71.
Vicentea, O., Boscaiu, M., Naranjoa, M.A., Estrellec, E., Bellésa, J. M., Soríanoc, P., 2004. Responses to salt stress in the halophyte Plantago crassifolia (Plantaginaceae). J. Arid Environ. 58, 463–481.
Vijayan, K., Chakraborti, S.P., Eresi, S., Ghosh, P.D., 2008. NaCl induced morpho-biochemical and anatomical changes in mulberry (Morus spp.). Plant Growth Regul. 56, 61–69.
Wilde, S.A., Corey, R.G., Lyer, J.G., Voigt, G.K., 1979. Soil and Plant Analysis for Tree Culture. Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta, p. 223.
Wu, X.X., Ding, H.D., Chen, J.L., Zhang, H.J., Zhu, W.M., 2010. Attenuation of salt-induced changes in photosynthesis by exogenous nitric oxide in tomato (Lycopersicon esculentum) Mill. L. seedlings. Afr. J. Biotechnol. 9, 7837–7846.