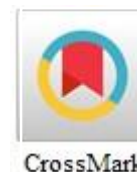




Eco-Morphological and Physiological Behaviours of *Atriplex halimus* L. Under Different Natural Habitats



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Abstract: *Atriplex halimus* is a widely distributed species in the Mediterranean coastal areas and can grow in saline and non-saline habitats. Plant leaves were collected from two habitats, non-saline (1.14 dSm⁻¹) and saline (30.63 dSm⁻¹) at Borg Alarab area on the North-Western coast of Egypt, to investigate the eco-morphological and physiological behaviour variations of *A. halimus* under different habitats. Leaf area and specific leaf area (SLA) were measured while moisture, chlorophylls a and b, carotenoids, Na⁺, K⁺, Ca⁺⁺ and Cl⁻ contents were determined. *A. halimus* leaves generate adaptive changes as plastic responds to salinity by reducing leaf area, SLA, chlorophylls a and b, and Cl contents but expressed an increase of phenyl-alanine ammonia-lyase (PAL) specific activity as well as Na⁺ and total phenol contents.

1 Introduction

Plants that grow naturally in semi-arid habitats have physiological and morphological adaptations that permit plants to deal with environmental stressors. When plants are grown in different ecological conditions, their resistance differs amongst individuals of the same species; for example, desert plants have distinct stress responses that differ from those of other plants. They have a variety of adaption features that allow them to tolerate and be protected from environmental challenges (Batanouny 2001). The shift in microtopography caused by various edaphic and climatic conditions may force populations of some species to evolve

local adaptive functional features (Hegazy 2001, Lobo et al 2003).

Atriplex halimus L. (Amaranthaceae) (Mediterranean saltbush) is widespread in arid and semi-arid regions of the Mediterranean basin. *A. halimus* is used as an energy crop, fodder, phytoremediation of contaminated soils, and soil remediation (Chisci et al 2001, Gharibeh et al 2023). It thrives on a wide range of soils, from fine to coarse, with varied salt levels (Walker et al 2014). It is economically significant; besides, its populations from various environmental conditions have been examined for the features that contribute to their adaptability to environmental stresses, including salinity, drought, severe temperatures and excess trace elements (Walker et al 2014). The present work aims to

identify the ecophysiological behavior variations of *A. halimus* in saline and non-saline habitats.

2 Materials and Methods

Plant leaves and associated soils were collected from two sites in Borg Alarab area. The GPS reading for the first site (roadside, non-saline habitat) is N: 31 00.728 and E: 29 44.614, while the second site (salt marshes, saline habitat) is N: 30 55.454 and E: 29 31.553.

Soil mechanical analysis, organic matter (O.M) content, electrical conductivity (EC), pH, and cations and anions were measured in soil paste according to Estefan et al (2013).

Leaf area (cm²) was measured (20 leaf) by ImageJ software. The leaves were then oven-dried for 24 hours at 70°C to estimate their dry weight. Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry mass (cm² g⁻¹) (Cutini et al 1998). The water content percentage was calculated as:

$$\%WC = (Fw - Dw / Fw) * 100$$

Where %WC is the water content percentage, Fw is the fresh weight and Dw is the dry weight.

Moran (1982) technique was used to extract chlorophylls (chl a and chl b) and carotenoids. The pigments were assessed using a UV/Vis spectrophotometer (T60 PG Instrument Limited, China) at 664, 647, and 470 nm for chl a, chl b, and carotenoids, respectively. The concentrations of chl a, b and carotenoids were measured according to Lichtenhaler and Wellburn (1983). The soluble proteins were estimated in fresh frozen leaves according to the method of Bradford's (1976). The specific activity of phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5) was estimated using the Lister et al (1996) technique. The total soluble phenolic compounds in leaves powder were estimated at 80% MeOH using the Folin-Denis technique according to Shahidi and Naczk (1995). Ions (Na⁺, K⁺, Ca⁺⁺, Cl⁻) were determined in dry leaves according to Estefan et al (2013). All data were statistically analyzed as a Completely Randomized Design (CRD) by SAS (2006). The significant differences between the means were compared using Tukey's Studentized Range (HSD) test at $p \leq 0.05$.

3 Results and Discussion

Data in **Table 1** revealed that *A. halimus* grows in alkaline soil, with a sandy loam texture. The EC was 1.14 dSm⁻¹ at site 1 (roadside habitat) and

30.63 dSm⁻¹ at site 2 (salt marshes habitat). The leaf area and SLA decreased significantly by 1.75 and 1.29-folds, respectively, at saline compared to non-saline habitat as represented in **Table 2**. The water content had no significant change between the studied habitats.

A. halimus is a wide ecological species, highly adapted to xeric and saline conditions along the Mediterranean zones. The decrease in leaf area and SLA are in agreement with those obtained by Bendaly et al (2016). They found the highest growth in *A. halimus* at 100 mM NaCl then decreased significantly at higher salinity. According to Reef and Lovelock (2015), a reduction in leaf area might be viewed as an adaptation strategy to salinity and drought that conserves water under stress conditions. De la Riva et al (2016) suggested that decreasing SLA contributes to nutrient retention, extended leaf life span, and protection against dehydration which is consistent with the prevailing harsh environmental conditions in site 2 (salt marshes habitat).

Our study explains the alteration in some physiological characters in *A. halimus* as a response to salinity. The water content is suggested as an index of water status in plant tissue (Abd El-Maboud et al 2023). The stability in water content can be explained by the accumulation of ionic composition in roots under salinity stress to keep the osmotic balance. Similarly, the water content in *Juncus rigidus* showed no significant change under 0, 200, and 400 mM NaCl treatments (Abd El-Maboud et al 2023).

The chl a, chl b and total chl were found to decrease in plants growing at saline by (1.36, 1.69, and 1.43-fold), respectively, while carotenoids showed no significant change. The total soluble phenols increased by 1.15-fold in leaves collected from saline compared with those in non-saline habitats as shown in **Table 2**.

Due to salinity stress in site 2, *A. halimus* responds by a reduction in growth parameters and a reduction in the photosynthesis process. The reduction in photosynthesis results in frustrated chlorophyll biosynthesis or impaired photosynthetic apparatus. Normally, photosynthetic pigments are the essential ingredient of energy metabolism and play a crucial role in the adaptation of plants in harsh environments (Rangani et al 2018). The reduction in chl a, chl b, and total chl by salinity implies a strategy to protect the photosynthetic system from salt-induced photo-injury and avoid excessive light absorption and excessive ROS biosynthesis or accelerate pigment degradation. Carotenoids are insensitive to salinity in salt marshes' habitat; they play an important role in photosynthesis by protecting chloroplasts from light oxidation and stabilizing chloroplast membranes. Carotenoids can also scavenge reactive oxygen species (ROS) protecting plants from stress-induced oxidative damage (Cazzonelli 2011).

Table 1. Physical and chemical analysis of soil supporting *A.halimus* at two different habitats

Soil physical analysis											
Habitat	Clay%	Silt%	Fine Sand%	Coarse Sand%	Texture	Moisture content (%)					
Roadside	16.41	24.05	7.95	51.59	Sandy loam	9.90					
Salt marshes	14.90	22.95	8.86	53.29	sandy loam	5.40					
Soil chemical analysis											
Habitat	ECe (dS/m)	TDS (ppm)	pH	O.M %	Soluble anions (meq/l)			Soluble cations (meq/l)			
					HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na+	K+
Roadside	1.14	728	8.37	0.8	2.39	7.62	1.37	1.99	2.18	6.60	0.60
Salt marshes	30.63	19600	8.16	1.64	4.31	205.2	96.75	53.6	58.8	177.6	16.2

Table 2. leaf area, SLA, Water content, chl. a, chl. b, total chl, carotenoids, total soluble phenols, K⁺, Ca²⁺, Na⁺, Cl⁻, in *A. halimus* leaves collected from two habitats

Parameters	Roadside	Salt marshes	LSD at 5%
Leaf area (cm ²)	2.36 ^a ± 0.55	1.35 ^b ± 0.31	0.2858
SLA (cm ² /g)	97.5 ^a ± 14.7	75.6 ^b ± 7.2	7.398
%Water Content	78.42 ^a ± 3.15	78.13 ^a ± 2.8	n.s
Chl. A (mg/g Fw)	0.68 ^a ± 0.011	0.5 ^b ± 0.011	0.0227
Chl. B (mg/g Fw)	0.22 ^a ± 0.029	0.13 ^b ± 0.19	0.0293
Ch. (a+b) (mg/g Fw)	0.9 ^a ± 0.16	0.63 ^b ± 0.18	0.0585
Car. (mg/g Fw)	0.15 ^a ± 0.016	0.13 ^a ± 0.019	n.s
PAL (unit mg-1 protein)	26287 ^b ± 1082	314078 ^a ± 19556	31397
Total soluble phenols (g/100g Dw)	2.82 ^b ± 0.075	3.24 ^a ± 0.078	0.1729
% Na ⁺	1.4 ^b ± 0.05	1.71 ^a ± 0.01	0.0962
% K ⁺	2.374 ^a ± 0.05	2.165 ^a ± 0.07	n.s
Na ⁺ /K ⁺ ratio	0.59 ^b ± 0.012	0.79 ^a ± 0.011	0.0684
% Cl ⁻	2.45 ^a ± 0.06	2.1 ^b ± 0.01	0.1983
% Ca ²⁺	0.33 ^b ± 0.03	0.485 ^a ± 0.09	0.1076

Data are shown as mean ± standard deviation, means followed by different letters are significantly different at (P≤.05) according to the LSD test. n.s. = non-significant.

The multiple increases in PAL-specific activity (11.95-fold) associated with the increase in total soluble phenols in *A. halimus* at salt marshes (**Table 2**) reflects how important salinity response mechanisms. PAL has a great role in secondary metabolites biosynthesis joining phenolic biosynthesis and plant growth regulation (Li et al 2019). Under stress conditions including salinity, PAL gene activity rises, and the PAL gene prompts the construction of phenolic compounds and tannins (Macias-Bobadilla et al 2020, Osman and El-Naggar 2022). In addition, total phenols increase the antioxidant system by developing the scavenging capacity against ROS (Waśkiewicz et al 2014). This increase in PAL activity in plants collecting from salt marsh habitats could explain the above-mentioned increase in total soluble phenols. This physiological response may be considered an important adaptive regulatory behavior in the harsh environments of *A. halimus* plants. Our results are in line with many authors (Abd El-Maboud and Abd Elbar 2020) in *Limoniastrum monopetalum*, Abd El-Maboud and Elsharkawy (2021) in *Sarcocornia fruticosa* and *S. perennis*.

The Na⁺ content increased by 1.21-fold in *A. halimus* grown under saline condition (salt marshes) compared with those in non-saline habitats (roadside). Simultaneously, there is no significant difference in K⁺ concentrations between the two *Atriplex* populations in the two different habitats (**Table 2**). Potassium has many physiological functions in plants including maintaining balance with Na⁺ which is considered a key salinity tolerance mechanism. Na⁺/K⁺ ratio was lower in plants collected from saline habitats than in plants grown in non-saline condition. This result explains that the halophyte *A. halimus* can select ion uptake even with a high concentration of Na in soil. In halophytes, the cellular Na⁺ and K⁺ uptake distinctly are unlinked. These halophytes can maintain stable rates of K⁺ uptake even under wide ranges of external Na⁺ and high rate of Na⁺ uptake (Flowers et al 1977, Glenn et al 1999). Consequently, it is unlikely that Na⁺ leaks into the halophyte cells via K⁺ carriers. The Ca²⁺ content in leaves of the studied species increased by 1.47-fold, while the Cl⁻ decreased by 1.16-fold in those in saline as compared to those in non-saline habitats. Under salinity stress, plant cells tend to accumulate Na⁺ and Ca²⁺ to adjust osmotic potential (Abd El-Maboud and Abdelbar 2020). Our results are supported by Pan et al (2016) who observed the increase of Na⁺ and

decrease of K⁺ as NaCl treatment increased in different tissues of *Atriplex canescens*.

4 Conclusion

Plastic responses within an individual's habitat along environmental gradients can cause adaptive changes in leaf characteristics. Our data showed that *A. halimus* can tolerate salinity by morphological changes including a decrease in leaf area and SLA, as well as physiological alterations expressed by a decrease in photosynthetic assimilation to enhance water use efficiency, and accumulation of Na ions. In addition, PAL activity increased significantly leading to an increase in the total soluble phenol accumulation.

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