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Morpho-anatomical differentiation of *Suaeda maritima* (L.) Dumort. 1827. (Chenopodiaceae) populations from inland and maritime saline area

Research Article

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Abstract: Morphological analysis of four populations of *Suaeda maritima* was undertaken in order to examine the variation between populations from inland and maritime saline area, as well as between the two subspecies. Variability and significance of morpho-anatomical differentiation were examined using principal component analysis (PCA), discriminant component analysis (DCA) and cluster analysis. Plants of each population exhibited halomorphic and xeromorphic characteristics. The results of PCA and DCA showed that *S. maritima* subsp. *prostrata* and *S. maritima* subsp. *maritima* could be clearly separated based on their quantitative anatomical characteristics. Based on our analysis, climate and the amount of salt and ions in the soil, are important factors that enhance the adaptive potential of *S. maritima*.

Keywords: *Halophytes • Suaeda maritima • Anatomy • Salinity*

1. Introduction

Salinity is an environmental stress that limits the growth and development of salt-sensitive plants, but promotes the growth of halophytes even at relatively high levels of NaCl [1]. Natural or primary salinity is a result of an accumulation of soluble salts in soil or groundwater over long geological periods. These salts are mainly derived from the weathering of parent minerals, which release a variety of salts, including chlorides, sulphates, carbonates and bicarbonates of sodium, magnesium and calcium [2]. Although soil salinity and sodicity are common phenomena for arid and semiarid regions, salt-affected soils have been recorded in nearly all the climatic regions of the world, and in a wide range of altitudes [3].

Maritime salines lie at the interface between land and sea and they are periodically flooded by sea water [4]. Inland saline-alkaline wetlands are distributed over the inland regions of arid or semi-arid climate.

Studies on the halophilic vegetation of arid and humid areas have been attracting an increasing amount of attention [5-8]. Areas that contain high concentrations of salt are boundaries for the distribution of halophytes. In addition, substrate salinization may well induce halophilic, xerophilic and heliomorphic characters in plants. Halophytes have adopted different strategies in order to survive periodic soil saturation. The succulent euhalophytes accumulate salt in their tissues, whereas crinochalophytes are able to excrete excess salt through salt glands and bladders. Despite these different processes of eliminating salt, certain modifications of this classification are known to exist [8,9].

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Plants that grow in saline soil have adapted various survival mechanisms that limit the concentration of sodium and chloride ions in plant tissues. In halophytes and glycophytes cytosolic enzymes are sensitive to these ions, and consequently, the plant accumulates sodium and chloride ions in the vacuole [1,5,10]. For example, succulent leaves of *Suaeda maritima* have large mesophyll cells in which the vacuoles, where Na^+ is accumulated, occupy 77% [11]. In a study with *S. maritima* conducted on its natural habitat in Serbia, sodium concentration of the cell sap exceeded even 800 mM, while the total salt content contributed to the osmotic potential in degree of up to 91% [12]. Wang *et al.* [13] provided clear evidence for differences in the characteristics of Na^+ uptake with increasing external concentration. In contrast to *S. maritima,* most glycophytes and some halophytes, have a strong selectivity for K^+ over Na^+, thereby limiting Na^+ uptake [14-17]. Also, under saline conditions, mesophyll resistance to the gaseous exchange increases as a consequence of structural changes in the mesophyll cells [18]. It has been suggested that the main reason for this increase is a reduction in the volume fraction of intercellular spaces [19]. Photosynthetic oxygen evolution in the chloroplasts of *S. maritima* was related to the ion content of the chloroplasts [20,21].

Anatomical structures of plant organs, especially of leaves, change, thus enabling plant adaptation to its environment. Many leaf traits have been recognized to provide a protection against various environmental conditions and stresses including drought, high air temperature and high concentration of salt in soil. Morpho-anatomical alterations of succulent halophytes include increase of cell volume, especially of spongy and water parenchyma, increase of leaf thickness and decrease in number of stomata [22]. Isolateral leaf anatomy is common in plants that are found in hot and arid environments because this variation minimizes heating of the leaves and reduces transpiration demands. This is true in desert environments and on shorelines, habitats for many species of *Suaeda* [6]. The leaf histological components seem to be an ideal model to studying of the relations between halomorphic and xeromorphic structures of plants and their habitat, although anatomy of other plant organs could give additional information [23].

S. maritima is an annual, succulent, salt-marsh plant, with semi-cylindrical leaves. It is found on most of the European sea coast and in inland saline areas of Russia, Central Europe, East Asia and the East India. *S. maritima* has been found in North and South America, and also in Australia. According to Ball [24], Ball and Akeroyd [25] and Jalas and Suominen [26], *S. maritima* in Europe is present as three subspecies: *S. maritima* subsp. *maritima*, *S. maritima* subsp. *salsa* (L.) Soó and *S. maritima* subsp. *pannonica* (G. Beck) Soó ex P. W. Ball. On the other hand, Soó [27] considered *S. maritima* located in Hungary and the Pannonian Plain is divided into *S. maritima* subsp. *maritima*, *S. maritima* subsp. *salsa* (L.) Soó and *S. maritima* subsp. *prostrata* (Pall.) Soó. Furthermore, Soó noted that the subspecies *pannonica* was a separate species, *S. pannonica* Beck. According to Boža and Vasić [28] while *S. maritima* subsp. *maritima* does not grow in Serbia, *S. maritima* subsp. *prostrata* was found growing in saline areas of the Pannonian Plain. In Montenegro, *S. maritima* subsp. *maritima* was found on the Adriatic coast.

Since *S. maritima* grows in different types of saline habitats, the first aim of our research was to analyze leaf and stem structure and the variability rate of geographically distinct populations from maritime and inland saline area. The second aim was to establish whether morpho-anatomical differentiation exists between populations of *S. maritima* subsp. *maritima* and *S. maritima* subsp. *prostrata*.

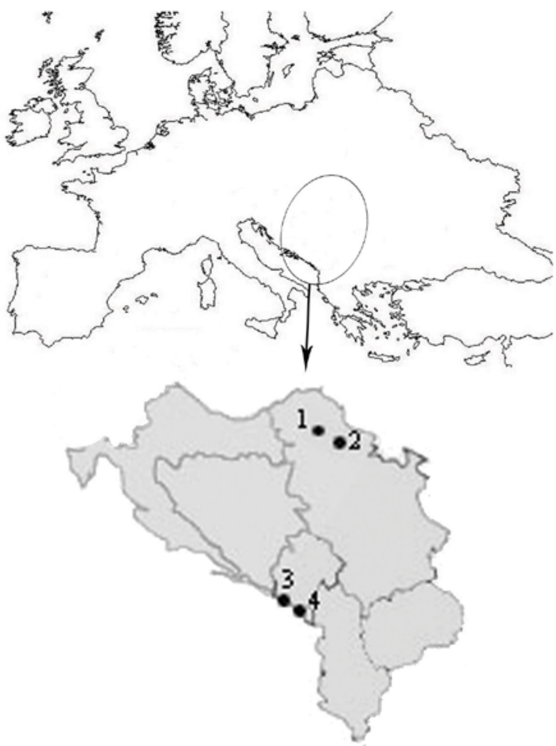

Figure 1. Map of Europe, with collecting sites indicated: 1- Slano Kopovo, 2-Rusanda, 3-Tivatska solila, 4-Ulcinj salina

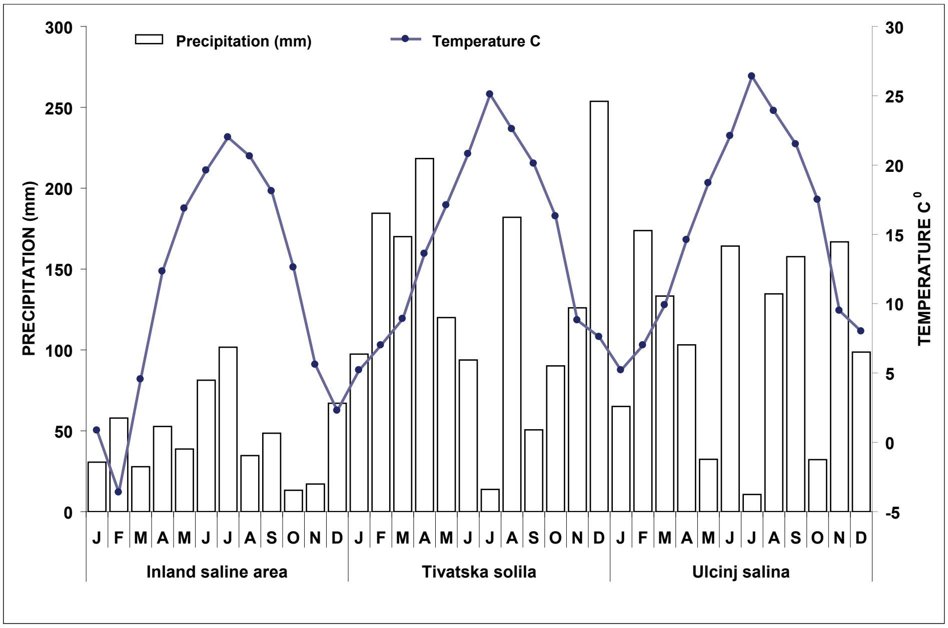

Figure 2. Mean monthly air temperature and total monthly rainfall during 2006 at collecting sites

2. Experimental Procedures

Soil and plant analysis was performed on samples from two locations from Mediterranean coast in Montenegro (Tivatska solila, Ulcinj salina) and two inland saline areas in Pannonian Plain in Serbia (Slano Kopovo, Rusanda) (Figure 1). Ulcinj salina is the greatest salina in Montenegro, Tivatska solila is ex salina and sites located in the Pannonian Plain are the most fertile grounds in Serbia.

The meteorological data was taken from Meteorological stations in vicinity of studied sites: Tivat and Ulcinj for maritime saline areas and one station, Novi Sad, for the two inland saline areas (Figure 2).

At each site, three cylindrical soil cores were taken from the top 30 cm and mixed. From each site, 300 g of soil was air-dried, crushed and sieved. pH value was determined in soil suspension with water (10 g : 25 cm^3), potentiometrically in a slurry system using an electronic pH meter. Content of total water soluble salts and pH value was determined in water-saturated soil paste (%).

Morpho-anatomical studies were conducted on plant samples, which were collected in August 2006, from four populations of *S. maritima*. Plants were identified at the Department of Biology and Ecology, University of Novi Sad. Voucher specimens were deposited in the Herbarium of the Department of Biology and Ecology, University of Novi Sad - BUNS (Table 1). Thirty plants of each population were used for anatomical investigations. For light microscopy observations leaf epidermal prints were made according to Wolf [29]. Leaf and stem segments from the middle part of the plants were separated and fixed in 50% ethanol. 25 μm thick cross-sections were made using Leica CM 1850 cryostat, at temperature -20°C. The structure of leaves and stems were observed and measurements made using Image Analyzing System Motic 2000. Stomata were counted on five randomly selected areas of the adaxial and abaxial surfaces and calculated per mm^2 of the leaf surface. Relative proportions were calculated for leaf and stem tissues, and expressed as a ratio to the whole cross-section area of each organ.

Data was statistically processed by analysis of variance. Means, standard errors and coefficients of variation were calculated using STATISTICA for Windows version 8.0. The significance of differences in measured parameters between the populations was determined using Duncan's and t-test ($P \leq 0.05$). We used Multivariate Discriminant Function Analysis in order to check the hypothesis that the analyzed sample was composed of groups which were differentiated from each other. The general structure of sample variability was established by Principal Component Analysis (PCA), based on correlation matrix. Overall differences between the compared groups are presented by Euclidian distances.

Sample	Collecting site	Date	Voucher Number
Suaeda maritima (L.) Dumort. 1827 subsp. prostrata (Pall.) Soó 1951	Serbia, Novi Bečej, Slano Kopovo, UTM 34T DR1 45	15.08.2006.	2-1997
Suaeda maritima subsp. prostrata	Serbia, Melenci, Rusanda, UTM 34T DR2 44	11.08.2006.	2-1999
Suaeda maritima (L.) Dumort. 1827 subsp. maritima	Montenegro, Tivat, Tivatska solila, UTM 34T CM2 10	08.08.2006.	2-1996
Suaeda maritima subsp. maritima	Montenegro, Ulcinj, Ulcinj salina, UTM 34T CM4 54	13.08.2006.	2-1998

Table 1. Voucher data for specimens used in the study

3. Results

The soil sampled from inland saline areas contained small amounts of salts (0.60 - 0.65%), whereas considerable amounts of salts were detected in soils at maritime areas (0.8 - 1.5%). The alkaline reaction was the highest at Rusanda locality (9.91) and the lowest in Ulcinj salina locality (7.50) (Table 2). In most saline environments, external Na^+ concentrations exceed concentrations of other ions. Concentrations of cations and anions were greater in maritime saline area, except for sulphate and carbonate ions, whose concentrations were much greater in inland saline area, especially at Rusanda.

The leaf cross-sections ranged from rounded to elliptical in shape (Figure 3A-D). The epidermal cells were fairly large, forming a single layer that had a relatively thin cuticle (Figure 3E). Paracytic stomata were observed on both leaf surfaces and were slightly sunken. Leaves also had isolateral palisade mesophyll structure. The mesophyll was differentiated into two distinct layers: palisade tissue below the epidermis and central water-storage tissue, containing vascular bundles. The palisade tissue consisted of 2-3 rows of elongated and densely arranged cells. Water storage cells were large and thin-walled. The main vascular bundle was in the centre of the leaf, surrounded by water storage parenchyma, while other bundles gradually became smaller towards the tip part of the leaf (Figure 3F,G).

The leaf cross-section area was significantly higher in plants from inland saline area (Table 3). The average leaf thickness was between 745 μm at Tivatska solila and 994 μm at Slano Kopovo. Populations from maritime saline area had higher proportion of epidermis. The average number of stomata per mm^2 ranged from 46.0 to 54.7 on adaxial leaf side and from 31.8 to 39.1 on abaxial leaf side. Stomata length and width on adaxial and abaxial leaf side were significantly higher in plants from inland saline area. Populations from inland saline had higher proportion of palisade tissue and larger palisade cells. The thickness of palisade tissue of abaxial leaf side showed significant differences between populations from Slano Kopovo and Tivatska solila, while there were no significant differences in the thickness of palisade tissue of adaxial leaf side, or in the proportion of water storage tissue between the populations. Duncan's test showed significantly lower proportion of vascular bundles with sclerenchyma in plants from Slano Kopovo.

	Inland saline area		Maritime saline area	
	Slano Kopovo	Rusanda	Tivatska solila	Ulcinj salina
% of salts in soil	0.65	0.60	1.50	0.80
Ph soil	8.83	9.91	7.68	7.50
Na^+	123.9	239.7	589.3	1131.3
K^+	0.18	0.65	6.5	28.8
Ca^{++}	1.9	1.0	42.9	67.2
Mg^{++}	3.9	0.8	89.4	201.9
CO_3^{2-}	0	2.5	0	0
HCO_3^-	5.9	52.3	1.1	2.1
Cl^-	7.8	7.8	51.2	131.2
HSO_4^-	60.6	96.3	61.0	63.6

Table 2. Percentage of salt, pH values and concentration of ions (meq/l) in the soil obtained from the plant root zones at examined localities

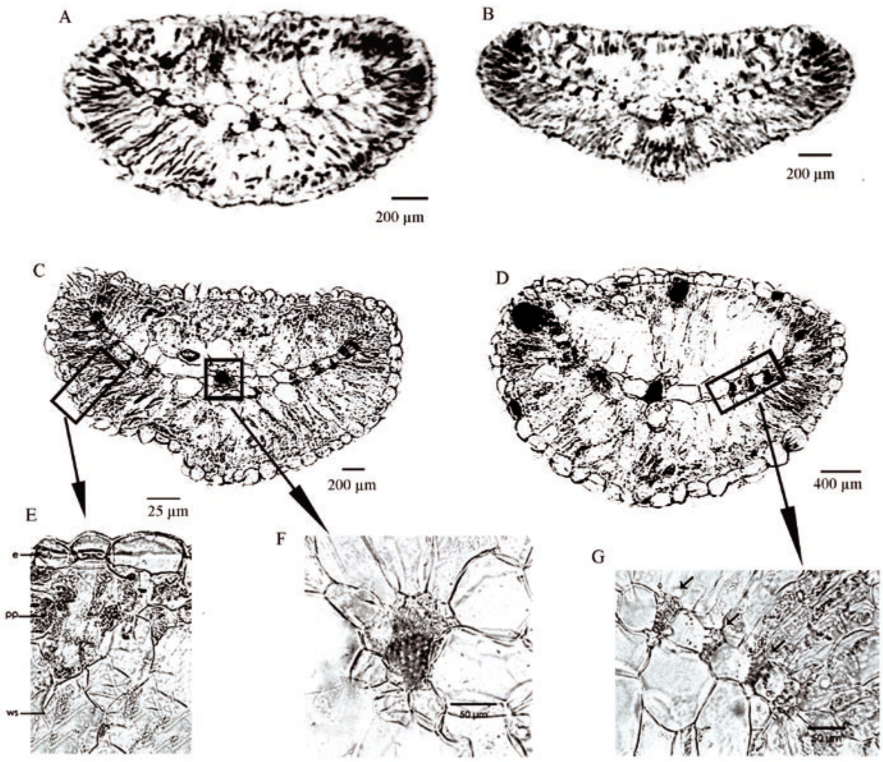

Figure 3. Cross-section of the leaf. A- Slano Kopovo, B- Rusanda, C- Tivatska solila, D- Ulcinj salina, E: e-epidermis, pp-palisade tissue, ws-water storage tissue, F-main vascular bundle, G-smaller vascular bundles

In the populations studied, the average stem height ranged from 14.8 cm to 20.6 cm (Table 3). The stem had a single epidermis layer, which was covered with thin cuticle (Figure 4). The outer part of stem cortex was differentiated into palisade tissue and collenchyma, both tissues forming strands that radially alternated each other (Figure 4E). Beneath collenchyma and palisade tissue, several layers of thin-walled parenchymatous cells were observed (Figure 4F). In the central cylinder, numerous collateral vascular bundles were arranged in a circle beneath well developed sclerenchyma tissue (Figure 4G). Pith parenchyma was compact, composed of relatively large parenchyma cells, with no cavity present.

Stem cross-section area, stem diameter, cortex thickness, total percentage of cylinder and proportion of collenchyma and pith parenchyma were significantly higher in plants from inland saline area, while populations from maritime saline area had significantly higher proportion of epidermis and cortex parenchyma (Table 3). The plants of the population from Slano Kopovo had significantly higher percentage of pallisade tissue than plants of other three sample sites. However, the highest proportions of vascular bundles with sclerenchyma and pith parenchyma were recorded in plants from Rusanda.

Populations from inland saline area had significantly higher percentages of leaf palisade tissue, stem collenchyma, stem vascular bundles and pith parenchyma, while populations from maritime saline area had higher percentages of leaf and stem epidermis, leaf vascular bundles and cortex parenchyma, according to the results of t-test (Figure 5).

Principal Component Analysis (PCA) defined four groups of characters that contributed to 65.88% of the total variation (Table 4). The first principal component accounted for 33.14% of the total variation observed. It was defined by the size of stomata, leaf and stem cross-section area, proportions of leaf palisade tissue, stem collenchyma, cortex parenchyma and pith parenchyma. The second principal component amounting to 15.65% of variation, was characterized by the variability of adaxial leaf side, cortex thickness and proportions of leaf vascular bundles and stem epidermis. While the third principal component explained 2.97% of variation due to the variability of palisade tissue thickness on adaxial leaf side, the fourth principal component contributed to only 1.82% of variation due to the variability in the number of stomata on abaxial leaf side. According to the type of variability, examined populations were grouped by PCA (Figure 6). The projection of the cases for the first

Organ		Character	I	II	III	IV
Leaf		cross-section area (mm^2)	1.7±0.2^{a} (32.1)	1.6±0.2^{a} (30.7)	0.7±0.1^{b} (32.4)	0.9±0.1^{b} (18.5)
		leaf thickness (μm)	994±51.4^{a} (16.4)	901±48.1^{a} (16.9)	745±35.4^{b} (15.0)	876±32.2^{a} (11.6)
		% epidermis	8.6±0.8^{b} (27.6)	9.7±0.5^{b} (17.8)	15.0±0.9^{a} (18.5)	14.9±0.9^{a} (19.4)
	Adaxial leaf side	number of stomata/mm^2	54.7±2.8^{a} (16.02)	47.5±3.0^{ab} (19.9)	46.0±2.4^{b} (16.4)	49.1±1.6^{ab} (10.2)
		stomata length (μm)	35.4±1.2^{a} (10.4)	37.4±2.1^{a} (17.4)	29.0±1.4^{b} (15.2)	28.5±0.9^{b} (9.5)
		stomata width (μm)	22.9±1.7^{a} (22.9)	24.5±1.1^{a} (13.6)	17.9±0.7^{b} (11.7)	18.2±0.4^{b} (6.8)
	Abaxial leaf side	number of stomata/mm^2	33.3±2.4^{ab} (23.2)	38.0±2.6^{ab} (22.0)	31.8±2.2^{b} (22.4)	39.1±1.9^{a} (15.7)
		stomata length (μm)	39.6±1.4^{a} (11.6)	41.0±1.3^{a} (10.4)	33.7±0.7^{b} (6.8)	32.1±0.8^{b} (8.2)
		stomata width (μm)	23.4±0.9^{a} (11.9)	25.1±0.8^{a} (10.3)	19.7±0.9^{b} (14.8)	18.9±0.6^{b} (10.3)
	mesophyll	pallisade tissue thickness on adaxial leaf side (μm)	220±14.1^{a} (20.4)	204±9.1^{a} (14.1)	189±10.1^{a} (16.9)	205±7.1^{a} (10.9)
		pallisade tissue thickness on abaxial leaf side (μm)	188.6±8.9^{a} (14.9)	179.7±7.9^{ab} (14.0)	153.4±10.9^{b} (22.6)	167±9.1^{ab} (17.3)
		% pallisade tissue	62.4±0.8^{a} (3.9)	61.3±0.7^{a} (3.6)	56.8±0.9^{b} (5.2)	57.2±1.0^{b} (5.4)
		cross-section area of pallisade cells (μm^2)	2906±180^{a} (6.2)	2668±287^{a} (34.0)	2047±244^{b} (37.6)	1778±76.6^{b} (13.6)
		% water storage tissue	28.9±0.5^{a} (5.6)	29.0±0.6^{a} (6.3)	28.1±0.5^{a} (5.8)	27.9±0.5^{a} (5.9)
		% vascular bundles	1.5±0.1^{b} (19.5)	1.8±0.1^{a} (25.5)	2.0±0.1^{a} (17.7)	1.9±0.09^{a} (14.34)
Stem		height (cm)	20.6±2.5^{a} (38.7)	19.1±1.2^{ab} (19.6)	14.8±0.9^{b} (19.4)	19.3±1.4^{ab} (22.3)
		cross-section area (mm^2)	2.8±0.2^{a} (17.6)	3.3±0.4^{a} (33.7)	1.2±0.1^{b} (19.2)	1.4±0.1^{b} (20.1)
		diameter (mm)	0.9±0.03^{a} (8.9)	1.01±0.1^{a} (19.4)	0.6±0.02^{b} (13.6)	0.7±0.02^{b} (10)
		% epidermis	5.3±0.2^{b} (9.8)	4.7±0.2^{b} (10.2)	6.1±0.2^{a} (13.1)	6.4±0.2^{a} (12.2)
	cortex	thickness (mm)	0.3±0.01^{a} (9.4)	0.3±0.02^{a} (18.0)	0.2±0.01^{b} (12.0)	0.2±0.01^{b} (12.2)
		% collenchyma	9.8±0.7^{a} (23.3)	8.9±0.7^{a} (24.8)	6.0±0.2^{b} (12.1)	6.8±0.2^{b} (10.8)
		% pallisade tissue	7.6±0.5^{a} (20.6)	5.7±0.4^{b} (20.0)	5.5±0.3^{b} (14.8)	6.1±0.2^{b} (10.4)
		% parenchyma	31.1±1.3^{c} (12.8)	29.7±0.8^{c} (8.9)	46.5±1.0^{a} (6.8)	41.8±1.1^{b} (8.4)
	cylinder	% v.bundles with sclerenchyma	10.3±0.7^{ab} (21.3)	11.6±0.8^{a} (0.2)	9.3±0.4^{bc} (12.7)	7.6±0.4^{c} (18.7)
		% pith parenchyma	22.2±1.5^{b} (21.9)	26.1±1.1^{a} (12.9)	14.0±0.6^{d} (13.9)	18.1±0.7^{c} (12.2)

Table 3. Leaf and stem anatomical characteristics (mean value ± standard error and coefficient of variation %)

I – Slano Kopovo, II – Rusanda, III – Tivatska solila, IV – Ulcinj salina. Different superscripts indicate that differences between localities are significant according to Duncan's test ($P \leq 0.05$)

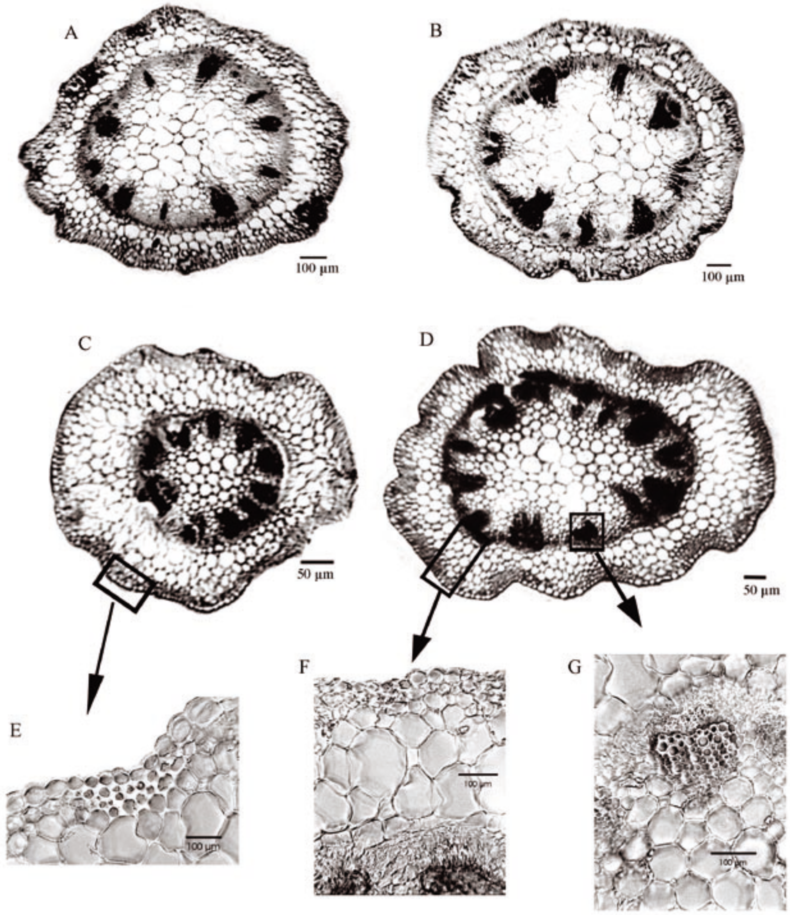

Figure 4. Cross-section of the stem. A- Slano Kopovo, B- Rusanda, C- Tivatska solila, D- Ulcinj salina, E-collenchyma, F-cortex, G-vascular bundle

two components showed that populations from inland and maritime saline areas could be clearly separated according to the type of variation of the examined parameters. It also showed higher variability of examined characters in populations from inland localities.

The results of the Multivariate Discriminant Analysis of the populations studied showed that continental (Slano Kopovo and Rusanda) and maritime populations (Tivatska solila and Ulcinj salina) formed two completely separate groups (Figure 7), supporting the PCA analysis. Morpho-anatomical separation between inland and maritime saline area populations were also observed on the basis of Euclidian distances (Figure 8).

4. Discussion

The analyzed populations grew under different climate conditions: continental-semi-arid and Mediterranean climate. Concentration of soil salt in inland saline area depends mainly of climatic conditions (temperature and precipitation). The duration of evaporation periods increases with elevation and thus salt becomes increasingly concentrated in the soil. The Pannonian Plain has a moderately continental climate with warm summers, cold and dry winters, the precipitation unevenly distributed in space and time, and very often dry periods during June, July and August. The salinity gradient increases during the dry and warm season. However, rainfall analysis for July 2006 showed that monthly precipitations were above long term average by 33% [30]. Considering soil and plant sampling in August and amount of precipitations in July, it might be possible that the precipitations influenced decrease in salt concentration in soil in that area compared to previous studies [3,31]. Detected values of salt concentrations in analyzed maritime saline areas were greater because of higher average temperature and lower precipitation in July. However, periodical flooding of sea water in maritime saline area can greatly influence the quality and quantity of salt found in the soil. This might explain why the compositions of salt in soil in maritime saline

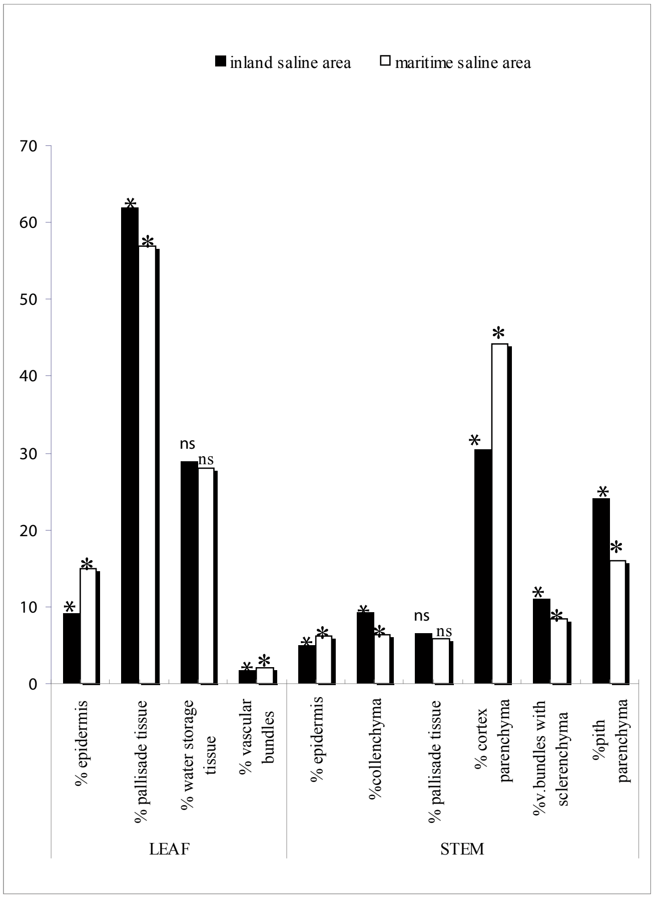

Figure 5. Comparison of leaf and stem tissue percentages in plants from inland and maritime saline area. * - differences between inland and maritime saline area significant; ns - differences not significant (t-test, P≤0.05)

areas are more similar to each other, compared to those of inland saline areas.

Leaf structural characteristics revealed that investigated populations of *S. maritima* were very similar and typically halomorphic. The leaves had a single layer of epidermis, two or three layers of palisade tissue and inner water-storage tissue, containing vascular bundles. According to Fisher *et al.* [6] the mesophyll of C_4 species of *Suaeda* is differentiated into three distinct layers: below the epidermis is palisade parenchyma, an inner chlorenchymatous sheath and central water-storage tissue. This specialized anatomy, so called *suaedoid* type, is typical for *S. californica, S. taxiflora, S. moquinii, S. tampicensis* and *S. conferta*. In contrast, in leaves of *S. calceoliformis, S. esteroa* and *S. linearis* distinct chlorenchymatous sheath was not observed. This anatomy, termed *austrobassioid* is typical for most C_3 plants of the genus. Water-storage tissue is more abundant in the *suaedoid* species, where it occupies at least 50% of the volume of the leaf, whilst it comprises less than 30% of the leaf volume in the *austrobassioid* species [6]. Our results confirm that *S. maritima* belongs to *austrobassioid* type of anatomy. All studied populations had less than 30% of the water-storage tissue and the special chlorenchymatous sheath was not observed. This is in accordance with previous work by Welkie and Caldwell [32], Carolin *et al.* [33] and Mateu [34]. Macromorphological and structural similarity of analyzed populations might be assumed as a strong indicator of some kind of morpho-anatomical conservatism of this species.

Longstreth and Nobel [35] reported that leaf succulence, leaf epidermal thickness and mesophyll thickness increased with increasing NaCl concentration, both in salt-tolerant and salt-sensitive plants of different genera. Hopkins and Blackwell [36] found that succulence and roundness of the cross-sections of *Suaeda* species leaves increased with increasing salinity of the habitat. According to Flowers *et al.* [37] growing of *S. maritima* in high concentration of salts induced an increase in leaf thickness and cell size and decrease in number of stomata per leaf unit area and stem stellar diameter. All these anatomical changes were assigned as halomorphic adaptations of plants

Characters			Factor 1	Factor 2	Factor 3	Factor 4
Leaf	leaf cross-section area		0.712042*	-0.410872	0.385409	-0.108707
	leaf thickness		0.492985	0.331723	0.573758	-0.166579
	% epidermis		-0.644659	-0.562869	-0.138459	-0.039817
	no. of stomata /mm^2 (adaxial)		0.449827	-0.719270*	0.098720	0.012000
	stomata length (adaxial)		0.664803	0.180331	-0.216196	-0.317704
	stomata width (adaxial)		0.719572*	0.135939	-0.079399	-0.096444
	no. of stomata /mm^2 (abaxial)		0.047224	-0.144704	0.227993	0.820192*
	stomata length (abaxial)		0.714444*	0.105135	-0.462323	-0.332989
	stomata width (abaxial)		0.745372*	-0.008710	-0.373355	-0.046600
	pallisade thickness (adaxial)		0.340329	0.313031	0.741557*	-0.185216
	pallisade thickness (abaxial)		0.459927	0.366324	0.685504	-0.135724
	% pallisade tissue		0.739028*	0.189013	0.276910	-0.110958
	area of pallisade cells		0.537132	0.335062	0.460539	-0.050506
	% water storage tissue		0.421446	-0.329284	-0.005005	0.183773
	% vascular bundles		0.206808	-0.873221*	0.136516	-0.024477
Stem	stem height		0.336839	-0.118631	-0.265257	-0.251246
	stem cross-section area		0.854086*	-0.273250	-0.064213	0.156537
	stem diameter		0.639198	-0.582501	0.077681	0.040112
	% epidermis		-0.209683	-0.725410*	0.295025	-0.207896
	cortex	thickness	0.569117	-0.759640*	0.065495	-0.098515
		% collenchyma	0.716837*	-0.150024	-0.287427	-0.022480
		% pallisade tissue	0.400142	-0.410370	-0.014705	-0.209905
		% parenchyma	-0.743449*	-0.389975	0.242748	-0.280357
	cylinder	% v.bundles with sclerenchyma	0.653480	-0.159379	-0.325637	-0.108474
		% pith parenchyma	0.713371*	0.157118	-0.106779	0.440222
cumulative percentages of the vectors			33.14	48.79	59.40	65.88

Table 4. Principal component analysis (PCA) of measured parameters. Factor coordinates of variables, based on correlations and cumulative percentages of the vectors

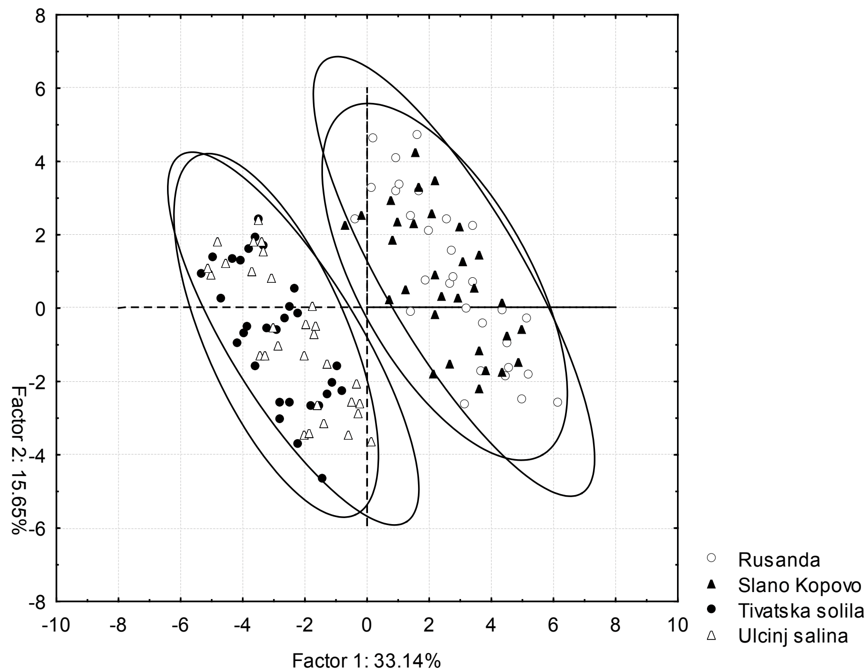

Figure 6. The projection of the cases of the first two components of the Principal Component Analysis

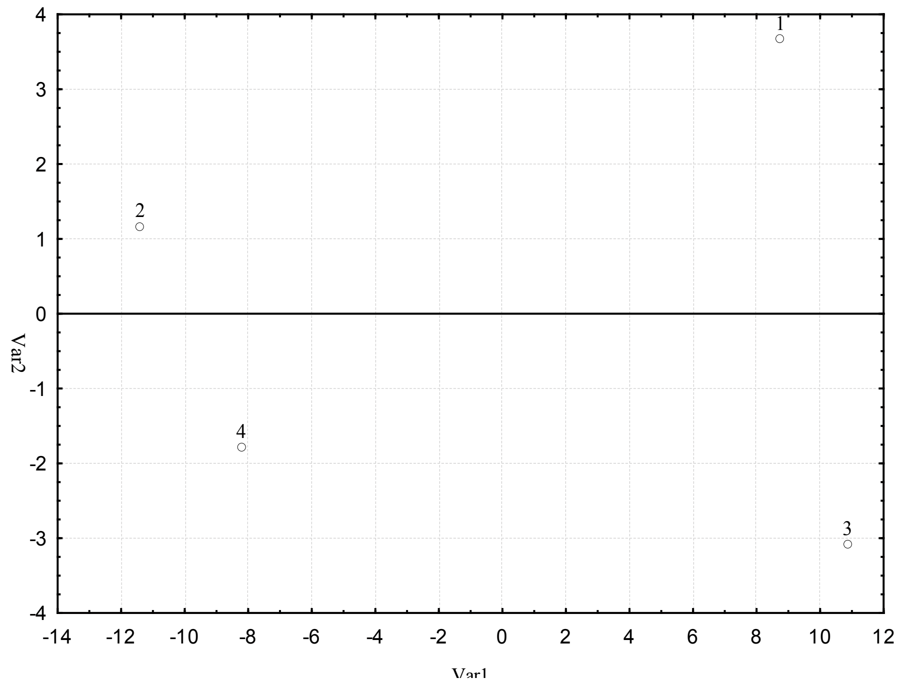

Figure 7. The results of the Multivariate Discriminant Analysis, projection of the first two factors. 1- Slano Kopovo, 2- Rusanda, 3- Tivatska solila, 4- Ulcinj salina

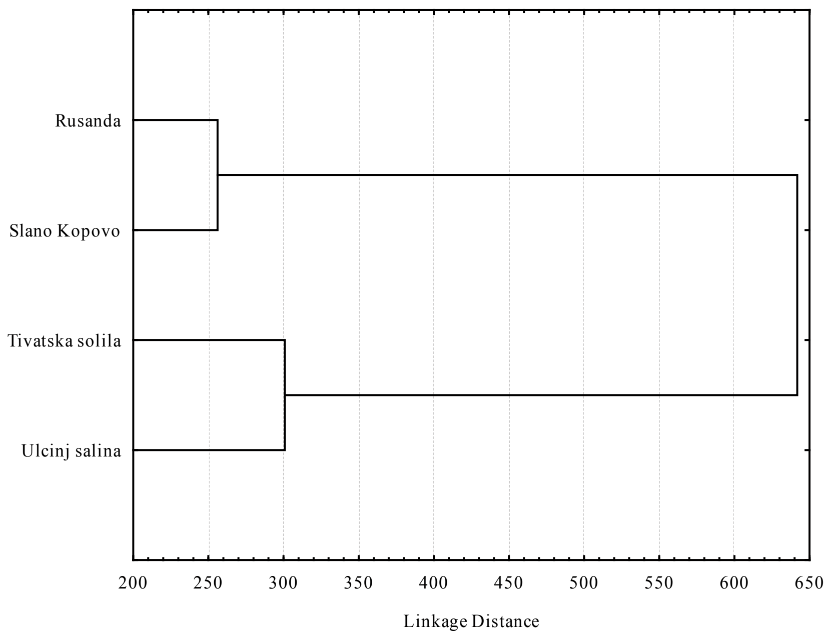

Figure 8. Euclidian distances between the analyzed populations of *S. maritima* subsp. *prostrata* (Rusanda and Slano Kopovo) and *Suaeda maritima subsp. maritima* (Tivatska solila and Ulcinj)

to high salt concentrations in the soil. Considering leaf characters, our data showed that leaves from from maritime saline plants were more rounded and had a significantly higher percentage of epidermis and lower number of stomata per mm^2 on adaxial leaf side, than the inland saline plants. On the other hand, plants from inland saline area had significantly larger leaf cross-section area and palisade cell size. Hajibagheri *et al.* [20] found that both the cuticle and cell wall of epidermal cells of *S. maritima* showed a considerable increase in thickness when grown in presence of sodium chloride. Higher values were recorded in plants from maritime salines potentially due to a thicker cuticle and an increase in the size of epidermal cells, following salt accumulation. Smaller stomata size, especially stomata width, was related to smaller stomatal pore width. With stomatal pore closure and reduction of stomatal frequency, plants adapt under a salt stress environment, in order to effectively economize water use [38]. Stomatal closure limits water loss and reduces transpiration, which minimizes the accumulation of toxic ions [39]. With respect to stem characters, plants from maritime populations had smaller stem stellar diameter, thinner cortex, and higher percentage of cortex parenchyma and stem epidermis. Wahid [40] also reported these stem characters as adaptations of desert halophytes in natural saline habitats.

The results of our study correspond to the previously described changes in the leaf and stem structure, induced by higher concentrations of soil salts. Comparison of leaf and stem anatomical characteristics between the plants from two saline areas indicated that plants from maritime saline area showed more halomorphic characteristics, which could be explained by the higher percentage of total salt in the soil. Furthermore, in maritime saline areas higher concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^{2-} were recorded, compared with inland saline area. Duan *et al.* [41] recorded plants being taller with increasing salinity of the habitat, which was not the case for our examined material. The observed reduction in growth is in line with the results published by Wahid [40]. Plants from inland saline area had higher number of stomata per mm^2 on adaxial leaf side, significantly larger proportions of leaf palisade tissue and thicker stem cortex. Also, the ratio of the stem cortex to stem diameter ranged from 0.269 (Rusanda) to 0.399 (Tivatska solila), which was within the usual values found in xeromorphic stems [42]. High concentration of sulphate ions in the soil induces xeromorphism in plants [22,43]. As the concentration of sulphate ions was greater in inland saline area, we propose that this was the reason why more xeromorphic characteristics were recorded in these plants. They must be regarded as favorable adaptive modifications, which help in protecting the mesophyll from excessive water loss and intense radiation in this continental semiarid environment.

Morpho-anatomical studies of *S. maritima* populations showed that each population had a combination of halomorphic and xeromorphic characteristics. Most quantitative parameters of the leaf and stem were similar to the parameters of desert species as observed by Voronkova *et al.* [9], indicating that the anatomical adaptive strategies of halophytes and desert plants were similar. Furthermore, our research has shown that populations from inland saline area showed higher variability of examined characters. Principal component analysis of the anatomical leaf and stem traits clearly separated *S. maritima* subsp. *prostrata* populations from inland saline area and *S. maritima* subsp. *maritima* from maritime saline area, due to the type of variation of the size of stomata, leaf and stem cross-section area, proportions of leaf palisade tissue, stem collenchyma, cortex parenchyma and pith parenchyma. The results of Multivariate Discriminat Analysis also showed that two subspecies could be clearly separated according to their quantitative anatomical characteristics. In conclusion, based on our analysis, climate and the amount of salt and ions in the soil, are important factors that enhance adaptive potential of *S. maritima*.

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