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Morphological and ecological differentiation of the fruit of *Fragaria vesca* L. (Rosaceae) from different habitats in Bosnia and Herzegovina

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***Abstract*:**

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30 individuals from each population of the *Fragaria vesca* in the fruiting phenophase were collected in the ecologically different habitats. Morphological variation was investigated using 10 populations and 300 individuals. All specimens were properly preserved in a Formalin-Acetic Acid mixture and stored in herbarium. The fruits of *Fragaria vesca* were investigated in order to determine the most variable and the most consistent morphological character, depending on the environmental factors under which they developed. Each population and its belonging individuals were labeled with special numbers. The percentage of variability for the height of fruit is 24.338% and for the width of fruit is 25.747%. However, considering high resource values of strawberries, it is necessary to emphasize that the size of the fruit follows the overall size of an individual.

Key words: *Fragaria vesca* L., fruits, morphological and ecological variability

***Apstrakt*:**

Huseinović, S., Osmanović, S., Terzić, Z., Šabanović, M.: ***Morfološka i ekološka diferencijacija plodova vrste Fragaria vesca L. (Rosaceae) na različitim staništima u Bosni i Hercegovini. Biologica Nyssana, 5 (2), Decembar 2014: 75-82.***

Na ekološki različitim staništima prikupljeno je po 30 individua iz svake populacije vrste *Fragaria vesca* u fenofazi plodonošenja. Morfološka varijabilnost je sagledana na osnovu 10 populacija i 300 jedinki. Sve jedinke su konzervirane u FO-a smjesu ili pohranjene u herbarijumu. Plodovi odabranih vrsta roda *Fragaria* (*Fragaria vesca*) su istraženi s ciljem da se odredi najvarijabilniji i najkonzistentniji morfološki karakter, u zavisnosti od ekoloških faktora pod kojima se razvijao. Utvrđen je stepen varijabilnosti morfometrijskog karaktera za parametar: maksimalna širina ploda (P1), kretao se u rasponu 24,338%, a za širinu ploda (P2) iznosi 25,747%. Na osnovu statističkih analiza utvrđeno je da plod jagode ne spada u grupu onih biljnih delova koji najviše variraju.

Ključne reči: *Fragaria vesca* L., plodovi, morfološka i ekološka varijabilnost

Introduction

Strawberry, genus *Fragaria* L., is a plant from the family Rosaceae and subfamily Rosoideae (Potter et al., 2007). It has great economic importance and therefore it has been investigated from different aspects - morphological (Harrison et al., 1997), systematic and biogeographic (Staudt, 1989, 2009), demographic (Angevine, 1983), genetic (Galletta & Maas, 1990), molecular (DiMeglio et al., 2014) and other. The genus *Fragaria* includes 20 wild species, three described naturally occurring hybrid species, and two cultivated hybrid species important to commerce (Hummer et al., 2011). The wild species are distributed in the north temperate and holarctic zones. The only species which is widespread in Europe, Asia and America is *Fragaria vesca* L. (Staudt, 1989, 2009). In Europe it is represented with 6 species (Tutin et al., 1968; Euro+Med, 2006). The genus *Fragaria is* represented by 3 species in Bosnia and Herzegovina - *F. vesca* L., *F. moschata* Weston and *F. viridis* Weston (Beck-Mannagetta, 1927; Euro+Med, 2006).

Fragaria vesca is the most widely distributed species. It is growing in a different natural habitats (forests, meadow, pasture and other) (Beck-Mannagetta, 1927) and therefore it is very variable. Up to now, the morphological studies of this species included only vegetative parts of the plant (Huseinović & Osmanović, 2010).

The objective of this study was to investigate morphological and ecological differentiation of the fruits of *Fragaria vesca* from different habitats in Bosnia and Herzegovina.

Material and methods

Plant material of the *Fragaria vesca* was collected in the fruiting phenophase from ten sites around Tuzla during 2013 (**Tab. 1**). During the selection of the research sites, efforts were made to cover populations developed in different plant communities.

Field research

The field research consisted of a determination of biotic habitat factors of each of the studied populations, establishing phenological data and collecting samples and herbarium material. During the fieldwork we also made an adequate photographic documentation.

Based on literature data (Redžić, 1997) we determined type of plant communities on each site.

Collecting samples of plant material

On the selected ecologically diverse habitats, living material of the species *Fragaria vesca* was collected. At least 30 individuals from the population in the fruiting phenophase were sampled from each locality. Morphological variability was observed on the basis of 10 populations and 300 individuals.

During the collection of plants in the field, keys for determination of plant species have been used (Domac, 2002; Aichele, 2004).

All specimens were properly preserved in a Formalin - Acetic Acid mixture, or stored in the herbarium.

As the collection of plant material was run at the time of fruiting, each population and its dependent individuals were marked with special numbers.

Laboratory studies

In addition to the morphometric and statistical processing of materials and data, the laboratory part of the study assumed also numerous activities: consulting the relevant literature, collecting and processing eco-climate data, creation of original illustrations, etc.

Table 1. List of plant collection sites

Site	Locality	Type of vegetation
1	Kladanj (Muška voda)	*Abieti-Fagetum fragarietosum vescae*
2	Gornja Tuzla	*Arrhenatherion elatioris*
3	Cerik-Ljubače	*Arrhenatherion elatioris*
4	Husino	*Querco-Pinetum nigrae*
5	Ilinčica II	*Agropyro-Rumicion* in zone *Tilio-Quercetum petraeae*
6	Krojčica	*Agrimonyo-Fragarietum*
7	Simin Han	*Arrhenatherion elatioris*
8	Ilinčica I	*Fragarion vescae* in zone *Querco-Carpinetum betuli*
9	Zlaća - bridge	*Fagetum montanum serpentinicum*
10	Zlaća - road	*Fagetum montanum serpentinicum*

Morphological studies of the species *Fragaria vesca* on the localities of Gornja Tuzla, Simin Han, Ilinčica I, Ilinčica II, Krojčica, Husino, Cerik, Zlaća (bridge), Zlaća (road) and Kladanj (Muška voda), were performed in the laboratories of the Faculty of Science in Tuzla.

The following morphological characters were determined: maximum width of the fruit (P1) and maximum height of the fruit (P2).

Characters of the fruit

Besides all the above mentioned characters we studied the fruit as well, as an essential characteristic of the gene expression.

According to its shape (P3), the fruit was placed into one of the following groups: rounded, globose, conical or elongated.

Statistical analysis

Evaluation and interpretation of individual character's variability, the degree of correlation or relationship between individual characters and ecological parameters, as well as analysis of the significance of the obtained results were performed in accordance with the relevant statistical and biological and ecological patterns contained in the available software packages (ANOVA, 2006; Statistics SPSS). By methods of descriptive statistics we determined: the arithmetic mean, range, variance, standard deviation and coefficient of variation of individual characters in specific patterns. The significance of the identified differences between the arithmetic means was determined by the method of testing the difference between the arithmetic means of small independent samples (P e t z , 1983).

Results

Fragaria vesca has a pronounced ecological valence in relation to a complex of environmental factors. It inhabits a variety of volcanic and sedimentary rocks, as well as the different stages and types of automorphic soils. It was noted also at the serpentine - peridotic substrate (locality M. voda, Kladanj). Its populations equally well developed on bare parent substrate, cleared land, fire sites, and at developed soil in hornbeam, pine and pine-oak forests. So far it has been recorded in all regions of the former Yugoslavia, including Bosnia and Herzegovina, and the first varieties have been created in North America and Chile, from where they were transferred to Europe in the 17th and 18th century.

Variability of morphometric characters

Comparative morphological analysis of plant material collected from different habitats showed a significant degree of variability in a series of characters of the *Fragaria vesca.*

Variability in character of the fruit

Observing these two characteristics (height and width of the fruit) on the total sample (whose number is 300 individuals for all ten sites) it can be seen that for the first property, the minimum and maximum values range from 4 to 18 mm, and for the second property they range from 4 to 14 mm. Percentage of variability for the fruit height is 24.338% and for the width of the fruit is 25.747% (**Tab. 2**).

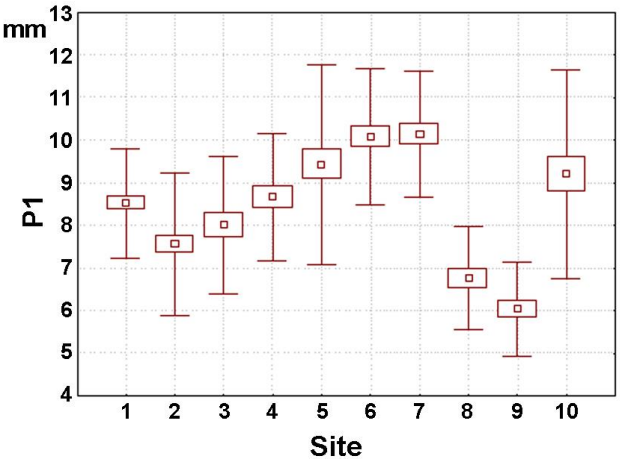

Fig. 1: Variations in height of the *Fragaria vesca* fruits by locality (1-10)

The height of the fruit (**Fig. 1**) is the largest in populations from the localities Ilinčica II (5), Krojčica (6), Simin Han (7) and Zlaća - road (10). Populations from the sites (6) and (7) represent communities on cleared land. At the given localities, in forest conditions, the light was limited. After cutting down the forest, there was a rapid expansion of the strawberries population due to the optimization of conditions of its ecological valence.

Fruit width (**Fig. 2**) is the largest in populations from the localities Krojčica (6), Simin Han (7) and Zlaća - road (10). These sites represent communities of open habitats and forest edges suggesting that these are the communities which by their ecological characteristics provide optimum conditions for the growth of strawberries.

Observing present forms of fruit by localities, it can be seen that the globose ones were present in the largest proportion at the site Krojčica (6), which is cleared land of a forest. The round fruits were

Table 2. Statistical data of the analyzed *Fragaria vesca* characters in all localities

Location	Variable	N	$\overline{X}$	$S\overline{x}$	X_{min}	X_{max}	Range	S	V (%)
Total	P1	386	8.486	0.105	4	18	14	2.065	24.338
	P2	386	7.459	0.098	4	14	10	1.920	25.747

Table 3. Significance in variations of fruit characters

significance			
Site 1	P1	P2	P3
P1		0.066	0.009
P2	0.066		0.000
P3	0.009	0.000	
Site 2	P1	P2	P3
P1		0.000	0.068
P2	0.000		0.127
P3	0.068	0.127	
Site 3	P1	P2	P3
P1		0.098	0.130
P2	0.098		0.163
P3	0.130	0.163	
Site 4	P1	P2	P3
P1		0.218	0.026
P2	0.218		0.000
P3	0.026	0.000	
Site 5	P1	P2	P3
P1		0.000	0.284
P2	0.000		0.427
P3	0.284	0.427	
Site 6	P1	P2	P3
P1		0.000	
P2	0.000		
P3			
Site 7	P1	P2	P3
P1		0.049	0.989
P2	0.049		0.124
P3	0.989	0.124	
Site 8	P1	P2	P3
P1		0.002	0.528
P2	0.002		0.194
P3	0.528	0.194	
Site 9	P1	P2	P3
P1		0.020	0.461
P2	0.020		0.040
P3	0.461	0.040	
Site 10	P1	P2	P3
P1		0.043	0.005
P2	0.043		0.728
P3	0.005	0.728	

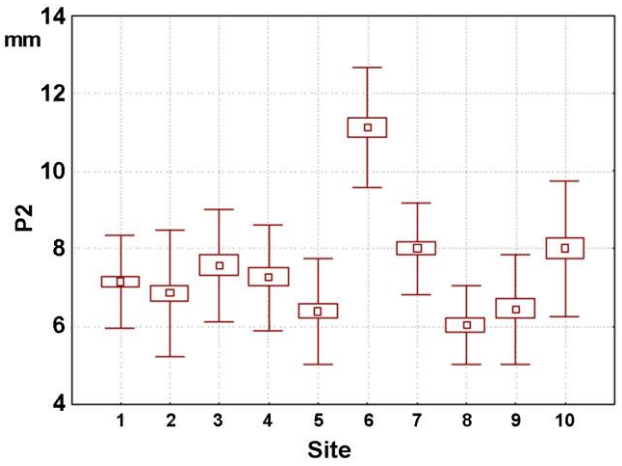

Fig. 2: Variations in width of the *Fragaria vesca* fruits by locality (1-10)

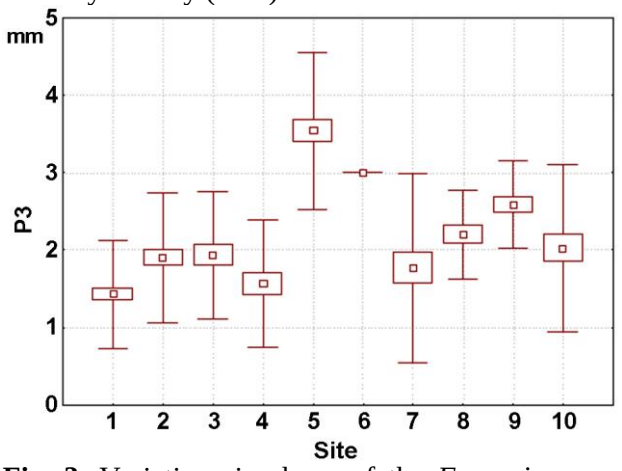

Fig. 3: Variations in shape of the *Fragaria vesca* fruits by locality (1-10)

present at all sites, with the exception of the site (6) and they prefer forest edges and meadows. The conical were present most in localities Kladanj (1), Zlaća - road (10) and Simin Han (7), which leads to the conclusion that they prefer cooler places. The strawberries with elongated fruits in the greatest proportion inhabit the site Ilinčica II (**Fig. 3, 4**).

Correlation in variations of fruit characters

We can conclude that in most cases there is a correlation between the width and shape of the fruit (**Tab. 3**, **Fig. 5**).

Ecological differentiation

Differences between morphological characteristics of populations originate from

differences in environmental factors prevailing in their habitats.

Determination of a high degree of variability of the analyzed characters indicates high inter-population variability, which further points to the possible influence of the present environmental factors.

The basis of the high degree of variability are genetic determinants of the species *Fragaria vesca*. Wide ecological valence in relation to the complex environmental factors resulted with spreading of this species in an environmentally very dynamic habitats, usually ecotones, where environmental factors of two different habitats collide (e.g. forests and meadows).

As a specific answer to this kind of dynamic (changeable) value of environmental factors, there is expressed variability of morphological and meristic characters. These features have the function of population's adaptation within the present environmental factors in order to achieve the best use of capacities and potentials of the habitats.

Given the very different types of habitats, and very different conditions in which the population can survive, the question is which habitat factors favor, and which limit the population to achieve its biological / ecological functions.

In the aim of shedding light on the role of individual factors from the complex, a cluster analysis was performed on the basis of the observed morphometric characters. Based on the character of the fruit **Fig. 6** shows high morphological similarity of populations from localities 8 and 9, i.e. from populations of the community habitats of *Fragarion vescae* in the zone *Querco-Carpinetum betuli* and *Fagetum montanum serpentinicum*. Morpho-logical characteristics of individuals of these populations are similar: individuals are of average height and have average values of the other monitored characters.

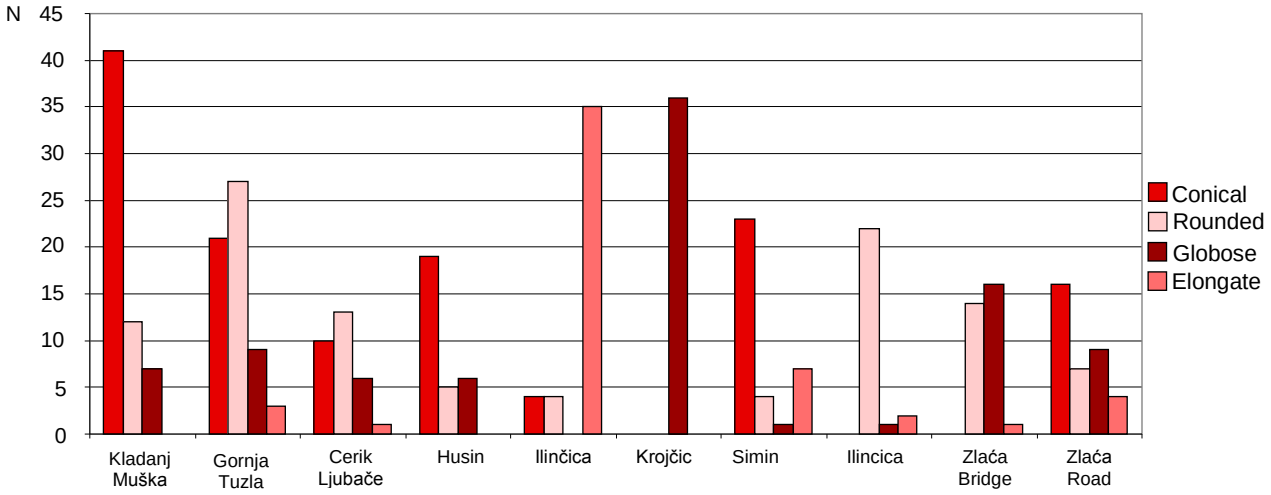

Fig 4. Presence of the four basic groups of the fruit shape by locality (N – number of individuals)

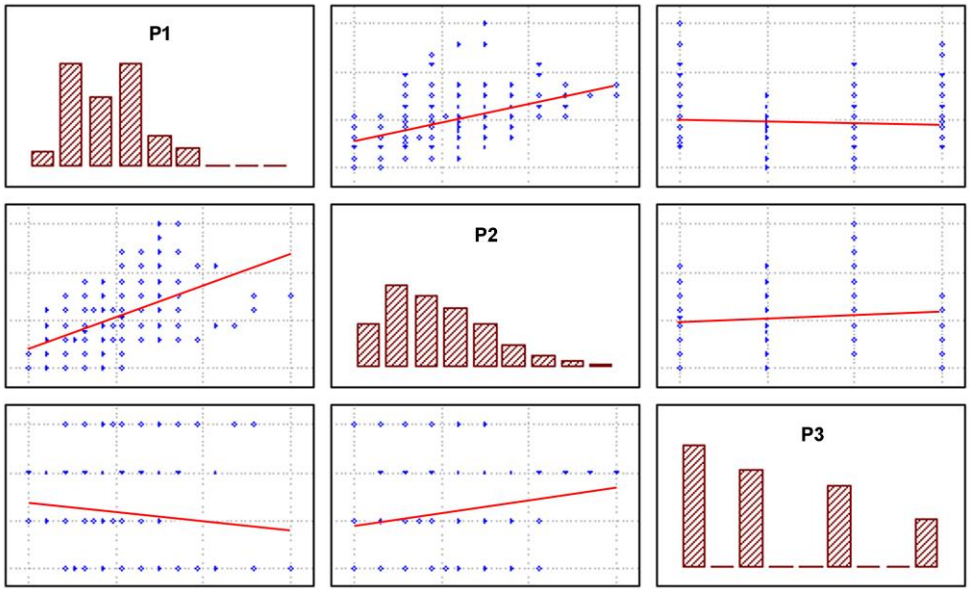

Fig. 5: Correlations for the height (P1), width (P2) and shape (P3) of the fruit *Fragaria vesca*

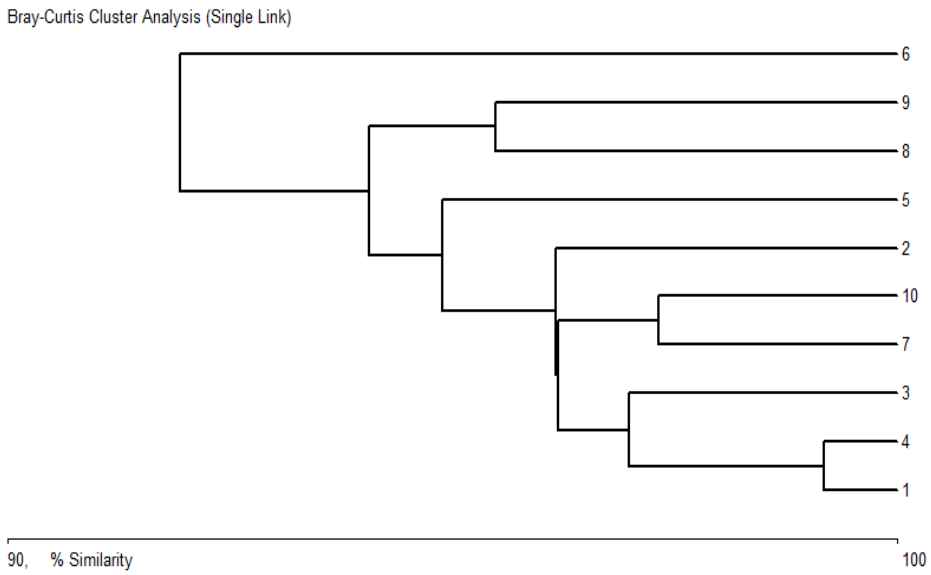

Fig. 6: Cluster analysis of the populations of *Fragaria vesca* in the investigated area based on the observed character of the fruit

The next group of very similar populations is made up of the populations from sites 1 and 4, and the related population from locality 10. The complex of factors that govern the habitats of these populations is heterogeneous. At the localities 1 and 10, vegetation of the ecosystem of beech-fir forests on serpentine geological substrate has been developed. In contrast to that, the site 4 has developed vegetation of the ecosystem of pine and oak. Although considering the temperature these are very different habitats, in ecological sense the sites are linked by having poor supply of water. In relation to the appearance of individuals in this group of populations (individuals are small, as well as most of individual characters), it can be assumed that the supply of water to habitats plays a major role in the possibilities of biomass production and dissemination of strawberries population.

The group of populations of meadow ecosystems of the alliance *Arrhenatherion elatioris* shows similarity with the populations developed in vegetation of fern colonies. Individuals are high in growth (stems are elongated), the leaves are large and petioles are small. In the dense meadow complexes, individuals of strawberry, looking for sufficient quantities of light and heat, in a competitive fight, "invest" their energy in stem elongation and increase in all vegetative parts of the plant body (sites 3, 7 and 2).

However, the greatest in growth are individuals that grow on the so-called cleared land (site 6, cleared land of the forest *Tilio-Quercetum petraeae)* and on the very edge of the forest (site 9, the edge of the forest *Fagetum montanum "serpentinicum.")* This fact indicates that the strawberry as a species is very capable that through rapid expansion occupy new habitats before other species use nutrients that forest land offers. However, this also points to the fact that the lack of light and heat in a given period of development (conditions prevailing in assembled forest communities), stand out as the limiting factor for the expansion of its populations.

A similar differentiation is shown also by the observed characters of the fruit.

Discussion

Variability in character of the fruit

(i) The largest coefficient of variation in the height of the fruit was recorded at the site 10 and amounts 26.618%, the smallest on the site 7 and amounts 14.573%.

(ii) In the variation of the width of the fruit, the largest coefficient of variation was recorded at the site 2 (23.863%) and the smallest amounts 14.667%, being recorded at the site 6.

(iii) The coefficient of variation of the height of the fruit was 24.338%, and of the width of the fruit was 25.747%.

Thus, the fruit does not fall into the group of those plant parts that vary the most.

However, due to the high resource values of strawberries, it is necessary to emphasize that the size of the fruit follows the overall size of individuals. As seen from the previous findings, the highest growth was achieved by individuals from the cleared land of the community *Tilio-Quercetum petraeae*, which indicates that strawberries in our conditions optimally thrive on mesophilic, well lit to somewhat shaded and relatively warm habitats of the mountain zone.

Morphological differentiation

The populations of species *Fragaria vesca* in the study area were not morphologically uniform, but they contain more or less differentiated morpho-eco-types (R e d ž i ć , 1990).

The fact that in the populations of different geo-biocenosis the same morphological types of plants occured, and that these populations differ among themselves, however, shows that the quantitative participation of individual types in them is different (B a š i ć , 2004; K o v a č i ć , 2006).

At the site of Muška voda, Kladanj, the size of specimens is relatively small compared to individuals from cleared land and open meadow habitats. The flowers are small, as well as the fruits, which are mostly elongated.

Individuals of the population at the locality of Gornja Tuzla developed within the community of *Arrhenatherion elatioris.* Flowers are also relatively small, as well as the fruits, which are mostly elongated. Variations within the population are relatively large.

Individuals of the population at the site Cerik - Ljubače (in the zone of the forest community *Querco-Carpinetum)* are characterized by the lowest average length and width of a rosette leaf midrib.

Individuals sampled at the site Husino developed within the community *Sambucetum nigrae* and are characterized by the fact that there have not been reported extremely high or low values for the observed characters.

Population individuals of the site Ilinčica II developed within the community *Fragarion vescae* by the edge of *Querco-Carpinetum betuli.* The specimens are large, but still far less than those on the site Krojčica, where maximum values for the most of the studied characters were recorded.

Individuals of the population at the site Simin Han developed within the community *Pteridio-Fragarietum vescae* and are very variable among them. On the site Ilinčica I, population has been developed within the community *Agropyro-Rumicion* in anthropogenized conditions in the zone of mesophilic meadows. They do not stand out by the high growth, nor by the size of the fruit.

Individuals of the population Zlaća (road) developed on the anthropogenized eutric cambisol within the community of dark deciduous forests, as well as their neighboring population. Individuals of both populations are characterized by relatively low growth.

Ecological differentiation

Group of populations from meadow ecosystems of the alliance *Arrhenatherion elatioris* showed similarity with the population developed in the vegetation of fern colonies and all other populations that are found in the open (non-forested) habitats (R e d ž i ć , 1987, 1988). Individuals were high in growth (stems are elongated), the leaves were large, while petioles were small. In dense meadow complexes, individuals of strawberry, looking for sufficient quantities of light and heat, in a competitive fight, "invest" their energy in stem elongation and increase in all vegetative parts of the plant body (sites 2, 3 and 7).

However, the greatest growth had the individuals that grew on the so-called cleared land (site 6, cleared land of the forest *Tilio-Quercetum petraeae)* and on the very edge of the forest (site 9, the edge of the forest *Fagetum montanum "serpentinicum").* This fact indicates that the strawberry as a species, is very capable to occupy new habitats by rapid expansion and before other species use nutrients that forest lands offer.

(i) As a specific answer to this kind of dynamic (changeable) values of environmental factors there is an expressed variability of morphological and meristic characters, which has the function of adaptation of populations to present environmental factors, that is, the function of the best use of habitat's capacities and potentials (E r i k s s o n et al., 2003).

(ii) The investigated morphologic characters are changing, and the emerged changes and differences among populations are not stable. Further, stronger environmental isolation does not lead to a stronger morphological differentiation of the same character or group of characters. Greater morphological differentiation is achieved by increasing the number of differentiating characters. In the second case, individual differential characters can lose that property and differentiation among populations is accomplished through other characters.

(iii) Since ecologically distant populations are simultaneously morphologically remote too, it could be assumed that those changes are still at the level of phenotypic variation. However, within the same population clearly separated groups of individuals (groups of clones) can be observed, among which there are no observed larger and clearer environmental differences. Similar clones are located in ecologically different populations.

Therefore, a detailed knowledge of morphological variability and ecological differentiation of populations within the species is

essential condition for the full overview of biological potential and evolutionary status in which the analyzed group of plants is located.

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