

VARIATION IN *VERBASCUM PHOENICEUM* L. SEEDS GROWN ON THE DIFFERENT TYPE OF SOILS

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ABSTRACT. The long-standing analysis of the species *Verbascum phoeniceum* L. belonging to the populations of various geological substrates (serpentine, andesite and lime) show the presence of morphoanatomic and ecophysiological differences among the plants of the studied populations. The study of the content and composition of lipids in various plant organs of cormophyta showed that the seed lipids could have taxonomic significance.

The aim of this paper is to determine, by means of gas-chromatography method, the content of fatty acids in the seed of *Verbascum phoeniceum* L. belonging to the populations of various geological substrata and the possibility to use these data in defining the taxonomic belonging of this species.

The results of the content of fatty acid in the seed of *Verbascum phoeniceum* L. from various geological substrata populations show that there are differences regarding the content of methyl-ester of oleic acid and methyl-ester of linoleic acid among the plants within all the examined populations.

INTRODUCTION

Verbascum phoeniceum L. (fam Scrophulariaceae), is a perennial, deciduous xerophyta that has a wide ecological valence in relation to geological substrate of the habitat. During our floristic studies, this species was found to exist within populations growing on serpentine, andesite and limestone mother rock. It was noticed that the specificity of ecological conditions prevalent to the serpentine habitat brought about a series of morphological-anatomical and physiological characters with plant organs of this species, by which such plants remarkably differ from those, belonging to the populations from the other geological substrates. Serpentine geological substrates at different localities may be composed of various base and ultrabase magma rocks showing considerable differences in mineral and therefore chemical composition, which may affect both chemical soil composition and characteristics of the plants growing on that stand. The analysis lasting several years (TATIĆ *et al.*, 1981, PETKOVIĆ *et al.*, 1997) indicated that

xeromorphoses were more pronounced in the plants *Verbascum phoeniceum* L. from the serpentine than those in the plants from the other geological bases.

Numerous researchers have shown that micromorphological characters of leaf, fruit and seed surfaces may be used as taxonomic markers at the level of genera, species and even lower taxonomic categories. Considering specific and biochemical characteristics of each species, the differences in the content of the fatty acids investigated indicate that environmental factors particularly edaphic ones may affect their biosynthesis.

MATERIAL AND METHODS

The aim of this paper is to determine, by means of gas-chromatography method, the content of fatty acids in the seed of *Verbascum phoeniceum* L. belonging to the populations of various geological substrata and the possibility to use these data in defining the taxonomic belonging of this species.

Investigations were conducted to examine the seed of *Verbascum phoeniceum* L (fam Schrophulariaceae) of the populations sampled from the following different geological substrates (Fig. 1 and Fig. 2):

- serpentine – the Ušće and Goč sites, the Village of Kamenica;
- andesite and dacite – the Golo Brdo site;
- limestone – the Grza site.

All populations colonized lokalities similar with regard to climatic and orografic ecological factors.

A comparative morphological analysis (of length and width) of the seed was conducted on a sample of 100 seeds of each of the populations investigated. The data obtained were statistically analysed.

The micromorphological stereo scanning method was used for the comparative organographic analysis. The micromorphology investigations included taking photographs seed on scanning microscope Jeol ISM.

Extraction of higher fatty acids was performed using 100 mg mature dry seeds of the plant species *Verbascum phoeniceum* of each of the populations investigated. The content of fatty acid esters was determined on a Varian 3400 Gas Chromatographer, using a 40 m long Superwax Capillary Column containing hydrogen as carrier gas.

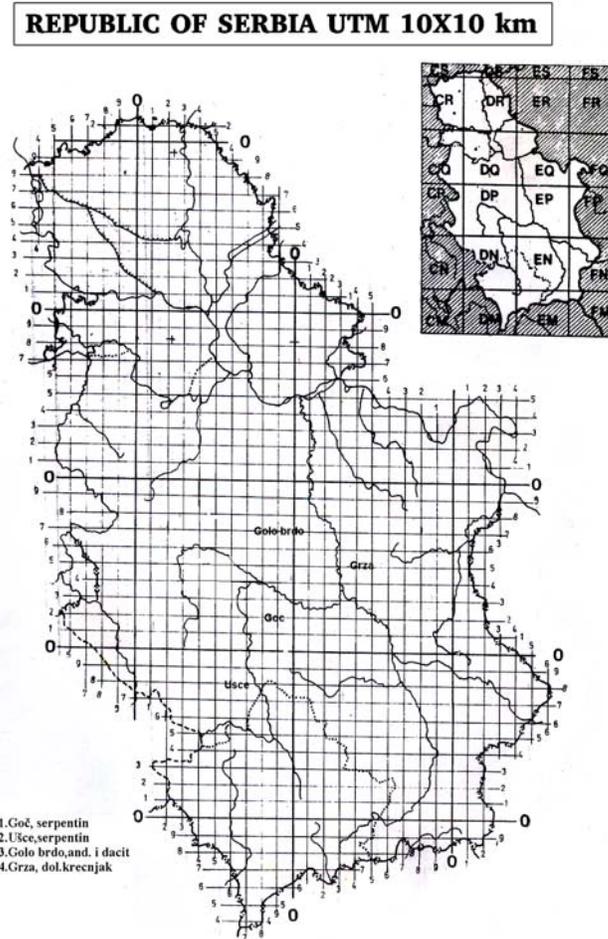


Fig.1 Distribution of *Verbascum phoeniceum* L. populations in Serbia



Ušće (serpentine)



Goč (serpentine)



Golo brdo (andesite and dacite)



Grza (limestone)

Fig. 2 Localities of *Verbascum phoeniceum* L.

RESULTS AND DISCUSSION

Seed length of the investigated plants of the species *Verbascum phoeniceum* L. ranges from 0.63 – 1.23 mm (Flora Europea gives data for 1 mm seed length), seed breadth varying from 0.42-0.9 mm (no literature data on this character available).

The largest seeds are recorded with the plants from dolomitic limestone (1.05 mm in length, 0.7 mm in width), and the smallest ones with the plants from Goč serpentine being 11% and 31 % shorter than the seeds from the Ušće serpentine and those from dolomitic limestone Grza that are the largest. The seed in the plants from serpentine are conical-prismatic in shape.

The micrographs (Fig. 4 and Fig.5) show differences in the sharpness of ridged wrinkles and in the density of their net.

- The seed surface is covered with ridge-forming nodular thickenings that are less pronounced in the plants from serpentine (Goč). The thickenings in the plants collected from the Golo Brdo site were found to form pronounced rounded ridges, as opposed to the interconnecting ones in the plants from the Grza dolomitic limestone site forming twisting ridges.

The following could be observed from the micrographs showing seed surface:

- The seed surfaces of the plants from the Ušće site were found to have main ridged thickenings more pronounced than those in the plants from the Goč (serpentine) site being barely noticeable. The surface of the thickenings and inter-ridge cells is reticulate with the meshes being larger in the plants from the Ušće site compared to those in the plants from the Goč site having a denser reticulate structure.

- In the plants from the Golo Brdo site, longitudinal thickenings and cells are pronounced with a small distance between them. The reticulate structure of the surface consists of dense meshes surrounded by pronounced ribbonlike thickenings.

- In the plants from Grza, longitudinal twisting nodular thickenings are evenly distributed and smaller than those in the plants collected from Golo Brdo. The reticulate structure is poorly developed, having frequently torn and irregular meshes framed by short wrinkles.

The seeds of the plants collected from the Golo Brdo site were found to be cubical in shape (with the seed index of 1.08 : 1) whereas those from Grza were elongated, the seed index being 1.5 : 1 (Tab. 2). Figure 1 presents the form and surface of the seed.

The final analysis of variance of the morphological traits of the seed is presented in Tab. 3. It shows the differences between the *Goč* (serpentin) – Ušće (serpentin), *Goč* (serpentin) – Golo Brdo (andezit i dacit), *Goč* (serpentin) – Grza (limestone), Golo Brdo (andezit i dacit) – Grza (limestone) and Ušće (serpentin) – Grza (limestone) populations and those between the *Goč* (serpentin) – Grza (limestone) and Golo Brdo (andezit and dacit) – Grza (limestone) populations to be statistically significant as regards the seed length and the seed width, respectively.

A comparative distribution of data for the seed length and width of the plants investigated can be seen in Graph 1.

The seed surface is covered with ridge-forming nodular thickenings (Figs. 4.a and 4.b) that are less pronounced in the plants from serpentine (*Goč* and Ušće). The thickenings in the plants collected from the Golo Brdo (andesite and dacite) site were found to form powerful rounded ridges, as opposed to the interconnecting ones in the plants from the Grza (limestone) site forming twisting ridges.

The seed of the plant species *Verbascum phoeniceum* L. is prismatic, and small. The surface of the seed coat is tuberculate with longitudinal wrinkles. The seed core consists of endosperm and embryo (Fig. 6). The differences in the anatomy of the plant species *Verbascum phoeniceum* L seed sampled on different geological substrates are negligible.

Table 4. presents the results of the determination of the content of fatty acid esters in the mature seed of the plant species *Verbascum phoeniceum* L. sampled from different geological substrates.

The results obtained show that no differences were found between the plants collected from the serpentine and those sampled from the dolomite limestone substrates in the content of esters of palmitic and stearic acids. The highest content of the acids in the seed was recorded with the plants from the andesite site.

The content of oleic acid ester was lowest in the plants sampled from the serpentine substrate (0.77 %), and almost three times (2.0 %) and almost four times (2.8 %) higher in the seeds of the plants collected from the andesite and those sampled from the dolomite limestone substrates, respectively.

The plants sampled on the serpentine substrate were also lowest in the content of linoleic acid ester (2.0 %). The content was three times (6.0 %) and five times (10.0 %) higher in the seeds of the plants sampled from the andesite and those from the dolomite limestone substrates, respectively.

Polymorphism within plant of *Verbascum phoeniceum* L. is based on their adaptation to different geological substrates.

Tab.1 A COMPARATIVE ANALYSIS OF THE MORPHOLOGICAL TRAITS OF THE *Verbascum Phoeniceum* L. SEED SAMPLED FROM DIFFERENT GEOLOGICAL SUBSTRATES AT THE GOC, USCE, GOLO BRDO AND GRZA SITES

MORPHOLOGICAL CHARACTER	GEOLOGICAL SUBSTRATES	LOCALITY	X_{min}	X_{max}	X_{sr}	SD	CV	VŠ
LENGTH SEEDS (mm)	<i>serpentine</i>	Goč	0.63	1.00	0.80	0.07	9.8	0.37
	<i>serpentine</i>	Ušće	0.63	1.11	0.89	0.10	11.9	0.48
	<i>andesite and dacite</i>	Golo brdo	0.63	1.06	0.87	0.09	10.7	0.43
	<i>limestone</i>	Grza	0.74	1.32	1.05	0.16	6.3	0.58
WIDTH SEEDS (mm)	<i>serpentine</i>	Goč	0.42	0.79	0.57	0.08	14.8	0.37
	<i>serpentine</i>	Ušće	0.47	0.79	0.63	0.08	13.9	0.32
	<i>andesite and dacite</i>	Golo brdo	0.47	0.79	0.60	0.07	11.6	0.32
	<i>limestone</i>	Grza	0.58	0.90	0.68	0.10	15.8	0.32

TAB. 2 A COMPARATIVE ANALYSIS OF THE RATIO BETWEEN THE LENGTH AND WIDTH OF THE PLANT SPECIES *Verbascum phoeniceum* L. SEEDS SAMPLED FROM DIFFERENT GEOLOGICAL SUBSTRATES AT THE GOC, USCE, GOLO BRDO AND GRZA SITES

MORPHOLOGICAL CHARACTER	GEOLOGICAL SUBSTRATES	LOCALITY	
LENGTH SEEDS	<i>serpentine</i>	Goč	1.40
	<i>serpentine</i>	Ušće	1.41
WIDTH SEEDS	<i>andesite and dacite</i>	Golo brdo	1.08
	<i>limestone</i>	Grza	*1.54

TAB. 3 THE FINAL TABLE OF THE ANALYSIS OF VARIANCE FOR THE MORPHOLOGICAL TRAITS OF THE *Verbascum phoeniceum* L SEED SAMPLED FROM DIFFERENT GEOLOGICAL SUBSTRATES AT THE GOC, USCE, GOLO BRDO AND GRZA SITES

Morphological characteristic	variability	SS	df	MS	F p 0.05	Scheff-ov test
LENGTH SEEDS	bg	2.2	4	0.55		*FGoč-Ušće=23.68 >F°
	wg	8.46	495	0.017	32.28	*FGoč-G.brdo=14.32>F°
	Total	10.66				**FGoč-Grza=154.7 >F° FUšće - G.brdo=1.16<F° *FUšće --Grza =57.3>F° *FG.brdo-Grza=74.8>F°
WIDTH SEEDS	bg	1.08	4	0.27		FGoč-Ušće=10.52 < F°
	wg	3.54	495	0.007	29.32	FGoč-G.brdo=2.63 <F°
	Total	4.62				*FGoč-Grza=35.38 >F° FUšće - G.brdo=2.63<F° FUšće --Grza =7.3 < F° FG.brdo-Grza=18.71 >F°

CONCLUSION

Based upon the results obtained of the study of the *Verbascum phoeniceum* seed following conclusions may be drawn:

- The smallest and the largest seeds were recorded with the plants sampled from the Goč (serpentin) substrate and those collected from the Grza dolomite limestone, respectively.
- The seed surfaces of the plants from the Ušće (serpentin) site were found to have main ridged thickenings more pronounced than in the plants from the Goč (serpentine) site. The ridge and inter-ridge surface is reticulate with the meshes being larger in the plants from the Ušće (serpentin) site compared to those in the plants from the Goča (serpentin) site having a denser reticulate structure.
- In the plants from the Golo Brdo (andesite and dacite) site, main ridges are pronounced with a small distance between them. The reticulate structure consists of dense meshes surrounded by pronounced ribbonlike thickenings.

- In the plants from Grza (dolomite limestone), main twisting ridges are regular and smaller than those in the plants collected from Golo Brdo. The reticulate structure is poorly developed, having frequently torn and irregular meshes framed by short wrinkles.
- The lowest content of fatty (oleic and linoleic) acids was registered with the seeds of the plants sampled from the serpentine geological substrate, indicating the effect of this minerally unfavourable substrate on their synthesis as well.
- The seeds of the plants sampled from dolomite limestone are richest in (oleic and linoleic) fatty acids, and the plants from the andesite-dacite substrate have the similar content. Probably owing to its physico-chemical properties, the andesite-dacite substrate has a favourable effect on the synthesis of palmitic and stearic acids the content of which is highest in the plants sampled from the substrate mentioned.
- Former morpho-anatomical and biochemical analyses had show the influence of the geological substrates, especially serpentine, on plants, which may differ in many characters from those growing on other types of mother rock.

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Fig. 3. The seed of the species *Verbascum phoeniceum* L. sampled from different geological substrates (x 100)



Fig. 3a Goč (serpentine)



Fig. 3b Ušće (serpentine)

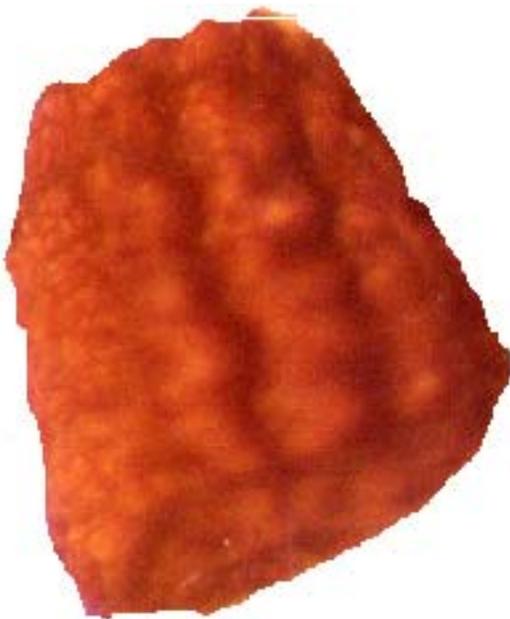


Fig. 3c Golo Brdo (andesite and dac)



Fig. 3d Grza (limestone)

Fig. 4. The seed surface of the species *Verbascum phoeniceum* L. sampled from different geological substrates



Fig.4a Goč (serpentine)

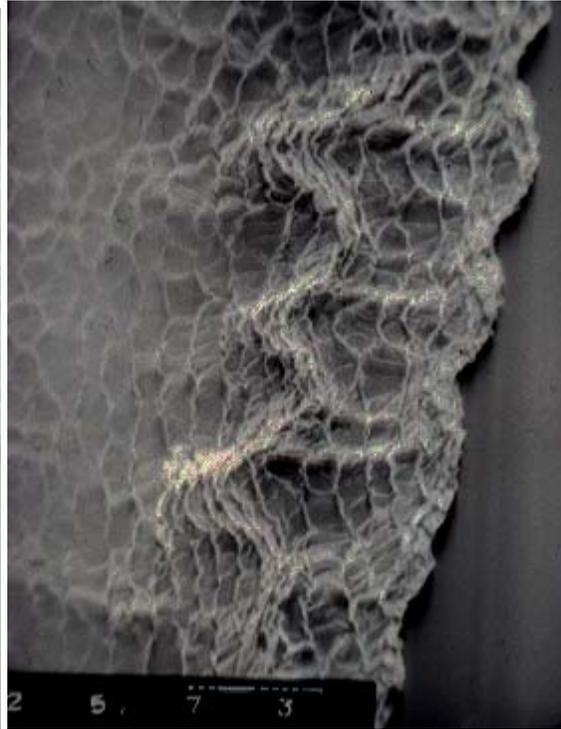


Fig. 4b Ušće (serpentine)



Fig. 4c Golo Brdo (andesite and dac)

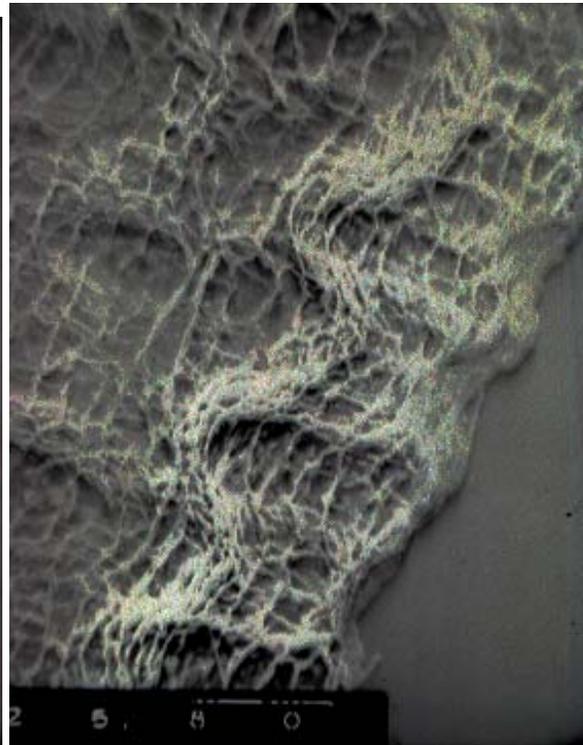


Fig. 4d Grza (limestone)

Fig.5. The seed surface of the species *Verbascum phoeniceum* L sampled from different geological substrates (x 1000)

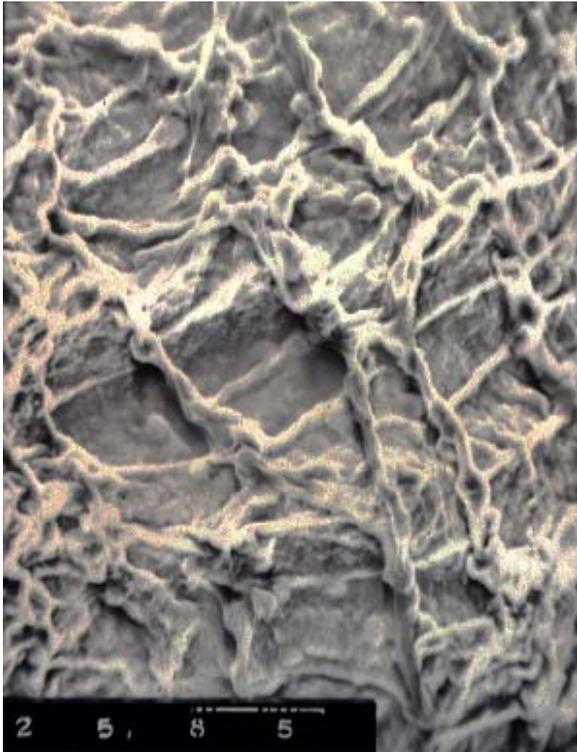


Fig. 5a Goč (serpentine)

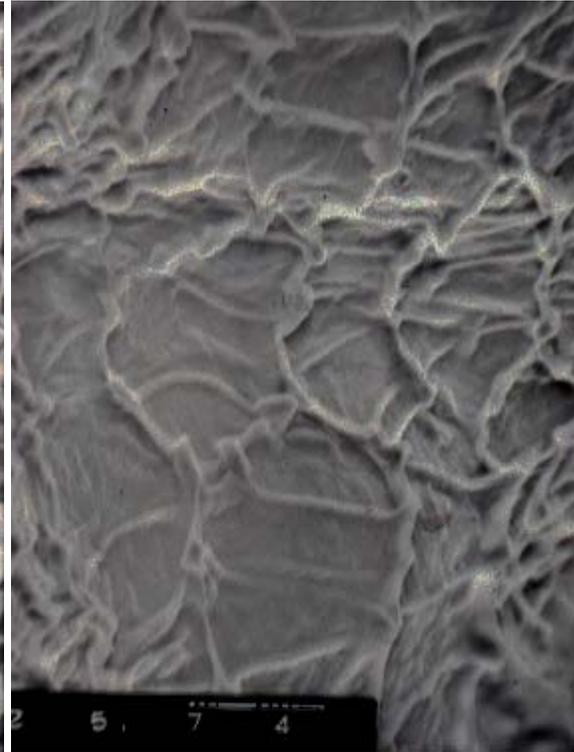


Fig. 5b Ušće (serpentine)



Fig. 5c Golo Brdo (andesite and dac)

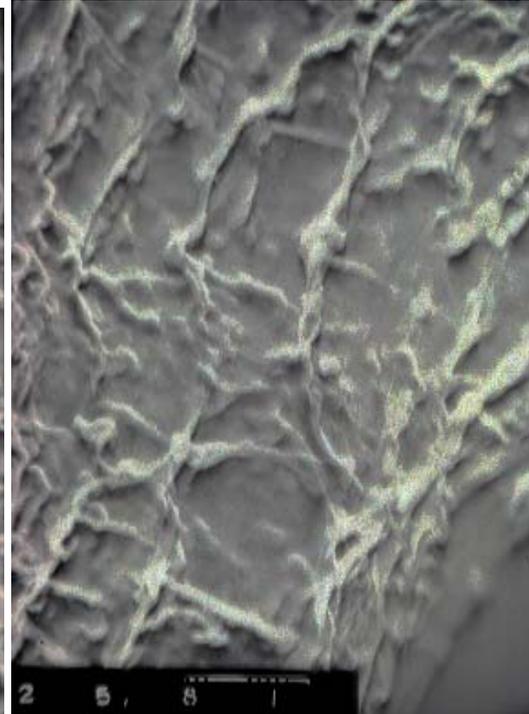


Fig. 5d Grza (limestone)

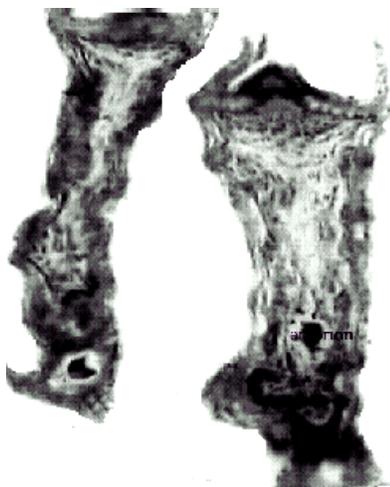
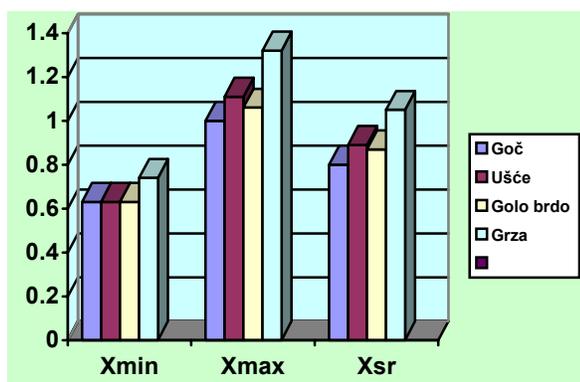


Fig.6. Anatomy of the seed of the species *Verbascum phoeniceum* L.

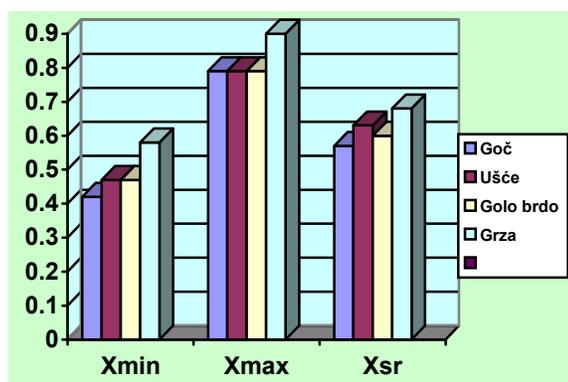
TAB. 4 A COMPARATIVE OVERVIEW OF THE FATTY ACIDS CONTENT IN THE SEEDS OF THE PLANT SPECIES L SAMPLED FROM DIFFERENT GEOLOGICAL SUBSTRATES

	<i>palmitic acid ester C 16</i>	<i>stearic acid ester C18</i>	<i>oleic acid ester C18</i>	<i>linoleic acid ester C18</i>
<i>serpentine</i>	0.81mg	0.39mg	0.77mg	2.0mg
<i>andesite and dac</i>	1.11mg	0.54mg	2.08mg	6.08mg
<i>limestone</i>	0.82mg	0.38mg	2.80mg	9.97mg

Graph 1. A comparative distribution of the data for the length and width of the *Verbascum phoeniceum* L. species seeds sampled from different geological substrates at the Goč, Ušće, Golo Brdo and Grza



Graph 1a. The seed length



Graph 1b. The seed width