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SAMPLES OF ECOLOGICAL-MORFOLOGICAL DIFFERENTIATION OF TRIFOLIUM PRATENSE L. IN VERTICAL PROFILE OF BREZOVICA (SHARRI MOUNTAIN, KOSOVO)

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Abstract

Plant samples of 13 populations of Trifolium pratense L. species were analyzed in vertical profile of Brezovica – Sharri Mountain (Kosovo), in order to assess individual, population and inter - population anatomical, morphological variability. Investigated populations were distributed in different altitudes. The research has shown that T. pratense L. species successfully climbs up to sub-alpine zone, thus demonstrating phenotype changes due to the change of the complex of ecological factors.

Key word: *Trifolium pratense L. , Populations, Differentiation, Altitude.*

1. INTRODUCTION

Knowing that *Trifolium* L. genus has wide distribution and shows high variability, many scientist have used certain species as object for various researches.

Trifolium pratense L. was research object of many scientists. According to the research of Taylor and Quesenberry (1996) *Trifolium pratense* L. is a temperate species originated from Southeast Europe and Asia Minor near the Mediterranean Sea. It is a short-lived perennial diploid species with seven pairs of chromosomes (Taylor and Quesenberry, 1996), insect pollinated and self incompatible. Therefore, red clover populations are heterogeneous and consist of heterozygous genotypes. This results in high levels of genetic variation within and among populations.

Leto *et al.*, (2004), Sato *et al.*, (2005), Voigt and Mosjidis,(2002) have stated that *Trifolium pratense* L. is an important forage legumes widely cultivated in most temperate regions around the world. Its success is based on the following characteristics: high seeding vigor, rapid growth, tolerance to acid and humid conditions, nitrogen fixing ability, and high nutritive value for ruminants.

Trifolium pratense L. is one of the most important forage legumes and a valuable component of permanent grassland as well as grass-clover mixtures in temperate regions (Herrmann et al., 2008). It is adapted to a wide range of soil types, environmental conditions, fertility levels, use patterns and management (Drobna, 2009). It is of high ecological value due to its ability to fix atmospheric nitrogen, allowing a reduction in the use of nitrogenous fertilizer (Abberton, Marshall,2005).

Using *Trifolium pratense* L. as an object for their research, Nowak et al., (1992) and Nelke et al., (1999) have analyzed the tolerance of this plant to abiotic stresses .

The reason for having this plant species as research object, was mainly due to its high morphological variability, ecological plasticity and wide ecological valence.

Despite species assessment approach towards molecular biological aspect, the classical methods are considered more fundamental and irreplaceable. Especially the methodology of morphological evaluations of plant species individuals results in a range of relevant answers to many current and open questions. Morphological variability is one of the key responses of each individual and population on quantitative - qualitative factors of environment, respectively ecosystems where they grow. Morphological variability is one of the best indicators of evolution and phylogeny interaction, and mutual harmonization of genotype and external environment.

Having in mind what was said above, the object of our reaserch was *Trifolium pratense* L . The research has covered considerable number of plant indiviudals from 13 populations, collected in different altitudes in vertical profile of Brezovica (Sharri Mountain –Kosovo).

Trifolium pratense L. is a very adaptable plant that adapts quickly, featuring its phenotype changes in response to change of environmental conditions, or change of biotic and abiotic factors. It has a very wide range of distribution and a broad ecological valence. Resides in areas at different altitude, and climbs up to sub-alpine zone (reaches the altitude of 1903 m).

Trifolium pratense L. species, given the wide geographical distribution and wide ecological valence, shows significant variability in relation to the dominant ecological factors, and may serve as a paradigm of unique samples of eco-morphological differentiation in the natural ecosystem.

The purpose of this research was to ascertain the form and level of individual, populations and inter population variability of *Trifolium* pratense L. species, depending on ecological factors where it grows.

The study of the impact of the altitude gradient, on variability of different plant species, has been the subject of study for many scientists.

Studies of altitude can be viewed as a "*natural experiment*" from what we can learn about the adaptations of plants and responses to different environmental conditions (Korner, 1999).

It means, that the change of altitude, necessarily results with changes of a range of environment factors (heat, wind, thickness and duration of snow cover, light intensity, etc.), which have an impact on plant organisms. Given what was said above, a number of scientists have researched the impact of altitude in terms of physiological, anatomical, morphological and ecological aspects.

Whittaker (1972) has concluded that altitude gradient has strong influence on the content and structure of plant cpmmunities.

Redžić (1991) has reserached the vegetation syngenesys in ecosystems of vertical profile of Ozreni Mountain near to Sarajevo.

Gaston (2000) has researched global biodiversity samples, and concluded that species diversity among others is the result of the altitude.

Caputa (1996) has came up with the conclusion that with increasing altitude, the feeding quality of forage (*Trifolium pratense* L.) improved due to a decreased content of crude fibers, lignin and cellulose.

Knežević et al. (1997) have determined higher degradability of *Trifolium pratense* L. cultivars cultivated in the mountain region (650 m altitude), than those cultivated in the lowland (123 m altitude).

2. MATERIALS AND METHODS

In order to have a more comprehensive and complete research on morphological differentiation of some parameters of some *Trifolium pratense* L. populations, considerable number of individuals (30) from 13 populations was researched. The research has the original experimental and field nature.

Samples or plant material is collected in different topo-geographical-ecological localities on vertical profile of Brezovica (Sharri Mountain) (Kosovo). For the sake of easier research, we have listed populations according to the altitude, nominated by capital letters (A, B, C. ..), and index shows the number of population that was initially collected. (A1, B3, C2, D4, E5, F6, G7, H8, I9, J10, K11, L12.). It means that A3 represents the lowest altitude (962 m) whereas L13 presents the highest altitude (1903 m).

Plant material was collected during the period from May to August 2009.The plant material was dried and placed in Herbarium and preserved in alcohol, making it ready for further research. Leaf epidermis was pealed with the manicure methodology which has served for measurements of trichoma length. During the measurements the Digital SWIFT Microscope and special lamp with magnifying glass were used, in order to come up with more qualitative results.

For the purpose of this research, these morphological parameters were measured (length of stem, number of flowers per inflorescence and trichoma length). The values obtained during measurements, were analyzed with statistical methodologies. The evaluation and interpretation of the level of variability of the above-mentioned morphological parameters, the degree of correlation, respectively connection between certain features and ecological parameters, as well as analysis of the result significance was made with Sigma program.

3. RESULTS AND DISCUSSION

Trifolium L. genus in the territory of Kosovo is differentiated into more than 30 taxons (species and subspecies), and from ecological point of view has wide ecological valence, especially Trifolium *repens* and *Trifolium pratense species.*

Investigations were made in the vertical profile of Brezovica - Sharri Mountain (2.500m altitude).



Figure 1. Brezovica View



Figure 2. *Trifolium pratense L. – part of M13 Population*

In 1986 a part of Sharri Mountain was declared a National Park. From floristic aspect, this mountain is among the richest mountains of Kosovo and Balkan Peninsula, and represents a mosaic of plant species where are present many endemic and endangered plant species.

So far in Sharri Mountain are found about 2,000 plant species and 68 plant communities. *Trifolium pratense* L. species is highly variable in terms of morphological, molecular-biological and biochemical ecological aspects. It has a high degree of adaptation to anthropogenic factor. Takes part in different plant communities with a wide phytocenological and ecological range : *Lolieto-Cynosuretum* (Br.-Bl. et De Leeuë 1937); *Molinion coeruleae* (Koch 1926): *Potentillo albae-Festucetum rubrae* Blažková (Blažková, 2007); *Arrhenatherion* (*Arrhenatherum elatius*) (Luquet 1926)

From the plant samples collected from 13 populations of *Trifolium pratense* L. species, at different altitudes in vertical profile of Sharri Mountain, or more specifically Brezovica, the level of morphological variability of some parameters (length of stem, number of flowers per inflorescence and trichoma length) was analyzed.. From the results shown in tables and charts below, we can see how plants respond by changing their phenotype due to the change of ecological factors (biotic and abiotic) that vary as a result of progressive increase of altitude.

Table 1 shows Variance analysis results of total stem length. It was conluded that the difference in the mean values of various populations is greater than would be expected by chance. Therefore it is a statistically significant difference (P = <0.001).

Mean value of total stem length of A3 Population where the samlpes were collected at 861 m above the sea level is 43,5 cm, whereas the mean value of stem length of Population M13 (1903m) is 20,2, which is half less than mean value of stem length in Population A3.

Based on the table presentation of obtained results and their chart presentation (Chart 1) there is a clear decressing trend of mean values in relation to the increase of altitude. This means that we can freely conlude that stem length is standing in negative correlation with increasing altitude.

n	Population	Total Stem Length			
		Average value(cm)	Standard Deviation	Maximal Value	Minimal Value
10	A3	43,350	4,594	49,000	37,300
10	B4	43,730	7,464	57,000	36,200
10	C2	35,390	4,437	42,000	29,200
10	D1	56,390	11,774	72,300	36,000
10	E5	37,270	5,622	48,000	31,000
10	F6	33,660	4,828	41,000	27,500
10	G7	38,400	7,726	53,000	26,000
10	H8	38,660	8,443	58,800	27,800
10	I9	41,520	7,977	54,000	28,000
10	J10	22,520	5,163	29,000	11,600
10	K11	29,920	5,362	39,000	20,200
10	L12	26,080	8,122	41,300	15,000
10	M13	20,120	4,830	26,700	14,000
P<0,001					
P=0,05					
t = 11.020					

Table 1. *Trifolium pratense* L - Total Stem Length

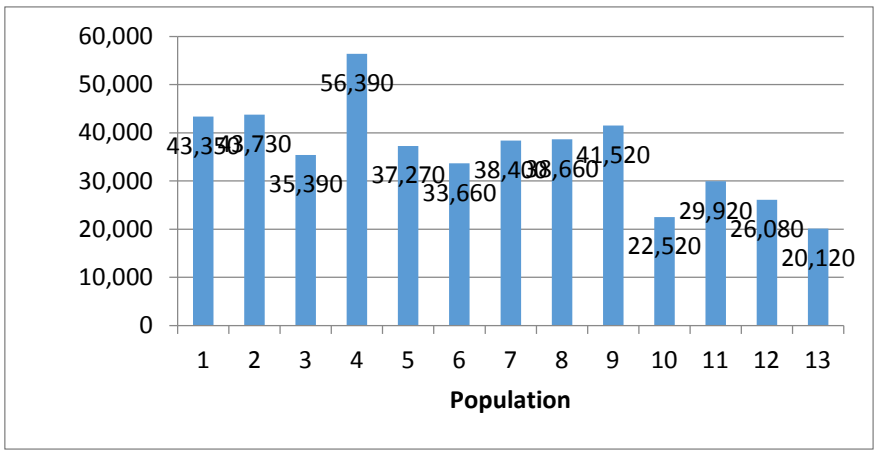


Chart 1. *Trifolium pratense* L. – Total Stem Length

Shortening of the stem length that occurs at high altitude allows the plant to avoid the detrimental impact of strong winds, which are present at high altitudes and also enables the condition improvement for photosynthetic activity, because with the shorter plants leaves stay closer to the warm soil surface (Korner & Chochrane, 1983).

Also Jun-feng & Zun-xiang (2006) have come to the conclusion that with increasing altitude maximum height of Picea w*ilsonii* , *P. meyeri* species and others get reduced.

In Figure 3, three plant individuals from different populations, collected at different altitudes are presented .

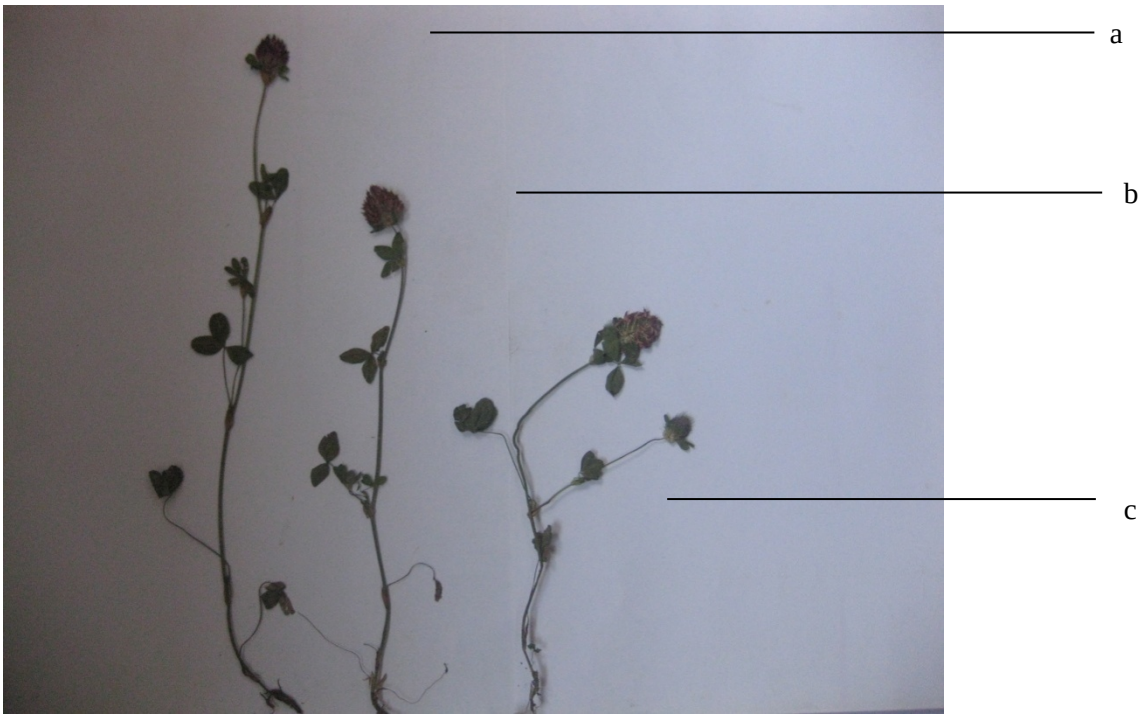


Figure 3. *Trifolium pratense L. Different lenght of plant stem from localities situated in different altitudes: a. Population D1 (962 m);b. Population I9 (1451 m); c. Population M13 (1903 m)*

From the figure are clearly visible changes that plant phenotype has experienced in response to various biotic and abiotic factors, which vary as a result of the increase of altitude.

The plant is experiencing environmental changes by manifesting changes in biochemical-physiological, anatomical and morphological level, which lead to changes in the phenotype of the plant . The plant in the picture labeled with a letter a belongs to Population D1 that was taken at an altitude of 962 m; plant labeled with the letter b belongs to I 9 Population (1451 m), while the plant labeled with the letter c belongs to population L13 (1903 m).

The picture is self-explanatory, and clearly shows reducing trend of stem length in correlation with increase of altitude, thus demonstrating the high variability and wide ecological valence.

It is said that for every 100 m increased altitude the air cools with the rate of 0.5 and 1.0 ° C. As far as the air rises in the mountains and cools, reduces the amount of water that holds. A typical air cooling of 1 ° C per 100 m above sea level increase causes a difference in the season of plant development, where at lower elevations it is shown long season of development, while in the mountains, in the higher altitudes there is a shortened season of plant development.

In addition, changes in altitude are followed with changes of temperature, precipitation and wind. As a result of these changes, the climate of the mountains is very different from the climate in low altitudes (Barry 1992; Whiteman 2000).

Although the investigated *Trifolium pratense* L. plant species is mesophyte type, it is proved that successfully copes with changes of ecological factors caused by the change of altitude gradient . Thus thanks to its ability to successfully cope with environmental changes, can be concluded that *Trifolium pratense* L. species is very suitable to be used as a model in various researches.

Table 2 presents obtained results from variance analysis of flower number per inflorescence. Obtained results allow us to make conclusion that difference in the mean values of various populations is greater than would be expected by chance. Therefore it is a statistically significant difference (P = <0.002).

Population A3 indicates that mean value of flower number per inflorescence is 67,8 whereas mean value of flower number per inflorescence of Population M13 amounts 58,2. Although mean values of some populations indicate decreasing and increasing oscilations in relation to the altitude gradient, again there is a reduction trend of mean value of flower number per inflorescence.

n	Population	Number of flowers per inflorescence			
		Mean value(cm)	Standard Deviation	Maximal Value	Minimal Value
10	A3	67,800	15,761	90,000	43,000
10	B4	75,500	12,695	97,000	49,000
10	C2	65,700	18,494	102,000	44,000
10	D1	80,200	28,436	118,000	40,000
10	E5	67,300	14,930	86,000	41,000
10	F6	69,900	11,090	88,000	57,000
10	G7	84,100	21,403	117,000	44,000
10	H8	94,400	11,481	110,000	74,000
10	I9	87,200	19,019	117,000	62,000
10	J10	72,000	35,793	156,000	28,000
10	K11	86,800	11,263	103,000	61,000
10	L12	79,100	16,816	105,000	46,000
10	M13	58,200	19,142	105,000	46,000
P<0,001					
P=0,05					
t = 1.224					

Table 2. *Trifolium pratense* L. – Number of flowers per inflorescence

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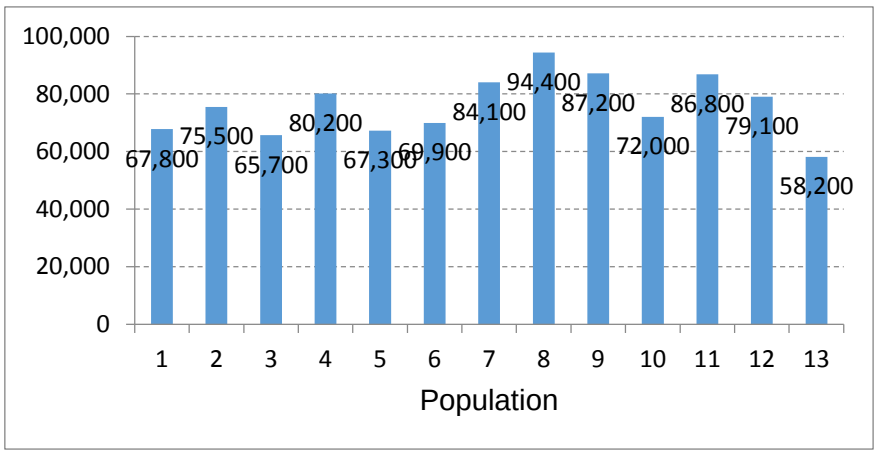


Chart 3. *Trifolium pratense* L. - Number of flowers per inflorescence



Figure 4. *Trifolium pratense* L.- Flower: *a. Corolla; b. Calyx*

Table 3 shows Variance analysis results obtained from measurements of trichoma length on the upper part of the leaf. Based on this results can be concluded that difference in the mean values of various populations is greater than would be expected by chance. Therefore it is a statistically significant difference (P = <0.019).

The mean value of trichome length on the upper part of the leaf in Population A3 is 997,79 µm, whereas in Population M13 is 850,21 µm. Table and chart (Table 3 & Chart 3) presented results indicate that there is a general decreasing trend of mean values in relation to the increasing altitude.

n	Population	Length of trichoma on the upper part of the leaf			
		Mean value(cm)	Standard Deviation	Maximal Value	Minimal Value
10	A3	997,795	293,520	1289,150	572,800
10	B4	772,790	154,683	929,650	490,850
10	C2	714,700	161,139	1099,000	485,950
10	D1	1061,335	420,277	1732,650	640,100
10	E5	842,040	259,697	1481,400	613,900
10	F6	618,685	251,346	1270,800	291,050
10	G7	871,255	192,313	1225,500	694,250
10	H8	847,135	226,446	1300,800	540,250
10	I9	868,425	293,564	1662,550	643,650
10	J10	822,180	364,194	1758,500	529,650
10	K11	888,070	263,197	1253,450	627,100
10	L12	878,248	159,536	1144,825	692,250
10	M13	850,214	207,467	1393,150	679,025
P<0,001					
P=0,05					
t = 1.298					

Table 3. *Trifolium pratense* L. – Length of trichoma on the upper part of the leaf

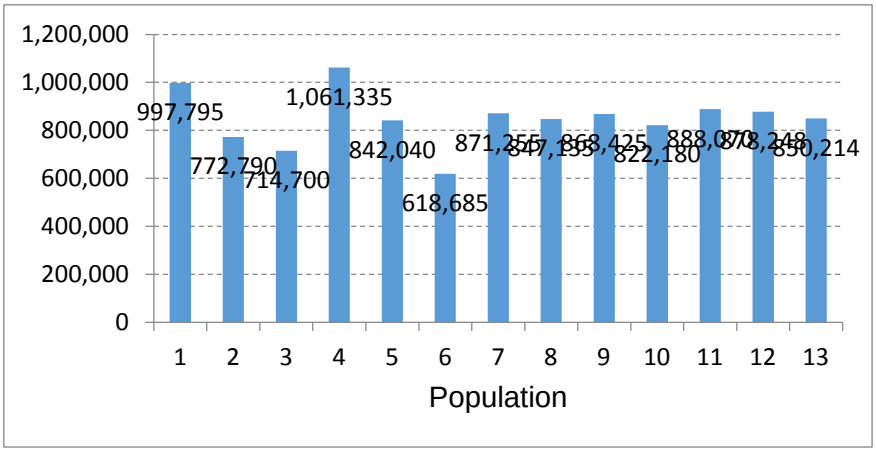


Chart 3. *Trifolium pratense* L. - Length of trichoma on the upper part of the leaf

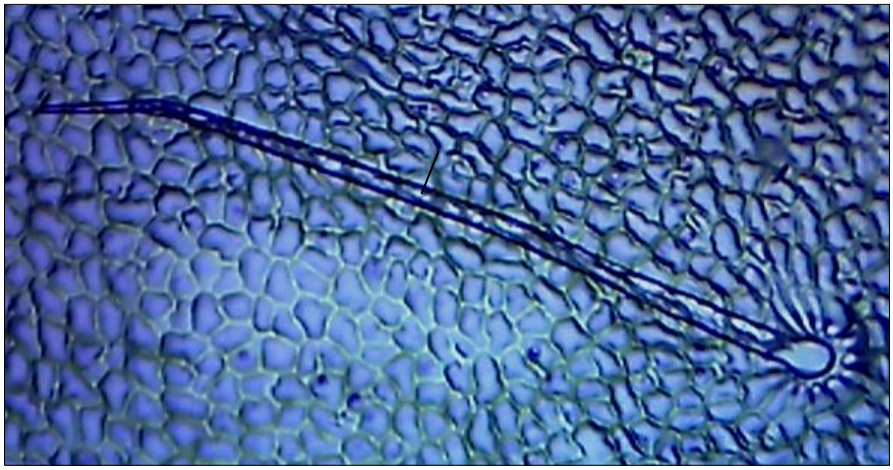


Figure 5. *Trifolium pratense* L.- *Trichoma (10x10)*



Figure 6. *Trifolium pratense* L.- *Trichoma (10x10)*

Trifolium pratense L. species is regularly higher on the upper part of the leaf than on the lower part of the leaf.

On the epidermis of upper and lower part of the leaf of *T. pratense* L. there are long, single-celled trichomes with no living content .

On the Table 4 obtained results from Variance analysis of trichome length on the lower part of the leaf , are presented. It was conclude that difference in the mean values of various populations is not greater enough . Therefore there is no statistically significant difference (P = <0.132).

Mean values of this parameter starting from Population A3 which was situated on the lowest altitude up to Population K11, with some oscilations, indicate the increasing trend,whereas there is a rapid reduction of mean values of this parameter in Population L12 and M13 which are situated in higher altitudes (Chart 4).

n	Population	Length of trichoma on the lower part of the leaf			
		Average value(cm)	Standard Deviation	Maximal Value	Minimal Value
10	A3	618,840	114,933	855,500	460,350
10	B4	639,520	171,369	870,050	425,250
10	C2	745,060	163,628	980,300	551,300
10	D1	769,805	192,435	1118,200	571,300
10	E5	678,535	134,833	987,300	488,650
10	F6	596,575	114,155	802,700	464,100
10	G7	683,190	72,612	791,150	582,200
10	H8	680,862	80,179	849,638	585,612
10	I9	638,719	82,065	791,669	552,000
10	J10	660,954	60,170	755,997	587,650
10	K11	670,908	67,703	800,730	590,375
10	L12	654,814	71,394	583,995	640,885
10	M13	599,505	107,288	755,450	454,200
P = 0,132					
t = 0.389					

Table 4. *Trifolium pratense* L. - Length of trichoma on the lower part of the leaf

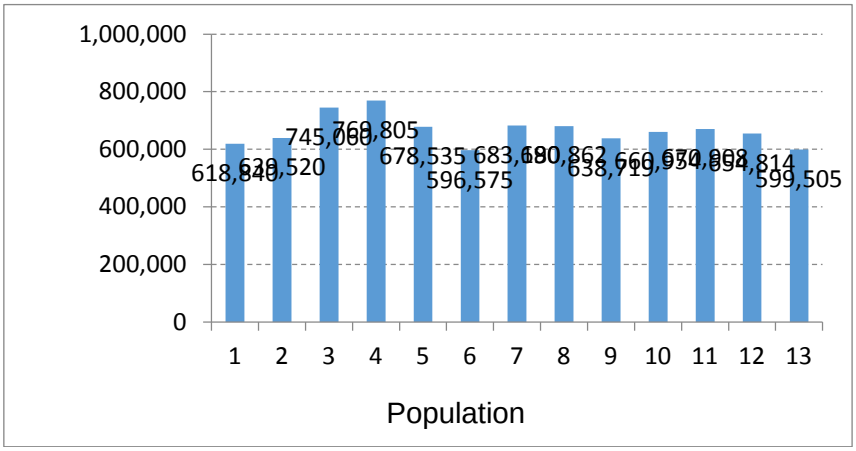


Chart 4. *Trifolium pratense* L. - Length of trichoma on the lower part of the leaf

4. CONCLUSIONS

At the very end we can conclude that investigated populations of *Trifolium pratense* L., showed statistically significant individual , population and inter population variability on most of investigated parameters .

Given the high variability that was found during the numerous analysis of researched parameters of *T. pratense* L. species, this paper contributes to the examination of species differentiation within this taxon in a new light, which eventually can lead to its taxonomic revision.

Assessment of morphological variability of certain parameters of plant species, distributed in different altitude s in mountainous environments can also be used to assess the effect that climate change is having in a particular location.

For most of investigated parameters was found that with increasing altitude, and therefore with the change of complex of ecological factors, that also condition the presence of different plant community types, comes to reduction of average values; this means that researched parameters exist in negative correlation with increasing altitude.

REFERENCES

Abberton M. T., Marshall A. H. 2005. Progress in breeding perennial clovers for temperate agriculture. Journal of Agricultural Science, 143 (2–3): 117–135

http://dx.doi.org/10.1017/S0021859605005101

Barry R. G. 1992. Mountain Weather and Climate. 2d Ed. Routledge, New York, 402 pp.

Caputa J. (1966): Forage production in relation to altitude. Proc. X. Int. Grassl. Congr., Helsinki: 846–851.

Drobna J. (2009): Yield and Forage Quality of Romanian Red Clover (*Trifolium pratense* L.) Varieties Studied in Slovakia; Print ISSN 0255-965X; Electronic ISSN 1842-4309 ; Not. Bot. Hort. Agrobot. Cluj 37 (1), 204-208

Gaston J. 2000. Global patterns in biodiversity. Biodiversity and Macroecology Group, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK Macmillan Magazines Ltd.

Herrmann D., Boller B., Studer B., Widmer F., Kölliker R.2008. Improving persistence in red clover: insights from QTL analysis and comparative phenotypic evaluation. Crop Science, 48 (1): 269–277.http://dx.doi.org/10.2135/cropsci2007.03.0143.

Knežević M., Leto J., Kozumplik V., Pejić I. (1997):Dry matter degradability of red clover (*Trifolium pratense* L.) grown at different altitudes tested with rumen fistulated rams. In: XVIII. Int. Congr., Saskatoon,Canada: 75–76.

Körner Ch. & Cochrane P. 1983. Influence of plant physiognomy on leaf temperature on clear midsummer days in the Snowy Mountains, south-eastern Australia. Acta Oecol,Oecol Plant 4:117-124.

Körner Ch. 1999. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems (SpringerVerlag; ISBN:3540650547/3540654380).

Leto, J., M. Knežević, K. Bošnjak, D. Mačešić, Z. Štafa, and V. Kozumplik (2004). Yield and forage quality of red clover (*Trifolium pratense* L.) cultivars in the lowland and the mountain regions. Plant, Soil and Environment. 50 (9):391-396.

Nelke M., Nowak J., Wright J.M., McLean N.L., Laberge S., Castonguay Y., Vezina L.P. : 1999. Enhaced expression of a cold-induced gene coding for a glycine-rich protein in regenerative somaclonal variants of red clover (Trifolium pratense L.) –Euphytica vol. 105 211-217 pp

Nowak J. , Matheson S.L., McLean N.L. , Havard P. : 1992. Regenerative trait and cold hardiness in highly productive cultivars of alfalfa and red clover – Euphytica vol. 59189-196 pp.

Redžić S. 1991. Ph. D. in Biology with doctoral thesis in the field of Ecology with the title "*Syngenesis of vegetation in ecosystems at vertical profile of mountain Ozren near Sarajevo*" at the Faculty of Science of the University of Sarajevo.

Sato, S., S. Isobe, E. Asamizu, N. Ohmido, R. Kataoka, Y. Nakamura, T. Kaneko, N. Sakurai, K. Okumura, I. Klimenko, S. Sasamoto, T. Wada, A. Watanabe, M. Kohara, T. Fujishiro, and S. Tabata (2005). Comprehensive structural analysis of the genome of red clover (*Trifolium pratense* L.). DNA Research. 12(5):301-364

Taylor N.L. , Quesenberry K.H. (1996) : Red Clover Science. Series: Current Plant Sciences and Biology in Agriculture: 28. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Voigt, P. W., and J. A. Mosjidis (2002). Acid-soil resistance of forage legumes as assessed by a soil-on-agar method. Crop Science. 42(5):1631-1639.

Whiteman C. D. 2000. Mountain Meteorology: fundamentals and applications. Oxford University Press, New York, 355 pp.

Whittaker R. H. 1972. Evolution and measurement of species diversity. Taxon 21: 213-251.