THE INTRASPECIFIC VARIABILITY OF SOME CYTOTAXONOMIC FEATURES IN *LUZULA LUZULOIDES* POPULATIONS (FAM. JUNCACEAE)

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Abstract. In this paper it was analysed the variability of some cytotaxonomic features of the mature leaves, depending on altitude and genotype, in two *Luzula luzuloides* subspecies (Juncaceae family), which vegetate in the North of Oltenia (Romania). The investigations were performed in six populations belonging to *Luzula luzuloides* ssp. *luzuloides*, from 400 – 1900 m altitude, and four populations of *L. luzuloides* ssp. *rubella*, from 1650 – 2228 m altitude. As cytotaxonomic features there were used the size of epidermal cells (length and width), and stomata (length and density), being performed 50 measurements for each feature, at the ten populations. The statistical data processing was made with the STATISTICA 10 software, ANOVA/MANOVA. The analysis of some cytotaxonomic (microhistological) features in on two subspecies of the species *Luzula luzuloides* (*Luzula* genus): *L. luzuloides luzuloides* and *L. luzuloides rubella* pointed out a significant intraspecific variability. Between the two subspecies, there were observed minor differences regarding the structure of the two epidermis (upper and lower). Statistical analysis revealed a higher intraspecific variability in *L. luzuloides luzuloides* populations, in comparison with *L. luzuloides rubella* ones, due to genotype, but with a major contribution of the various environmental conditions. In both epidermis (upper and lower), the cells length is significantly influenced by the environment, less by genotype, while the cell width, the stomata size and density have a certain genetic determinism. This fact can be the explanation of the significant differences for these characters between the two subspecies.

Keywords: *Luzula luzuloides luzuloides, L. luzuloides rubella;* cytotaxonomic features (epidermal cells and stomata), genotype and altitude effect.

Rezumat. Variabilitatea intraspecifică a unor caractere citotaxonomice în populaţii de *Luzula luzuloides* (Fam. Juncaceae). În această lucrare a fost analizată variabilitatea unor caractere citotaxonomice la frunzele mature, dependent de altitudine şi genotip la două subspecii de *Luzula luzuloides* (Fam. Juncaceae), care vegetează în nordul Olteniei (România). Investigaţiile au fost efectuate în şase populaţii de *Luzula luzuloides* ssp. *luzuloides*, de la 400 m – 1900 m altitudine şi patru populaţii de *L. luzuloides* ssp. *rubella*, de la 1650 m – 2228 m altitudine. Drept indici citotaxonomici au fost folosite mărimea celulelor epidermale (lungime şi lăţime) şi stomatelor (lungime şi densitate) fiind efectuate 50 măsurători pentru fiecare caracter, la zece populaţii. Valorile biometrice au fost prelucrate statistic cu programul STATISTICA 10, ANOVA/MANOVA. S-a remarcat o variabiliatate intraspecifică semnificativă, în urma analizei caracterelor citotaxonomice a celor două subspecii ale speciei *Luzula luzuloides* (genul *Luzula*). Intre cele două subspecii au fost observate diferenţe minore, în ceea ce priveşte structura celor două epiderme (superioară şi inferioară). Analiza statistică a relevat o variabilitate intraspecifică mai mare la populaţiile de *L. luzuloides luzuloides*, în comparaţie cu cele de *L. luzuloides rubella*, datorată pe de o parte genotipului, dar cu o contribuţie majoră a condiţiilor de mediu diferite. Lungimea celulelor din ambele epiderme este influenţată semnificativ de mediu, mai puţin de genotip, în timp ce lăţimea celulelor, dimensiunea şi densitatea stomatelor au un determinism genetic cert. Acest fapt poate fi explicaţia diferenţelor semnificative înregistrate pentru aceste caractere, între cele două subspecii.

Cuvinte cheie: *Luzula luzuloides luzuloides, L. luzuloides rubella*, caracteristici cito-taxonomice (celule epidermale şi stomata), efectul genotipului şi al altitudinii.

INTRODUCTION

The *Luzula* genus belonging to *Juncales* order, *Juncaceae* family, the perennials plants spread especially in the temperate regions, the Arctic and higher elevation areas in the tropics. In Romania flora, there are 11 species (four with two subspecies) and two genotypes with an uncertain presence (*L. sieberi* and *L. nivea*), (CIOCÂRLAN, 2009). The recent researches performed about the genus *Luzula*, conduct to some taxonomic modifications (KIRSCHNER & KAPLAN, 2001; BAČIČ et al., 2007; ROMO & BORATINSKI, 2011). Thus, in KIRSCHNER and KAPLAN's opinion (2001), *Luzula alpinipilosa* represents a new combination. The *Luzula* taxa are very similar morphologically, few qualitative and quantitative features being registered between them (KIRSCHNER, 2002). *Luzula* is a monocot genus with holocentric chromosomes.

Luzula luzuloides (Lam.) Dandy et Wilmott [sin. *L. nemorosa* (Poll.) E. Meyer; *L. albida* (Hoffm.) DC] is a perennial species, native to Central Europe, from the Balkans to Fennoscandia. Frequently, it vegetates in subalpine (juniper) level and in evergreen oak. This species has also been introduced to the British Isles and other parts of Europe and to the north-eastern and eastern United States. After the inflorescence shape and tepals colour, two subspecies were described.

Luzula luzuloides ssp. *luzuloides*, with lax inflorescence, whitish tepals, met in the oak forest zone (evergreen oak and beech sublevel).

Luzula luzuloides ssp. *rubella* (Hoppe ex Mert. et Koch) Holub [sin. ssp. *cuprina* (Rochel) Chrtek et Křisa], with inflorescence ± contracted and reddish tepals, spread in boreal (spruce fir) and subalpine level (juniper tree) level.

The morphological features of the two subspecies were reviewed by KIRSCHNER (2002). A detailed presentation of the chromosomes and genome size in this genus was reviewed by MALHEIROS & GARDI (1950),

NORDENSKIÖLD (1951), MADEJ & KUTA (2001). A comprehensive study performed on seven *Luzula* ssp. by BOZEK et al. (2012) concluded that "*Luzula* exhibits considerable genomic flexibility and tolerance to large, genome-scale changes". Then, it can be considered that is a genus in evolution, very interesting from the genetic point of view. The two subspecies belonging to *Luzula luzuloides* species are diploid (2n=12 AL), having a primary karyotype: *Luzula luzuloides rubella* (GARCIA-HERRAN, 2001); *Luzula luzuloides luzuloides* (MADEJ & KUTA, 2001).

In the present paper, there were analysed some cytotaxonomic features of the epidermal cells from the mature plants (at flowering), represented through the shape and size (length and width) of the epidermal cells, as well as the stomata length and density.

MATERIAL AND METHODS

Biological material. The cytotaxonomical investigations were performed on ten *Luzula luzuloides* populations, six belonging to *Luzula luzuloides* ssp. *luzuloides* genotype and four to *Luzula luzuloides* ssp. *rubella*. These populations vegetate at different altitudes in the same geographical region, namely the mountain region from the North of Oltenia, the Carpathian Mountains (Romania). The altitude was higher for *L. luzuloides* ssp. *rubella*, in comparison with *L. luzuloides* ssp. *luzuloides* (Table 1). There were collected whole plant specimens (5 for each location) during the interval June – July.

Table 1. Harvesting area altitude.

Species	Location	Altitude (m)
Luzula luzuloides ssp. luzuloides	Coşava peak	1900
	Balota sheepfold, Furnicelu saddle	1850
	Polovrăgeni valley	900
	Slătioara, Măgura Slătioarei hill	767
	Roeşti, Ciocâltei village	450
	Comăneşti hill	400
Luzula luzuloides ssp. *rubella*	Urdele peak	2228
	Ursu peak	2124
	Balota sheepfold, Furnicelu saddle	1850
	Rânca Mountain	1650

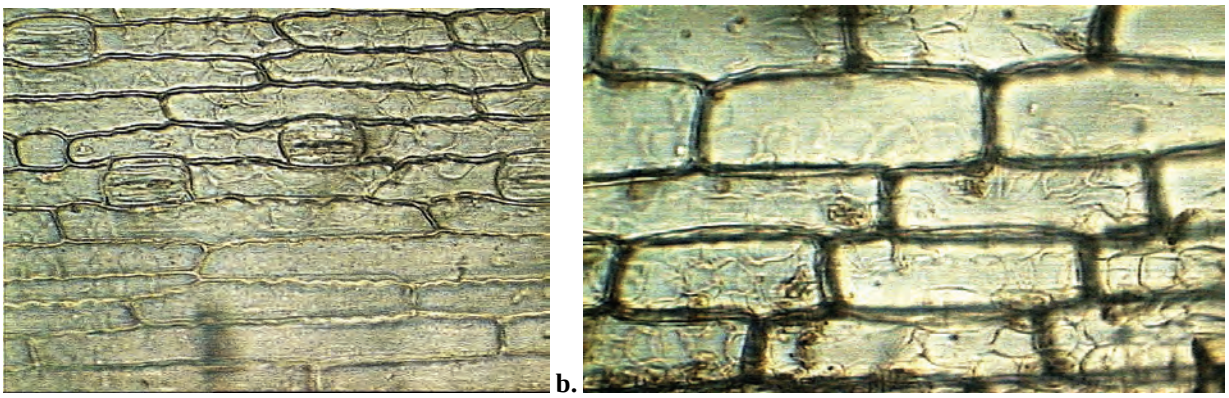
Work method. The biometrical observations were effected on the mature plants (at flowering stadium), being analysed the shape and the size (length and width) of the epidermal cells, as well as the stomata length and density, from both sides of the leaf. Fragments of epidermis (1 cm^2) were carefully removed from the middle of the leaf blade. The biometrical observations were performed on fresh slides, with a ZEISS micrometre at a NIKON optical microscope. For every feature there were performed 50 observations, to 5 collected plants for each population. The data were statistically processed with STATISTICA 10 soft (ANOVA/MANOVA, DUNCAN test, correlations).

RESULTS AND DISSCUSIONS

Epidermal cells shape and size.

Epidermal cell shape.

The shape of the cells on the two sides of the leaf presented differences in the two *Luzula luzuloides* subspecies. The upper epidermis is made up of rectangular cells, covered with a thick cuticle, the right walls having a constant width in *L. luzuloides* ssp. *rubella* or slightly wider at the middle cell part in *L. luzuloides* ssp. *luzuloides* (Figs. 1a, 2a). The lateral walls, usually with perpendicular position on the cell length, present a slightly rounded edge (Figs. 1a, 2a). The stomata are absent on the upper epidermis.



a. **b.**

Figure 1. *Luzula luzuloides* ssp. *luzuloides*. Slătioara, Măgura Slătioarei hill, 767 m altitude (a. lower epidermis; b. upper epidermis).

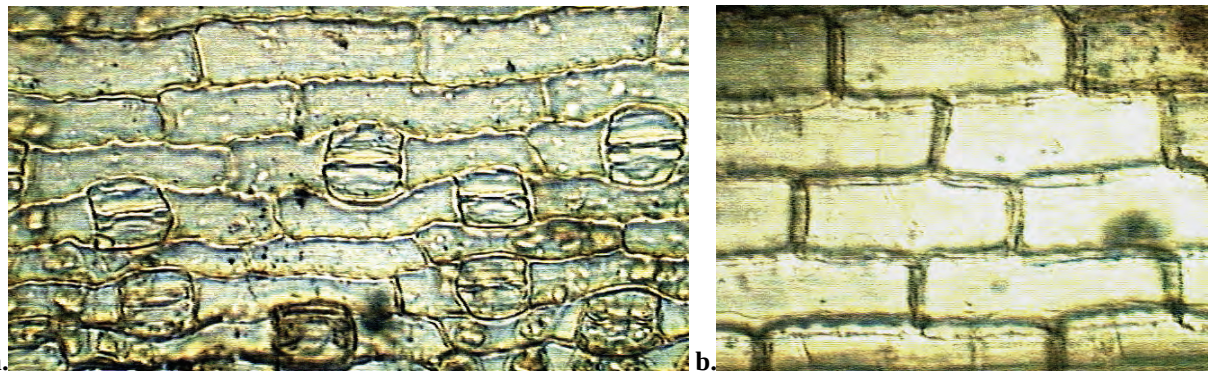


Figure. 2. *Luzula luzuloides* ssp. *rubella*. Ursu peak, 2124 m altitude (a. lower epidermis; b. upper epidermis).

The lower epidermis, in two subspecies, is made up of epidermal cell rows with stomata, which alternate with cells rows without stomata (Figs. 1b, 2b).

Between the analysed *Luzula* populations, there were recorded differences regarding the number of the cell rows with or without stomata, which alternate, as well as the number of the epidermal cell situated among two stomata from the same row. These differences depend on genotype, altitude, leaf size, the cytotaxonomic feature, a/o (Table 3; Figs. 3-6). An exception was observed in *L. luzuloides* ssp. *luzuloides* populations collected from the lowest altitude (400-450 m), in which the cell rows without stomata are absent (Fig. 5). Similarly, in other *Luzula* species, on the lower epidermis, it was reported the presence of the rows with and without stomata (*Luzula wahlenbergii* and *Luzula piperita*, HÄMET-AHTI, 1965). The cell size from the upper epidermis is bigger in comparison with the cell size from the rows with stomata.

The size of epidermal cells.

The analysis of variance revealed that the dimensions of the epidermal cells and stomata are significantly influenced by the altitude of the site, subspecies (with some exceptions), as well as by the interaction of both factors.

The length of the cells in both epidermis (upper and lower) is a character influenced significantly by the environmental factor, while the width of the cells has also a genetic determinism (Table 2). Thus, it can be explained the higher intraspecific variability for cell size in *L. luzuloides luzuloides*, species spread from 400 to 1900 m altitude, from hills to alpine regions (in habitats with very different environment conditions), in comparison with *L. luzuloides rubella*, which vegetates in regions over 1500 m altitude. Duncan test shows the significance of the differences between populations and underlines this intraspecific variability (Figs. 3-5).

Table 2. Analysis of Variance (Fisher test) for the main features of the epidermal cells.

Character	**Factor**	**Analysis of Variance (1 = altitude ; 2 = genotype) (Marked effects are significant at p < 0.05000; n =500)**		
		F	P	Sign.
Upper epidermis cell length	1	**28.2750**	**0.000000**	***
	2	1.18819	0.276576	(-)
	1x2	**26.6766**	**0.000000**	***
Upper epidermis cell width	1	**46.6007**	**0.000000**	***
	2	**32.76842**	**0.000000**	***
	1x2	**48.5559**	**0.000000**	***
Lower epidermis cell length (rows of cells with stomata)	1	**6.8669**	**0.000000**	***
	2	1.06882	0.302050	(-)
	1x2	**6.4969**	**0.000000**	***
Lower epidermis cell width (rows of cells with stomata)	1	**23.8597**	**0.000000**	***
	2	**7.03591**	**0.008417**	**
	1x2	**22.5116**	**0.000000**	***
Stomata length	1	**164.9352**	**0.000000**	***
	2	**69.73360**	**0.000000**	***
	1x2	**148.7899**	**0.000000**	***
Stomata density	1	**60.7311**	**0.000000**	***
	2	**88.20021**	**0.000000**	***
	1x2	**57.5189**	**0.000000**	***
Lower epidermis cell length (rows of cells without stomata)	1	**6.9307**	**0.000001**	***
	2	0.04693	0.828682	(-)
	1x2	**7.3611**	**0.000000**	***
Lower epidermis cell width (rows of cells without stomata)	1	**60.2178**	**0.000000**	***
	2	1.58371	0.209460	(-)
	1x2	**93.2826**	**0.000000**	***

Table 3. The variability of some cytotaxonomic features of epidermal cells and stomata (mean values in µm) in the populations from two *Luzula luzuloiudes* genotypes.

Genotype	Cell length	Cell width	Stomata length	Stomata density[c]
Luzula luzuloides ssp *luzuloides*				
Upper epidermis	52.8 – 104.0	28 – 32.3	-	-
Lower epidermis[a]	55.4 – 76.3	15.5 – 20.0	28 - 38	50 - 146
Lower epidermis[b]	115.8 – 163.0	18.5 – 23.4		
Luzula luzuloides ssp. *Rubella*				
Upper epidermis	64.0 – 95.0	30.0 – 42.0	-	-
Lower epidermis[a]	45.0 – 89.0	18.0 – 21.3	30 - 42	120 - 204
Lower epidermis[b]	123.0 – 152.0	15.5 – 26.0	-	-

Legend: [a] – rows of cells with stomata; [b] – rows of cells without stomata; [c] – number of stomata per mm^2.

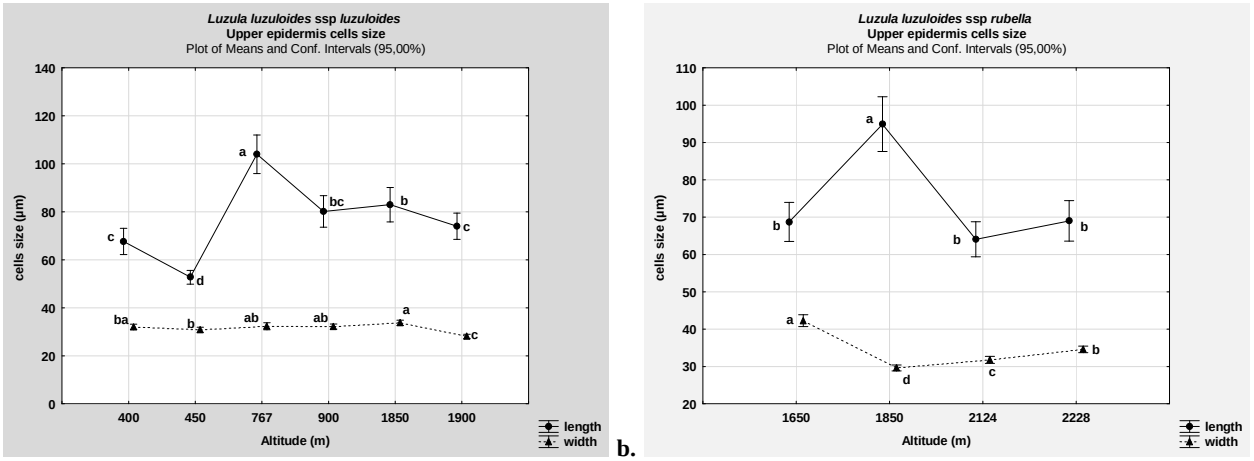


Figure 3. Upper epidermis cell size in *Luzula luzuloides* ssp. *luzuloides* (a) and *Luzula luzuloides* ssp. *rubella* (b).

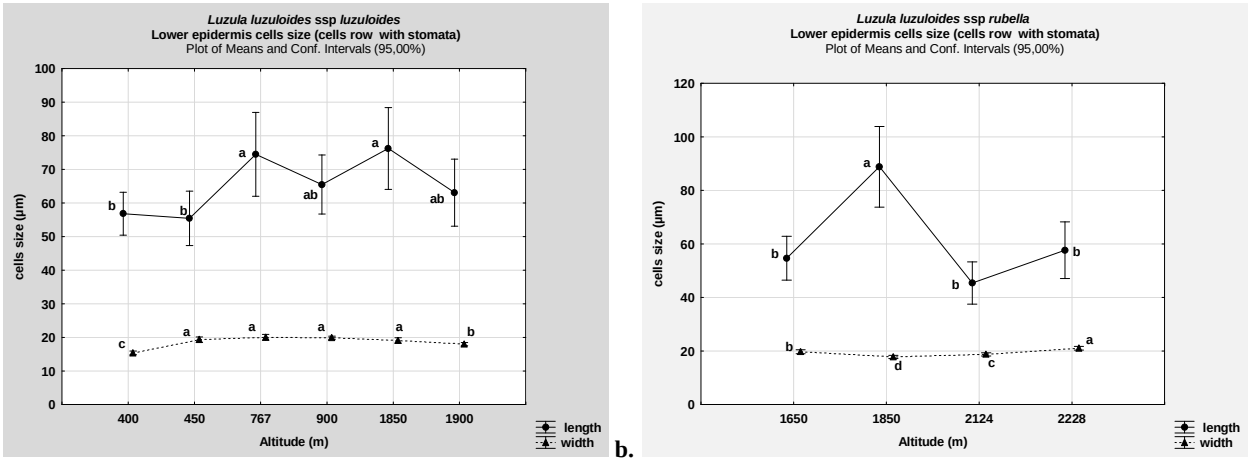


Figure 4. Lower epidermis cell size (rows with stomata) in *Luzula luzuloides* ssp. *luzuloides* (a) and *Luzula luzuloides* ssp. *rubella* (b).

Table 4. The significance of the differences between the means for the main characters of cells and stomata in the two *L. luzuloides* ssp.

Species	Upper epidermis		Lower epidermis			
	Cell length (µm) Mean ± St.dev.	Cell width (µm) Mean ± St.dev.	Cell length (µm) Mean ± St.dev.	Cell width (µm) Mean ± St.dev.	Stomata length (µm) Mean ± St.dev.	Stomata density/mm^2 Mean ± St.dev.
Luzula luzuloides ssp *luzuloides*	76.93 ± 22.5 **a**	31.53 ± 3.5 **b**	65.26 ± 27.3 **a**	18.61 ± 2.4 **b**	32.28 ± 4.1 **b**	106.6 ± 40.3 **b**
Luzula luzuloides ssp. *rubella*	74.18 ± 19.5 **a**	34.54 ± 5.6 **a**	61.64 ±33.0 **a**	19.32 ± 2.1 **a**	36.60 ± 4.8 **a**	153.1 ± 44.3 **a**

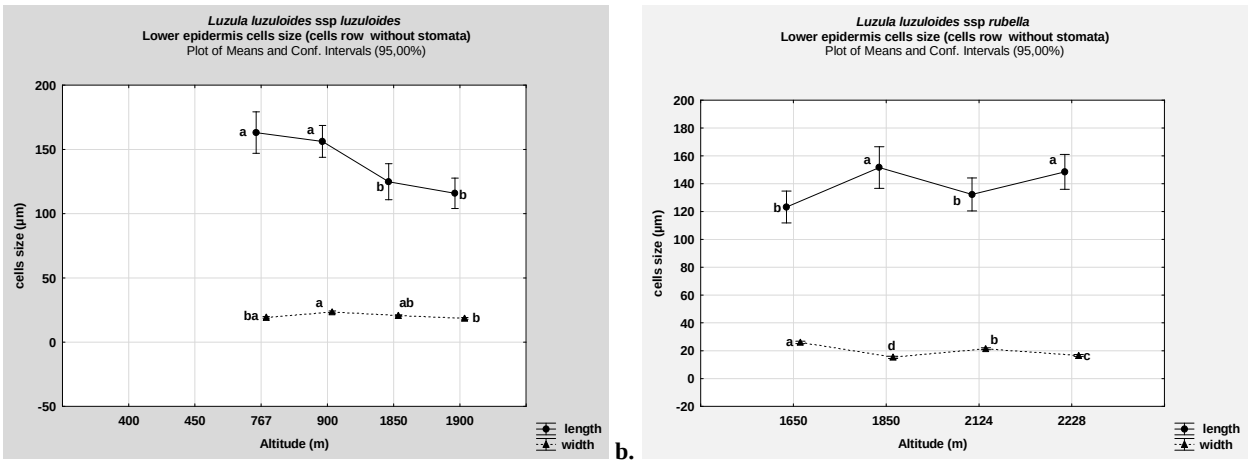


Figure. 5. Lower epidermis, cell size (cell row without stomata) in *Luzula luzuloides* ssp. *luzuloides* (a) and *Luzula luzuloides* ssp. *rubella* (b).

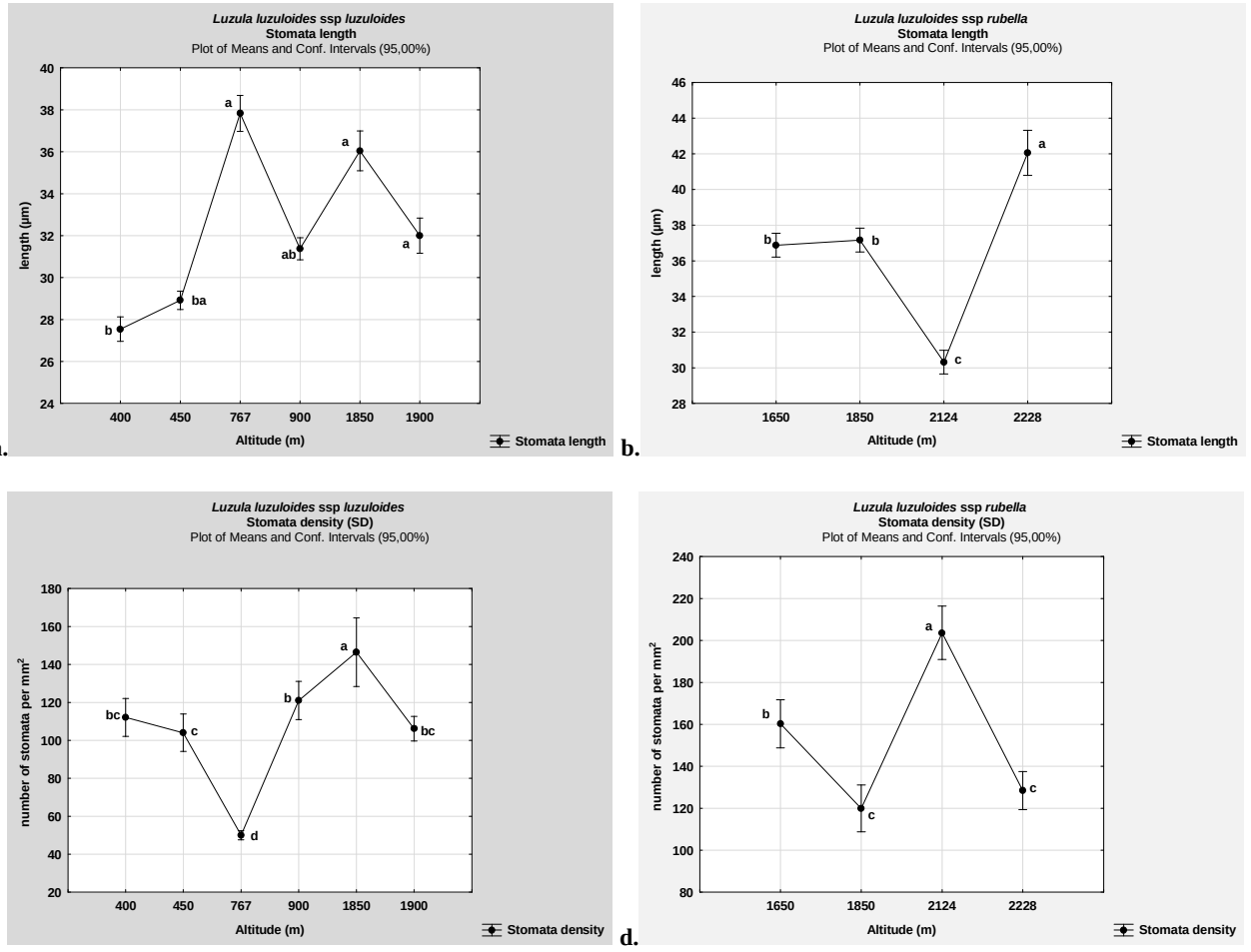


Figure 6. Lower epidermis, stomata length and density in *Luzula luzuloides* ssp. *luzuloides* (a. stomata length; c. stomata density) and *Luzula luzuloides* ssp. *rubella* (b. stomata length; d. stomata density).

The highest values of the epidermal cells, on both sides of the leaf, were recorded at the plants which are native from the station situated at an altitude slightly higher as compared to the extreme values for the two subspecies: 767 m for *L. luzuloides luzuloides* and 1850 m for *L. luzuloides rubella* (Figs. 3-5). Generally, the epidermal cell and stomata size, recorded significantly higher values in the *Luzula luzuloides rubella* subspecies, except the cell size from the rows without stomata (Table 4).

The implication in taxonomy of different cytotaxonomic features was reported by different specialists: AGBAGWA & NDUKWU (2007) in *Cucurbitaceae*; in sub-tropical species (AHMAD et al., 2010; PĂDURE in

Nepeta sp. (2006, flower and inflorescence); *Persicaria* sp. from Pakistan (YASMIN et al., 2010); also, MUSTAPHA (2000) uses the same leaf feature (epidermal cell and stomata size) in an experiment performed in *Urginea indica*. In previous researches, CORNEANU & POPESCU (1981) point out the presence of some structural features of the epidermal cells, confirming the plant allegiance in two related *Fritillaria* species (*F. meleagris* and *F. orientalis* [sin. *F. montana* and *F. tenella*]). A similar situation was presented in the *Dianthus* genus (SANDA, 1972; SIMEANU & CORNEANU, 1978; CORNEANU & SIMEANU, 1980).

Analysing some morphological features at three *Luzula* genotypes spread in the Northern Hemisphere, HÄMET-AHTI (1965) established that these three are distinct genotypes. In monocotyledons, STEBBINS & KHUSH (1961) analysed the stomata complex and its bearing in phylogeny. The 192 analysed species (belonging to 49 families) were classified in four categories. The *Juncaceae* family with the two genera (*Juncus* and *Luzula*), is in the first category, having 4 or more subsidiary cells surrounding the guard cells. As a synthesis, CARRIÈRE (2002) performed a list with the arctic vascular plants which can be identified through the use of some microhistological features.

The size and stomata density.

Stomata length is dependent both on environment conditions and genotype (Table 2). In *L. luzuloides luzuloides*, the variability of this character is lower than in *L. luzuloides rubella* (Table 3; Fig. 6a, b). The difference calculated between the mean values of the stomata length registered in the two subspecies is significant, *L. luzuloides rubella* recorded the highest value (36.60 ± 4.8 µm), (Table 4). In the *L. luzuloides luzuloides* populations, native at lower altitudes (under 500 m), there were recorded lower values for stomata length in comparison with the values recorded in the populations which vegetate at higher altitudes; the highest value was recorded in the population from 767 m altitude. This population manifests the greatest adaptation to the environment (on the basis of the stomata and epidermal size values).

Stomata density is dependent mainly on genotype and less on environment, even both factors have a significant influence on this character (Table 2; Figs. 6c, d). The differences registered between populations, as well as between subspecies are statistically significant (Table 4; Figs. 6c, d). The intraspecific variability of the character is also high. In *L. luzuloides rubella* the stomata density recorded a significant higher value (153.1 ± 44.3/ mm^2 **a**) in comparison with *L. luzuloides luzuloides* (106.6 ± 40.3/mm^2 **b).**

CHMURA et al. (2005) established that the concentration of different atmosphere components is dependent on altitude and other variables. The paleontological studies pointed out different values of the CO_2 concentration in different geological eras of our planet. In a review, ROYER (2001) analysed the relationship between atmospheric CO_2 concentration and stomata density (SD) or stomata index (SI), using 285 previously published SD and 145 SI responses to variable CO_2 concentrations from a pool of 176 C3 plant species. ROYER (2001) reported "a positive relationship between CO_2 and SD/SI in only 12% of cases. Thus, CO_2 appears to inversely affect stomata initiation, although the mechanism may involve genetic adaptation and, therefore, is often not clearly expressed under short CO_2 exposure times". In *Luzula luzuloides* populations analysed in the present paper, it can be established a correlation between stomata density and altitude of origin for the two species. In this case, the stomata density on leaf surface can present a genetic determinism.

Table 5. Correlations.

Variable	Correlations (Marked correlations are significant at p <0.05000; N=500)							
	UE cell length	UE cell width	LE[a] cell length	LE[a] cell width	Stomata length	Stomata density	LE[b] cell length	LE[b] cell width
Altitude	**-0.3874**	-0.0118	-0.1242	-0.1184	0.1114	**0.5211**	**-0.2442**	**-0.2779**
	p=0.000	p=0.856	p=0.055	p=0.067	p=0.085	**p=0.000**	**p=0.000**	**p=0.000**
Ssp.	**-0.2627**	**0.2978**	**-0.1301**	0.0215	**0.2665**	**0.4654**	-0.0140	-0.0813
	p=0.000	**p=0.000**	**p=0.044**	p=0.741	**p=0.000**	**p=0.000**	p=0.829	p=0.209
UE cell length	1.0000	**-0.2483**	**0.1765**	**-0.1584**	0.1224	**-0.4305**	**0.1741**	**-0.1923**
	p= ---	**p=0.000**	**p=0.006**	**p=0.014**	p=0.058	**p=0.000**	**p=0.007**	**p=0.003**
UE cell width		1.0000	**-0.1281**	**0.2527**	**0.2286**	**0.1576**	-0.0355	**0.4508**
		p= ---	**p=0.047**	**p=0.000**	**p=0.000**	**p=0.015**	p=0.584	**p=0.000**
LE[a] cell length			1.0000	**-0.1330**	0.0905	**-0.1894**	0.1246	**-0.1893**
			p= ---	**p=0.039**	p=0.162	**p=0.003**	p=0.054	**p=0.003**
LE[a] cell width				1.0000	**0.2676**	-0.0581	**0.1802**	0.1203
				p= ---	**p=0.000**	p=0.370	**p=0.005**	p=0.063
Stomata length					1.0000	**-0.2251**	**0.1502**	**-0.2954**
					p= ---	**p=0.000**	**p=0.020**	**p=0.000**
Stomata density						1.0000	**-0.1813**	**0.2429**
						p= ---	**p=0.005**	**p=0.000**
LE[b] cell length							1.0000	-0.0765
							p= ---	p=0.,237
LE[b] cell width								1.0000
								p= ---

Legend: UE = Upper epidermis; LE = Lower epidermis; [a] – rows of cells with stomata; [b] – rows of cells without stomata.

Correlations between different features.

The analysis of the correlations between different cytotaxonomic features of the leaves, as well as their values depending on genotype or altitude, evidenced the environmental conditions influence on the microhistological features. Their existence underlined their value in the genotype characterization (Table 5).

There were recorded positive correlations between: the length of the epidermal cell, in the upper epidermis – the length of the epidermal cell, in the lower epidermis; the cell width in the upper epidermis – the cell width in the lower epidermis; stomata size and density. Negative correlations were registered between: the cell length – the cell width; stomata length – stomata density.

The two factors: altitude (environment) and subspecies (genotype), involved in variability are significantly correlated with the analysed characters. Altitude is positively correlated with stomata density and is negatively correlated with the length of the epidermal cell, in the upper epidermis, as well as with the size of the lower epidermis cells (rows without stomata). The genetic determinism of the stomata size and density is shown again by the very significant correlation between subspecies and these characters (r =0.2665, for stomata length; r = 0.4654 for stomata density). Thus, in the characterization of these genotypes, the microhistological features present a great importance, similarly to the morphological features.

CONCLUSIONS

The analysis of some cytotaxonomic (microhistological) features in on two subspecies of the species *Luzula luzuloides* (*Luzula* genus): *L. luzuloides luzuloides* and *L. luzuloides rubella* pointed out a significant intraspecific variability.

Between the two subspecies, there were observed minor differences regarding the structure of the two epidermis (upper and lower). The populations of *L. luzuloides rubella* as well as *L. luzuloides luzuloides*, which vegetate at higher altitude (over 500 m) presented in lower epidermis cell rows with stomata, which alternate with cell rows without stomata. This particularity was not found in the populations collected from lower altitude.

Statistical analysis revealed a higher intraspecific variability in *L. luzuloides luzuloides* populations, in comparison with *L. luzuloides rubella* ones, due to genotype, but with a major contribution of the various environmental conditions.

In both epidermis (upper and lower) the cell length is significantly influenced by the environment, less by genotype, while the cell width, the stomata size and density have a certain genetic determinism. This fact can be the explanation of the significant differences, for these characters, between the two subspecies. All these observations supported statistically, proved the genomic flexibility of a species in evolution.

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