RESEARCH ARTICLE **(Open Access)**

Preeliminary data on morfo-histological study of two populations of *Leucojum ionicum* Tan, Mullaj, Sfikas, Strid.

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Abstract

In this study *Leucojum ionicum* was considered in two habitats never studied before: Dhërmi and Palasë. The morphological and histological traits were studied, and comparison between these two populations was made. The technique used for the analysis of epidermal structures is the standard technique of nail polish, which consists in obtaining stomatal traces in the upper and lower epidermis. From the analysis of morphological traits it resulted that in both habitats *L. ionicum* appears with the same morphological traits only with small differences in leaf area. Comparing the distribution of stomata, was observed variation between the two populations. In Palasa individuals, distribution of stomata results a little bit higher than in Dhërmi individuals. SI (stomatal index) and SD (stomatal density) results higher in Palasa population with small differences from Dhermi population. We encountered also variation in stomatal morphology. Stomata in individuals of Dhërmi population appears larger in size and with angles being more distinct than stomata in the individuals of Palasa population. This explains the reason why the population of Palasa had a higher number of stomata/field.

Keywords: Leucojum ionicum, morphological traits, histological traits, stomatal index, stomatal density, stomata.

1. Introduction

L. ionicum = *Leucojum valentinum* Pau *subsp* ***vlorense*** Paparisto et Qosja is a herbaceous, bulbous perennial plant, part of the family Amaryllidaceae. Until today is found on the islands of western Greece and in Eastern Spain. Its location in Albania is declared by botanists in 1977. From subsequent studies of this species taken in the area Vlorë-Jonufër resulted that a subspecie of *L.valentinum* Pau was in Albania, named *subsp. vlorense* from Prof.Xh. Qosja and Prof. K. Paparisto. Studies at chromosomal and cytological level were carried out for *L. valentinum* Pau *subsp* ***vlorense*** in three habitats; Kalaja, Orikum and Dukat [20]. Other researchers have estimated morphologically this specie, and together with the widespread populations in Greece, named it *Leucojum ionicum* Tan, K. et al (2004). In this work *L. ionicum* is being studied for the first time in two new habitats: Dhërmi and Palasë. These two new locations of this specie were found and suggested from Prof. L. Kashta. The results in this paper are preliminary data in the framework of PhD thesis. We have analysed intraspecific diversity at morphological and histological level between two populations of *Leucojum ionicum* in Palasë and Dhërmi areas. The histological indicator considered in study is the apparatus of stomata. Analysis of the number, type and dimensions of stomata is used as a rapid detector for observation of the existence of changes in the populations of a specie. Some authors consider foliar epidemis as a bioindicator of environmental quality [2]. Stomatal frequency differs greatly from specie to specie [15]. Stomatal density and stomatal size demonstrate marked phenotypic plasticity. It may vary according to environmental factors, such as light, atmospheric humidity, water

availability and atmospheric concentration of CO_2 [11]. But these indicators are not considered as the only and definitive, but associated with others like trichome, chromosomes and other leafy parameters create a framework of a cytotype [20]. For the classification and nomination of types of stomata it is based on the nomenclature of the name suggested by Prabhakar (2004) [16].

2. Material and Methods

Specie L. ionicum is collected during expeditions in the period September 2014 which coincides with the time of flowering. During the collection of plant material, is examined the land where is taken the specie and plant association. Were also realised morphological measurements of specie and are compared the populations of the two areas as regards the morphological traits. Plants are planted in pots for subsequent studies. Was made histological evaluation of epidermal structures through colloidal technique with nail polish [5], which consists in obtaining the stomatal traces in the upper and lower epidermis. Preparations were observed using a microscope PARALUX connected to a camera Sony PARALUX with objective magnification 40x and eyepiece 10X. Photography was realized with Apple iphone directly in ocular tube as well as through the Microgiciel program. Through the lens tube was conducted count of stomatal cells / field, total number of cells / field, was determined type of stomata, was conducted count of stomata / mm^2 and measured dimensions of stomatal cells. Measurements of dimensions of stomatal cells was realized through Microgiciel program. The stomatal index, which indicates the proportion of stomata relative to leaf surface, is also a reliable taxonomic character. This is because it is independent of the changes in epidermal cells size brought about by environmental factors [14]. Stomatal index was calculated according to Cutter (1969) [6] by fomula: $SI = \frac{S}{E+S} \times 100$

Where,

SI- Stomatal Index.

S- No. of stomata per unit area.

E- No. of epidermal cells per unit area.

3. Results and discussion.

3.1 Ecology and morphology

Plants of the species *L. ionicum = valentinum* Pau *L. subsp. vlorense* Paparisto et Qosja in Dhërmi, are located near the source of Potami in the side of the street, in a limited space of 2-3 m^2. The terrain where was found a few individuals is rocky terrain filled with little humus with high humidity being that were located on the side of a stream. Their identification was easy, because they were in the flowering period (September 2014). Dhërmi population is located in the geographical coordinates 40.155376°N 19.639420°E, 100 m above the sea level. In Palasë *L. ionicum* is located in Palasa village in the side of the street. Same as here, Palasa population plants were located in a limited space, rocky and humid terrain with geographical coordinates:40.162809°N, 19.625999°E, 200 m above the sea level.

3.2 Morphological data of L. ionicum Pau Dhërmi and L. ionicum Pau Palasë

Dhërmi individuals have white flowers of small size, with 6 petals, 6 stamens and 1 pistil that bloomed in mid August (individuals bloomed in vase). The flowers contain 3 narrow petals and 3 wide petals alternating with each other. The scape in *Leucojum* is variously hollow and solid [12], appears before the leaves and reaches a length up to 32 cm. The leaves are filiform and reach a length up to 34 cm and an average width of 2,5 mm being that Dhërmi population was located near a water stream. Acording to Fraser et al (2008) [9] an increase in water supply cause a reduction in leaf area. Inflorescences have an average of 4-7 flowers every scape (individuals bloomed in vase). The fruit is a little pressed capsule at the top. Dimensions of the bulb have an average of 2.4 cm in length and 1.5 cm in width.

Individuals of Palasa population have small white flowers that bloom by mid-August (individuals

bloomed in vase). The scape have an average of 25 cm in length and inflorescences have 4-7 flowers every scape (individuals bloomed in vase). Leaves are filiform and reaches a length up to 40 cm and an average width of 3mm. Fruit is a little pressed capsule at the top. Dimensions of the bulb have an average of length and 1.8 cm in width.

Morphological features of Leucojum ionicum presented in the previous paragraph are summarized in Table 1.

Table 1. Summary of the data of morphological traits of *L. ionicum* in two habitats

Specie *L. ionicum*	Average (cm) length of leaves	Average (cm) width of leaves	Average(cm) length of bulb	Average(cm) width of bulb	Number of flowers
Dhërmi	34	2.5	2.4	1.5	4-7
Palasë	40	3	2.7	1.8	4-7

Figure 1. *L. ionicum* a) Dhërmi (Potami Source); b) in the vase, c) bulbs Photo M. Shehu

Figure 2. *Leucojum ionicum* a) Palasë b) bulbs (Photo M. Shehu)

3.3Epidermal structures

Modifications in stomatal density, distribution and morphology on a foliar surface can be considered as significant traits in plants [4]. Observations are realised in the upper and lower epidermis of the leaves of *L. ionicum* in Palasa and *L. ionicum* in Dhërmi. In many species, stomata are present on both leaf surfaces (i.e. in amphistomatic leaves), whereas in others they are restricted to the abaxial surface (hypostomatic leaves) [17]. In this study, it was observed that the leaves are amphystomatic type, where stomatal cells are present both in the upper and

the lower epidermis of the leaf. Epidermal cells are elongated and located parallel to the axis of the leaf. Generally the stomata of monocotyledones are parallel to the axis of the leaf [8]. Stomatal cells are tetracytic type, surrounded by 4 subsidiary cells, two lateral cells and two polar cells with parallel axis to the axis of stomatal cells [16, 18]. Comparing the distribution of stomata, was observed variation between the two populations. In the upper epidermis distribution of stomata / field at Palasa population (20-21 stomata / field) it is higher than in Dhërmi population (17-18 stomata / field). While in the lower epidermis we noted small difference in the distribution of stomata between the two populations. Individuals of Palasa have 22 - 23 stomata / field, and in individuals of Dhërmi are observed an average of 21-22 stomata / field. In figure 3 we may see comparative data for two populations of L. ionicum where are clearly distinguished these differences. Acording to Çağlar et al. (2004) [7] there was a trend towards increasing stomatal density at higher altitudes. Palasa population is at higher altitudes than Dhërmi population. In the figure 4 is shown that stomatal density and stomatal index is largest in population of Palasa. According to Loranger and Shipley (2010) [13], thought that there is a relationship between stomatal density and morphological and physiological features of the leaf at both intra and interspecific levels. Also stomatal conductance is associated with leaf age, decreasing in older leaves under the influence of a stress compared with young leaves [19]. Leaves developed in low intensity light conditions have lower stomatal densities than leaves developed in sunny areas [10]. Palasa population is more exposed to sunlight (south-west direction) than Dhërmi population (west direction). Acording to Fraser et al (2008) [9] an increase in water supply resulted in a reduction in stomatal density.

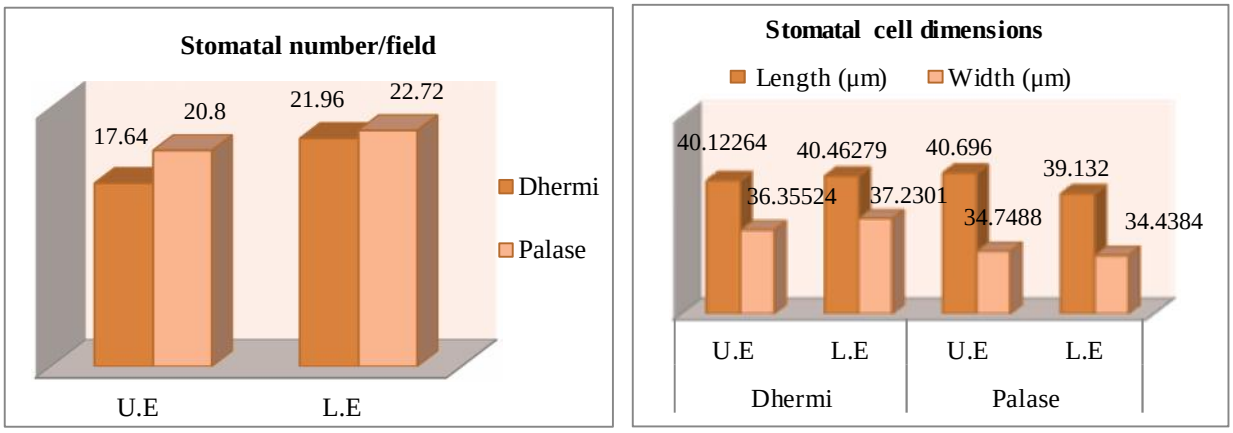

Figure 3. a) The distribution of stomata b) size of the stomata for two populations of *L. ionicum*

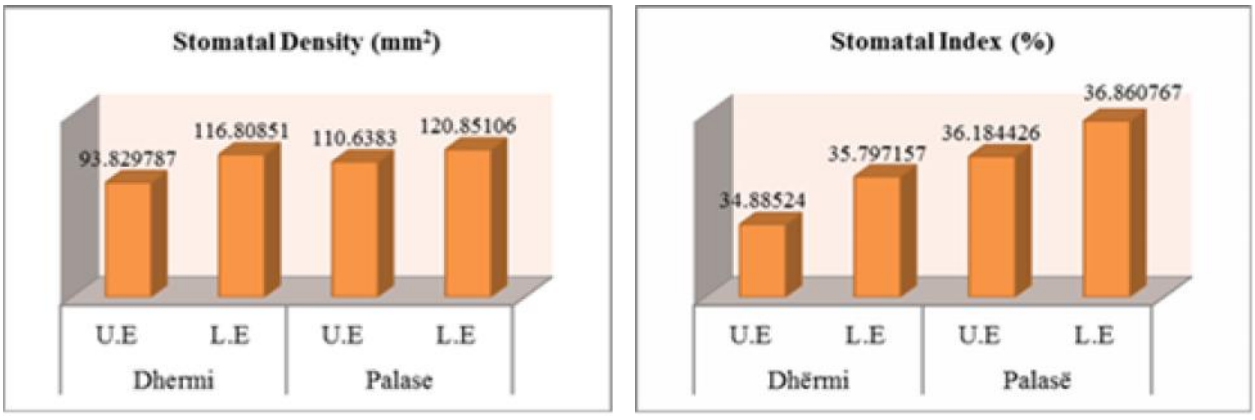

Figure 4. Stomatal density and stomatal index for L. ionicum Dhërmi and Palasë.

Table 2 summarizes the data on the type of stomata, dimensions (length / width) of stomatal cells, stomatal density / mm^2, stomatal index, standard deviation and coeficient of variation between these twopopulations. The figure 5 shows distribution of stomata in the lower epidermis of L. ionicum in two habitats.

Table 2. Summary of the data of epidermal traits of *L. ionicum* in two habitats

Specie *L. ionicum*		Epidermal surface area	Type of stomata	Stomatal number/field	Length (µ)	Width (µ)	SI (%)	SD (mm^2)
Dhërmi	Sd	Upper	Tetracytic	17.64 ± 1.83	40.12±3.18	36.35 ± 4.63	34.88±2.56	93.82±9.73
		Lower	Tetracytic	21.96± 3.46	40.46±4.57	37.23± 3.19	35.79± 4.49	116.80±18.45
	Cv(%)	Upper		10.37	7.93	12.74	7.35	10.37
		Lower		15.79	11.31	8.59	12.54	15.79
Palasë	Sd	Upper	Tetracytic	20.8± 2.71	40.69±2.67	34.74± 2.72	36.18± 2.9	110.63±14.43
		Lower	Tetracytic	22.72±2.91	39.13± 5.51	34.43±3.56	36.86±4.07	120.85±15.52
	Cv(%)	Upper		13.04	6.56	7.82	8.03	13.04
		Lower		12.84	14.08	10.34	11.04	12.84

Cv: Coeficient of variation
Sd: Standard deviation

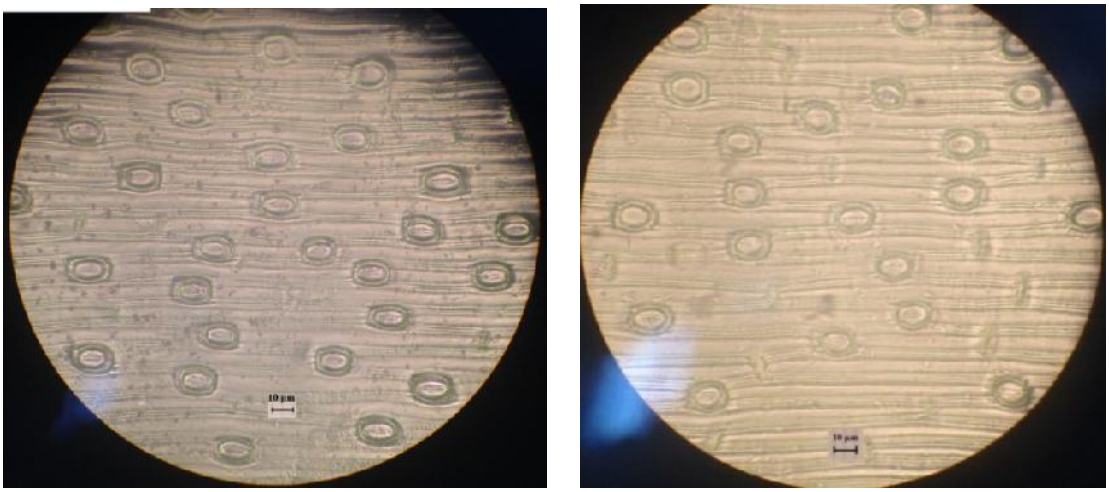

Figure 5. Distribution of stomata in the leaf epidermis of *L. ionicum* a) Palasë, b) Dhërmi

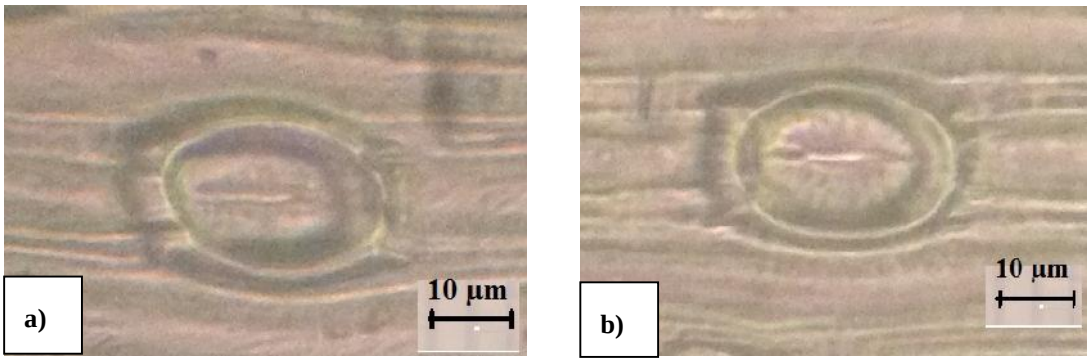

Figure 6. Stomata morphology of *L. ionicum* a) Palasë b) Dhërmi

Variation was found in the morphology of the stomata too. As seen in Figure 6 stomata in individuals of population of Dhërmi appears larger in size and with angles being more distinct than stomata in individuals of Palasa population. According to Metcalfe & Chalk (1988) [14] dhe Beerling & Woodward (1997) [3], Abdulrahaman et al (2009

[1]stomata with large size provide lower stomatal density while stomata with small size give high stomatal density. This explains the fact that population of Palasa have a greater number of stomata /field.

4. Conclusions

From the analysis of morphological traits it resulted that in both habitats *L. ionicum* appears with the same morphological traits, only with differences in leaf area, thought that are caused by enviromental factors. Comparing the distribution of stomata, was observed variation between the two populations. Individuals of Palasa have higher stomatal frequency than Dhërmi individuals in the lower epidermis although this difference is small. In the upper epidermis distribution of stomata is higher at Palasa population than in Dhërmi population. Variation was found in the morphology of the stomata too. In individuals of population of Dhërmi stomatal cells appears larger in size and with angles being more distinct than stomata in individuals of Palasa population. In some studies stomatal density and size are dependent by enviromental factors. But these indicators are not considered as the only and definitive, but associated with others like trichome, chromosomes and other leafy parameters create a framework of a cytotype [20].

5. Acknowledgements

I am grateful to **Prof. Dr. Lefter Kashta** for disponibility and support during expedition September 2014 for plant material collection.

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