

Ecophysiological study of some coastal dune species of Zemmouri El Bahri (Algeria)

Sadjia Rabhi*, Réda Djebbar, Aicha Belkebir

University of Science and Technology Houari Boumediene, Faculty of Biological Sciences, Laboratory of Biology and Physiology of Organisms, BP 32, El Alia, Bab Ezzouar 16111, Algiers, Algeria

Abstract – Species of coastal dunes are subjected to several environmental stress factors such as drought, high temperature, light intensity as well as salinity, which led to development of specific morphological, physiological and biochemical adaptation mechanisms. Studying these strategies allows explaining the survival of these species in such hostile and stressful conditions. In this study we compared some parameters (morphological, physiological and biochemical) of two Brassicaceae species *Matthiola tricuspidata* (L.) W.T.Aiton, *Cakile maritima* Scop., and two Fabaceae species *Lotus creticus* L. and *Ononis variegata* L., harvested from their natural environment in coastal dunes of Zemmouri El Bahri (Algeria), with the aim to understand their mechanisms of adaptation. The results revealed that *C. maritima* showed the highest relative water content, leaf area, leaf mass area, and succulence index. Based on the highest levels of total phenols, flavonoids and anthocyanins as well as high contents of photosynthetic pigments, *M. tricuspidata* can be classified as “homoiochlorophyllous” plant. The dry mass content varied considerably among species, with the highest values observed in *L. creticus* and the lowest in *C. maritima*. As both Fabaceae species *L. creticus* and *O. variegata* showed high chlorophylls, carotenoids, total phenols and flavonoids could also be classified as “homoiochlorophyllous” species. The relatively high levels of total phenols, total flavonoids, anthocyanins and carotenoids implies a biochemical adaptation that allows these plants to maintain necessary photosynthetic activity under a water deficiency condition.

Keywords: adaptation, anthocyanins, flavonoids, photosynthetic pigments, relative water content, total phenols

Introduction

Coastal dunes are characterized by high ecological diversity, environmental heterogeneity, and variability of species (Martinez and Psuty 2005), with close interaction between biotic and abiotic factors (Rucco et al. 2014). Vegetation in the sand dunes is also influenced by a strong seasonality with two distinct periods being rainy cold winters and dry hot summers, the latter characterized by high irradiance and little or no precipitation. Consequently, plants experience intense stress during summer due to drought, large evaporative demand and high irradiance at high temperatures (Antunes et al. 2018). The ecological conditions of coasts are often stressful or even critical, their flora grows under unfavourable environmental conditions including low capillarity, salt spray, and dryness, water holding capacity, high light intensity, extreme temperature, sand burial, deficiency of nutrients and negative action of violent wind

(Gratani et al. 2009). These abiotic stress factors generate secondary osmotic and oxidative stresses, which then negatively influence and induce changes in the normal growth, development, and metabolism of the plant (Król et al. 2014). In spite of all these constraints, these environmental conditions promote the development and evolution of some particular species belonging to different botanical families. Many plant species have adapted on genetic, morphological and physiological level, which help them partially or completely withstand these stress conditions (Gad et al. 2002). These established strategies are an important step that could explain the preservation and survival of these species in such extreme and hostile environmental conditions. To explore these strategies and in order to value plant resources native to saline biotopes, we have chosen four species belonging to two important families Brassicaceae and Fabaceae.

* Corresponding author e-mail: sadjiarabhi@gmail.com

The genus *Matthiola* W.T.Aiton (Brassicaceae) contains 50 species of annual and biannual herbs and perennial subshrubs, characterized by a silicle that features horns or swellings in its apical end (Sánchez et al. 2006). *Matthiola tricuspidata* (L.) W.T.Aiton is a small annual plant characterized by a sturdy stem softly drooping, sinuous leaves, large flowers and a potential oilseed (Demiral 2003); this species is widespread on the sandy and rocks of the coast (Quezel and Santa 1962). *Cakile maritima* Scop. is an annual, succulent and halophyte plant (Ksouri et al. 2007), growing in sandy habitats along the North Atlantic Ocean, the Mediterranean Sea coasts, the Canary Islands and in southwestern Asia (Clausen et al. 2000). It is usually positioned in front of the pioneer dune and is fixed by its developed roots, it has fleshy and pinnatifid leaves (Parisod and Baudière 2006). This plant has an ecological value as its roots bind dunes, potential economical use as its seeds contain oil (40% of dry weight), and therapeutic value (Ciccarelli et al. 2010).

Among the Fabaceae two species have also been selected: *Ononis variegata* L. and *Lotus creticus* L. The genus *Ononis* L. comprises about 75 species occurring in Europe, Central Asia, and Africa (Wollenweber et al. 2003), being generally perennial herbs and shrubs (Wdowiak-Wróbel et al. 2017). Many species of *Ononis* genus carry glandular trichomes that are more or less viscent, and contain flavonoids in several cases (Wollenweber et al. 2003). The genus *Lotus* L. comprises between 120 to 130 species of perennial and annual native plants, distributed mainly in the Mediterranean region (Sandral et al. 2010). In this genus, *L. creticus* is considered an important perennial species due to its ability to stabilize dunes (Sandral et al. 2010).

Plants living under hostile conditions synthesize higher amounts of protecting specialized metabolites such as phenolic compounds with free-radical quenching activity which can be primarily related to the substitutions on the aromatic ring and the structure of the side chain (Abdeen et al. 2015). These compounds are involved in various plants physiological and developmental processes such as growth regulation, pigmentation, reproduction, and resistance to pathogens. They protect plants against adverse factors including drought, UV radiations, infection and physical damage (Slatnar et al. 2016). Various biotic and abiotic factors generate oxidative stress that can be very harmful to cells (Xiao et al. 2014). In such situations, antioxidant molecules intervene and decrease the deleterious effect of free radicals (Apel and Hirt 2004). Thus, we evaluated the contents of total phenols, total flavonoids (as one of the largest groups of plant phenols) and anthocyanins (a sub class of flavonoids) in the leaves of the four selected plants growing on the coastal dunes of Zemmouri El Bahri, Algeria. Moreover, major photosynthetic pigments and certain morphological parameters were also determined as they are usually affected by drought and other environmental conditions as well. In this paper, we compared morphological and physiological traits of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata* with the aim to explore their possible adaptive mechanisms to environmental stress.

Materials and methods

Study site and plants materials

The study is conducted in the coastal dunes of East-Algiers (Zemmouri El Bahri), located between 3°32', 3°38' E and 36°48', 36°50' N geographical coordinates. The area is characterized by a Mediterranean climate, with an annual rainfall mean of 579 mm. The air temperature mean of the coldest months reaches 9.3 °C whereas the mean maximum air temperature of the warmest month goes up to 28.9 °C, with a drought period spreading over five months, a sub-humid bioclimatic zone and a variant hot winter (Hanifi et al. 2007). The species *M. tricuspidata*, *C. maritima*, *L. creticus* and *O. variegata* were collected at the floral stage during April and May from the study station (Sand dunes of Zemmouri el Bahri). The leaves were freshly harvested, washed, dried, finely ground and then stored in a dry dark place.

Morphological parameters of the leaf

The morphological leaf traits were determined by the method described by Ciccarelli et al. (2010). The leaves from three individual plants per each selected species were collected, and weighed to determine the fresh weight (FW). Leaf projected area (LA) was obtained with a Canon Scan using a Meserium Software area and expressed in cm². Plant material was dried at 70 °C to constant weight (dry mass, DM).

Leaf dry matter content (LDMC) was calculated as the ratio of leaf dry mass (DM) to fresh mass (FM), expressed in mg g⁻¹ (Li et al. 2005):

$$\text{LDMC} = \frac{\text{DM}}{\text{FM}}$$

Leaf mass per area (LMA) was expressed as the ratio of dry mass (DM) to leaf area (LA), expressed in mg cm⁻² (Ogaya and Penuelas 2007):

$$\text{LMA} = \frac{\text{DM}}{\text{LA}}$$

Succulence index (SI) is the ratio of the difference between the dry mass (DM) and fresh mass (FM) to the leaf area (LA), expressed in mg cm⁻² (Gratani et al. 2009):

$$\text{SI} = \frac{\text{DM-FM}}{\text{LA}}$$

Where DM corresponds to: dry mass, FM: fresh mass, LA: leaf area.

Physiological parameters

Leaf relative water content (RWC) was evaluated using the equation introduced by Ladigues (1975):

$$\text{RWC}(\%) = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right] \times 100$$

Where FW corresponds to: fresh weight, DW to: dry weight and TW to: full turgor weight, measured after soak-

ing the leaves in water for 24 hours at room temperature and in the presence of light. The dry weight was estimated after drying the leaves at 60 °C for 48 hours (Clarck and Mac-Caig 1982).

To extract the photosynthetic pigments chlorophyll *a* (Chl *a*) chlorophyll *b* (Chl *b*), total chlorophylls (Chl *a+b*) and total carotenoids (Car), 0.05 g of fresh leaf samples were ground in 5 mL of acetone solution (80%). The homogenate was centrifuged at 3000×g for 10 min at 4 °C. The supernatant was recuperated and the absorbance was measured at 647 nm, 663 nm and 470 nm for Chl *a*, Chl *b* and Car, respectively. The amounts of pigments present in the extract were calculated using the equation of Lichtenthaler (1987):

$$\text{Chla} = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$\text{Chlb} = 21.5 \times A_{647} - 5.1 \times A_{663}$$

$$\text{Chla} + \text{b} = 7.15 \times A_{663} + 18.71 \times A_{647}$$

$$Cx + c = \frac{1000 \times A_{470} - 1.82 \times \text{Chla} - 85,02 \times \text{Chlb}}{198}$$

Biochemical parameters

Sample extract was obtained by stirring 1g of dry leaf powder with 7 mL of pure methanol and 3 mL deionized water for 30 min. The extracts were then kept for 48 hours at 4 °C, filtered through N°1 Watman filter paper, evaporated under vacuum to dryness and then stored at 4°C until analysis (Ksouri et al. 2007).

Total phenolic contents were determined using the Folin-Ciocalteu reagent, according to the method of Singleton and Rossi (1965). A volume of 0.025 mL of sample extract was added to 3.975 mL of distilled water before adding 0.25 mL of the Folin-Ciocalteu reagent. The mixture had the ability to react for 3 minutes before an amount of 0.75 mL of 20% Na₂CO₃ was added. After incubation for 40 minutes at 40 °C, the absorbance was measured at 760 nm. There has been a sequence of three assays, and the phenolic contents was expressed as Gallic acid equivalent in milligrams per gram of dry weight (mg GAE g⁻¹ DW).

Total flavonoids were measured using a colorimetric aluminum chloride (AlCl₃) assay (Lamaison and Carnet 1990, Chang et al. 2002). Volume of 1.5 mL of each extract were mixed with 1.5 mL of 2% AlCl₃ 6H₂O. The preparation was shaken and the absorbance was read at 440 nm after 10 minutes at room temperature. The assay was repeated three times and the total flavonoid contents were expressed

as rutin equivalent in milligrams per gram of dry weight (mg ERu g⁻¹ DW).

The anthocyanins content was determined according to the procedure described by Gould et al (2002) as follows; anthocyanins were extracted by adding 2 mL of acidified methanol 1% HCl (V/V) to 50 mg of dry leaves. The supernatant aliquot was retrieved and the absorbance was measured at 530 nm. Total anthocyanin was calculated by endoresing the following formula:

$$T_{\text{anthocyanin}} = \frac{\text{Absorbance} \times 449.2 \times \text{Dilution factor}}{29600 \times \text{Weight(g)}}$$

The anthocyanin content was expressed in mg of Cyanidin-3-glucoside equivalent per gram of dry weight.

Statistical analysis

The statistical study was performed by Excel and Statistica v.8 (StatSoft, Inc., Tulsa, OK). Results were analysed by one-way ANOVA, followed by Tukey test to compare means. The differences were held significant if P < 0.05. All experiments were carried in triplicates and expressed ± SD (n = 3).

Results

Plant morphological study

There were significant differences between species for all morphological traits studied. Leaf succulence index (SI) shows different values in the four species (Tab. 1). *Cakile maritima* had the largest leaf area and the highest succulence index (12.51 ± 0.03 cm⁻², 131.37 ± 0.83 mg cm⁻², respectively), though both species of Brassicaceae showed high leaf area and a high succulence index. Fabaceae family species have narrow leaves, while *L. creticus* leaves exhibit the lowest leaf succulence (22.86 ± 0.56 mg cm⁻²). Leaf mass area (LMA) was also evaluated, this parameter varied from 6.55 ± 0.45 mg cm⁻² (*L. creticus*) to 8.52 ± 0.74 mg cm⁻² (*C. maritima*). Leaf dry matter content (LDMC) indicates that Fabaceae species have the highest values with 219.51 mg g⁻¹, 147.64 mg g⁻¹ for *L. creticus* and *O. variegata*, respectively. Among the Brassicaceae family species, *C. maritima* leaves show the lowest LDMC (58.52 ± 0.53 mg g⁻¹). Leaf area (LA) of *C. maritima* was significantly higher, but the lowest LDMC was found there. On the other hand, the leaves of *L. creticus*, having the smallest foliar surfaces but highest dry matter accumulation (Tab. 1).

Tab. 1. Leaf area (LA), leaf dry matter content (LDMC), leaf mass area (LMA), and succulence index (SI) of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*. Numbers present average of three replicates ± standard deviation. Different letters within each column mean significant differences at P < 0.05 (Tukey's HSD test).

Species	LA (cm ²)	LDMC (mg g ⁻¹)	LMA (mg cm ⁻²)	SI (mg cm ⁻²)
<i>Matthiola tricuspidata</i>	10.68 ± 0.95 ^a	91.15 ± 0.33 ^a	7.39 ± 0.29 ^a	57.39 ± 0.78 ^a
<i>Cakile maritima</i>	12.50 ± 0.02 ^b	58.52 ± 0.53 ^b	8.52 ± 0.74 ^a	131.37 ± 0.83 ^b
<i>Lotus creticus</i>	0.57 ± 0.02 ^c	219.51 ± 0.54 ^c	6.55 ± 0.45 ^b	22.86 ± 0.56 ^c
<i>Ononis variegata</i>	0.54 ± 0.01 ^c	147.64 ± 0.90 ^d	6.84 ± 0.29 ^b	39.14 ± 0.74 ^d

Plant physiological responses

The relative water content (RWC) was significantly higher in *C. maritima* (77.14%) than other species in which that parameter ranged from 63.10 to 65.15% (Fig. 1).

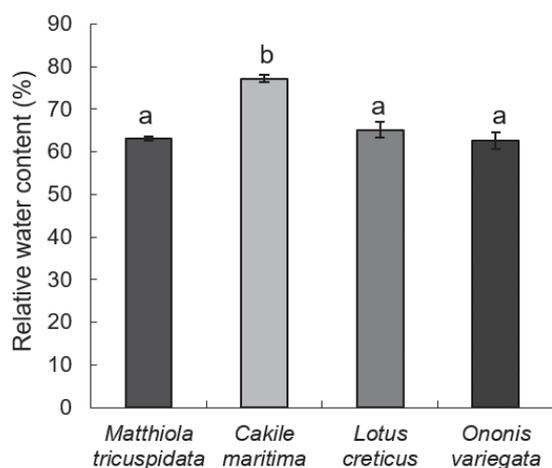


Fig. 1. Relative water content (%) in leaves of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*. Data are mean values of three replicates \pm standard deviation. Different letters are significantly different at $P < 0.05$ (Tukey HSD test).

The variations of photosynthetic pigments were shown in Fig. 2A. Among the four species, *M. tricuspidata* and *L. creticus* showed the highest levels of chlorophyll *a* (1.02 ± 0.04 mg g⁻¹ FW and 1.11 ± 0.09 mg g⁻¹ FW), total chloro-

phylls (1.39 ± 0.1 mg g⁻¹ FW and 1.34 ± 0.12 mg g⁻¹ FW, respectively) and carotenoids content (0.41 ± 0.06 mg g⁻¹ FW, 0.47 ± 0.08 mg g⁻¹ FW), while the lowest content of chlorophyll *a*, total chlorophylls and carotenoids were obtained for *C. maritima*. The content of Chl *b* was similar among all species and the Chl *a/b* ratio varied from 2.82 (*M. tricuspidata*) to 2.33 (*L. creticus*). The chlorophyll/carotenoid ratio was in the range of 3.42 to 2.78 (Fig. 2B).

Plant biochemical responses

The results of total phenolic compounds and total flavonoids in plant extracts are presented in Tab. 2. The results obtained show that the content of total phenolic compounds varied between different species; *M. tricuspidata* had the highest total phenolic compounds with 72.72 ± 0.45 mg GAE g⁻¹ DW, and the lowest were found in *C. maritima* (24.17 ± 0.25 mg GAE g⁻¹ DW). The quantities of total phenolic were 40.73 ± 0.77 mg GAE g⁻¹ DW and 50.01 ± 0.37 mg GAE g⁻¹ DW for *L. creticus* and *O. variegata*, respectively. Flavonoid content also varied between species; *M. tricuspidata* showed the highest content (7.36 ± 0.09 mg ERu g⁻¹ DW), and the lowest were found in *C. maritima* (1.93 ± 0.02 mg ERu g⁻¹ DW) which is 4 times lower than that of *M. tricuspidata*. The values recorded for Fabaceae species were 5.86 ± 0.21 mg ERu g⁻¹ DW and 3.7 ± 0.08 mg ERu g⁻¹ DW for *L. creticus* and *O. variegata*, respectively.

The results of anthocyanin contents of species present some notable differences (Fig. 3). Leaves of *M. tricuspidata* displayed the highest levels of anthocyanins with 0.54 ± 0.06

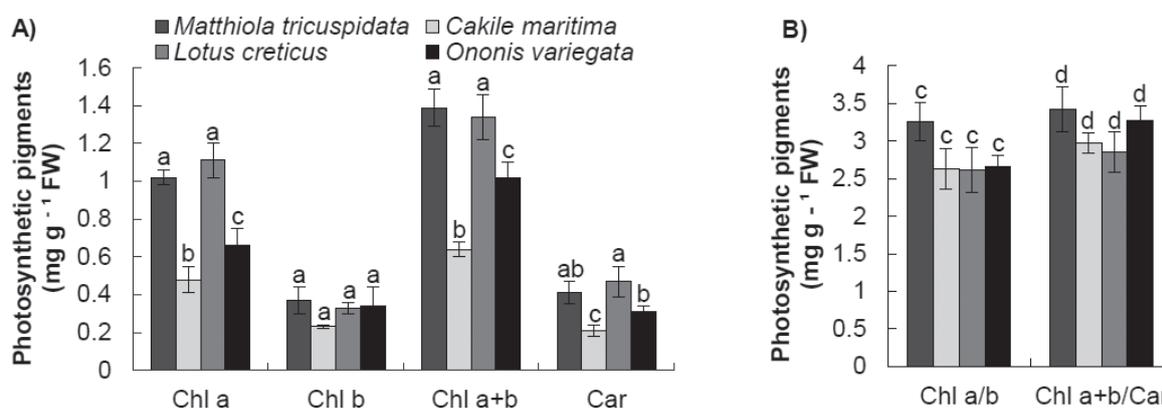


Fig. 2. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophylls (Chl *a*+*b*), total carotenoids (Car) contents (A), and ratio of chlorophyll *a* to chlorophyll *b* (Chl *a/b*) and total chlorophyll to carotenoid ratio (B) in leaves of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*. Data are mean values of three replicates \pm standard deviation. Different letters are significantly different at $P < 0.05$ (Tukey HSD test).

Tab. 2. Phenolic contents (total phenols, total flavonoids) in leaf methanolic extracts of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*. Numbers present average of three replicates \pm standard deviation. Different letters within each column are significantly different at $P < 0.05$ (Tukey's HSD test).

Species	Total phenols (mg EAG g ⁻¹ DW)	Total flavonoids (mg ERu g ⁻¹ DW)
<i>Matthiola tricuspidata</i>	72.72 ± 0.45^a	7.36 ± 0.09^a
<i>Cakile maritima</i>	24.17 ± 0.25^b	1.93 ± 0.02^b
<i>Lotus creticus</i>	40.73 ± 0.77^c	5.86 ± 0.21^c
<i>Ononis variegata</i>	50.01 ± 0.37^d	3.70 ± 0.08^d

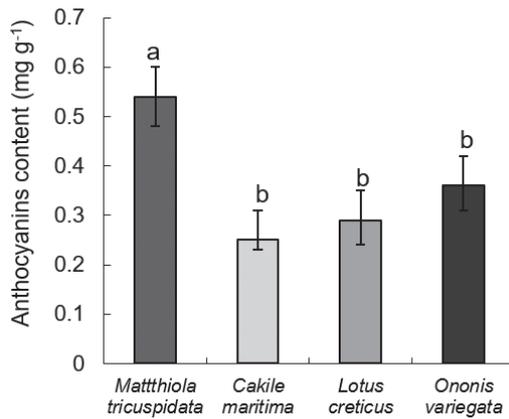


Fig. 3. Anthocyanins contents in leaves of *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*. Data are mean values of three replicates \pm standard deviation. Different letters are significantly different at $P < 0.05$ (Tukey HSD test).

mg g⁻¹ DW, compared to other species. The values of those compounds were 0.36 ± 0.05 mg g⁻¹ DW for *O. variegata*, 0.29 ± 0.05 mg g⁻¹ DW for *L. creticus* and 0.25 ± 0.02 mg g⁻¹ DW for *C. maritima*.

Discussion

The results of the study show that *Matthiola tricuspidata*, *Cakile maritima*, *Lotus creticus* and *Ononis variegata*, living on the sand dunes have different adaptive strategies for morphological, physiological and biochemical to survive in hostile conditions of the coastal dunes. Drought, salinity and UV ray's intensity, affect the photosynthesis, growth and development of species (Shah et al. 2017). Relative water content is probably the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit (Tátrai et al. 2016). *Cakile maritima* showed the highest relative water content (RWC), a large leaf area (LA), leaf mass area (LMA) and succulence index (IS), but its Chl *a*, Chl *b* and total chlorophyll contents were the lowest. The high LMA represents a morphological character of Mediterranean species that plays a protecting role for plants exposed to drought stress (Gratani et al. 2009). The values of leaf mass area (LMA) and index of succulence (IS) are similar to those reported in some species of the Mediterranean coastal dunes (Gratani et al. 2009). *Cakile maritima* is a halophyte and succulent plant, which is naturally tolerant under salinity and drought conditions (Ksouri et al. 2007), with taproots very developed allowing it maximum water absorption, and fleshy leaves, ensuring the accumulation of large tissue water reserves (Parisod and Baudière 2006). The survival of plants in circumstances of water deficiency is dependent on their osmotic adjustment (Baroowa and Gogoi 2015). Different studies have indicated that the drought stress decreases Chl *a*, Chl *b* and the total chlorophyll content of the various crops (Hailemichael et al. 2016). In this regard, *C. maritima* could be classified as a "poikilochlorophyllous" species (Challabathula et al. 2018), which reduce their chlorophyll concentration to absorb less

light energy and thus have less excess electrochemical energy, and therefore their needs for antioxidant defence are less important. Content of phenolic compounds in *C. maritima* is close to that reported by Meot-Duros et al. (2008): 22.24 mg EAG g⁻¹ DW and Ksouri et al. (2007): 42.84 ± 7.67 mg EAG g⁻¹ DW. The total content of flavonoids in plants from the Brassicaceae family ranged from 0.72 EC.g⁻¹ DW to 15.38 EC.g⁻¹ DW (Grigore and Oprica 2015), which are close to those reported in this study.

The flavonoid content found in *Lepidium crassifolium* (2.37 EC.g⁻¹ DW) was similar to that in *C. maritima* (1.93 mg ERu g⁻¹ DW). Ivan and Oprica (2013) also reported total flavonoids levels comparable to those in our results. *Cakile maritima* adopts a "drought avoidance" strategy to resist drought, which is defined as the ability of the plant to maintain a relatively higher water content in tissues despite reduced water content in the soil (Omprakash et al. 2017). In comparison with *C. maritima*, *M. tricuspidata* had the lower relative water content and higher content of Chl *a* and Car. The levels of total phenols, total flavonoids, and anthocyanins were the highest with respect to other species. Based on those results, *M. tricuspidata* can be classified as one of the "homoiochlorophyllous" species, which maintain their chlorophyll pool relatively high under high salinity and light intensity (Challabathula et al. 2018). As a large part of the light energy captured and converted into electrochemical energy at the reaction centers would then be in excess, these species need to have efficient protective mechanisms against the free radicals formed. The carotenoids (xanthophylls) are necessary for photoprotection of photosynthesis and they play an important role as a precursor in the signaling during plant growth under abiotic/biotic stress (Ola and Abd El-Maboud 2013). This could explain the high levels of antioxidant molecules such as carotenoids, total flavonoids and anthocyanins that we have recorded in *M. tricuspidata*. The chlorophyll content results reported in this study were similar to those found in plants growing in coastal dunes (Frosi et al. 2017). Many studies have shown that the concentrations of specialized metabolites in plant exposed to drought are higher than in those grown under well-watered conditions (Al-Gabbiesh et al. 2015).

The results of total phenol contents of species are in accordance with studies on several halophytes (Ksouri et al. 2007, Ivan and Opricã 2013). The increase of content in phenolic compounds in the different tissues under the effect of salinity has been recorded in a certain number of plants (Ksouri et al. 2007, Abideen et al. 2015). Plants react to various environmental factors by increasing the production of polyphenols, particularly flavonoids (Anket et al. 2019). These molecules have important properties such as the protection of tissues against the harmful effects of UV radiations and are an important antioxidant activity (Xiao et al. 2014).

The two species of Fabaceae family have different morphological strategies, *L. creticus* and *O. variegata* have smaller leaf areas (LA), lower Leaf mass area (LMA), and succulence index (SI). The two species show a combination of

high leaf dry matter content (LDMC) and low leaf mass area (LMA). The species with high LDMC and low LMA show a slow growth rate, with long leaf lifespan and more efficient conservation of nutrients (Saura-Mas and Lloret 2007). The reduced leaf area is a strategy to conserve water and control excessive transpiration (Melo Júnior and Boeger 2016). The species with low LMA (i.e. high specific area, SLA), is characterized by high photosynthetic capacity per unit mass (Gratani et al. 2009). Since both *L. creticus* and *O. variegata* displayed high chlorophylls, carotenoids, total phenols and flavonoids contents, they could also be classified as “homiochlorophyllous” species. The results of total phenolics and flavonoids contents were similar to those found by Sayari et al. (2016).

Thus, *M. tricuspidata*, *L. creticus* and *O. variegata* use the “drought tolerance” strategy to resist water deficit, which involves maintaining cell turgor by osmotic adjustment, the elevation of protoplasmic resistance (Supratim et al. 2016), the extension of antioxidant capacity, and development of desiccation tolerance (Osmolovskaya et al. 2018).

Conclusion

This paper shown the various strategies adopted by the four species under unfavorable environmental conditions present in coastal dunes. On the morphological and physiological level, *C. maritima* has a high water potential (high RWC) with a succulent character, which is an adaptive trait that comprises a large leaf area and water reserve. In comparison, *M. tricuspidata*, *L. creticus* and *O. variegata* show lower relative water content, have relatively high chlorophyll contents and fight the consequent higher oxidative damage by greater accumulation of antioxidant molecules such as phenolic compounds and carotenoids. In addition, *L. creticus* and *O. variegata* showed reduced leaf area and a high leaf dry matter content, i.e. leaf morphological adaptations enabling protection against water deficit and limitation of water losses.

References

- Abideen, Z., Qasim, M., Rasheed, A., Adnan, M.Y., Gul, B., Ajmal Khan, M., 2015: Antioxidant activity and polyphenolic content of *Phragmites karka* under saline conditions. *Pakistan Journal of Botany* 47(3), 813–818.
- Al-Gabbiesh, A., Kleinwächter, M., Dirk, S., 2015: Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. *Jordan Journal of Biological Sciences Review* 8(1), 1–10.
- Anket, S., Babar, S., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019: Review response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* 24, 2452.
- Antunes, C., Pereira, A.J., Fernandes, P., Ramos, M., Ascensão, L., Correia, O., Máguas, C., 2018: Understanding plant drought resistance in a Mediterranean coastal sand dune ecosystem: differences between native and exotic invasive species. *Journal of Plant Ecology* 11(1), 26–38.
- Apel, K., Hirt, H., 2004: Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *The Annual Review of Plant Biology* 55, 373–399.
- Baroowa, B., Gogoi, N., 2015: Changes in plant water status, biochemical attributes and seed quality of black gram and green gram genotypes under drought. *International Letters of Natural Sciences* 42, 1–12.
- Challabathula, D., Zhang, Q., Bartels, D., 2018: Protection of photosynthesis in desiccation-tolerant resurrection plants. *Journal Plant Physiology* 227, 84–92.
- Chang, C., Yang, M., Wen, H., Chern, J., 2002: Estimation of total flavonoids contents in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10, 178–182.
- Ciccarelli, D., Balestri, M., Pagni, A.M., Forino, L.M.C., 2010: Morpho-functional adaptations in *Cakile maritima* Scop. subsp. *maritima*: comparison of two different morphological types. *Caryologia* 63, 411–421.
- Clarck, J.M., Mac-Caig, T.N., 1982: Excised leaf water relation capability as an indicator of drought resistance of Triticum genotypes. *Canadian Journal Plant Science* 62, 571–576.
- Clausing, G., Vickers, K., Kaderiet, J.W., 2000: Historical biogeography in a linear system: genetic variation of sea rocket (*Cakile maritima*) and sea holly (*Eryngium maritimum*) along European coasts. *Molecular Ecology* 9, 1823–1833.
- Demiral, M.A., 2003: Determination of salt tolerance of stock (*Matthiola tricuspidata*) as a potential oil crop. *Turkish Journal of Agriculture and Forestry* 27, 229–235.
- Frosi, G., Harand, W., Teixeira de Oliveira, M., Pereira, S., Cabral, P.S., Montenegro, A.A., Santos, M.G., 2017: Different physiological responses under drought stress result in different recovery abilities of two tropical woody evergreen species. *Acta Botanica Brasiliica* 31, 153–160.
- Gad, M.R.M., El-Hadidy, M.E.A., El-Nabarawy, A.A.A., 2002: Comparative study on the adaptation of some natural plants grown under macronutrients limitation at North Sinai sand dunes (Egypt). *Annals of Agricultural Science* 57, 81–90.
- Gould, K.S., Neill, S.O., Vogelmann, T.C.A., 2002: A Unified explanation for anthocyanins in leaves? In: Gould, K.S., Lee, D.W. (eds.), *Anthocyanins in Leaves. Advances in Botanical Research*, vol. 7, 167–192. Academic Press, Amsterdam.
- Gratani, L., Varone, L., Crescente, M.F., 2009: Photosynthetic activity and water use efficiency of dune species: the influence of air temperature on functioning. *Photosynthetica* 47, 575–585.
- Grigore, M.N., Oprică, L., 2015: Halophytes comme source possible de composés antioxydants, dans un scénario basé sur l’agriculture menacées et la crise alimentaire. *Iranian Journal of Public Health* 44, 1153–1155.
- Hailemichael, G., Catalina, A., González, M.R., Martin, P., 2016: Relationships between water status, leaf chlorophyll content and photosynthetic performance in tempranillo vineyards. *South African Journal for Enology and Viticulture* 37, 149–156.
- Hanifi, N., Kadik, L., Guittonneau, G.G., 2007: Analyse de la végétation des dunes littorales de Zemmouri (Boumerdès, Algérie). *Acta Botanica Gallica* 154, 143–151.
- Ivan, M., Oprică, L., 2013: Study of polyphenols and flavonoids contents of some halophytes species collected from Dobrogea region, *Bulletin of the Transilvania University of Brasov Series II, Forestry. Wood Industry. Agricultural and Food Engineering* 6, 121–128.
- Krøl, A., Amarowicz, R., Weidner, S., 2014: Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under con-

- tinuous of long-term drought stress. *Acta Physiologiae Plantarum* 36, 1491–1499.
- Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C., Abdelly, C., 2007: Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry* 45, 244–249.
- Ladigues, P.V., 1975: Some aspect of tissue water relations in three populations of *Eucalyptus viminalis* Labill. *New Phytology* 69, 501–513.
- Lamaison, J.L.C., Carnet, A., 1990: Teneurs en principaux flavonoïdes des fleurs de *Crataegus monogyna* Jacq. et de *Crataegus laevigata* (Poir.) DC. en fonction de la végétation. *Pharmaceutica Acta Helvetia* 65, 315–320.
- Lichtenthaler, H.K., 1987: Chlorophylls and carotenoids. Pigments of photosynthetic biomembranes. *Methods in Enzymology* 148, 350–382.
- Li, Y., Johnson, D.A., Su, Y., Cui, J., Zhang, T., 2005: Specific leaf area and leaf dry matter content of plants growing in sand dunes. *Botanical Bulletin of Academia Sinica* 46, 127–134.
- Martinez, M.L., Psuty, N.P., 2005: Coastal Dunes. *Ecology and Conservation*, 388. *Ecological Studies* 171, Springer-Verlag, Berlin.
- Melo Júnior, J.C.F., Boeger, M.R.T., 2016: Leaf traits and plastic potential of plant species in a light-edaphic gradient from restinga in southern Brazil. *Acta Biológica Colombiana* 21, 51–62.
- Meot-Duros, L., Le Floch, G., Magné, C., 2008: Radical scavenging, antioxidant and antimicrobial activities of halophytic species. *Journal of Ethnopharmacology* 116, 258–262.
- Ogaya, R., Penuelas, J., 2007: Leaf mass per area ratio in *Quercus ilex* leaves under a wide range of climatic conditions. The importance of low temperatures. *Acta Oecologica* 31, 168–173.
- Ola, H.A.E., Abd El-Maboud, M., 2013: Anatomical and physiological responses of three species of *Suaeda* Forssk. ex Scop. under different habitat conditions. *Journal of Applied Sciences Research* 9, 5370–5379.
- Omprakash, R., Gobu, P., Bisen, P., Baghel, M., Chourasia, K.N., 2017: Resistance/Tolerance mechanism under water deficit (drought) condition in plants. *International Journal of Current Microbiology and Applied Sciences* 6, 66–78.
- Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., Olga, A., Keltsieva, O.A., Zhukov, V., Tikhonovich, I., Tarakhovskaya, E., Frolov, A., Wessjohann, L.A., 2018: Methodology of drought stress research: Experimental setup and physiological characterization. *International Journal of Molecular Sciences* 19, 4089.
- Parisod, C., Baudière A., 2006 : Flore du littoral sableux : Description et conservation de la plage rousillonnaise en tant que théâtre écologique de l'évolution. *Bulletin de la Société vaudoise des Sciences Naturelles* 90, 47–62.
- Quezel, P., Santa, S., 1962 : Nouvelle flore d'Algérie et des régions désertiques méridionales, Tome 1, 433–435. Centre National de la Recherche Scientifique, Paris.
- Rucco, M., Bertoni, D., Sarti, G., Ciccarelli, D., 2014: Mediterranean coastal dune systems: Which abiotic factors have the most influence on plant communities? *Estuarine, Coastal and Shelf Science* 149, 213–222.
- Sánchez, J.L., Domina, G., Caujapé-Castells, J., Ciccarelli, D., 2006: The Sicilian circumscription of the genus *Matthiola* (Brassicaceae): population genetic insights from isozymes. *Bocconea* 19, 243–252.
- Sandral, G., Degtjareva, G.V., Kramina, T.E., Sokoloff, D.D., Samigullin, T.H., Hughes, S., Carmen, M., Valiejo-Roman, C.M., 2010: Are *Lotus creticus* and *Lotus cytisoides* (Leguminosae) closely related species? Evidence from nuclear ribosomal ITS sequence data. *Genetic Resources and Crop Evolution* 57, 501–514.
- Saura-Mas, S., Lloret, F., 2007: Leaf and shoot water content and leaf dry matter content of Mediterranean woody species with different post-fire regenerative strategies. *Annals of Botany* 99, 545–554.
- Sayari, N., Saidi, M.N., Assaad, S., Ellouz-Chaabouni, S., Bougatef, A., 2016: Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of *Ononis natrix* leaves extracts. *Free Radicals and Antioxidants* 6, 23–31.
- Shah, S.H., Houborg, R., McCabe, M.F., 2017: Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* 7, 61.
- Singleton, V.L., Rossi, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16, 144–158.
- Slatnar, A., Mikulič-Petkovšek, M., Veberič, R., Štampar, F., 2016: Research on the involvement of phenolics in the defence of horticultural plants. *Acta Agriculturae Slovenica* 107, 183–189.
- Supratim, B., Venkategowda, R., Anuj, K., Pereira, A., 2016: Plant adaptation to drought stress. *F1000 Research* 5, 1554.
- Tátrai, Z.A., Sanoubar, R., Pluhár, Z., Mancarella, S., Orsini, F., Gianquinto, G., 2016: Morphological and physiological plant responses to drought stress in *Thymus citriodorus*. *International Journal of Agronomy*, 8.
- Wdowiak-Wróbel, S., Marek-Kozaczuk, M., Kalita, M., Karaś M., Wójcik, M., Malek, W., 2017: Diversity and plant growth promoting properties of rhizobia isolated from root nodules of *Ononis arvensis*. *Antonie van Leeuwenhoek* 110, 1087–1103.
- Wollenweber, E., Dörra, M., Riverab, D., James, N., Roitman, N.J., 2003: Externally accumulated flavonoids in three Mediterranean *Ononis* species. *Zeitschrift für Naturforschung C* 58(11–12), 771–775.
- Xiao, J., Muzashviti, T.S., Georgiev, M.I., 2014: Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnology Advances* 3.