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Morphological, anatomical and physiological analyses of *Vicia narbonensis* subsp. *serratifolia* (*Fabales, Fabaceae*)

Abstract

Boža, P., Krstić, B., Merkulov, L., Krstić, L. & Pajević, S.: Morphological, anatomical and physiological analyses of *Vicia narbonensis* subsp. *serratifolia* (*Fabales, Fabaceae*). — Fl. Medit. 13: 219-229. 2003. — ISSN 1120-4052.

Most of the measured values of stem, leaf, flower and pod morphological characters are in agreement with data from literature. Since leaflet index value and length of inflorescence pedicel data were not found in the available literature, our results may contribute to better understanding of morphological features of this taxon. The anatomical analyses point to amphistomatal leaflet structure with numerous and smaller stomata on abaxial side, numerous glandular hairs, well developed leaflet spongy tissue and stem vessel elements. Mineral element concentrations are very high, especially K in all plant organs, N in leaves, P in pods and Ca in stem.

Introduction

Vicia narbonensis subsp. *serratifolia* belongs to section *Faba* (Ball 1968), or to subgen. *Vicia*, sect. *Vicia* by Diklić (1972). This plant is a close relative of *V. faba* that is currently grown as a vegetable and *V. sativa*, which is a fodder crop. The results gained by complex investigations of *Vicia narbonensis* subsp. *serratifolia* may be useful in solving taxonomical problems of genus *Vicia*, as well as in breeding and selection of cultivated species (Boža & al. 1993; Boža & al. 1996; Marin & al. 1998).

As a complex species, *Vicia narbonensis* is widely distributed and belongs to sub-mediterranean floral element, but was introduced in Eastern Africa and Northern America (Soó 1966). There are two subspecies in Yugoslavia. Subsp. *narbonensis* is distributed in the southern part and is characteristic for the Mediterranean floristic subregion. It reaches the Southeast parts of Serbia in the north (Niš, Vlasotince) (Diklić 1972) (Fig. 1). Subsp. *serratifolia* grows in continental parts of Yugoslavia and reaches Hungary, floristic province Pannonicum in the north (Ball 1968). Towards the south, it spreads up to Pirot and Niš in Yugoslavia, where areals of those two subspecies overlap (Diklić 1972) (Fig. 1).

Subspecies *serratifolia* can be considered as a continental vicar of subsp. *narbonensis* that grows in areas under the Mediterranean influence.

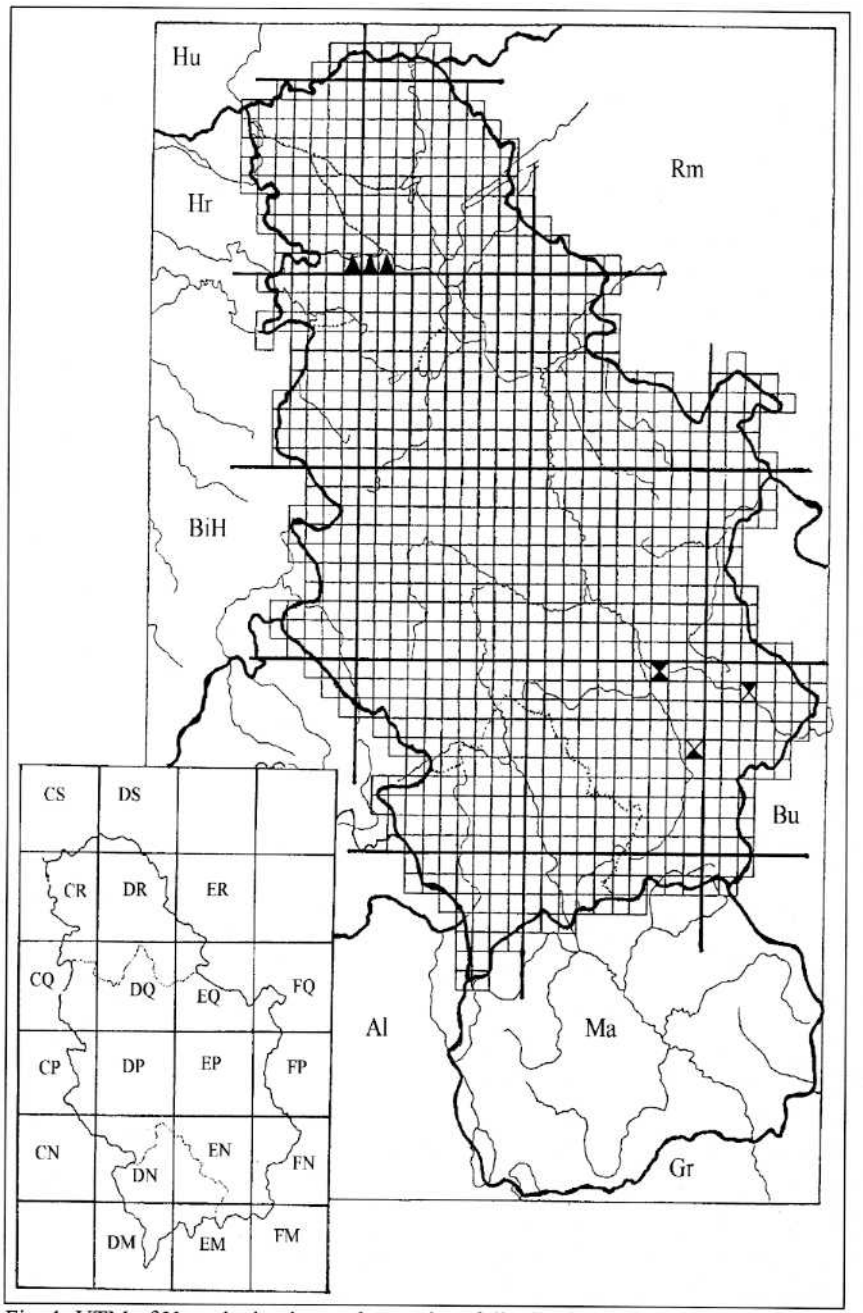

Fig. 1. UTM of Yugoslavia: the northest point of distribution of *V. narbonensis* subsp. *narbonensis* in Yugoslavia (Niš EN 79, Vlasotince EN 95) (⊠); the southest point of distribution of *V. narbonensis* subsp. *serratifolia* in Yugoslavia (Niš EN 79, Pirot FN 28) (⊠); localities on Fruška Gora where analysed plants were collected (Hopovo DR 00, Stražilovo DR 10, Osovlje CR 90, Paragovo DR 00) (▲).

Material and methods

Plant material was collected from Hopovo, Fruška Gora, at flowering (Fig. 1).

For morphological investigations 30 herbarium specimens were used. Plant height, leaflets length and width, stipule length, length of inflorescence and flower pedicels, pod length and width, number of leaflet pairs, number of flowers in inflorescence and number of grains in pod were determined. The gained values were compared with literature data.

Ten plants were used for anatomical investigations. Characteristics of leaflet epidermal tissue, number of stomata per mm^2 and stomata size were examined. Prints of leaflet epidermal tissue were made after Wolf (1954). For anatomical structure analysis of blade and stem, medium lateral leaflets of leaf rachis of pinnate leaf and central stem portion were used. Transections were made using freezing microtome. Leaflet thickness, stem diameter, tissue thickness and cell sizes of examined organs were measured.

For the purpose of physiological investigations plant material was dried and minced.

Concentrations of mineral elements in leaf, stem and pod were analyzed. Nitrogen concentration was determined using standard micro-Kjedahl method and phosphorus spectrophotometrically, by ammonium-vanadate-molybdate method (Gericke & Kurmies 1952). Concentrations of K, Ca and Na were assayed by flame photometry method (Sarić & al. 1990).

Photosynthetic pigment concentrations were determined after Wetsttein (Sarić & al. 1990), while net photosynthesis rate and dark respiration rate polarographically with Clark's electrode, by measuring the amount of released oxygen, and its uptake in dark (Wolker 1990).

Results

Morphological investigations – The analyses of herbarium specimens show that plant height is between 23.0 and 53.5 cm, pinnate leaf is consisted of 3-4 leaflet pairs that are 3.4-4.2 cm long and 1.9-3.0 cm wide, the number of flowers in inflorescence is 3-7 and their length varies from 1.8 to 2.6 cm. The length of pods is between 4.0 and 6.7 cm. They consist 4-9 grains, 8-10 mm in diameter (Gams 1924), or 6-8 mm by Schermann (1967). These values are in agreement with data from the literature (Games 1924; Hayek 1927; Fedčenko 1948; Schermann 1967; Ball 1968; Diklić 1972; Kuzmanov 1976) (Table 1).

Stipule length data of up to 1.2 cm (Fedčenko 1948; Kuzmanov 1976) differs from our measurements showing somewhat higher values of up to 1.4 cm. In relation to literature data, lower values were measured for pod width (0.6-1.1 cm).

According to our analysis inflorescence pedicel length varied from 0.3 to 0.8 cm (x = 0.5 cm). No literature data dealing with this parameter value were found. Leaflet index value is 1.40-1.95 (x = 1.6).

Among all morphological characteristics, particularly interesting is the variability of leaflet dentation. At Fruška Gora we found plants with leaflet margin dentate almost to the leaf base (Fig. 2a) and plants whose leaflets were dentate only at the leaflet apex (Fig. 2b).

This variability can be considered as an intermediary character between subsp. *narbonensis* and subsp. *serratifolia.*

Anatomical investigations – The analyses of leaflet epidermal tissue showed that it

Table 1. Morphological characteristics.

	Measured values	Average values	Gams (1924)	Hayek (1927)	Fedèenko (1948)	Schermann (1967)	Ball (1968)	Dikliæ (1972)	Kuzmanov (1976)
plant height /cm/	23-53.5	42	30-60	-	40-75	-	20-60	20-60	40-60 (100)
number of leaflet pairs	3-4	3	3-4	2-3	2-3	-	1-3	(2) 3 (4)	2-3 (4)
leaflet length/cm/	3.4-4.2	3.8	3-5	-	2-4.5	-	2-5	2.5-5	2-4.5
leaflet width /cm/	1.9-3	2.5	2-3 (4)	-	1.5-2.5	-	1-4	2-3	1.5-2.5
leaflet index	1.40-1.95	1.6	-	-	-	-	-	-	-
stipule length/cm/	0.8-1.4	1.2	-	-	do 1.2	-	-	-	1.2
number of flowers	3-7	4	1-2 (6)	2-6	3-5 (7)	-	1-6	2-6	3-7
flower length /cm/	1.8-2.6	2.2	1.5-3	-	2-2.3	-	-	2.2-2.6	-
inflorescence pedicel length /cm/	0.3-0.8	0.5	-	-	-	-	-	-	-
pod length /cm/	4-6.7	5.4	3-6	-	4-6.5	4	3-7	5	4-7
pod width /cm/	0.6-1.1	0.9	1-1.5	1	-	-	1-1.5	1.2	1-1.5
number of seeds	4-9	6	4-6	-	5-8	4-6	4-8	4-6	5-10
seed diameter /mm/	-	-	8-10	-	-	6-8	-	-	-

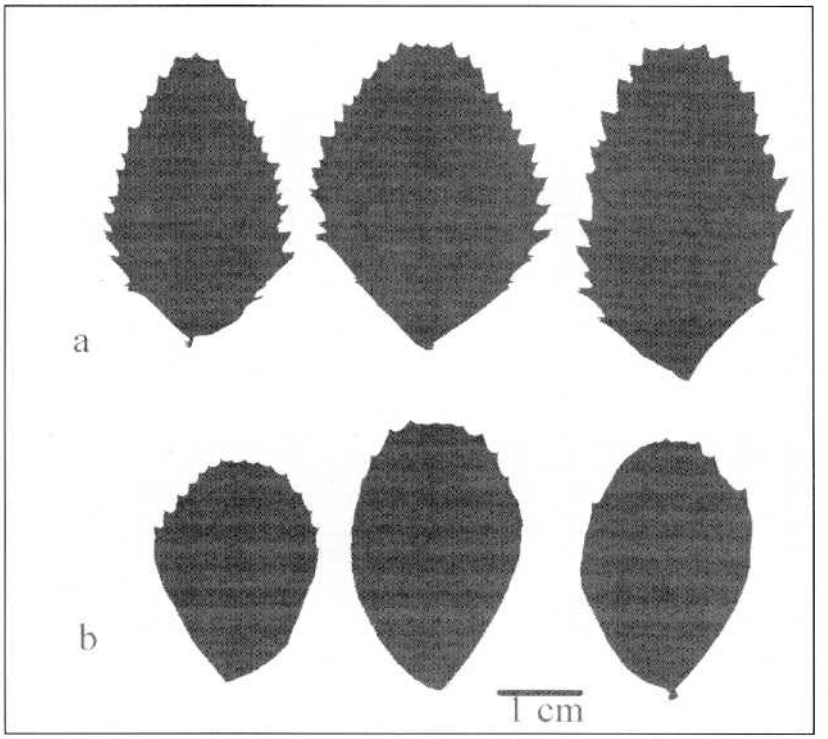

Fig. 2. Leaflet margin: a. dentate to ¾ of leaflet size; b. dentate to ↓ of leaflet size.

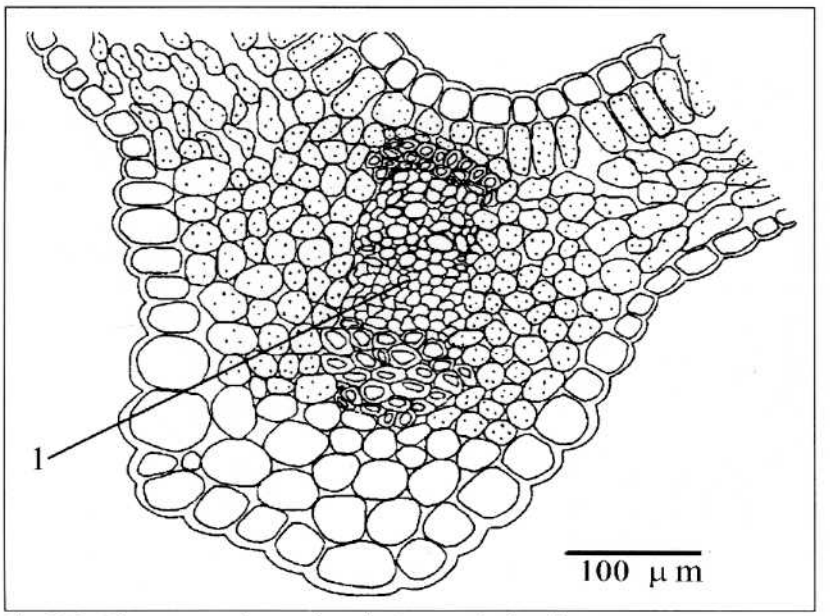

Fig. 3. Leaflet cross section, main vein: 1. vascular bundle.

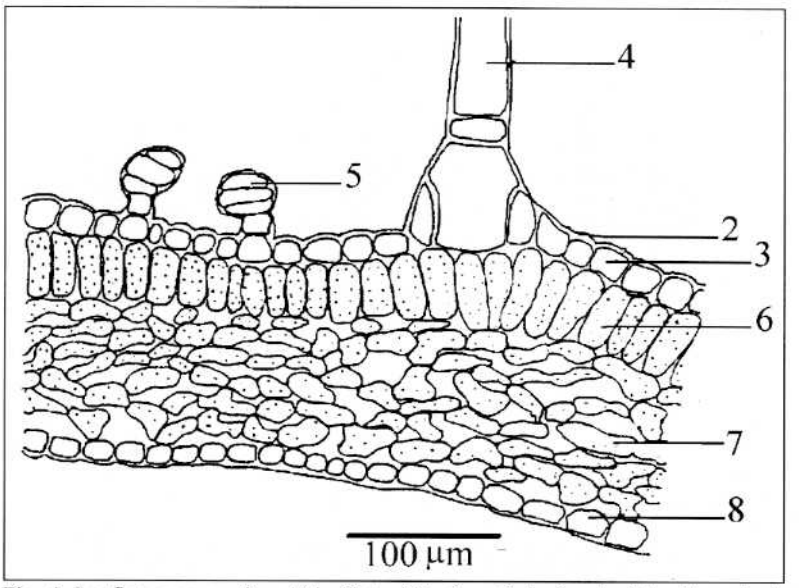

Fig. 4. Leaflet cross section, ¼ leaflet width: 2. cuticle; 3. adaxial epidermis; 4. non-glandular hair; 5. glandular hair; 6. palisade tissue; 7. spongy tissue; 8, abaxial epidermis.

consisted of cells with wavy anticlinal walls. On both leaflet surfaces non-glandular and glandular hairs occur, but more numerous adaxially. Non-glandular hairs are large, rare and mostly short, present at the main vein and leaflet margins. They are composed of the basal cell surrounded by cells raised above epidermis surface, the smaller cell above it and the elongated terminal cell. Glandular hairs are more numerous. They are consisted of the basal cell, a more or less elongated stalk cell and the multicellular round or oval head (Fig. 4).

Stomata are paracytic (Metcalfe & Chalk 1950), occurring on both epidermises. The average number of stomata on adaxial epidermis is 99/mm^2, their length and width being 31.5 µm and 24.0 µm respectively (Table 2). Stomata are smaller and more numerous abaxially (174/mm^2).

Thickness of dorsiventral leaflets is 199µm (Fig. 4, Table 3). In its transection, the main vein that is 461 µm high and 359 µm wide is convex abaxially (Fig. 3). Only one vascular bundle, 210 µm high and 168 µm wide, with mechanical tissue close to phloem and xylem occurs in the main vein. Vessel diameter is 18 µm. Epidermal cells are tabular in shape (Fig. 4). One layered palisade tissue, with relatively small cylindrical cells is 53 µm thick (Table 3). Spongy tissue is composed of 5 layers of cells irregular in shape. Among them,

Table 2. Number and size of leaflet stomata.

Stomata number / mm^2		Stomata size (µm)			
Ade	Abe	Ade		Abe	
		length	width	length	width
99	174	31.5	24.0	28.6	23.6

Ade-adaksial epidermis, Abe-abaksial epidermis

Table 3. Leaflet anatomical characteristics (μm).

Main vein		Main vein vascular bundle	
height	461	height	210
width	359	width	168
Vessel diameter	18	Leaflet thickness	199
Adaxial epidermis cells		Palisade tissue	
height	22.8	tissue thickness	53
width	27.0	cell height	49.9
cuticle thickness	2.9	cell width	17.3
Abaxial epidermis cells		Spongy tissue	
height	22.2	tissue thickness	82
width	29.7	cell height	20.6
cuticle thickness	2.8	cell width	32.7

Table 4. Stem anatomical characteristics (μm).

Stem diameter		Parenchyma cells	
bigger	3744	height	73.3
smaller	3278	width	76.3
Number of vascular bundles		Sclerenchyma	
bigger	14	height	200
smaller	4	width	220
Bigger vascular bundles		Epidermal cells	
height	346	height	42.3
width	365	width	40.6
Vessels		cuticle thickness	5.6
height	45.5		
width	33.7		

large intercellulars occur. This tissue is 82 μm thick. Small colateral vascular bundles, enveloped with sheath parenchyma cells, are present in mesophyll.

Cross section of stem shows its round to ovate shape with two protuberances and main and intermediate ribs, like in other *Vicia* species (Fig. 5a). Two opposite ribs are prominent, whereas four intermediary ribs are inconspicuous. One-layered stem epidermis is composed of almost spherical cells, with thick cuticle (Fig. 5b). Non-glandular and glandular hairs of the same anatomy as those on leaves are present in epidermis, more numerous on ribs. Primary cortex is rather thin, made of few cell layers. Peripheral layers are composed of smaller cells, rich in chloroplasts. Colenhyma is observed subepidermally in the main ribs, as well as in two protuberances. Starch sheath is visible only above vascular bundles.

In the cortex, closer to the epidermis, lacunas that vary in number and size can be detected.

Central cylinder is well developed. In its periphery circularly arranged 4 smaller and 14 larger vascular bundles are observed (Table 4). Both protuberances contain one smaller vascular bundle. Above phloem, groups of sclerenchyma fibers 200 μm high and 220 μm wide occur (Table 4). Ray cells and perimedullar zone cells are with thicker, lignified cell walls. Parenchyma cells of cylinder enlarge towards stem central part, where they get ripped and form central cavity.

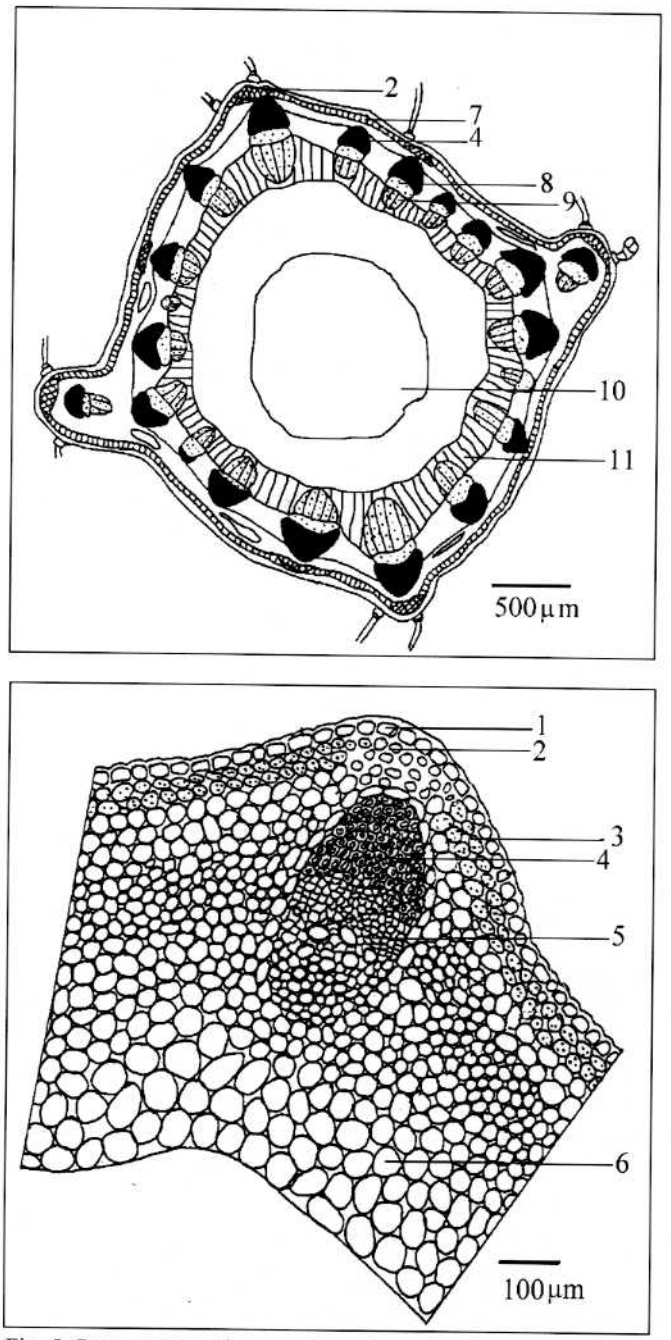

Fig. 5. Stem cross section: a. schematic review; b. structure detail. 1. epidermis; 2. colenchyma; 3. chlorenchyma; 4. sclerenchyma; 5. vascular bundle; 6. parenchyma; 7. cortex; 8. phloem; 9. xylem; 10. cavity; 11. perimedullar zone.

Table 5. Element concentration and ash content in organs.

Plant organ	Element concentration (mg%)					Ash (%)		
	N	P	K	Ca	Na	total	soluble	unsoluble
leaf	4950^{a}	319^{c}	2017^{b}	2034^{a}	73^{a}	9.88^{b}	8.65^{b}	1.23^{a}
stem	1725^{c}	181^{c}	3217^{a}	1640^{b}	77^{a}	11.76^{a}	10.77^{a}	0.95^{a}
pod	3203^{c}	429^{a}	1917^{b}	1333^{c}	67^{ab}	7.50^{cd}	6.67^{c}	0.83^{a}
Average	3293	310	2383	1669	72	9.71	8.70	1.00
LSD 5%	121	25	264	166	14	0.70	0.64	0.34
1%	172	36	375	236	20	0.99	0.91	0.48

Table 6. Rate of net photosynthesis, dark respiration and concentration of chloroplast pigments in leaves.

Rate of net photosynthesis (μmol $O_2g^{-1}h^{-1}$)	Rate of dark respiration (μmol $O_2g^{-1}h^{-1}$)	Content of chloroplast pigments (mg/g dry matter)			
		chl a	chl b	chl (a+b)	carotenoids
15.62	8.12	4.11	0.68	4.80	3.72

Physiological investigations - In analyzed samples total ash content and content of N, P, K, Ca and Na in leaf, stem and pod were determined. The highest ash content is recorded in stem (11.76%), lower in leaves and the lowest in pods (7.50%) (Table 5). The average ash content in plant is 9.71%. The analyses of element concentrations in plant organs showed that the highest nitrogen concentration was found in leaves (4950 mg%), then in pods and stems. Phosphorus concentration was the highest in pods and lowest in stem. Stem contains the highest amounts of potassium, while equal potassium content is found in leaves and pods. The highest calcium concentration is found in leaves, stem and pods respectively. Sodium content is low, showing no significant differences among plant organs, in average 72 mg%.

Net photosynthetic rate is 15.62 μmol $O_2g^{-1}h^{-1}$, while dark respiration rate is 8.12 μmol $O_2g^{-1}h^{-1}$ (Table 6). Ratio of those two values is 1.9:1.

Chloroplast pigment concentrations were also measured. The highest concentrations were recorded in chlorophyll a (4.11 mg/g dry weight), followed by carotenoids and chlorophyll b (Table 6).

Discussion

On the basis of morphological analyses we can conclude that the values of most of measured leaf, stem, flower and pod characters were in agreement with data from literature. Stipule length on examined material ranged from 0.8 to 1.4 cm, which were higher values when compared with the literature data (1.2 cm) (Fedčenko 1948; Kuzmanov 1976). Pod length varies from 1 to 1.5 cm (Gams 1924; Ball 1968; Kuzmanov 1976), while we found somewhat lower values of 0.6-1.1 cm. Since leaflet index value and inflorescence pedicel

length data were not found in the available literature our results may contribute to a better understanding of morphological features of this taxon.

Morphological comparation of examined subspecies with *V. sativa* would be unreal, because, in from evolutionary point of view, they belong to different adaptive lines. *V. sativa* is closely correlated with *V. angustifolia*.

The results of anatomical analyses of vegetative organs of this taxon, compared with the results of similar analyses of other species that belong to the genus *Vicia* (Burduja & al., 1970; Burduja & al., 1971; Merkulov & al., 1996), point to the same type of internal structure, which is characteristic of all species from this genus. However, quantitative differences between taxa are very significant. *V. narbonensis* subsp. *serratifolia* has a prominent main vein on abaxial leaflet side, with a big vascular bundle. Stomata are larger, especially on adaxial epidermis, when compared with other examined taxa from sect. *Vicia*, but more numerous and smaller on abaxial epidermis. Non-glandular hairs are rare, while glandular are numerous. This could be explained by the Mediterranean origin of this taxon.

When comparing examined subspecies with *V. sativa* we can conclude that *V. sativa* has less stomata on adaxial (54/mm^2), and greater on abaxial epidermis (209/mm^2) (Merkulov & al. 1996). Stomata are smaller on both leaf sides. Those differences could be explained by different ecological conditions on growing fields. Non-glandular hairs are more dense, while glandular hairs are equally numerous. Those taxa are similar by numerous glandular hairs and rather small cells of the palisade tissue, but they differ in number of cell layers in the spongy tissue (five and three cell layers respectively). Similarity of these two taxa is also in the presence of numerous and large vascular bundles. All comparisons were given according to data in Tables 2, 3 and 4 and data earlier published in Merkulov & al. 1996.

The analyses of mineral element concentrations in leaf, stem and pod showed differences between the plant organs. Total ash content, potassium and sodium concentrations were the highest in stem. The highest nitrogen and calcium amounts were recorded in leaves, while phosphorus in pods. Concentrations of sodium did not vary significantly between plant organs. Ratio of net photosynthetic rate and dark respiration rate was favorable. Of the investigated pigments, higher concentrations were obtained for chlorophyll a, then for chlorophyll b and carotenoids. Comparing the results of these analyses with the results of similar analyses of other *Vicia* species (Krstić & al. 1995), we can conclude that *V. narbonensis* subsp. *serratifolia* is very rich in mineral elements. It has the highest potassium concentrations in all plant organs and nitrogen concentrations in leaves. Phosphorus content in pods and calcium content in stem are significantly higher than in other species of this genus. High values had also been recorded for phosphorus content in leaves and stems and calcium content in pods. When comparing with *V. sativa* it is obvious that mineral element concentrations are higher in all examined plant organs of *V. narbonensis* subsp. *serratifolia*, except N concentration in pods and Ca concentration in leaf (Krstić & al. 1995).

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