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ANATOMICAL FEATURES OF SEAGRASS *POSIDONIA OCEANICA* (L.) DEL. GROWING IN MONTENEGRO (ADRIATIC SEA)

SUMMARY

Anatomical features of *Posidonia oceanica* leafs were not described for the South Adriatic Sea, so fare. Study was performed on three *P. oceanica* meadows in the Bay of Boka Kotorska (Montenegro) and anatomical characteristics of sheaths and leaf blade are presented.

Although the rarity of sexual reproduction in *P. oceanica* and the isolation of certain populations, suggests that particular clones may have differentiated locally, on the base of anatomical characteristics three investigated populations of *P. oceanica* in the Adriatic Sea are not significantly different from others in different areas of Mediterranean basin.

Key words: *Posidonia oceanica*, Adriatic, Montenegro, anatomy

INTRODUCTION

In the Mediterranean Sea a great number of scientific projects have been dedicated to *Posidonia oceanica* (L.) Del. an endemic seagrass that plays a major ecological, sedimentary and economic role (Molinier and Picard 1952, Pergent, 1990, Mazzella *et al.* 1998, Duarte, 1999, Waycot *et al.* 2009, Dural *et al.* 2013). Meadows of this seagrass are recognized by the European Habitat Directive (92/43/CEE) as a priority habitat and a plant is protected by several international conventions (Barcelona Convention 1976, Bern Convention 1979) and in Montenegro, as well as in many other countries, *P. oceanica* is protected by national law (Službeni list 76/06). Furthermore, seagrass in general and *P. oceanica* meadows in particular, are considered to be appropriate for biomonitoring, because of their wide distribution, reasonable size, easy collection, abundance and sensitivity to modifications of littoral zone (Pergent-Martini *et al.* 2005).

Beside very intensive ecological studies of *P. oceanica* and seagrasses in general, recently, different types of research has been directed towards their phylogeny, population genetic and chemical composition (Dolenc Koce *et al.* 2003, Dumay *et al.* 2004, Haznedaroglu and Zeybek 2007). It has been found out that seagrasses contain several compounds in their secondary metabolism in which they differ from terrestrial plants and also not known from other taxonomic groups. Some of these compounds might be of interest for commercial purpose, so the further analysis, especially in pharmaceutical sciences are of great interest (Papenbrock, 2012, Haznedaroglu and Zeybek 2007).

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Up to now, for the coast of Montenegro (Adriatic Sea) there are several reports about distribution, phenological characteristics and heavy metals concentration in the *Posidonia oceanica* meadows (Špan and Antolić, 1983, Mačić and Sekulić 2001, Mačić and Boža 2001, Mačić *et al.* 2011, Mačić, 2012) that could be compared with studies performed in the whole Mediterranean and used as descriptor for assessing a good ecological status of coastal zone. But, beside lepidochronological analysis performed in some parts of Mediterranean basin (Pergent, 1990), anatomical features of this species are less known and in the south Adriatic Sea not studied at all. Because of all that, the objectives of this study were to describe anatomical features of *Posidonia oceanica* leafs collected from the Bay of Boka Kotorska (Adriatic Sea) and to compare data from these populations with others in Mediterranean.

MATERIAL AND METHODS

The plant samples were collected by SCUBA diving at 3 locations in the Boka Kotorska Bay (Kotor N 42.466151° E 18.762448°, Tivat N 42.448683° E 18.686076° and Herceg Novi 42.448056° E 18.537580°). In each location 20 orthotropic (erect) shoots were collected from 6m depth. Furthermore, 20 orthotropic shoots were collected from deepest pars of the meadows, so, for Kotor location it was at 10 m, for Tivat at 7m and for Herceg Novi at 22 m depth. The anatomical examination was performed on transverse section of adult sheats and leaf blade made by cryomicrotome. Cutting was performed 20-30 mm from the base for sheats and 10-20 mm from the base of the leaf blade (Crouzet, 1984). Treatmen with Sudan III was used to determine lipids, while tannins cells were collored by shafranin (Švob, 1974). Anylisis of variance (ANOVA) was performed and factors were represented by the stations and depth.

RESULTS AND DISCUSSION

The cross section of the sheaths has a characteristic shape of the letter "C" because of two lateral flaps tightly folded over the younger leaf. Compering with the central part of the sheets, these lateral flaps are vary thin and thay are representing up to 70-78% of the overall breadth of the sheat. Unlike the sheets, the cross section of the leaf is almost of the same thickness in each part of the blade.

The epidermis of the sheaths is formed by one quadrangular cell layer were the cells are elongated in the direction of growth and covered by a thin cuticule. On the cross section of the sheaths epidermal cells are quadrangular or poligonal and they did not have chloroplasts (Fig. 1). Adaxial (ventral) epidermis consists of the bigger cells compering to the abaxial (dorsal) epidermis (Tab. 1). Furthermore, for the adaxial cells equal thicknes of cell walls was observed, while abaxial cells had a thicker cell wals on the surface.

Epidermis of the leaf blade is formed also by one quadrangular cell layer, elongated in the direction of growth and rich in chloroplasts.

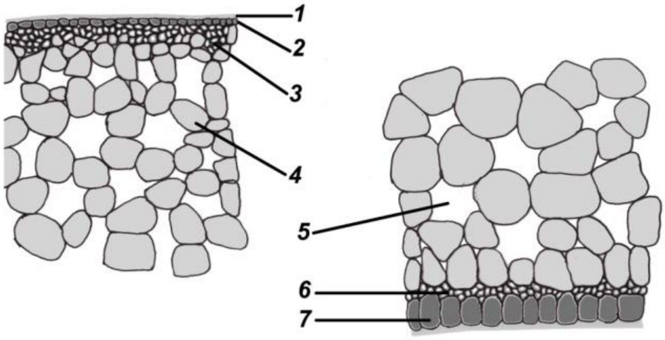


Figure 1. Scheme of sheaths cross section: 1. cuticula, 2. dorsal epidermis, 3. sclerenchyma bundle, 4. messophyll, 5. air lacunae, 6. sclerenchyma layer and 7. ventral epidermis

The highest concentration of chloroplasts and also other organels in *P. oceanica* leafs are situated in epiderms. This is adaptation of the plant to the reduced amount of the light in the water environment and also adaptation for the easier exchange of the gases (Kojić, 1984, Papenbrock, 2012). Tipical for aquatic plants, also in *P. ocanica* leaves there are no stomata and leaf surface is covered by a thin cuticule layer with a wax.

Before, it was considered that waxed cuticule could stop the flow of gas and nutrients, but detail research showed the porose structure of cuticule and its role in the gas and ion exchange with the surrounding water (Gibson, 1984, Haznedaroglu, Akarsu 2009). Dimensions of the epidermal cells (Tab. 1) were equal on the both sides of the blade. Contrary to the situation with the sheats, environmental impact is practicaly the same on the both sides of the blade, so the cell walls are eqaly thick on both sides (Fig. 2).

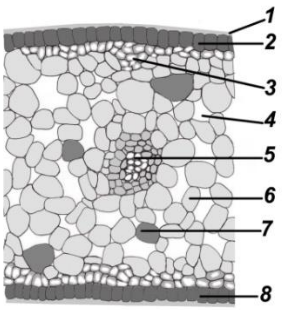


Figure 2. Cross section of the leafe blade in central part: 1. cuticula, 2. dorsal epidermis, 3. fibre bundle, 4.air lacunae, 5. vascular bundle, 6. messophyll, 7. tannin cells and 8. ventral epidermis

In this survey only significant difference in length of epidermal cells were between samples from 22m depth with those from 6m and 7m depth. Mean walues of dimensions (wide and high) for leaf blade and sheats epidermis (abaxial and adaxial) are shown in Table 4 and 5. Compering to the data with other locations from Algeria and France (Semroud *et al.* 1992) there is no significant difference, although more detail analisis of material sampled from the same depth would be needed for more precise conclusions.

Below the epidermis is mechanical tissue, represented by lignified cells. These cells are very elongated and with a very thick cell wall. On the abaxial side of sheats this type of cells are grouped in the fibre bundles, while on the adaxial side are mostly distributed in one layer, as well as in the leaf blade (Fig. 1 and 2). In the mesophyll tissue there was no fibre bundles and practicaly their possition close to the surface of the lafes is favorable for the resistance of the leafe. Number of fibre bundles ranges withouth significant difference from 10 to 20 with average of 14.

The messophyll tissue is represented by oval cells of approximately same dimensions and withouth diferentiation to the palisade and spongy layer. These cells have a thin cell walls and compering to the epidermis smaller number of chloroplasts. Between messophyll cells there are many air lacunae, so, this type of tissue is also called aerenchyma (Figure 1 and 2). It is generally assumed that aerenchyma is adaptation of the aquatic plants specially to the lack of oxigen in the deeper layers of the water, but also they are considered important in seagrass photosynthesis (Terrados, 1999; Kuo and den Hartog 2006). Beside that, funcion of the air lacunaes is also to allow more or less vertical possition of the very long leafs. In that way plant is awoiding deposition of the sediments and other material on the leaf and also it is capable to use maximum quantity of the light.

Table 1. Range of dimensions for epidermis cells on different locations (*Semroud et al. 1992)

PART OF LEAF	TISSUE	DIMENSIONS IN μm	LOCALITY		
			Algiers La Marsa*	Montenegro Boka Kotorska	France Port-Kros*
sheaths	abaxial epidermis	high	8-21	14-19	7-13
		wide	10-18	10-19	12-20
	adaxial epidermis	high	23-52	19-29	28-43
		wide	15-20	16-25	15-30
blade	epidermis	high	17-24	7-18	12-24
		wide	3-9	6-10	4-10

Vascular bundles are located in the messophyll tissue and compering to the vascular land plants they are reduced (Fig. 2). Central vascular boundle is sligtly bigger than others and positioned more to the dorsal side. Xylem cells are present in this central boundle, but in most of the others xylem is absent. This reduction of xylem tissue, tipical for the aqatic plants, is interpreted as a result of the loss of functional need (mechanical and conductive) in plants with a constant supply of water and supported by the aquaeous medium (Kuo and den Hartog 2006). In the sheats from 13 to 17 vascular bundles were counted, while in the leaf was slighetly less, from 13 to 15. Comparison for numbers of vascular bundles in Boka Kotorska Bay and data from literature are shown in Table 2.

Tab. 2. Number of vascular bundle in the leafs from different locations (* - Semroud *et al.*, 1992)

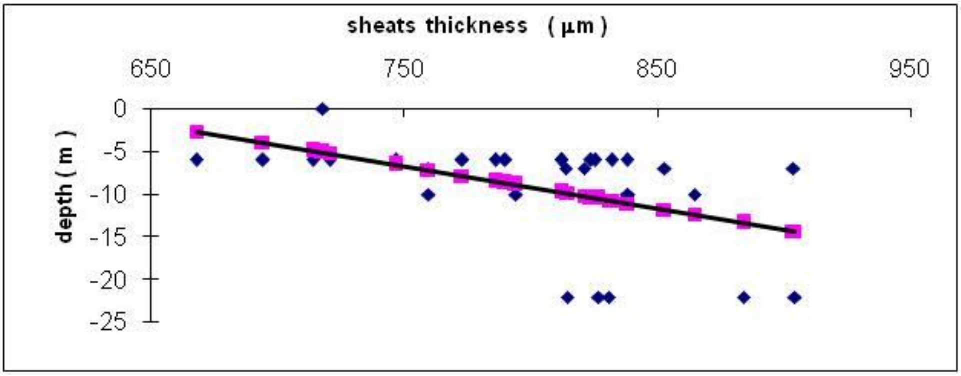
PART OF LEAF	LOCALITY		
	Algiers (La Marsa)*	France (Port-Cros)*	Montenegro Boka Kotorska
sheaths	19-21	13-17	13-17
blade	15-17	14-15	13-15

Close to the vascular bundles and to the surface of the leafs are numerous cells with tannin (Fig. 2). Vacuolas of these cells are of granulate structure and they are collored with Sudan III indicating presence of lipids. The tannin cells are specialized in production of phenolic compounds and they play an important role in the protection of plant against predators, competitors and pathogens (McMillan 1984, Agostini et al. 1998, Haznedaroglu and Zeybek 2007). Pergent (1990) reported a significant increase in the number of tannin cells with the age of the rhizome, but some authors (Dumay et al. 2004, Pergent et al. 2008) reported that the production of secondary metabolites and the number of tannin cells largely increased when the degree of interaction with invasive algae Caulerpa taxifolia increased. Although allelopathy is well known in the terrestrial environment, for the marine plants is not very well studied (Kojić, 1984, Dumay et al. 2004). Furthermore, concentration of different metabolites differ due to the metabolism of the plant in different condition (Cuny et al. 1995, Haznedaroglu and Zeybek 2007). Because of that, although the plant might be a source of compounds to be investigated for anti-HIV, antitumor, antioxidant and antibacterial activities further studies will be needed to confirm the potential use in pharamceutical and other purposes (Anselmi et al. 2004, Cardenas et al. 2006, Haznedaroglu and Zeybek 2007).

Tab. 3. Levels of significance in thickness of sheaths and blade for samples from different depth (ns-not significant, * significant for P < 0,05)

LEAF		DEPTH					
		6m-7m	6m-10m	6m-22m	7m-10m	7m-22m	10m-22m
sheaths	length	ns	*	*	*	ns	*
	wide	*	*	*	ns	ns	ns
leaf blade	length	ns	ns	ns	ns	ns	ns
	wide	ns	ns	ns	ns	ns	ns

The mean thickness of sheats collected in this survey shows significant variation between different depts, while differences in the blade thickness were not significant (Table 3). Furthermore, it is noted that thickness of sheats increases with depth (Graf 1) and coeficient of corelation was r=0,45 what was in accordance with the literature data that the mean thickness varies between sites depending on three factors: depth, water movement and locality (Pergent 1990).



Graf. 1. Function of the mean thickness of the scales according to the depth at Boka Kotorska Bay

Tab. 4. Some of the parameters for anatomical characteristics of *P. oceanica* sheaths on cross section.

PARAMETERS	LOCALITY AND DEPTH					
	Kotor		Tivat		Herceg Novi	
	6 m	10 m	6 m	7 m	6 m	22 m
Wide (µm)						
$\overline{x}$	8693	8729	8936	9730	8769	9721
St. er.	207	155	200	112	231	66
min	8017	8279	8318	9471	8135	9532
max	9274	9196	9484	10021	9445	9897
Thickness (µm)						
$\overline{x}$	754	819	815	830	740	852
St. er.	23	18	11	23	26	17
min	694	759	773	759	668	814
max	825	864	838	903	812	904
Number of vascular bundle						
$\overline{x}$	14.8	15.6	15.0	16.6	15.2	16.4
St. er.	0.6	0.6	0.3	0.2	0.6	0.2
min	13.0	14.0	14.0	16.0	13.0	16.0
max	17.0	17.0	16.0	17.0	17.0	17.0
Main vascular bundle. -high (µm)						
$\overline{x}$	136.48	137.28	143.88	146.16	123.54	149.22
St. er.	5.36	3.46	1.91	1.65	4.02	1.43
min	126.40	131.60	139.30	141.90	113.50	145.90
max	156.10	150.90	149.00	150.90	134.20	153.20
Main vascular bundle - wide (µm)						
$\overline{x}$	96.76	102.96	110.93	112.98	107.76	113.38
St. er.	3.77	5.02	4.48	3.20	5.30	2.34
min	86.40	91.60	98.04	105.00	95.50	108.20
max	108.40	117.40	122.60	122.50	121.30	119.70

Tab. 5. Some of the parameters for anatomical characteristics of P. *oceanica* leaf blade on cross section

PARAMETERS	LOCALITY AND DEPTH					
	Kotor		Tivat		Herceg Novi	
	6 m	10 m	6 m	7 m	6 m	22 m
Wide (µm)						
$\bar{x}$	8711	9316	9285	9466	8386	9067
St. er.	116	144	379	227	231	149
min	8449	8803	7964	8960	7689	8750
max	9130	9576	10283	1023	8999	9445
Thickness (µm)						
$\bar{x}$	357	406	400	421	379	382
St. er.	24	33	33	26	14	34
min	301	301	288	353	340	314
max	423	497	471	510	419	497
Number of vascular bundle						
$\bar{x}$	13.0	13.0	13.4	13.8	14.0	13.6
St. er.	0.0	0.0	0.2	0.4	0.4	0.4
min	13.0	13.0	13.0	13.0	13.0	13.0
max	13.0	13.0	14.0	15.0	15.0	15.0
Main vascular bundle - high (µm)						
$\bar{x}$	139.06	137.00	140.35	148.09	141.13	133.14
St. er.	2.56	3.54	4.27	2.62	4.72	6.18
min	132.87	131.58	129.00	141.90	126.42	113.58
max	147.06	150.93	149.64	156.09	150.93	152.22
Main vascular bundle - wide (µm)						
$\bar{x}$	102.43	104.49	109.13	108.88	113.72	108.10
St. er.	3.23	5.90	5.95	6.72	5.98	3.88
min	94.17	86.43	95.46	86.43	96.75	92.88
max	109.65	117.39	125.13	125.13	127.71	113.52

CONCLUSIONS

This study shows the first data of the anatomical characteristics of *P. oceanica* leafes on the Montenegrin coast (Adriatic Sea). Although the rarity of sexual reproduction in *P. oceanica* and the isolation of certain populations, suggests that particular clones may have differentiated locally in several basis (Semroud *et al.* 1992, Papenbrock, 2012) for the moment it seems that 3 investigated populations of *P. oceanica* in the Adriatic Sea are not significantly different from others in different areas of Mediterranean basin.

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Vesna MAČIĆ

ANATOMSKE KARAKTERISTIKE MORSKE TRAVE *POSIDONIA OCEANICA* (L.) DEL. U CRNOJ GORI (JADRANSKO MORE)

SAŽETAK

Anatomske karakteristike listova morske trave *Posiodonia oceanica* do sada nisu opisivane za južni Jadran. Istraživanje je urađeno na 3 naselja morske trave *P. oceanica* u Bokokotorskom zalivu (Crna Gora) i prikazane se anatomske karakteristike rukavaca i liske.

Iako rijetkost polnog razmnožavanja kod ove morske trave i izolacija pojedinih populacija, sugerišu da neki klonovi mogu da se mjestimično izdiferenciraju, na osnovu anatomskih karakteristika ispitivanih pupulacija *P. oceanica* u Jadranskom moru može se zaključiti da one nisu značajno različite od drugih u Sredozemnom moru.

Ključne riječi: *Posidonia oceanica*, Jadransko more, Crna Gora, anatomija