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Comparative leaf and stem anatomy of *Tamarix tetrandra* (*Tamaricaceae*) species from different habitats

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Farklı habitatlarda yayılış gösteren *Tamarix tetrandra* (*Tamaricaceae*) türünün karşılaştırmalı yaprak ve gövde anatomisi

Abstract: In this study, the leaf and stem structure of *Tamarix tetrandra* Pallas ex. Bieb. species, which are distributed in riparian and salt marshes, were examined in terms of anatomical and micromorphological aspects. Specimens of the species have been preserved in 70% alcohol for anatomical study. Herbarium specimens were used for micromorphological studies. The studies showed that there were differences in anatomy and micromorphology. It was found that the stomata were embedded in the epidermis in the samples distributed in the salt marsh. In addition, stem epidermal cell length, sclerenchyma cell diameter, sieve tube cell diameter, and pith cell diameter were found to be greater in riparian species. In the correlation analysis, a positive correlation was observed between leaf lower surface stomata width and upper epidermis cell width, and between stem sclerenchymatic cell diameter and upper epidermis cell width in species distributed in salt marshes. The studies did not find intensive salt accumulation in the stem and leaf structures of the species that spread in the salt marsh. Salt uptake is thought to be inhibited in these species.

Key words: Anatomy, micromorphology, riparian, salt marsh, *Tamarix tetrandra*

Özet: Bu çalışmada nehir kenarında ve tuzlu bataklıklarda yayılış gösteren *Tamarix tetrandra* Pallas ex. Bieb. türünün yaprak ve gövde yapısı anatomik ve mikromorfolojik yönden incelenmiştir. Türe ait örnekler anatomik incelemeler için %70'lik alkol içerisinde stok örnek haline getirilmiştir. Mikromorfolojik incelemelerde herbaryum örnekleri kullanılmıştır. Yapılan incelemelerde anatomik ve mikromorfolojik yönden farklılıklar olduğu belirlenmiştir. Tuzlu bataklıkta yayılış gösteren örneklerde stomaların epidermise gömülü olduğu tespit edilmiştir. Ayrıca nehir kıyısında yayılış gösteren türlerde ise gövde epidermis hücre boyu, sklerenkima hücre çapları, kalburlu boru hücre çapları ve öz hücre çaplarının daha büyük olduğu saptanmıştır. Yapılan korelasyon analizinde tuzlu bataklıkta yayılış gösteren türlerde yaprak alt yüzey stoma en ile üst epidermis hücre en arasında ve gövde sklerenkima hücre çap ile üst epidermis hücre en arasında pozitif korelasyon gözlemlenmiştir. Yapılan incelemelerde tuzlu bataklıkta yayılış gösteren türlerin gövde ve yaprak yapılarında yoğun bir tuz birikimine rastlanmamıştır. Bu türlerde tuz alınımının engellendiği düşünülmektedir.

Anahtar Kelimeler: Anatomi, mikromorfoloji, nehir kıyısı, *Tamarix tetrandra,* tuzlu bataklık

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1. Introduction

Tamarix L. (*Tamaricaceae*) is naturally distributed in Asia, Europe, and northeastern and southwestern Africa (Sheidai et al., 2019). It is mostly found in temperate and subtropical regions. It is represented by approximately 70-75 species worldwide (Villar et al., 2019). There are 8 species of this family in Türkiye (Güner et al., 2012), one of which is endemic (*Tamarix duezenlii* Çakan & Ziel.). *Tamarix tetrandra* Pall. ex M.Bieb., one of these species, has a very wide distribution.

Plants are capable of adapting to various environmental conditions. Salty soils create different ecological living conditions for most plants (Dölarslan and Gül, 2012). While many plant species cannot adapt to very low levels of salinity (glycophytes), some plant species can adapt to high levels of salinity (halophytes) (Doğru and Canavar, 2020). This situation can be morphological as well as anatomical. Most plants cannot tolerate high levels of salt in the soil (Glenn and Brown, 1999). Plants (halophytes) adapted to these environments have developed adaptation mechanisms at the cell, tissue and whole plant level (Aslamsup et al., 2011). These mechanisms can be classified as osmotic tolerance, ion excretion, and tissue tolerance (Tiryaki, 2018).

The salt tolerance of plant species can vary depending on the environment. Even plants of the same species can have different levels of salt tolerance (Doğru and Canavar, 2020). The osmotic potential of water in saline soils can decrease and become unavailable to plants (physiological drought) (Marschner, 2011). Halophytes use the salts that accumulate in their leaves to compensate for the low osmotic potential of the soil (Lauchli and Epstein, 1984). Leaf cells store salts in vacuoles and maintain a level that does not affect enzyme activity and metabolic activities in organelles and cytoplasm (Lauchli and Epstein 1984). This is very important for plants adapted to high salinity soils. Several studies have been carried out to determine the anatomical characteristics of *Tamarix*. Some of these have focused on the leaf structures (Abbruzzese et al., 2013; Alaimo et al., 2013) of the species and others on the stem (Waly, 1999; Oladi et al., 2017) structures.

The aim of this study was to determine the anatomical and micromorphological characteristics of the *Tamarix tetrandra* Pall. ex M.Bieb. species found in different habitats. In this context, we compared the leaf and stem structures of *Tamarix tetrandra* found in riparian and salt marshes from an anatomical and micromorphological point of view.

2. Materials and Method

Samples of *Tamarix tetrandra* Pallas ex. Bieb. were collected in April in the Çarşamba (Aşıklı) district of Samsun. Some of the collected plants were herbarium samples, and some of them were made into stock samples in 70% alcohol (MKA 201, MKA 202, OMUB, 8222). Stem and leaf sections of the species were examined and measured using the Zeiss AxioLab A1 microscope and the Zeiss Axiocam 105 imaging system. After taking the anatomical sections, an average of 30 measurements were taken for each character (Table 1) using the same microscope. Glycerine (50%) was used for section examinations. In addition, the leaf surfaces of the study species were also examined from a micromorphological point of view.

Micromorphological studies were carried out to determine the general appearance of the epidermis and stomata on the leaf surfaces (measurements of stomatal and epidermal cell sizes were taken from superficial leaf sections using the Zeiss Axiocam 105 camera imaging system). Scanning electron microscopy (SEM) was used to determine surface characteristics. For scanning electron microscopy (SEM), the samples were attached to the stubs with double-sided carbon tape and coated with 12.5-15 nm gold-palladium (SEM coating system, SC7620). Examinations and photographs were made in a JEOL JMS-7001F scanning electron microscope (SEM) with a voltage of 5-15 KV. Statistical analyses were also carried out on the anatomical characteristics of the taxa. PAST 4.04 packages were used for the analysis (averages and correlation).

3. Results

The leaves of both habitat samples have a scale-like shape. In addition, there is a vascular bundle in the centre of the leaf structure in both habitat samples. *Tamarix*, which spreads along the riparian, has a single layer of epidermis, the cortex layer has 5-7 rows. Just below the cortex there is a sclerenchymatic sheath structure. The stem centre consists of parenchymatic cells. In salt marsh samples, a single layer of epidermis was identified in the stem structure. In addition, the cortex layer consists of 4-8 rows of elongated and cylindrical cells. In addition, the centre of the stem is made up of parenchymatic (cylindrical) cells. There is a sclerenchymatic sheath layer beneath the cortex.

The anatomical studies of the leaves compared the salt marsh and riparian species. It was found that the length and width of the lower epidermal cells and the width of the stomata on the lower surface of the leaves were greater in the *Tamarix tetrandra* species that spread in the salt marsh (Fig1, Fig. 3). It was observed that the leaf upper epidermis cell length and width and leaf lower surface stomata length were larger in the species distributed along the riparian. The leaves of species found in both habitats have a single layer of epidermis (Fig. 1). In addition, the mesophyll has a unifacial leaf type formed by parenchymatic cells. In the anatomical examinations made on the stem, it was determined that the stem epidermis cell width and trachea cell diameters were larger in species distributed in salt marshes. It was found that stem epidermis cell length, sclerenchyma cell diameter, sieve tube cell diameter, and pith cell diameter were greater in the riparian species (Fig. 2).

The micromorphological studies revealed conical papillae structures on the upper leaf surfaces of the species found in both habitats. The swelling was observed on the tops of the papillae structures on the upper surface of the leaves of the species that spread along the riparian. In addition, reticulated ornaments were identified on the surface structures of the papillae (Fig. 3). The upper parts of the papillae structures of the salt marsh species have a pointed end. Flat wavy ornamentation was found in the surface structures of the papillae. In the examinations made on the lower surface of the leaves, it was determined that there were reticulate ornaments on the surface structures of the species that spread along the riparian. In addition, epidermis cell structures and stomata were clearly observed on the lower surface. In salt marsh species, the stomata are prominently embedded in the epidermal layer. Moreover, the stomata are closed in both samples examined (Fig. 3). Crystal-like structures and amorphous structures were observed on the stomata margins and epidermis surfaces. Wavy ornaments were also found on the lower leaf surfaces of the samples examined (Fig. 3).

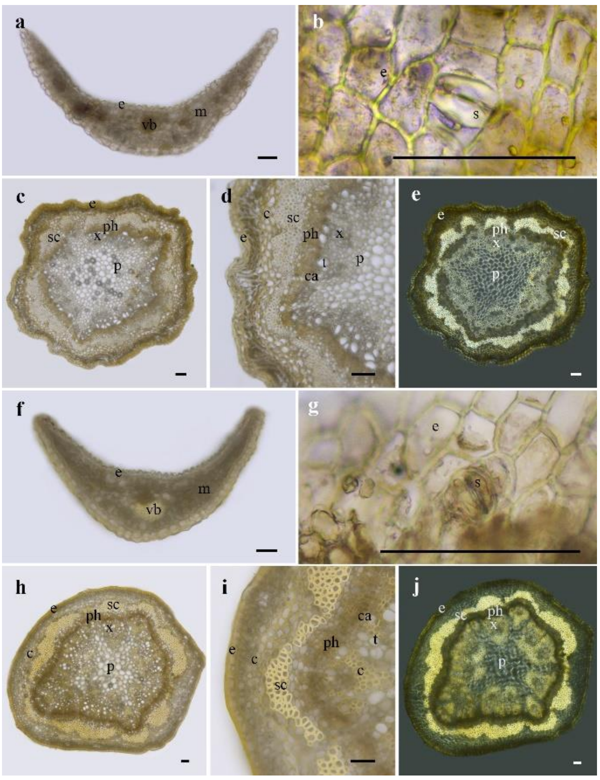

Figure 1. Stem and leaf anatomy of riparian and salt marsh specimens of *Tamarix tetrandra*. a-e: Riparian specimens (a-b leaf, c-e stem), f-j: Salt marsh specimens (f-g: leaf, h-j: stem). e: epidermis, vb: vascular bundle, m: mesophyll, sc: sclerenchyma, c: cortex, ph: phloem, x: xylem, ca: cambium, t: trachea, p: pith, s: stomata, scale bar: 100µm

Table 1. Measurements and characters of *Tamarix* from different habitats

Character/Habitat	Riparian samples		Salt marsh samples	
	Mean (µm)	Std. error	Mean (µm)	Std. error
Leaf lower epidermis cell length	32,030	1,011	28,397	1,616
Leaf lower epidermis cell width	24,518	1,249	20,502	0,504
Leaf upper epidermis cell length	26,771	1,358	33,621	1,477
Leaf upper epidermis cell width	15,612	0,397	28,358	0,861
Stomata length	21,983	0,623	27,419	1,040
Stomata width	16,645	0,580	16,462	0,878
Stem epidermis cell length	16,096	0,664	16,225	1,017
Stem epidermis cell width	13,558	0,468	12,957	0,504
Stem sclerenchyma cell diameter	8,486	0,462	9,531	0,648
Stem trachea cell diameter	11,977	0,754	10,323	0,641
Stem sieve tube diameter	4,494	0,168	5,326	0,200
Stem pith cell diameter	24,729	0,847	34,027	1,205

Correlation analysis was performed on anatomical data for samples from both habitats. In the correlation analysis, positive correlations were observed between leaf lower epidermis cell length and upper epidermal cell width, leaf lower epidermis cell length, and stem sclerenchyma cell diameter in riparian species. A negative correlation was determined between the stomata length and stem sieve tube diameter (Fig. 4). On the other hand, a positive correlation was observed between leaf upper epidermis cell width and stomata width and leaf upper epidermis cell width and stem sclerenchyma cell diameter in salt marsh species. There was also a negative correlation between leaf lower epidermis cell width and leaf upper epidermis cell width, stem epidermis cell length, and stem pith cell diameter (Fig. 4).

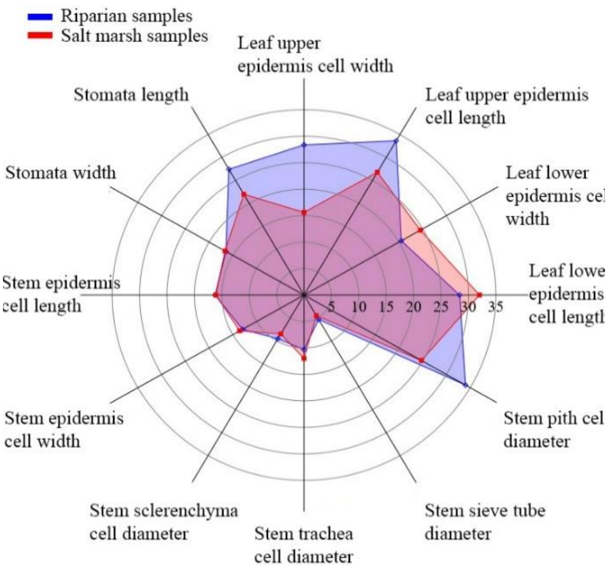

Figure 2. Mean of anatomical values of salt marsh and riparian samples.

4. Discussions

In this study, the leaf and stem structure of *Tamarix tetrandra*, which is distributed in the riverside and salt marsh area, were examined in terms of anatomy and micromorphology. Areas with abundant water but high soil salinity are similar to areas with low water availability (Munns and Tester, 2008). Although the salt marshes in our study area have high water availability, they have low water potential for *Tamarix* (and other species) that occur in this habitat.

Plants are able to adapt to salt marshes (areas) by performing certain functions. The first is the inhibition of salt uptake or storage in the protoplast (Dölarslan and Gül, 2012). Intense salt accumulation was not observed in the stem and leaf structures of salt marsh species. Salt intake is

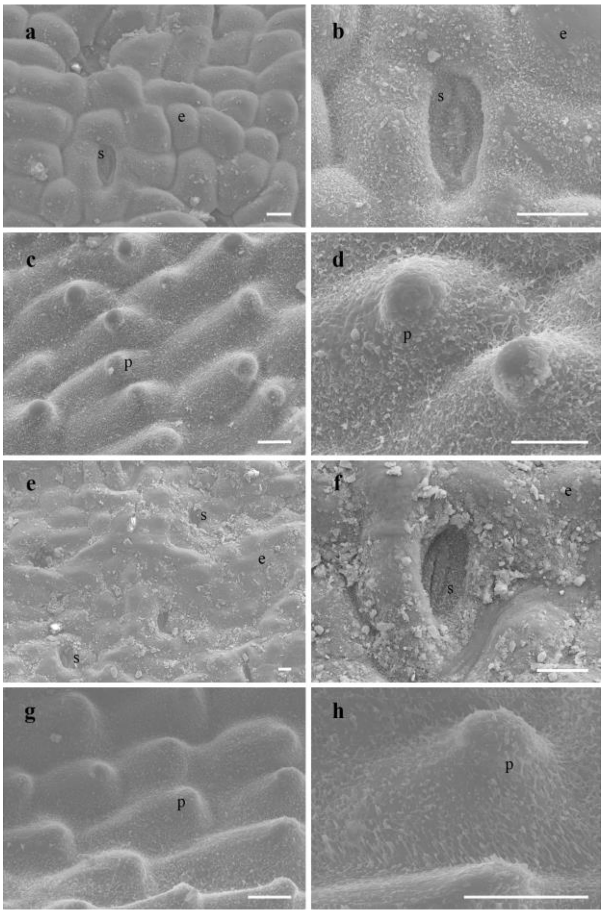

Figure 3. SEM image of salt marsh and riparian samples for micromorphological study. a-d: Riparian specimens (a-b: lower epidermis, c-d: upper epidermis), e-h: Salt marsh specimens (e-f: lower epidermis, g-h: upper epidermis). e: epidermis, s: stomata, p: papillae, scale bar: 10 µm

	2	3	4	5	6	7	8	9	10	11	12
1	0,02	-0,03	0,52	-0,08	-0,25	-0,06	-0,12	0,45	0,24	-0,16	0,25
2		0,03	-0,10	-0,15	-0,20	-0,35	-0,30	0,10	-0,19	0,11	0,20
3			-0,08	0,35	0,12	-0,15	0,01	-0,19	0,00	-0,26	0,08
4				0,19	-0,15	-0,26	-0,22	0,26	-0,18	-0,23	0,17
5					-0,06	0,27	0,32	-0,24	-0,35	-0,45	-0,16
6						-0,05	-0,02	-0,33	-0,14	0,16	0,07
7							0,14	-0,08	-0,02	0,02	-0,21
8								-0,17	0,23	-0,09	-0,18
9									-0,06	0,30	0,38
10										0,00	0,01
11											0,32

-1 -0,33 0,33 1

	2	3	4	5	6	7	8	9	10	11	12
1	0,00	-0,11	0,22	0,11	0,41	0,15	-0,14	0,24	0,03	0,09	-0,36
2		0,11	-0,64	0,40	-0,29	-0,14	0,25	-0,39	0,02	0,27	-0,10
3			-0,17	0,34	-0,32	-0,11	0,14	-0,40	-0,05	0,08	-0,01
4				-0,27	0,50	0,03	-0,33	0,53	-0,33	-0,35	0,08
5					0,20	-0,10	0,14	-0,38	-0,28	0,31	-0,24
6						-0,22	-0,43	0,34	-0,30	0,09	0,07
7							0,60	0,15	0,24	-0,01	-0,47
8								-0,34	0,12	0,00	-0,15
9									0,02	-0,48	-0,22
10										0,16	-0,24
11											-0,09

-1 -0,33 0,33 1

Figure 4. Correlation analysis for anatomical characters (The first analysis is for riparian samples and the second analysis is for salt marsh samples). 1: Leaf lower epidermis cell length, 2: Leaf lower epidermis cell width, 3: Leaf upper epidermis cell length, 4: Leaf upper epidermis cell width, 5: Stomata length, 6: Stomata width, 7: Stem epidermis cell length, 8: Stem epidermis cell width, 9: Stem sclerenchyma cell diameter, 10: Stem trachea cell diameter, 11: Stem sieve tube diameter, 12: Stem pith cell diameter.

thought to be inhibited in these species. It is noted that some characters are lost during the adaptation phase of plants to saline environments (Grigore and Toma, 2017, 2021). In the examined specimens of *T. tetrandra*, no loss of anatomical characteristics was observed in the leaf and stem structure. According to Grigore and Toma (2021), salt-secreting structures are rarely observed in *Tamarix* species growing in saline soils. In our study, no salt glands were found, especially in the samples distributed in salt marshes.

Micromorphological studies showed that the stomata were embedded in the epidermis. This condition is known as enhanced xeromorphic properties to reduce sweating. For species that spread in saline environments, this mechanism is also very important. The first of the changes that can occur in a short time when plants are under salt stress is the closing of stomata and the slowing down of carbon assimilation (Doğru and Canavar, 2020). In addition, stomata closure prevents transpiration and causes a decrease in stomatal conductance (Munns and Tester, 2008). It has been suggested that papillae structures are concentrated around stomata, reducing airflow (Maricle et al., 2009). Both of our habitat samples are located on the papillae leaf upper surface. However, no stoma was detected on the upper surface of the examined species. In a micromorphological study of *Tamarix* species, Abbruzzese et al., (2013) observed salt glands on the leaf surfaces of the species. Salt glands were not found in our micromorphological studies. This may be due to differences in species or environmental conditions.

Salinity causes differentiation in the transmission system (Flowers and Colmer, 2015). In our study, we found that the stem tracheal cells of salt marsh species were larger in diameter, while the sieve tube cells were smaller. *Tamarix* can show differences in development and distribution even over short distances in response to changes in soil salinity (Mumcu and Korkmaz, 2021). Salty environments have a negative effect on stem development in plants, but slow down vascular development (Zafar et al., 2015). It was determined that trachea diameters were larger and sieve tube diameters were smaller in salt marsh samples compared to riparian samples. There are smaller xylem diameters in *Tamarix*, especially in fast-drying areas (Long et al., 2021).

Tamarix has the ability to adapt to its environment. Therefore, it can have an active distribution in different habitats. It reveals anatomical differences in adaptation to the environment. In particular, the change in stomatal sizes, the stomatal structures embedded in the epidermis, and the differences in tracheal cell diameters clearly reveal this situation. Anatomical and micromorphological studies of the small leaf structures have shown that the anatomical structures can vary between species occurring in different habitats.

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