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Seasonal Effects on Some Eco-Morphological and Physiological Characters of *Tamarix nilotica* (Ehrenb) Bunge Growing Naturally in Egyptian Northern Coastal Salt Marshes

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Abstract: *Tamarix nilotica* is a perennial halophyte growing naturally at the Mediterranean coastal salt marshes. Vegetative parts were collected in two successive winters and summers to investigate the seasonal effects on the ecomorphological and physiological responses of *T. nilotica*. The results indicated that either in winter or summer seasons, *T. nilotica* can reduce the effect of soil salinity by excreting salts outside its body through salt glands. Summer season was characterized by low content of soil moisture (due to rare rainfall), high soil EC, high light intensity and high temperature; therefore, plant induced certain morphoanatomical changes in leaves and stem to face the previously mentioned adverse conditions. The most remarkable changes to reduce transpiration process was found by decreasing leaf area and increasing cuticle thickness and mesophyll tissue thickness. In addition, the most marked physiological changes in summer were the significant increase in total phenols, proline, free amino acids and total soluble sugars. These compounds can work as osmotic regulators and/or antioxidants. These features enhance the defensive mechanism against dehydration and permit *T. nilotica* to tolerate the stress conditions in salt marsh habitat.

1 Introduction

The climate of Egypt varying from semiarid to extremely arid climate; it is divided into three types: the Red Sea climate, the Mediterranean climate of the northern coastal areas and the desert climate (El-Marsafawy et al 2019). The Northwestern section of the Mediterranean coast (Mariut coast) runs from the Libyan border to Alexandria. The area is distinguished by semiarid conditions with seasonal drought in warm summer months and cold with sudden high rainfall in the winter months. The flora in the Northwestern coast is comparatively rich and it contains around 50% of the total flora of Egypt (Zahran and Willis 2009, Abd El-gawad and Shehata 2014). There are various types of habitats in the Western Mediterranean region such as salt marshes which is a widespread habitat in the area. Predominant plant species in this habitat are adapted to dominant salinity or drought stress. Salt marshes are remarkable habitat, inhabited by a considerable number of halophytes which have many economic uses. (El- Shaer and El-Morsy 2008, Abd el-gawad and Shehata 2014).

The ability of plants to adapt to stressful environments is related to the plasticity of morpho-anatomical structure combined with the physiological factors. Studies of plant adaptation to environmental stresses are important for understanding the adaptive mechanisms of plant species. It is indispensable to quantify the tolerance variances among halophytic species to salinity as a foundation for valuating their potential

utilization (Salsinha et al 2020). One of the distinctive plants of salt marches is *Tamarix nilotica* which is halophytic shrub or tree belongs to the family of Tamaricaceae. Tamarix plants can secrete salts by salt glands as a mechanism of salt tolerance. *T. nilotica* has many pharmacological activities, including the effects as antidiabetic, antioxidant, anti-tumor, antimicrobial and antiviral activity. The wood can be used to make charcoal, as a fuel, and sometimes used for some inferior carpentry and to stabilize sand (Brock 1994, Abdelgawad 2017). *T. nilotica* is rich in bioactive compounds e.g. phenols, ascorbic acid and natural pigments (Abd El-Maboud 2019). This work aims to study the morphological, physiological and biochemical adaptations of *Tamarix nilotica* collected from salt march under stress conditions.

2 Materials and methods

2.1 Collection of plant materials from the study area

Fresh shoot samples of *Tamarix nilotica* were collected randomly from 20 plants during the end of winter (wet season) in March and the end of summer (dry season) in September for two successive years 2018-2019. Samples were collected from salt marsh habitat of the Northwestern coast of Egypt at the city of Borg Al-Arab; the geographical position system (GPS) reading perusing is 30°55.259 N, 29°31.425 E.

2.2 Climate data

According to Abdel Moghith et al (2015), the study area belongs to subtropical Mediterranean climate. Climate data of the study area was taken from Borg Al-Arab meteorological station, the central laboratory for agriculture climate. Maximum and minimum average values of temperature (°C), rainfall (mm/month), the relative humidity (%) and solar radiation (MJ/m^2/day) were documented for the selected area.

2.3 Soil analysis

Soil samples were collected from the study area at a depth of 0-50 cm. Mechanical analysis was executed by the method described by Kilmer and Alexander (1949). Electrical conductivity in soil water extract (1:5) was determined; pH values, cations including (sodium and calcium) and anions including (chloride, bicarbonate and Sulphate) were determined according to Estefan et al (2013). The results of cations and anions were expressed as meq/L.

2.4 Morphological and anatomical examinations define location of samples

Fresh middle parts of the wintry and summery newly shoots were separated to assay the morphological and anatomical traits of *Tamarix nilotica* as follows: 40 leaves were used to measure the leaf area (mm^2) by ImageJ software. For the anatomical features, small samples (about 0.5 to 0.1 cm in length) were cut from the wintry and summery shoots and fixed in FAA solution (Formalin, Acetic acid, 50% ethyl alcohol 5:5:90 by volume) for 24 h. The Paraffin method of Abd Elbar (2017) was used. Serial of transverse sections at10-12 µm were made by LEICA rotary microtome model RM 2125 RTS. The sections were double-stained with safranin and fast green. Anatomical observations were performed using a LEICA light microscope model DM-500 supplied with a digital camera. The following measurements were taken: the cuticle thickness, epidermis thickness, palisade tissue thickness on both abaxial and adaxial surfaces, the spongy tissue thickness and the lamina thickness was measured (in the middle zone).

2.5 Physiological parameters

2.5.1 Determination of water content and degree of succulence

Water content was determined according to the following equation:

Water content= 100 ((Fresh weight–Dry weight)/Fresh weight) the degree of succulence was calculated according to the method described by Hoolbrook and Putz (1996), as initial fresh weight/dry weight ratio.

2.5.2 Determination of photosynthetic pigments

To extract chlorophylls (ch. A and ch. B) and carotenoids, Moran (1982) method was used as follows: 0.1g of *T. nilotica* frozen shoots was grinded by a mortar and pestle with 10 ml *N*,*N*-dimethyl formamide. The resulting extract was left in the dark overnight. The pigments were determined 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 chlorophyll A, B concentrations were determined by using Lichtenhaler and Wellburn (1983) formula, while the carotenoid concentration was calculated using Shlyk (1971) formulas:

Chlorophyll a = 9.784 * A_{664} – 0.990 * A_{647} = mg/L
Chlorophyll b = 21.426 X A_{647} – 4.650 X A_{664} = mg/L
Carotenoids = [(4.695 × A_{470}) - 0.268 (Chl. a + b)] = mg/L

The data were stated as mg/g fresh weight.

2.5.3 Determination of total phenols

Known weight of shoot (0.5 g) was mixed with 5 mL ethanol 80% and kept for 24 h at 0˚C. Sample extraction was repeated three times, then filtered. To determine total phenols content, Singleton and Rossi (1965) method was followed. Absorbance was documented at 650 nm in UV/Vis spectrophotometer. The concentration of total phenols was determined using a standard curve of catechol and calculated as mg catechol/g fresh weight**.**

2.5.4 Determination of proline

The concentration of proline was estimated according to the method of Troll and Lindsley (1955) modified by Petters et al (1997). The color was documented at 515 nm in UV/Vis spectrophotometer. The concentration of proline was calculated using L-proline as a standard curve and expressed as µmole proline/g fresh weight.

2.5.5 Determination of total soluble sugars

Fresh parts of the shoot were used to extract the soluble sugars with ethanol 80% according to **AOAC (2007)**. The total soluble sugars were determined by the method of Dubois et al (1956). The absorbance of the extract was documented at 490 nm in UV/Vis spectrophotometer. Total soluble sugar concentration is estimated using D-glucose as the calibration standard and calculated as mg/g fresh weight.

2.5.6 Determination of free amino acid

Free amino acids were extracted from the shoot with ethanol 80% according to AOAC (2007). It is determined by the method of Jayaraman (1981). The absorbance was documented at 570 nm in a UV/Vis spectrophotometer. Free amino acids concentration is estimated using glycine as the calibration standard and calculated as mg/g fresh weight.

2.6 Statistical analysis

Obtained results were analyzed by statistical one-way analysis of variance (ANOVA) using (Costat software, Version 6.4, 2008) computer program. The significance between means was tested by LSD at 5% significant level.

3 Results and Discussion

3.1 Climate data

In accordance with metrological data of the study area during 2018 to 2019 **(Table 1),** the region is characterized by higher temperature, light intensity and solar radiation in summer than in winter. The winter season was rainy while the summer season was almost rainless. So, the climate of the region belongs to the warm coastal desert.

3.2 Soil characters of the study habitat

Table 2 shows that the soil texture of the site of *Tamarix nilotica* was sandy clay loam. Also, the habitat of the investigated species was alkaline (pH ranging between 9.8 and 10). The soil moisture content was lower in summer. In general, electrical conductivity was high and higher in summer, indicating a high salinity. Also, sodium, chloride and calcium increased in summer.

3.3 Morphological and anatomical responses

T. nilotica is a perennial shrub that has many thin branches **(Fig 1 A)** with chestnut bark. The young branches are cylindrical and solid while cladophyll has reddish-green color. The leaf is scaly, simple, sessile, whorled, oblong-lanceolate, has green color, entire margin with acute apex **(Figs 1 B&C)**. The lamina cross-section of both the winter and summer seasons **(Figs 2 A, B, C & D)** revealed that the leaf consists of a uniseriate epidermis. The epidermal cells of the adaxial surface have tangentially elongated shapes while the abaxial cells are slightly isodiametric. Some epidermal cells are papillose (especially the adaxial cells). Several stomata and salt glands were observed (**Figs 2 E&F)**. The mesophyll differentiates into palisade tissue toward adaxial and abaxial surfaces and isodiametric chlorenchymatous cells. The palisade cells of the abaxial surface (facing the light) are more elongated than the adaxial ones (**Figs 2 A&B)**. The midvein has a vascular bundle that consists of approximately 2 or 3 rows of xylem elements, phloem and phloem fibers (**Figs 2 C&D)**. Data in **Table 3** show

Table 1. The climate data of the study area in winter and summer season

	2018						2019					
	average temperature (°c)						average temperature (°c)					
	max.	min.	mean	Solar Radiation ($MJ/m^2/day$)	Relativ Humidity (%)	Rainfall (mm/ month)	max.	min.	mean	Solar Radiation ($MJ/m^2/day$)	Relative Humidity (%)	Rainfall (mm/ month)
January	18.11	10.25	13.73	12.53	69.10	40.98	17.08	7.82	11.83	13.42	61.45	10.13
February	20.72	10.82	15.18	15.06	65.21	11.60	18.92	8.71	13.17	15.68	64.10	10.56
March	20.72	10.82	15.18	15.06	55.56	1.27	20.63	10.55	15.02	19.72	63.52	18.06
April	27.06	14.49	20.15	24.43	54.67	5.63	24.58	12.83	18.22	24.54	56.36	4.26
May	30.88	18.70	24.19	26.95	54.13	0.02	31.42	17.31	23.86	27.52	46.09	0.01
June	32.97	21.21	26.71	29.54	51.97	0.00	32.84	21.51	26.77	28.80	56.96	0.00
July	33.93	22.81	27.86	29.47	58.14	2.42	34.17	23.06	28.15	29.42	55.89	0.00
august	33.66	23.51	27.99	27.18	59.98	0.00	34.31	23.09	28.19	27.36	58.09	0.00
September	32.26	22.33	26.58	23.34	60.57	0.00	31.64	21.79	26.07	23.61	60.46	0.00
October	28.98	19.82	23.69	18.96	61.38	3.06	29.89	20.20	24.32	18.54	62.52	25.34
November	24.46	16.06	19.69	14.23	63.13	20.73	26.57	16.54	20.73	14.90	61.77	0.09
December	19.38	12.60	15.52	12.60	67.21	11.25	20.31	12.54	15.78	11.89	67.61	28.67
Annual	26.93	16.95	21.37	20.78	55.78	96.96	26.86	16.33	21.01	21.28	59.57	97.12

Table 2. Physical and chemical properties of soil supporting *T. nilotica* in winter and summer seasons

Sand %	Silt %	Clay %	texture
47	25.5	27.5	Sandy clay loam

Season	Moisture content (%)	pH	EC (ds/m)	SO_4^{-2} (meq/L)	CL^- (meq/L)	HCO^-_3	Na^+ (meq/L)	Ca^+ (meq/L)
Winter	26	10	47.84	61.68	371.19	2.12	269.57	66.67
Summer	17.5	9.8	54.28	202.98	426.27	2.36	410	74.24

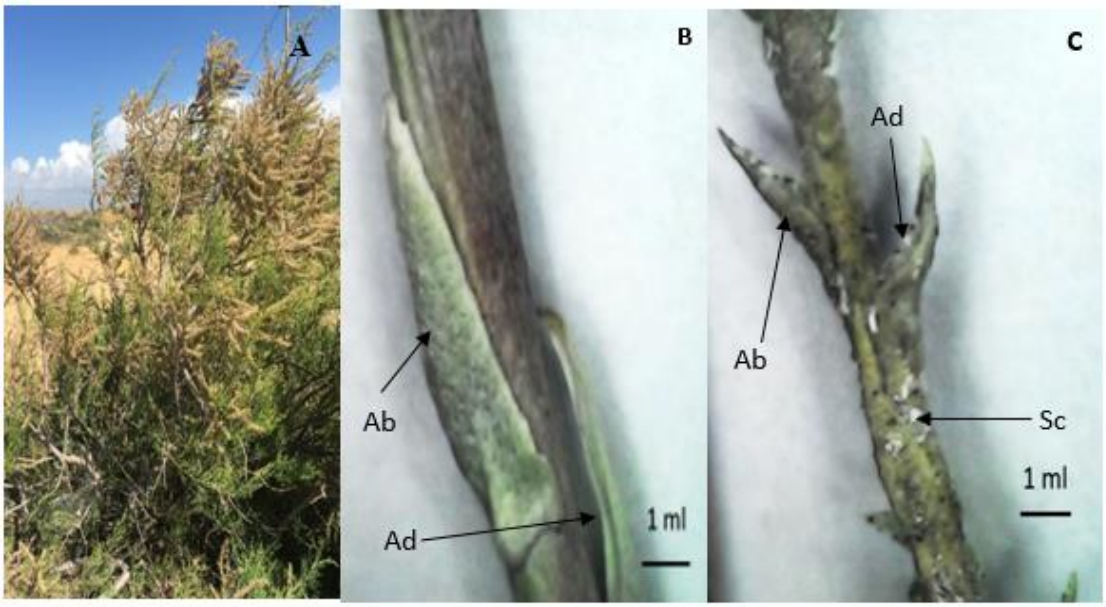

Fig 1. A *T. nilotica* in its habitat, B wintery shoot, arrow reveals to wintery leaf, C summery shoot, arrows reveal to Ad: adaxial surface of a leaf, Ab abaxial surface of a leaf, and Sc: salt crystals scattered on the summery stem

that there are significant decrease in the average leaf area, leaf length and width of summery leaf. Also, there are significant increase in leaf thickness (**Figs 2 A&B)**, spongy tissue thickness, palisade tissue on adaxial and abaxial surfaces and cuticle thickness of abaxial surface in summery leaves. In addition, the palisade tissue cells had a more columnar shape in summer (**Figs 2 B&D**).

Generally, both the wintry and summery stem of *T. nilotica* **(Figs 2 G & H)** consist of uniseriate epidermis. The epidermis cells have a slightly isodiametric shape and some cells are papillose. Several stomata and salt glands were observed (**Fig 2 G)**. Under the epidermis, there is the cortex that consists of outer layers of palisade tissue and inner non-photosynthetic parenchyma tissue followed by a vascular cylinder which includes collateral vascular bundles. It is noticeable in summery stem that each vascular bundle contains phloem and 1-3 radial rows of xylem elements and phloem fibers **(Fig 2 H)**. In the summery stem, the cuticle is thicker, palisade cells are more elongated and vascular bundles are more lignified **(Figs 2 G & H**).

3.4 Chemical parameters

Table 4 reveals that there is a significant decrease in water content percentage and degree of succulence. While there is a significant increase in total phenols, proline, free amino acids and total soluble sugars in the summer season. A reversible relationship was observed between water content and each of proline, free amino acid and total soluble sugar concentrations. On the other hand, seasonal changes had non-significant changes in the chlorophyll A, B and carotenoid concentrations in both winter and summer seasons.

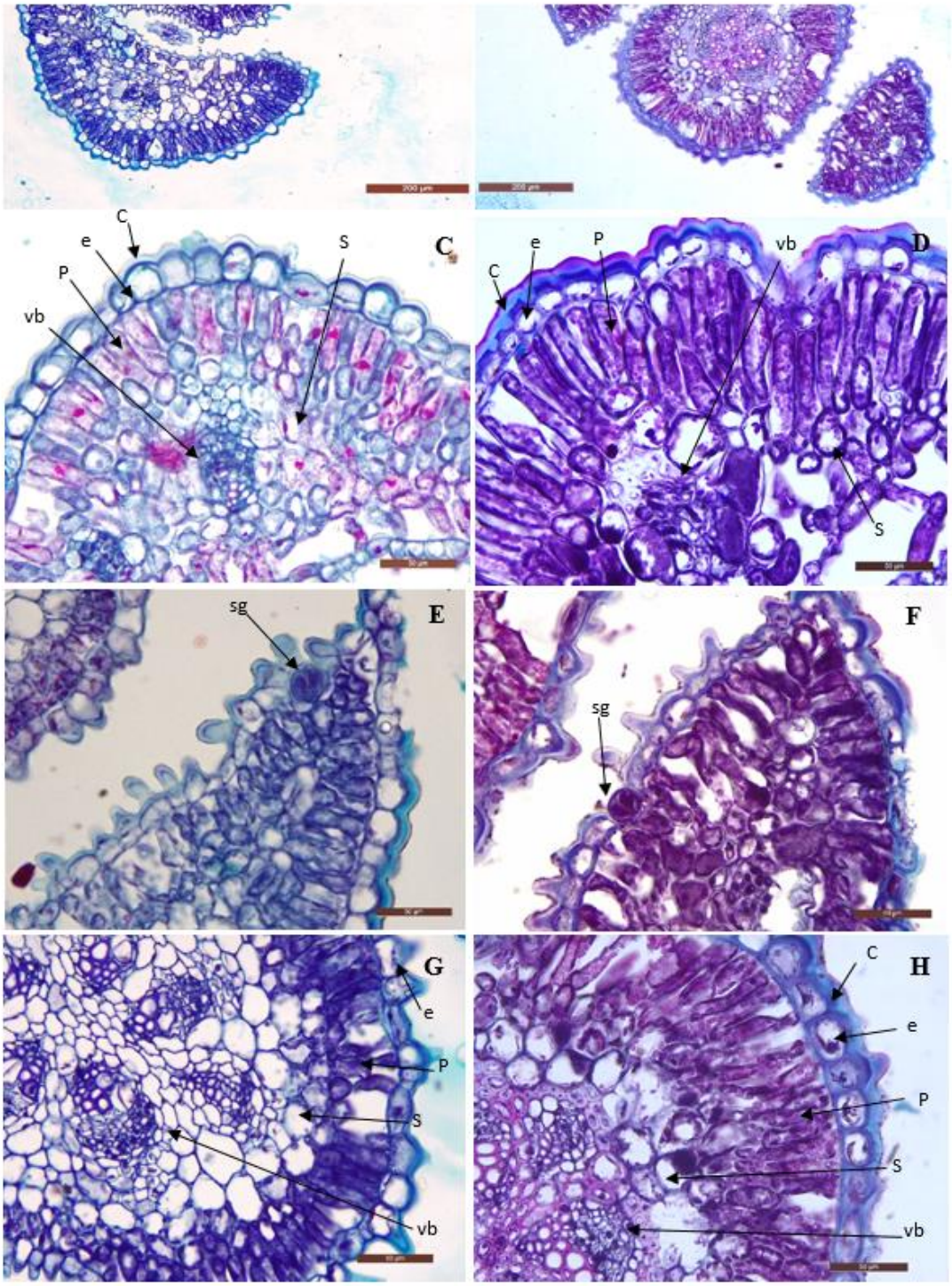

Fig 2. Cross-sections of *T. nilotica* young shoots. A. the wintery shoot, B the summery shoot. C, D, E and F magnifications of leaf transections. C, E reveal the wintery leaf with moderate thick cuticle and obvious intercellular spaces in the mesophyll layers. D and F illustrate transactions in the summery leaf. Note the thicker cuticle and compacted elongate palisade cells. G, H magnifications of stem transections. G. the wintry stem structure, H the summery stem structure, note increasing in lignification in xylem elements and phloem fibers surrounds the vascular bundles also increasing in cuticle thickness covered the summery stem and accumulation of tannins in cortex cells. c: cuticle, arrows reveal to e: epidermis, p: palisade tissue, s: spongy tissue, vb: vascular bundle, sg: salt gland.

Table 3. Morpho-anatomical characteristics of *T. nilotica* leaves in different seasons

Characters	Winter	Summer	LSD at 5%
Leaf length (mm)	$1.9^{a} \pm 0.64$	$1.3^{b} \pm 0.49$	0.25
leaf width (mm)	$0.58^{b} \pm 0.19$	$0.07^{a} \pm 0.02$	0.06
Leaf area (mm2)	$1.31^{a} \pm 0.52$	$0.95^{b} \pm 0.37$	0.20
Leaf thickness (µm)	$235.9^{b} \pm 19.3$	$297.8^{a} \pm 40.2$	27.08
Adaxial surface			
Thickness of cuticle (µm)	$3.64^{a} \pm 0.73$	$3.75^{a} \pm 0.7$	n.s
Thickness of epidermis (µm)	$19.11^{a} \pm 3.2$	$18.93^{a} \pm 3.4$	n.s
Radial diameter of epidermis cell (µm)	$14.82^{b} \pm 3.1$	$15.67^{a} \pm 2.2$	1.72
Tangential diameter of epidermis cell (µm)	$18.35^{a} \pm 3.1$	$18.00^{a} \pm 2.9$	n.s
Palisade thickness (µm)	$33.58^{b} \pm 6.2$	$48.86^{a} \pm 6.1$	3.95
Abaxial surface			
Thickness of cuticle (µm)	$5.70^{b} \pm 1.6$	$8.29^{a} \pm 1.1$	0.94
Thickness of epidermis (µm)	$24.89^{b} \pm 3.6$	$28.79^{a} \pm 4.2$	2.51
Radial diameter of epidermis cell (µm)	$19.44^{a} \pm 3.5$	$18.00^{a} \pm 2.1$	n.s
Tangential diameter of epidermis cell(µm)	$19.93^{a} \pm 4.6$	$21.54^{a} \pm 2.5$	n.s
Palisade thickness (µm)	$47.49^{b} \pm 6.1$	$58.26^{a} \pm 9.6$	5.12
Spongy thickness (µm)	$69.00^{b} \pm 11.3$	$78.41^{a} \pm 5.2$	8.62

Data in **Table 3** are shown as average ± standard deviation, averages with the different letters in rows are significant statistical by LSD test ($p \leq 0.05$), n.s: non-significant

Table 4. physiological and chemical parameters of *tamarix nilotica*

	Winter	Summer	LSD at 5%
Water content (%)	$67.8^{a} \pm 1.5$	$54.3^{b} \pm 4.5$	7.6
Degree of succulence	$3.16^{a} \pm 0.2$	$2.21^{b} \pm 0.2$	0.46
Chlorophyll a (mg/g)	$0.57^{a} \pm 0.06$	$0.55^{a} \pm 0.09$	ns
Chlorophyll b (mg/g)	$0.29^{a} \pm 0.02$	$0.28^{a} \pm 0.01$	ns
Carotenoids (mg/g)	$0.26^{a} \pm 0.01$	$0.23^{a} \pm 0.01$	ns
Total phenols (mg/g)	$137.32^{b} \pm 5.8$	$148.9.3^{a} \pm 1.3$	9.7
Proline (mg/g)	$0.96^{b} \pm 0.09$	$2.46^{a} \pm 0.05$	0.16
Total soluble sugars (mg/g)	$2.94^{b} \pm 0.6$	$6.9^{a} \pm 0.8$	1.59
Free amino acid (mg/g)	$15.3^{b} \pm 0.7$	$60.4^{a} \pm 3.5$	5.7

Data in T**able 4** are shown as mean ± standard deviation, means with the different letters in columns are statistically significant according to LSD test ($p \leq 0.05$), n.s: non-significant

Climate and soil analyses (**Tables 1, 2**) show that *T. nilotica* has been exposed to more salinity, drought and light intensity in the summer season. Plants growing in saline soils may face many abiotic stresses they may suffer from water stress and accumulation of some toxic ion concentrations such as chloride and sodium (Koyro et al 2009).

Tamarix growing naturally among halophytes was found to be a quite long-living plant resistant to a diversified of environmental stresses such as salinity and drought (Saïdana et al 2008). The present study shows that *Tamarix nilotica* has the ability to survive under environmental stresses. A set of adaptation mechanisms includes morpho-anatomical adaptations such as existence of salt glands on aerial parts. These salt glands may remove a lot of quantities of salts by secretion to the plant surface (Koyro et al 2009). Moreover, *T. nilotica* is characterized by some xeromorphic properties such as decreasing leaf area, increasing cuticle thickness in stem and abaxial leaf surface in the summer season. Hameed et al (2010) reported that xeromorphic features have appeared in many halophytes to survive under environmental stresses. Reduction in leaf area aims to limit

water loss *via* transpiration since smaller leaves cause diminishing the need for evaporative cooling. In addition, small leaves can avoid the harmful effect of increasing solar radiation. Also, the increase of cuticle thickness reduces the harmful effect of drought, salinity, and high radiation *via* limited water loss caused by transpiration. Thus, limits reaching salts in the photosynthetic tissues (Reef and Lovelock 2015, Koyro et al 2008).

The present results indicated a significant increase in the lamina thickness in the summer season due to an increase in both palisade and spongy tissue thickness. Galmés et al (2013) suggested that the increase in the thickness of palisade and the spongy could improve the distribution of chloroplasts and then increase the chloroplast area and number of CO_2 assimilation sites per leaf area unit. On the other hand, according to Vogelmann and Martin (1993), most leaves developed under conditions of increased light intensity are comparatively thick and have a well-developed palisade tissue with a high amount of columnar cells. These columnar cells facilitate more uniform light distribution to chloroplasts within the leaf. This may allow improving the vertical position of the chloroplasts and may respond to internal carbon dioxide concentration in the leaf. Thus, improving the entire photosynthesis process that may depend on the balance between the concentrations of both light and carbon dioxide. This balance could be achieved partly by controlling the level of palisade development. The anatomical results showed that the increase in the palisade tissue thickness was due to the palisade cells becoming more columnar not due to the increase in the palisade cell raw number.

Increasing the lignification in the summery stem of *T. nilotica* can be explained according to Abd Elbar (2015) as increasing lignification provides mechanical support to the cell wall. The presence of sclerenchyma tissue surrounding the vascular bundle may confer resistance of phloem against destruction caused by abiotic stresses.

Chlorophyll (Chl) is the most important chloroplast component for photosynthesis. Since there is a direct proportion between the chlorophyll concentration and the photosynthetic rate. While Carotenoids (Car) are important for photosynthesis photoprotection and they play a substantial role during plant growth under abiotic stresses. Generally, stressful environments cause a decrease in the pigments concentrations (Ashraf And Harris 2013, Ansari et al 2019). There was no significant change in the concentration of chlorophyll A and B and carotenoids due to seasonal change.

Usually, the reactive oxygen species (ROS) are formed due to oxidative damage from environmental stresses. ROS can cause inactivation of enzymes, damage to the cell membrane and alter gene expression**.** Various organic molecules, including phenols and proline can be used to scavenge the ROS (Abobatta 2020). Phenolics are known as antioxidants that help to prevent cellular damages induced by oxidative stress. They can directly scavenge molecular species of active oxygen or act as metal chelators (Bautista et al 2016). This could explain the significant increase of total phenols in the summer season. Since lignin is synthesized from phenolic precursors (Selmar 2008), this may explain the increase in lignification in summery stem.

A higher value of proline in shoot parts of *T. nilotica* in the summer season was observed. It may be used for several purposes including function as antioxidants by facilitating ROS scavenging, ensuring osmotic adjustment as well as protection of plasma membrane integrity, cellular structures and proteins. Proline can be used as a source of energy or reducing power. These results are in agreement with those reported by Per et al (2017) and Ansari et al (2019). Likewise, there was a significant increase in both free amino acids and total soluble sugars in the summer season in the shoot parts of *T. nilotica.* According to Yeo (1998), Couee et al (2006) and Abobatta (2020) organic molecules, such as free amino acid and soluble sugars improve the cell sap concentration and maintain the resistance of plants by acting as osmotic regulation. They have a stabilizing effect on the cell membrane, proteins and enzymes as an Osmo protectant.

4 Conclusion

Tamarix nilotica growing naturally in Egyptian salt marches has the ability to withstand the seasonal environmental stresses by many adaptations mechanisms. These mechanisms include: salt secretion by salt glands as well as reducing water loss and increasing water use efficiencies by reduction of scaly leaves size, increasing cuticle, epidermis thickness and palisade tissue thickness. In addition to the physiological adaptive mechanisms (osmotic adjustment and antioxidant activity), by increasing concentrations of proline, amino acids and total soluble sugars.

Abbreviations: Chl, chlorophyll; Car, Carotenoids; ROS, Reactive Oxygen Species.

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