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Original scientific paper

ANALYSIS OF INTER – POPULATION VARIABILITY OF SCOTS PINE (Pinus sylvestris L.) USING MORPHOMETRIC MARKERS

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The aim of this study is analysis of inter-population variability of Scots pine (*Pinus sylvestris* L.) using morphometric parameters of cones and seedlings originating from seven populations in Serbia.The analysis of 1960 cones and their seeds was performed as well as the analysis of morphometric parameters of seedlings (height and root neck diameter) that were produced from this seed. Based on the obtained results the significant

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differences were noticed in the values of analyzed characters for each population separately. The biological similarity or distance regarding the analyzed characters showed a significant population differentiation. By presenting the preliminary knowledge on the genetic variability of the study populations, the reported results will contribute to better understanding of the significance of the analyzed characters in the population differentiation.

Key words: cones, Scots pine, seeds, seedlings, variability

INTRODUCTION

The variability of cones, seeds and seedlings of major forest tree species has long been the research subject of Forestry scientists and experts. There are numerous papers in the country and abroad that deal with this, for the Forestry, very important scientific field. Thanks to the results of the domestic researchers TOŠIĆ (1991), LUČIĆ *et al.* (2007), MATARUGA *et al.* (2007, 2011), and abroad CUNNINGHAM *et al.* (1991), CHAISURISRI *et al.* (1992), SORENSEN *et al.* (1993), BARNETT (1998), ALÍA *et al.* (2001), URBANIAK *et al.* (2003), PARKER *et al.* (2006), WAHID *et al.* (2006), ANDROSIUK *et al.* (2011), MATTI (2011), the knowledge is gradually complemented and the relationship between the genetic constitution of populations and the variability of seeds, cones and seedlings is closer explained.

Multi-decade study of the natural variability of cones and seedlings aimed at defining a lower taxonomic belonging within the species, the links between provenances, environmental conditions and determination of the genetic constitution of parental individuals. Many authors have dealt with studying the characters of pine cones, while recently with notable results are distinguished MATARUGA (2003), PARKER *et al.* (2006), WAHID *et al.* (2006) and LUČIĆ (2007). The results of their work show great diversity. However, the shape of cones is very characteristic mark of some subspecies. Variability in size of cones and seedlings depending on the population, the genotype and the collection year of Scots pine were studied by CUNNINGHAM *et al.* (1991), TOŠIĆ (1991), URBANIAK *et al.* (2003) and MATTI (2011). The results indicate a large interpopulation and individual variability of these characters and the genetic determination of this variability.

The study results of variability of cone and seedling morphometric characters, are used for the preliminary knowledge on the genetic variability of the study populations.

It has to be pointed out that recently there have been significant results in the analysis of inter-population variability of Scots pine (*Pinus sylvestris* L.) in Serbia using other types of markers (biochemical and molecular) LUČIĆ *et al.* (2011a, 2011b, 2011c), which together with the research in this paper provides a basis for better understanding of genetic variability of the analyzed species.

MATERIALS AND METHODS

In each of seven locations (Table 1), 100 cones per tree were collected. The total number of trees was 98, per 15 trees for each location, except in Bukovik-

Aleksinački population – where 8 trees were selected (a small number of trees in the field). Then twenty cones per tree were taken by random sampling method for processing at the laboratory of Institute of Forestry

Cone opening was done in the dryer at the temperature of 40 °C. Dehusking of seeds was done by hand while cleaning of seeds from inert matter was done in "van". After that, the seeds were grouped by trees and locations and stored in a refrigerator at temperatures between 3 ° C and 5 ° C (Figure 1).

Figure 1. Overview of processed seeds prepared for analysis and planting in containers

The basic morphometric parameters of cone (length and width) were measured by caliper with an accuracy of 1 mm. Also, the number of seeds was determined for each cone.

In March 2009, the processed seeds were sown in containers of type Jukosad. In October 2010, at 2 +0 seedling age, were measured the height and the root neck diameter. Measurements were made by caliper with an accuracy of 1 mm in the measurement of height, and 0.1 mm in diameter measurement. In the unique manual were written values of measured parameters. The measurement of all 2,445 seedlings was accomplished, while the number of seedlings for each population separately is shown in Table 1. Figures 2 and 3 show the seedlings in April and September 2010.

After the measurements of morphometric parameters of cones, seeds and seedlings, the results were analyzed using software *Statistica.*

Figure 2. Pictures of seedlings, April 2010

Figure 3. Pictures of seedlings, September 2010

RESULTS AND DISCUSSION

Cones and seeds

Data obtained from cone length and width measurements as well as number of grains were analyzed using software *Statistica*. The main statistical indicators of analyzed characters are presented in Table 1.

Table 1. Mean values of basic statistical indicators, as well as minimum and maximum values of Scots pine (Pinus sylvestris L.) cones.

Population	Parameter	Arithmetic mean	min	max	Standard deviation	Coefficient of variation	Σ of seeds
FMU „Dubočica Bare“	Cone length *(mm)*	42.92	28	60	5.49	12.79	
60 a.	Cone width *(mm)*	21.16	2	30	2.77	13.09	
FE „Golija“ Ivanjica	Number of seeds	20.34	0	44	10.26	50.44	6103
FMU "Šargan", 25 b.	Cone length *(mm)*	38.66	26	57	5.99	15.49	
FE "Užice" Užice	Cone width *(mm)*	19.69	2	26	2.56	13.00	
	Number of seeds	11.68	0	40	7.74	66.27	3457
FMU "Radočelo-Crepuljnik" 4b.	Cone length *(mm)*	40.75	30	55	5.00	12.27	
	Cone width *(mm)*	19.24	2	25	2.33	12.11	
FE "Stolovi" Kraljevo	Number of seeds	22.34	4	42	8.75	39.17	6701
FMU "Jablanička reka" 33 d.	Cone length *(mm)*	40.75	30	55	5.00	12.27	
	Cone width *(mm)*	19.24	2	25	2.33	12.11	
FE "Rasina" Kruševac	Number of seeds	22.34	4	42	8.75	39.17	6701
FMU"Bukovik-Aleksinački", 23b, 24g,	Cone length *(mm)*	46.67	31	63	6.27	13.43	
	Cone width *(mm)*	22.08	17	29	2.43	11.01	
FE "Niš", Niš	Number of seeds	19.09	0	52	11.99	62.81	3055
FMU "Kaluđerske bare",1a	Cone length *(mm)*	41.79	3	66	6.03	14.43	
	Cone width *(mm)*	20.03	2	29	2.56	12.78	
NP Tara, Bajina Bašta	Number of seeds	17.05	0	47	9.82	57.60	5138
FMU "Zlatar I",22a,	Cone length *(mm)*	42.48	28	66	5.73	13.49	
FE "Prijepolje",	Cone width *(mm)*	20.28	2	29	2.64	13.02	
Prijepolje	Number of seeds	17.01	0	47	10.48	61.61	3502

Based on basic statistical parameters of analyzed characters it can be concluded that mean values of cone length and width were the highest in the population **V**-Bukovik Aleksinački (46.67 mm and 22.08 mm), the smallest cone

length was measured in the population **II** Šargan (38.66 mm) and the smallest cone width was measured in the population **IV** Jablanička reka (19.24 mm). The mean number of grains was the highest in the population **IV** Jablanička reka (22.34 pcs.) and it was the lowest in the population **II** Šargan (11.68 pcs.).

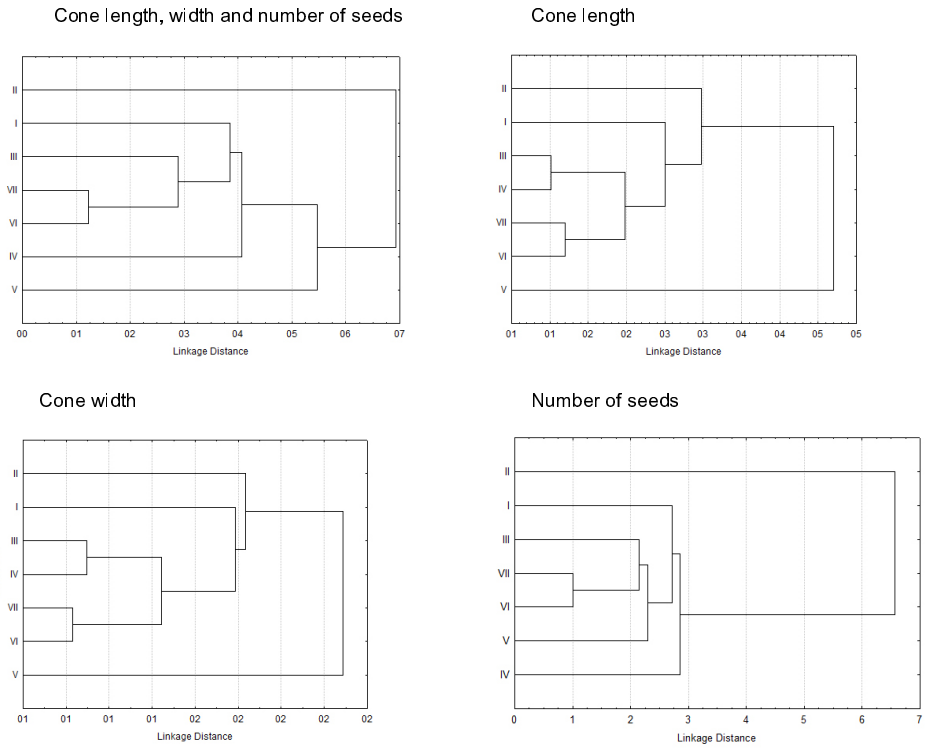

Figure 1. Dendrograms of cluster analysis for the analyzed characters

Cluster analysis was made for all three analyzed characters together, as well as individually for each character, Figure 1. Based on determined mutual biological similarity or distance regarding these three characters it was ascertained the following:

— In the case when all three characters were analyzed together, the greatest distance occurred between the populations **II** Šargan and **V** Bukovik- Aleksinački, while the shortest distance occurred between the populations **VII** Zlatar and **VI** Kaluđerske bare;

— If we take into account the cone length as a factor of variability, the most distant populations are **II** Šargan and **V** Bukovik- Aleksinački, while on the shortest distances are interconnected the populations **III** Radočelo - Crepuljnik and **IV** Jablanička reka, as well as **VII** Zlatar and **VI** Kaluđerske bare;

— Regarding the cone width as a factor of variability, the situation is the same as when it was considered the cone length, ie.— the greatest distance occurred between the populations **II** Šargan and **V** Bukovik - Aleksinački, while the shortest distance occurred between the populations **III** Radočelo - Crepuljnik and **I**V Jablanička reka, and **VII** Zlatar and **VI** Kaluđerske bare;
— When we take into consideration the grain number per cone, it can be noticed the great distance of the population **II** Šargan over others, and the connection of the populations **VII** Zlatar and **VI** Kaluđerske bare on the shortest distance.

From the above it is clear that there is a great distance of the populations **II** Šargan and **V** Bukovik - Aleksinački, both among themselves and relative to other, and on the opposite side expressed similarity of trees of the populations **VII** Zlatar and **VI** Kaluđerske bare.

Seedlings
Results of analysis of seedling morphometric parameters are shown in Table 2.

Table 2. Basic statistical indicators for the height and diameter of seedlings

Population		Character	No of measur.	Arithm. mean	min	max	Standard deviation	Coeff. of variation
I	FMU „Dubočica Bare“ 60 a.FE „Golija“ Ivanjica	height	335	6,64	2,50	17,3	1,70	25,60
		diameter	335	2,19	1,00	5,1	0,64	29,22
II	FMU "Šargan", 25 b. FE "Užice" Užice	height	355	7,67	3,60	13,2	1,98	25,81
		diameter	355	2,05	1,20	3,7	0,46	22,44
III	FMU "Radočelo-Crepuljnik" 4b.FE "Stolovi" Kraljevo	height	402	6,15	2,60	10,3	1,43	23,25
		diameter	402	2,05	1,00	3,7	0,49	23,90
IV	FMU "Jablanička reka" 33 d.FE "Rasina" Kruševac	height	336	7,56	2,70	15,3	2,00	26,46
		diameter	336	2,10	1,00	3,8	0,51	24,29
V	FMU "Bukovik-Aleksinački", 23b, 24g, FE "Niš", Niš	height	351	7,85	1,00	15,3	1,99	25,35
		diameter	351	1,97	0,90	4,8	0,50	25,38
VI	FMU"Kaluđerske bare",1a NP Tara, Bajina Bašta	height	340	7,46	3,30	17,5	2,13	28,55
		diameter	340	2,06	0,90	4,8	0,57	27,67
VII	FMU "Zlatar I",22a, FE"Prijepolje", Prijepolje	height	326	7,13	2,30	13,8	1,92	26,93
		diameter	326	2,11	1,00	4,0	0,51	24,17

In the analyses of inter-population genetic diversity of trees when the seedlings are used as their indicator, we equally analyze the range of variation of height and root neck diameter although in the researches is not yet completely

resolved the dilemma which character can be more reliable to use for this purpose. In Scots pine seedlings, the analysis of variance of the root neck diameter shows that the differences that occur between populations as per the average values are not statistically significant, and that "population" as a source of variability did not have a significant impact on this character, LUČIĆ (2012), Table 3. This result stipulated that the further analyses of the interpopulation variability are conducted by analyzing the variability of seedling height.

Table 3. Analysis of variance for the root neck diameter

Population		Arithmetic mean	1
V	FMU "Bukovik-Aleksinački", 23b, 24g, FE "Niš", Niš	1,969386	****
II	FMU "Šargan", 25 b. FE "Užice" Užice	2,038053	****
III	FMU "Radočelo-Crepuljnik" 4b. FE "Stolovi" Kraljevo	2,048139	****
IV	FMU "Jablanička reka" 33 d. FE "Rasina" Kruševac	2,080595	****
VI	FMU "Kaluđerske bare",1a NP Tara, Bajina Bašta	2,080753	****
VII	FMU "Zlatar I",22a, FE "Prijepolje", Prijepolje	2,123068	****
I	FMU „Dubočica Bare“ 60 a. FE „Golija“ Ivanjica	2,193921	****

Nominal values of statistical parameters for analyzed metric characters of seedlings-height and root neck diameter (Table 2), show that the lowest mean value for the seedling height of 6.15 cm had seedlings of the population **III** Radočelo - Crepuljnik, while the highest were seedlings from the population **V**, with height of 7.85 cm. In absolute values, the minimum seedling height of 1 cm was measured on a sample of the population **V**, while the maximum height of 17.5 cm was measured on a sample of the population **VI** Kaluđerske bare.

Populations **I** Dubočica Bare and **III** Radočelo-Crepuljnik are mutually similar while with other populations are connected at greater distances, Figure 2. At the greatest distance in relation to the other populations is the population **II** Šargan.

Comparing the results obtained in the analysis of variability of seeds, cones and seedlings in this paper and in papers TOŠIĆ (1991), ALÍA *et al.* (2001), URBANIAK *et al.* (2003), ANDROSIUK *et al.* (2011), it can be concluded that inter-population variability depends on the type of the analyzed character.

The results obtained in the analysis of inter-population variability using morphometric, biochemical and molecular markers on the same populations LUČIĆ

et al. (2011a, 2011b, 2011c) show differences that are directly dependent on the type of markers that were used, which is explained by the significant influence of stand conditions on the analyzed morphometric characters.

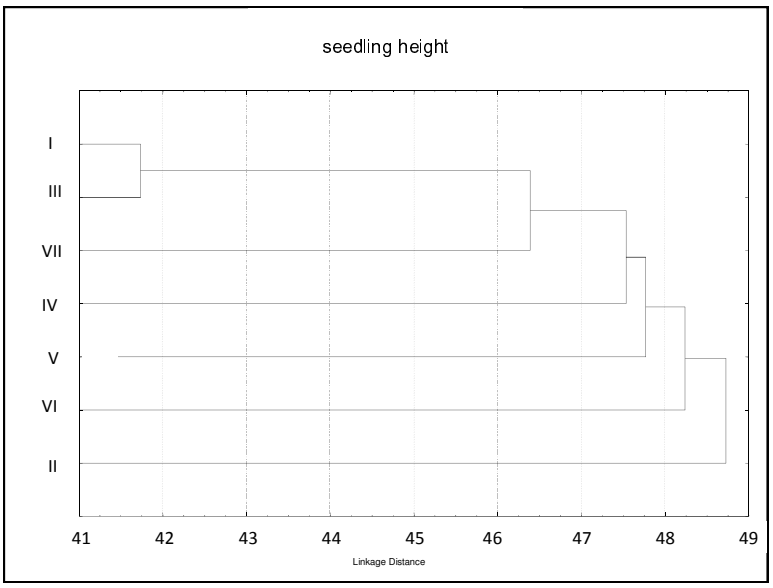

Figure 2. Cluster analysis dendrogram of seedling height

CONCLUSION

The populations were significantly different by values of analyzed characters. The results indicate a large inter-population variability of these characters and the genetic determination of this variability.

Considering the complexity of the flowering biology and seed-bearing of Scots pine, the results of performed statistical analyses of population variability for variability of cone length and width as well as grain number in them, should only be used as approximate indicators.

Special attention was given to the results obtained in the analysis of morphometric characteristics of seedlings. The analysis of morphometric characters of two-year old seedlings (height and root neck diameter) gave significant results. Comparative analysis of abovementioned quantitative characters of two-year old Scots pine seedlings was carried out aiming to determine the character of genetic variability. It is generally known that on the range of variation of metric characters of seedlings produced in similar conditions, affects only the genetic basis of seed from which they originate, which is why the obtained values of performed analysis

are good indicators of the genetic potential of the analyzed populations. Starting from this perspective, the results of performed analyses of variability in height and root neck diameter of two-year Scots pine seedlings, were used in the study of inter-population variability.

According to the character and quantity of variability of cones, seeds and seedlings, the spontaneous genetic variability in populations of Scots pine indicates that they are characterized by balance model of population genetic structure. According to this model there are more alleles of dominant, "wild" type in them. For many alleles-genes, and maybe for most loci, is presented a series of alleles with different frequencies.

The results presented in this paper, contribe to a preliminary knowledge of genetic variability of the study populations.

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ANALIZA MEĐUPOPULACIONOG VARIJABILITETA BELOG BORA (*Pinus sylvestris* L.) UPOTREBOM MORFOMETRIJSKIH MARKERA

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U okviru ovog rada prikazani su rezultati analize međupopulacionog varijabiliteta belog bora (*Pinus sylvestris* L.) upotrebom morfometrijskih parametara šišarica i sadnica koje vode poreklo iz sedam populacija u Srbiji. Izvršena je analiza 1960 šišarica i njihovog semena, kao i morfometrijskih parametara sadnica (visina i prečnik u korenovom vratu) koje su proizvedene iz tog semena. U okviru ovog rada izvršeno je blizu 11 000 merenja. Analiza dobijenih vrednosti je urađena u programskom paketu *Statistica*. Komparativnom analizom dobijenih rezultata u proučavanju varijabilnosti morfometrijskih karakteristika šišarica, semena i sadnica može se zaključiti da postoji značajna međupopulaciona varijabilnost koja je u direktnoj zavisnosti od vrste analiziranog svojstva. Ovom analizom proverena je međusobna bliskost ili udaljenost po pitanju analiziranih svojstava, pri čemu je došlo do značajne diferencijacije populacija. Dobijeni rezultati predstavljeni u ovom radu, daju doprinos boljem razumevanju značajnosti analiziranih svojstava na diferencijaciju populacija, dajući preliminarno upoznavanje genetičkog varijabiliteta proučavanih populacija.

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