ANTIOXIDANT ACTIVITIES OF PLANT SPECIES ADAPTED TO COASTAL DUNE OF ZEMMOURI EL BAHRI (ALGERIA)

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Abstract. Coastal dunes are a globally distributed ecosystem characterized by strong internal gradients in disturbance and abiotic stress. When plants are subjected to these environmental stresses, the balance between the production of reactive oxygen species (ROS) and the quenching activity of antioxidants is upset, often resulting in oxidative damage to lipids, protein, and nucleic acids. Plants possess several antioxidant systems that protect them from these cytotoxic effects. In the present work we reported for the first time the results of the study of the antioxidant systems in leaves of ten plant species characteristic of coastal dunes by the measurement of the antioxidant activity of superoxide dismutase (SOD), guaiacol peroxidase (GPOX) and catalase (CAT), as well as total non-enzymatic antioxidant capacity and proline content. Our study results showed a varied response in studied species to abiotic stress in coastal dune. This variation in the response of the studied species with respect to oxidative stress is an intrinsic character. Thus, the following species *Pancratium maritimum, Diotis maritima, Ononis variegata, Cakile maritima* and *Orlaya pumila* were more adapted under stress. The tolerance of these species to abiotic stress firmly linked to higher capacity of CAT, SOD activity and/or GPOX therefore a low MDA content and efficient non enzymatic activity (Total non-enzymatic antioxidant capacity and proline). However other species (*Ammophila arenaria*, *Agropyrum junceum*, *Lotus creticus* and *Salsola kali*) were characterized by a low effective antioxidant defense, with high MDA. We can also conclude that *Eryngium maritimum* is the less affected species with oxidative stress with low MDA and antioxidant activity.

Key words: coastal vegetation; antioxydant enzymes; non-enzymatic antioxidant; proline; malondialdehyde.

INTRODUCTION

The coastal dunes are part of unique ecosystems which are at the spatial transition between continental / terrestrial and marine/aqueous environments. They are distributed worldwide in association with sandy beaches, producing a wide range of coastal dune forms and dimensions related to spatial and temporal variations in sediment input and wind regime [32, 56]. Thus, one of the most outstanding features in these ecosystems is their broad distribution and ecological diversity (in terms of geomorphological dimensions, environmental heterogeneity, and species variability). Dune vegetation is typically arranged in distinct zones along the coast–inland gradient, which are influenced by several abiotic factors including wind, waves, tide, soil salinity, sand grain size, coastal dynamics, and dune morphology [1, 34]. These zones include the embryonic dunes, foredunes (shifting, also called white dunes), semi-fixed dunes and fixed dunes (stable, also called grey dunes). Effects of climate and spatial position on plant community structure differ between shifting and stable dunes [36] embryonic and foredunes are more dynamic due to coastal winds and occasional flooding by sea storms, whereas beyond the foredunes, sand becomes more stable and ecological conditions are more benign. Sandy dune vegetation is well adapted to strong environmental gradients and zonation, allowing for a wide variety of functional groups to coexist within a relatively small area [12, 16].

In Mediterranean regions, apart from these factors, sand dune vegetation is also influenced by strong seasonality, with two distinct periods: rainy, cold winters and dry, hot summers with high irradiance. Thus, plants experience intense stress in the summer, caused by drought, large evaporative demand and high irradiance at high temperatures [12, 16]. In coastal dunes, plants survive to the negative impact of environmental adverse conditions by using one of the three adaptive strategies: escape, avoidance or tolerance to desiccation [38]. Escape strategy occurs when plants develop rapidly and reproduce before drought conditions become severe. The avoidance strategy entails traits that enable plants to resist adverse conditions by preventing their deleterious effects (via enhanced water uptake and reduced water loss) whereas tolerance consists in traits that enable plants to endure adverse conditions (via osmotic adjustment, antioxidant capacity and desiccation tolerance [55]. Regardless, the adaptation of specific plant species to periods of drought stress is not limited to the use of a single mechanism but can use both avoidance and tolerance traits as a mean to survival [50].

Edaphic drought and salinity represent major constraints for coastal vegetation. Plant adaptation or tolerance to these stresses involves complex morphological, physiological, and biochemical responses, which are based on a series of conserved mechanisms, such as protection of the photosynthetic machinery, maintenance of cellular osmotic balance, the accumulation of numerous stress-related proteins and activation of antioxidant systems [26, 27, 49, 51].

When facing different abiotic stresses, plants quickly accumulate the common reactive oxygen species (ROS) as the first layer of defense [10, 70]. ROS generally refers to incompletely reduced oxygen species, including the well studied singlet oxygen

($^1O_2$), superoxide anions (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH*) [15, 48, 62]. ROS have high chemical activity and a relatively short half-life. Due to their inherent features, all types of ROS can damage macromolecules such as proteins, membrane lipids and nucleic acids, eventually leading to cell death [22, 53]. These ROS activate anti-oxidative systems which maintain proper ROS homeostasis in the cell. However, when too many ROS are produced, ROS-associated injury or cell death cannot be avoided [10, 19, 45, 65]. Plants primarily deal with oxidative stress via an endogenous defensive mechanism consisting of different enzymatic and non-enzymatic antioxidants [47].

Major ROS-scavenging enzymes include ascorbate peroxidase (APX), superoxide dismutase (SOD), guaiacol peroxidase (GPOX) and catalase (CAT) [31]. Superoxide dismutase (SOD), leads the frontline defense in the antioxidant system by dismutation O^{2-} into H_2O_2 and reducing the possibility of OH^- formation [28]. In the antioxidant defense system, catalase (CAT) is a tetrameric heme-containing enzyme for ROS detoxification, which converts 26 million H_2O_2 molecules into H_2O in 1 minute [42]. Guaiacol peroxidase (GPOX) breaks down H_2O_2 by oxidation of co-substrates such as phenolics or other antioxidants [31]. Further, this function may be achieved by a concerted action of low molecular weight non-enzymatic antioxidants such as α-tocopherol, ascorbate, glutathione, and phenolic compounds [63]. Other than being an osmoprotectant, proline can act as a potent nonenzymatic antioxidant. As a singlet oxygen quencher [3] and scavenger of hydroxyl radicals [64], proline may be important in preventing oxidative damage caused by ROS. The peroxidation of lipids in biological membranes is the most obvious symptom of oxidative stress in plants. Malondialdehyde (MDA) is one of the final products of oxidative modification of lipids and is responsible for cell membrane damage including changes to the intrinsic properties of the membrane, such as fluidity, ion transport, loss of enzyme activity and protein cross–linking. These changes eventually result in cell death [61].

Little literature references can be found dealing with studies of sand dune vegetation in Algeria. Some old works [66, 71] and relatively recent [30, 44, 58] described the syntaxonomical classification and phytosociological aspects of the main plant groups. There are other works that studied the floristic characteristics of species coastal dune [59]. And more recently, we mention the study of Rabhi et al. (2021) [57], who compared morphological and physiological traits of four plant species in Zemmouri coastal dune.

In the present work we reported for the first time the results of the study of the antioxidant systems in leaves from ten plant species characteristic of coastal dunes by the measurement of the antioxidant activities of peroxide dismutase (SOD), guaiacol peroxidase (GPOX) and catalase (CAT), as well as total non-enzymatic antioxidant capacity and proline content. The consequences of oxidative stress faced by plants will be assessed through the measurement of the content of malondialdehyde (MDA).

The study area located in Zemmouri El-Bahri (East of Algiers), which represents a good model of study in view of its rich flora. Improved understanding of the mechanisms adaptive of coastal plants is required for to better protect this very fragile ecosystem which plays an important role in maintaining the ecological balance of the environment.

MATERIALS AND METHODS

General characteristics of the study area

The study was 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. Zemmouri belongs administratively to the district of Boumerdes and is limited to the north by the Mediterranean Sea, to the South by National Route 24, west by the port of Zemmouri El-Bahri and east by Isser river (Fig. 1). The topography is mainly flat, the

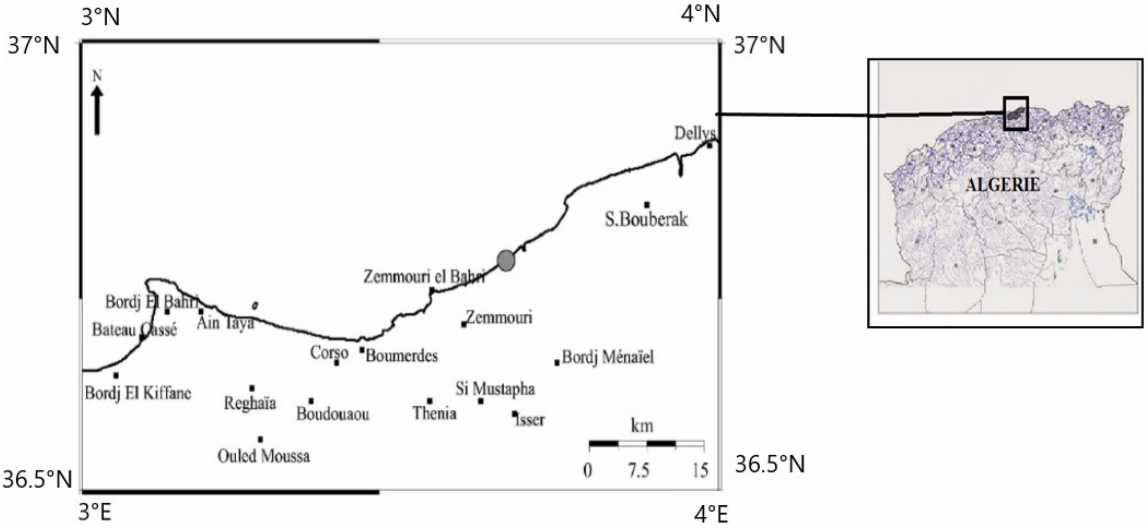

Figure 1. Geographical location of the study area *Zemmouri El-Bahri* (*District of Boumerdes*).

Table 1. List of studied species-(APGIII classification), Raunkier's life forms and Biogeographical types.

N°	Letter code	Species	Family	life forms	Biogeographical types
1	Aa	*Ammophila arenaria* (L.) Link	Poaceae	Ge	Med
2	Aj	[2] (L.) [Syn. *Elytrigia juncea* (L.) Nevski subsp. *Juncea*]	Poaceae	Ge	Atl-Med
3	Cm	*Cakile maritima* (L.) Maire	Brassicaceae	Th	Eur- Med
4	Dm	*Diotis maritima* (L.) [Syn. *Otanthus maritimus* (L.) Hoffmanns & Link]	Asteraceae	Ch	Atl-Med
5	Em	*Eryngium maritimum* (L.)	Apiaceae	He	Eur- Méd.
6	Lc	*Lotus creticus* (L.)	Fabaceae	He	Med
7	Op	*Pseudorlaya* [Syn. *Orlaya pumila* (L.) Grande subsp. *Pumila*]	Apiaceae	Th	Med
8	Ov	*Ononis variegata* (L.)	Fabaceae	Th	Med
9	Pm	*Pancratium maritimum* (L.)	Amaryllidaceae	Ge	Circ-Med
10	Sk	*Salsola kali* (L.)	Chenopodiaceae	Th	Paleo-Temp.

Raunkier's life forms: Ch, Chamaephytes; Hc, Hemicryptophytes; Ge, Geophytes; Th, Therophytes. Biogeographical types: Atl-Med., Atlantic-mediterranean; Eur-Med., Euro-mediteranean; Circ-Med., Circum-mediterranean; Med., mediterranean; Paleo-Temp., Paleo-temperate.

average altitude is 20 m. The hydrographic network is made up of three following main rivers*:* wadi Bergouga, wadi Merdja and wadi Isser. The soil is siliceous. The psammophila vegetation of the littoral is of azonal type, related essentially to the edaphism [59]. 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. The zone is charactered by subhumid bioclimate with hot winter variant [30]. Winds are light to moderate and their strength never exceeds 3. The prevailing wind direction varies between west and north-west in winter, autumn and spring period but in summer the wind direction varies between north-east and east.

Sampling

This study concerned ten plant species (Table 1) which were subjectively harvested along a sea-forest transect in April 2017. The leaf samples destined for the study of enzymatic activity were collected and immediately frozen in liquid nitrogen and stored at -80°C. The samples destined for measurement of proline and MDA content were collected, cleaned, dried in the shade at ambient temperature and stored in air-dried conditions until further use.

Biochemical study of plant species

Extraction and determination of free proline

The content proline in the leaves was determined according to the method described by Troll and Lindlsey (1955) [67] and modified by Magné and Larher (1992) [40]. Sample of 50 mg dry mass of leaves was homogenized with 1 mL of distilled water at 90°C during 30 minutes. After centrifugation at 12000 rpm for 10 min, 500 µL aliquot of the supernatant was mixed with 1 ml of the reagent mixture (60 mL glacial acetic acid, 40 mL distilled water and 1 g ninhydrin) and heated in sealed test tubes at 95°C for 30 minutes. After cooling down, 3 mL toluene was added to each sample. Proline content was read on a spectrophotometer at 520 nm and expressed as $mg.g^{-1}$ DM (dry mass). For this purpose, a standard curve of proline at 10 $\mu g.mL^{-1}$ was plotted. For this purpose, a standard curve prepared using 0, 1, 2, 4, 6, 8 and 10 $\mu g\ mL^{-1}$ of ascorbic acid was plotted.

Total water soluble protein extraction and assay

The soluble proteins were assayed according to Bradford method [14]. For their extraction, 100 mg of leaves were cold ground in 1 mL of extraction buffer (Tris-HCl pH 8.1). Centrifugation was then carried out at 12000 rpm for 20 min at 4 °C. The supernatant was analyzed to measure the protein content by a colorimetry technique using coomassie blue or Bradford's reagent. For this purpose, a standard curve was plotted using 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 and 2 $mg\ mL^{-1}$ of BSA.

This extract was used for measuring CAT and SOD activities.

Catalase (Cat) activity assay

The spectrophotometric assay for measuring catalase activity was carried out according to Anderson et al. (1995) [6]. CAT activity was determined by following the decomposition of H_2O_2 at 240 nm. The reaction medium consisted of 50 mM potassium phosphate buffer at pH 7 (2 mL) and protein extract containing the enzyme (50 µL). The reaction was initiated by the addition of 10 mM H_2O_2 (Extinction coefficient ε = 36 $mM^{-1}\cdot cm^{-1}$). The activity was expressed in nmoles of degraded H_2O_2 per minute and per mg of protein (µmol H_2O_2 $min^{-1}\cdot mg^{-1}\cdot$prot).

Superoxide Dismutase (SOD) activity

The measure of SOD activity was carried out according to the slightly modified technical of Marklund and Marklund (1974) [41]. The evaluation of the autooxidation of pyrogallol was carried out by differential measurement between a control and an assay. The control contained 2.1 mL of 50 mM Tris-HCl buffer at pH 8.2, 1 mM EDTA. The reaction was initiated by adding 100 µL of pyrogallol at 1 mM. The

increase in absorbance at 420 nm was due to the autooxidation of the pyrogallol. The absorbance variation was measured every 30 seconds for four minutes. The assay contained 2 mL of 50 mM Tris-HCl buffer at pH 8.2, 1 mM EDTA and 100 µM of the plant extract. The reaction was initiated by adding 100 µL of 1 mM pyrogallol. The activity of SOD was expressed in units of SOD per minute and per mg of protein.

Guaiacol peroxidase (GPOX) activity

The activity of guaiacol peroxidase was determined according to the method of MacAdam et al. (1992) [39] slightly modified by Boucelha et al. (2019) [13]. This technique is based on the increase in the absorbance at 470 nm due to the polymerization of the guaiacol to tetragaiacol (oxidation) giving an orange coloration in the presence of hydrogen peroxide. The extraction of the enzyme was carried out from 150 mg of leaves, cold crushed in 2 mL of 0.1 M potassium phosphate buffer, pH 6.5. A centrifugation of 20 min at 12000 rpm at 4° C was then carried out. The activity was measured on 700 µL of the enzyme extract to which were added 2.5 mL of the same phosphate buffer used for extraction, 36 mM guaiacol and 10 mM H_2O_2. The reaction begins upon the addition of 100 µL H_2O_2 to the reaction mixture. The activity was monitored as a function of time and expressed in µmoles of oxidized guaiacol per minute and per mg of protein, using the tetragaiacol molar extinction coefficient (ε = 26.6 $mM^{-1}\cdot cm^{-1}$).

Total non-enzymatic antioxidant capacity (TNEAC)

The total non-enzymatic antioxidant capacity was estimated by the method of Prieto et al. (1999) [54]. Samples of 150 mg of leaves were ground in 1 mL of pure methanol. Subsequently, the extracts were macerated at 4° C for 24 h, with stirring. For the estimation, 200 µL of sample extract was mixed with 1 mL of ammonium molybdate reagent. The tubes were incubated in a water bath at 95° C for 90 min. After cooling the mixture to room temperature, the absorbance of the solution was measured at 695 nm. TNEAC was expressed in mg equivalents of ascorbic acid (AAE) per g of fresh vegetable matter (FW). For this purpose, a standard curve of ascorbic acid at 300 $\mu g\cdot mL^{-1}$ was plotted.

Measurement of lipid peroxidation by determination of Malondialdehyde (MDA)

Lipid peroxydation was determined in terms of malondialdehyde (MDA) content by measuring the concentration of MDA, based on the method described by Alia et al. (1995) [4]. The leaf samples (50 mg) were weighed and homogenized in 2 mL of 0.1 % trichloroacetic acid (TCA) solution. The mixture was centrifuged at 12000 rpm for 20 minutes at 4 °C. Then, 0.5 mL of the supernatant was mixed with 0.5 mL of 0.5 % thiobarbituric acid (TBA) in 20 % TCA. The reaction mixture was heated in a water bath at 95 °C for 30 minutes, cooled to room temperature and then centrifuged at 1000 rpm for 10 min. The absorbance of supernatant at 532 nm was determined and nonspecific absorbance of supernatant at 600 nm was subtracted from it. The MDA content was calculated by using the extinction coefficient of ε = 155 $mM^{-1}\cdot cm^{-1}$ and expressed as nmol of MDA per g of FM (fresh mass).

Statistical Test

All experiments were performed in triplicate, each being repeated at least three times, means are plotted as histograms using Excel 2016. Standard errors (Student t-test at $p \leq 0.05$) are shown in the form of bars in the histograms.

RESULTS

Free proline content in leaves

The results of the free leaf proline content showed that most studied species had a low proline content. In fact, the content of 7 species did not exceed 2 $mg\cdot g^{-1}$ DM. The highest proline content was observed in *Ammophila arenaria* (3.83 $mg\cdot g^{-1}$ DM), while the lowest value was recorded in *Salsola kali* (0.28 $mg\cdot g^{-1}$ DM) (fig. 2).

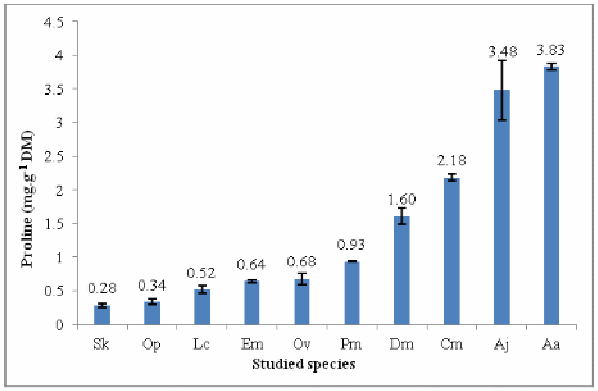

Figure 2. Free proline content in the leaves of the studied species. *For abbreviations (names of species), see table 1*

Catalase activity

The species which were characterized by the highest CAT activity are: *Diotis maritima* (45 nmol H_2O_2 $min^{-1}\cdot mg^{-1}$ prot*)* and *Orlaya maritima* (40 nmol H_2O_2 $min^{-1}\cdot mg^{-1}$ prot). On the other hand, weak catalase activities were recorded in *Ammophila arenaria* (3.2 nmol H_2O_2 $min^{-1}\cdot mg^{-1}$ prot) and *Agropyrum junceum* (3.9 nmol H_2O_2 $min^{-1}\cdot mg^{-1}$ prot) (fig. 3).

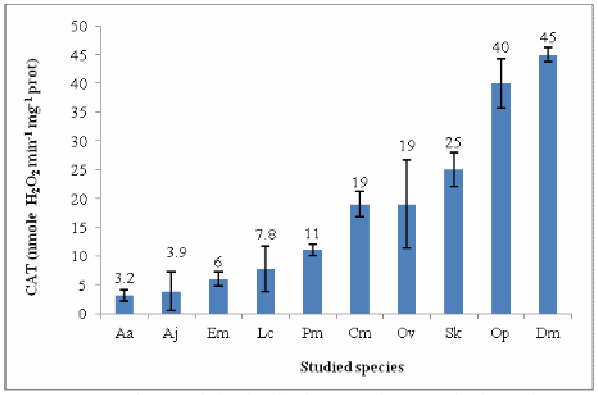

Figure 3. Catalase activity in the leaves of the studied species. *For abbreviations (names of species), see table 1*

Superoxide Dismutase activity

Our results showed that a large fluctuation in SOD values. A higher level of activity observed in *Lotus creticus* and *Salsola kali* with SOD activity that exceeds 18 EU. $min^{-1}\cdot mg^{-1}$ prot. On the other hand, very low values were recorded in the two geophytes *Agropyrum junceum* (1.78 EU. $min^{-1}\cdot mg^{-1}$ prot.) and *Ammophila arenaria* (0.61 EU. $min^{-1}\cdot mg^{-1}$ prot.) (fig. 4).

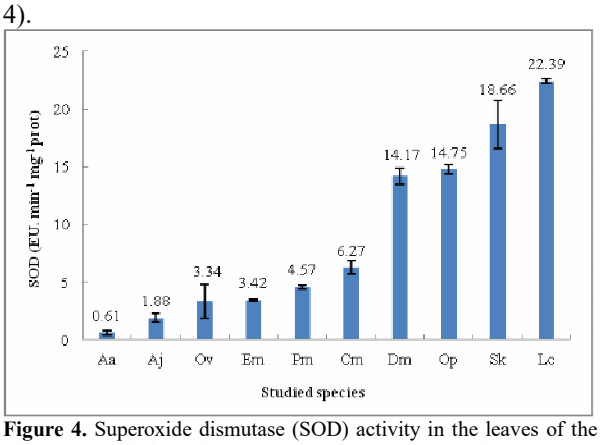

Figure 4. Superoxide dismutase (SOD) activity in the leaves of the studied species. *For abbreviations (names of species), see table 1*

Guaiacol peroxidase (GPOX) activity

A large difference was recorded between the GPOX values measured in the studied species (fig. 9). Thus, one value was remarkably high compared to the others, it was recorded in *Ononis variegate* (44 nmoles of tetraguaiacol formed $min^{-1}.mg^{-1}$ of protein). For other species, the activity was very low, especially for the following species: *Salsola kali, Asparagus acutifolius, Cakile maritima* and *Orlaya maritima,* with values varied between 0.042 and 0.24 nmoles of tetraguaiacol formed $min^{-1}\cdot mg^{-1}$ of protein (fig. 5).

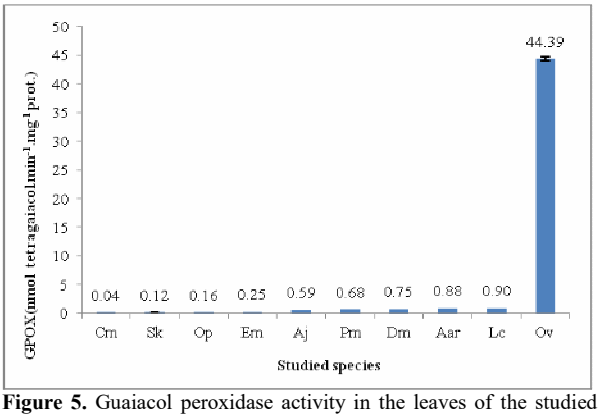

Figure 5. Guaiacol peroxidase activity in the leaves of the studied species. *For abbreviations (names of species), see table 1*

Total non-enzymatic antioxidant capacity (TNEAC)

The values of non-enzymatic antioxidant oscillates between a maximum value of 125.17 AAE $mg\cdot g^{-1}$ DM and a minimum value 3.47 AAE $mg\cdot g^{-1}$ DM obtained from *Diotis maritima* and *Eryngium maritimum*, respectively (fig 6).

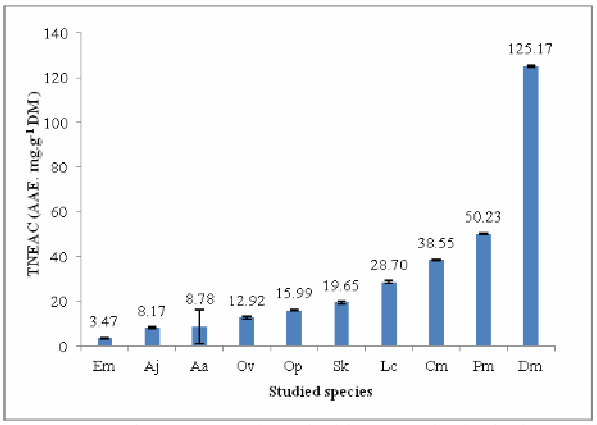

Figure 6. Total non-enzymatic antioxidant capacity in the leaves of studied species**.** *For abbreviations (names of species), see table 1*

Study of lipid peroxidation by assaying Malondialdehyde (MDA)

The MDA contents for most of the species studied fluctuated between 5 $nmole\cdot g^{-1}$ FM and 15 $nmole\cdot g^{-1}$ FM. The highest value was recorded in *Agropyrum junceum* with 17.96 $nmole\cdot g^{-1}$ FM of MDA. Whereas, the lowest value was recorded in *Diotis maritima* with 4.97 $nmoles\cdot g^{-1}$ FM (fig. 7).

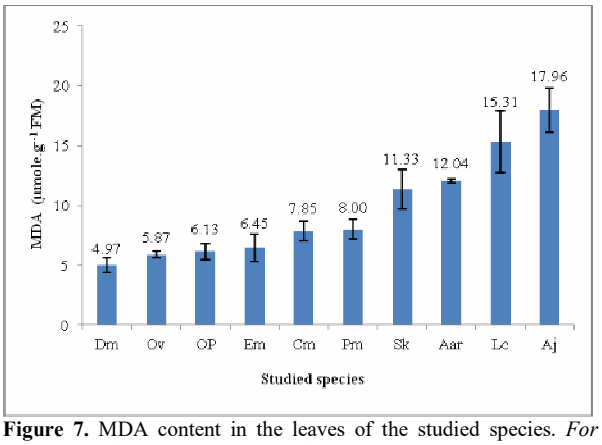

Figure 7. MDA content in the leaves of the studied species. *For abbreviations (names of species), see table 1*

DISCUSSION

The studied plants differ in their enzymatic and non-enzymatic antioxidant capacity, even if they are developed in the same environment and undergoing the same stressful conditions. Our results did not show a relationship between the different parameters measured (proline, non-enzymatic antioxidant activity) and the position of the species along the sea-forest transect, with the upper beach and embryonic dune vegetation closer to the sea and hence more exposed to salt spray, winds and silting up than mobile dunes and especially backdunes progressively less exposed to these harsh environmental constraints [17, 20, 23, 24]. It is obvious that the species installed on the littoral dune exhibit stress tolerance both on the embryonic dune and in the semi-fixed dune because of water scarcity, high temperatures, and high irradiance are often limiting factors also in backdune areas. Therefore, the response

of the studied species with respect to oxidative stress is an intrinsic character. The level of antioxidant enzymes activity varied with plant species, type and intensity of environmental stress and stage of development [5, 46].

MDA is an indicator of cellular oxidative damage in plants exposed to abiotic stresses and, then, low MDA content appears to be a characteristic of drought tolerant plants. Thus, MDA level is a function of the plant's ability to protect themselves against oxidative agents [8].

Eryngium maritimum species from embryonic dunes, presented the lowest MDA content and the lowest antioxidant activities, especially non-enzymatic activity. These results agree with those reported by Meot-Duros et al. (2008) [43] who evaluated the capacity of three halophytic species from coastal dune (*Eryngium maritimum* L., *Crithmum maritimum* L. and *Cakile maritima*). These authors demonstrated that *Eryngium maritimum* exhibited the lowest phenolic and total antioxidant capacity level. We can conclude that this species was not stressed since it is adapted due to its morphological characteristics. According to Ivanova et al. (2015) [35], *Eryngium maritimum* is one of the most salt tolerant plants. They have also been shown that the existence of *E. maritimum* in saline and dry soils is made possible thanks to others adaptive mechanisms by the specific microstructure of leaf, adaxial and abaxial surfaces of which have well-developed cuticle and stomata slit placed below the surface of the epidermis. The presence of a large amount of saturated fatty acids provides decrease of membrane permeability and better resistance against soil salinity. The optimization of water uptake by *Eryngium maritimum* is linked to their highly developed root system [9]. The roots can grow down about 4 meters into the sand [60].

We also noticed that the cell membrane integrity was little affected in the following species: *Pancratium maritimum, Diotis maritima, Ononis variegata, Cakile maritima Orlaya pumila* and *Ononis variegata* which may mean greater ability to avoid oxidative stress damages. These species used a different mecanisms for this. Thus, *Pancratium maritimum* showed a high nonenzymatic antioxidant value and a relative low enzymatic antioxidant activities. As for *Orlaya pumila, Diotis maritima* and *Cakile maritima,* in addition of high or moderate nonenzymatic antioxidant capacity, they showed relatively high CAT and SOD activities. *Ononis variegata* showed the highest GPOX activity. The other species are characterized by a relative membrane alteration with low antioxidant activity and a high proline content (*Agropyrum junceum* and *Ammophila aenaria*) or despite a high or moderate antioxydant activity (*Salsola kali* and *Lotus creticus*).

Ahmadizadeh et al. (2011) [2] suggested that genotypes with high SOD and CAT activities are the most resistant to the action of stress and show a more efficient antioxidant system. The role of GPOX remains regular but secondary in the mobilization of H_2O_2 because it has the low capacity to eliminate ROS compared to CAT [29]. According to Zlatev et al. (2006), high GPOX activity could be a sign either of the severe oxidative stress or an efficient stress response mechanism [72]. According to Hosseinkhani Hezave et al. (2015) [33], the activity of GPOX varies considerably depending upon plant species and stress condition. Another exception was recorded in *Pancratium maritimum* which was characterized by a strong TNEAC and a low enzymatic activity compared to other studied species. It seems, for this species, that the non-enzymatic antioxidant mechanisms were sufficient to protect against oxidative stress. Non-enzymatic antioxidants mainly act in concert to scavenge the ROS and stabilized the lipid membrane by interacting with the polyunsaturated acyl groups of lipids for increasing plant survival under harsh environmental conditions [68].

According to Arbona et al. (2008) [7], plants with high levels of MDA without causing any symptoms can withstand the effect of oxidative stress. In our study, the strongest accumulation of MDA was recorded in *Ammophila arenaria* and *Agropyrum junceum* which were characterized by the weakest antioxidant defense system both enzymatic and non-enzymatic. In these Poaceae species, proline which was highly accumulated could be involved in antioxidant activity. The adaptation of these xerophytes and halophytes to the water stress, saline stress and silting up is visible also on the morphological and anatomical levels. These species are characterized by fibrous root and the rhizome is well developed allowing a good fixing of the plant in the sand and a good water provision [18, 19]. In *Ammophila arenaria*, the bulliform cells, located at the base of the crypts, ensure an osmoregulation which controls the movement of the leaf blade [18]. This leaf rolling is an adaptation to the drought [21, 52]. The upper epidermis contains stomata, which are inserted in the stomatal crypts which, with existing hairs, reduce movement of air, which decreases the transpiration [14, 21, 52].

A strong accumulation of MDA in *Lotus creticus* and *Salsola kali* was related to an imbalance in their enzymatic antioxidant defense system. For *Lotus creticus*, it was recorded a low catalase activity, this could generate an overaccumulation of H_2O_2 which is harmful for the membrane integrity. Several authors showed that CAT plays a major role in reducing the average H_2O_2 content, which are harmful for cell integrity [25, 69]. However, in some cases and under stressful conditions, CAT activity is reduced due to sensitivity to stress [37]. Benkaddour (2014) [11] showed that salt stress caused a progressive inhibition of catalase activity (CAT) in the leaves of three varieties of wheat with increasing doses of salt. In *Salsola kali*, a high MDA content could result from the imbalance of intracellular ROS levels, either due to their overproduction or due to inefficiency of antioxidant mechanisms despite the higher antioxidant activity.

In conclusion, our study results showed a varied response of studied plant species to abiotic stress in coastal dune. This variation in the response of the studied species with respect to oxidative stress is an intrinsic character. Thus, the following species *Pancratium maritimum, Diotis maritima Ononis variegata, Cakile maritima* and *Orlaya pumila* can be considered as more adapted to this environment. The tolerance of these species to abiotic stress is firmly linked to higher capacity of CAT, SOD and/or GPOX activities, a low MDA content also and an efficient activation of non enzymatic antioxidant systems (TNEAC and proline). On the other hand, other species (*Ammophila arenaria*, *Agropyrum junceum*, *Lotus creticus* and *Salsola kali*) were characterized by a less effective antioxidant defense, with high MDA. Finally, we can consider that *Eryngium maritimum* is the least affected species by oxidative stress generated by abiotic stress.

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Received: August 19, 2021
Accepted: January 11, 2022
Published Online: January 15, 2022
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House