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Original scientific paper

ANALYSIS OF INTER-LINE VARIABILITY OF BALD CYPRESS (*Taxodium distichum* L. Rich.) JUVENILE SEEDLINGS USING MORPHOMETRIC MARKERS

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In this paper are presented results of the analysis of inter-line variability of morphometric characters of Bald cypress juvenile seedlings at the level of 20 half-sib lines. Obtained results contribute to better understanding of relationship of analyzed characters, as well as the influence of analyzed characters to the differentiation of half-sib lines. Based on these results, differences were observed in the values of the analyzed characters for each half-sib line individually. The determined values of seedlings' size indicate good genetic and adaptive potential of this species, which can be considered as a starting point for the mass production of quality planting material and its use in Serbia.

Key words: Bald cypress, genetic potential, seedlings, variability.

INTRODUCTION

Bald cypress is monoecious, long-lived, deciduous conifer. It belongs to the family *Taxodiaceae* and genus *Taxodium* that includes three species (DENNY and ARNOLD, 2007). In Serbia, there is only *Taxodium distichum* (L.) Rich. Bald cypress capability for establishment of forest cultures on lowland and floodplains in our country was recorded in the 1950s. At the beginning of the 1970s, STILINOVIĆ and TUCOVIĆ concluded that in our environmental conditions Bald cypress can be considered as a species of rapid growth, one of the few conifer species that

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may be suitable for introduction of conifers on lowland and floodplains where it can achieve high productivity (POPOVIĆ *et al*., 2012).

Nevertheless, on the territory of the Republic of Serbia this species has practically never got out from the range of horticultural activities. Researches on the variability and adaptive potential of Bald cypress in our conditions which have been published until now refer to trees that grow individually and in smaller or larger groups mainly in the city green areas (DRAŽIĆ and BATOS, 2002; NINIĆ-TODOROVIĆ and OCOKOLJIĆ, 2001, 2002; ŠIJAČIĆ-NIKOLIĆ *et al*., 2011). The possibility of wider application of Bald cypress as a forest species should be based on an assessment of its genetic and adaptive potential as well as quantity and quality of yield, primarily at the level of the existing Bald cypress seed stand.

MATERIALS AND METHODS

Seed material used for the research originated from the Bald cypress seed stand near Bačka Palanka, registration number S 01.10.01.01, which is managed by FE Novi Sad, FMU Bačka Palanka. On the basis of phenotypic characteristics and abundance of yield in 2010, 20 test trees were selected and per 200 cones were collected from each of them. Cones were put into separate bags and taken for processing at the laboratory of Institute of Forestry. Cone opening was done in the dryer at the temperature of 40°C, and seed cleaning was done manually. In April 2011 the seed sowing was perform in containers type Bosnaplast 12, in manner 10 containers for each test tree. In October 2011 at 1 +0 seedling age by method of random sampling 30 seedlings per each half-sib line were selected and height and root collar diameter were measured for each of them. In October 2012 at 2 +0 seedling age, also by method of random sampling 30 seedlings per each half-sib line were selected and height and root collar diameter were measured for each of them. Measurements were made by a caliper with an accuracy of 1 mm in the measurement of height, and 0.01 mm in the measurement of root collar diameter.

The obtained data were processed in a computer program Statistica.

RESULTS AND DISCUSSION

ONE-YEAR-OLD SEEDLINGS

In Table 1 is shown the variability of morphometric characters of one-year-old seedlings at the level of 20 Bald cypress half-sib lines.

Based on the statistical indicators (Table 1), it can be concluded that the highest mean value of root collar diameter shows the half-sib line number 1 (4.23 mm), while the smallest mean value of root collar diameter has the half-sib line number 3 (3.36 mm). The highest mean value for parameter height has the half-sib line number 6 (52.1 cm), while the smallest mean value for parameter height has the half-sib line number 2 (40.7 cm). The values of the root collar diameter and height coincide with the values that in their researches obtained other authors. Research results of VANN and MEGONIGAL (2002) show that one-year-old seedling reach height of about 50 cm and root collar diameter of about 4 mm. MAZHER *et al.* (2006) report that one-year-old seedlings of Bald cypress reach height up to 35 cm.

Table 1. Variability of morphometric characters of one-year-old seedlings at the level of 20 Bald cypress half-sib lines

Half-sib lines	Parameter	Average (mm)	Min	Max	Standard deviation	Coefficient of variation
1	diameter	4.23	2.96	5.19	0.56	13.28
	height	48.5	38.8	56.6	5.31	10.96
2	diameter	3.59	2.33	5.11	0.72	20.01
	height	40.7	30.9	51.9	6.87	16.87
3	diameter	3.36	2.57	4.56	0.53	15.84
	height	42.8	29.7	60.0	7.34	17.15
4	diameter	3.77	2.17	5.56	0.74	19.54
	height	46.0	36.4	58.6	5.61	12.20
5	diameter	3.37	1.96	5.44	0.85	25.15
	height	40.9	25.9	56.7	8.59	20.98
6	diameter	3.95	2.95	5.1	0.52	13.13
	height	52.1	41.3	71.2	6.44	12.37
7	diameter	3.75	2.09	5.25	0.99	26.53
	height	49.2	27.5	70.1	12.12	24.66
8	diameter	3.76	2.68	5.2	0.64	16.95
	height	44.4	29.1	54.2	6.60	14.87
9	diameter	3.84	2.6	4.95	0.72	18.68
	height	45.0	33.6	55.4	6.16	13.70
10	diameter	4.07	2.69	5.83	0.69	16.89
	height	51.2	32.2	67.4	7.62	14.89
11	diameter	3.81	1.98	5.3	0.87	22.69
	height	44.8	18.1	63.1	10.75	24.01
12	diameter	3.82	2.51	5.78	0.91	23.93
	height	45.3	32.1	61.7	8.47	18.69
13	diameter	4.02	2.37	5.52	0.88	21.78
	height	45.6	27.2	61.3	9.17	20.10
14	diameter	3.90	2.78	6.13	0.87	22.25
	height	50.1	36.8	64.7	7.76	15.50
15	diameter	4.11	2.42	5.89	0.78	18.99
	height	49.2	37.6	64.8	6.44	13.07
16	diameter	3.93	3.11	5.52	0.55	13.90
	height	47.3	37.0	66.8	5.78	12.21
17	diameter	4.08	3.08	4.92	0.55	13.55
	height	49.6	32.2	62.9	7.20	14.53
18	diameter	3.71	2.7	4.82	0.57	15.49
	height	40.8	33.5	54.4	4.85	11.88
19	diameter	3.42	2.1	5.88	0.86	25.28
	height	43.4	26.7	57.7	9.57	22.05
20	diameter	4.10	2.65	5.21	0.69	16.80
	height	48.6	32.1	58.2	7.41	15.25

Based on the statistical indicators (Table 1), it can be concluded that the highest mean value of root collar diameter shows the half-sib line number 1 (4.23 mm), while the smallest mean value of root collar diameter has the half-sib line number 3 (3.36 mm). The highest mean value for parameter height has the half-sib line number 6 (52.1 cm), while the smallest mean value for parameter height has the half-sib line number 2 (40.7 cm). The values of the root collar diameter and height coincide with the values that in their researches obtained other authors. Research results of VANN and MEGONIGAL (2002) show that one-year-old seedling reach height of about 50 cm and root collar diameter of about 4 mm. MAZHER *et al.* (2006) report that one-year-old seedlings of Bald cypress reach height up to 35 cm.

By analyzing the values of coefficient of variation as an indicator of statistical set homogeneity, we can conclude that for the parameter root collar diameter the most homogeneous is the half-sib line number 6 (13.13) and the most heterogeneous is the half-sib line number 7 (26.53). For the parameter seedling height, the most homogeneous is the half-sib line number 1 (10.96) and the most heterogeneous is the half-sib line number 7 (24.66).

Table .: Analysis of variance for morphometric characters of one-year-old seedlings

Root collar diameter	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
Between groups	35.4273	19	1.8646	3.42	0.0000
Within groups	316.004	580	0.544835		
Total	351.431	599			
Height of seedlings	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
Between groups	6873.67	19	361.772	6.08	0.0000
Within groups	34537.2	580	59.5469		
Total	41410.9	599			

The analysis of variance for observed characters of one-year-old Bald cypress seedlings was performed in order to determine the existence of statistically significant differences (Table 2). The results of the analysis of variance showed the statistically significant differences at level $p < 0.05$ between the root collar diameter and seedling height of 20 Bald cypress half-sib lines.

When we observe the mean values of root collar diameter, the half-sib lines are grouped into 3 homogeneous groups by which is confirmed the variability of root collar diameter of 20 Bald cypress half-sib lines. In the homogeneous group with the largest root collar diameter are half-sib lines 1, 15 and 20, and in the group with the smallest root collar diameter are half-sib lines 3 and 5 (Table 3).

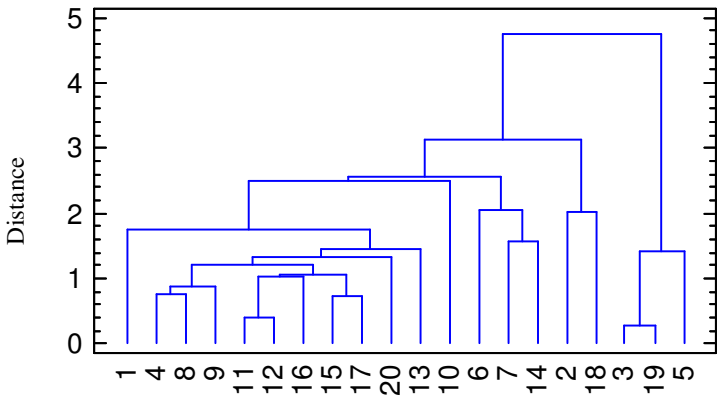
When we observe the mean values of seedling height, the half-sib lines are grouped into 5 homogenous groups by which is confirmed the variability of seedling height of 20 Bald cypress half-sib lines. In the homogeneous group with the highest seedling height are half-sib lines number 6, 10 and 14, and in the group with the smallest seedling height are half-sib lines 2, 18 and 5 (Table 4).

Based on the root collar diameter and seedling height the cluster analysis was performed for both observed characters together, Graph 1. With this analysis was tested the mutual biological similarity or distance regarding these two characters, and it was concluded that

the greatest distance occurred between half-sib lines number 1 and 5, while the shortest distance occurred between half-sib lines number 3 and 19.

Table 3. Tukey HSD test for root collar diameter

Half-sib lines	Mean	**1**	**2**	**3**
3	3.36	****		
5	3.37	****		
19	3.42	****	****	
2	3.59	****	****	****
18	3.71	****	****	****
7	3.75	****	****	****
8	3.76	****	****	****
4	3.77	****	****	****
11	3.81	****	****	****
12	3.82	****	****	****
9	3.84	****	****	****
14	3.90	****	****	****
16	3.93	****	****	****
6	3.95	****	****	****
13	4.02	****	****	****
10	4.07		****	****
17	4.08		****	****
20	4.10			****
15	4.11			****
1	4.23			****



Graph 1: Dendrogram of cluster analysis for measured characters of one-year-old seedlings

Table 4. Tukey HSD test for height of seedlings

Half-sib lines	Mean	1	2	3	4	5
2	40.73	****				
18	40.85	****				
5	40.93	****				
3	42.82	****	****			
19	43.43	****	****	****		
8	44.37	****	****	****	****	
11	44.76	****	****	****	****	
9	44.98	****	****	****	****	
12	45.32	****	****	****	****	****
13	45.62	****	****	****	****	****
4	45.97	****	****	****	****	****
16	47.34	****	****	****	****	****
1	48.45		****	****	****	****
20	48.57		****	****	****	****
7	49.15		****	****	****	****
15	49.25		****	****	****	****
17	49.55		****	****	****	****
14	50.07			****	****	****
10	51.19				****	****
6	52.07					****

TWO-YEAR-OLD SEEDLINGS

In Tabele 5 is shown the variability of morphometric characters of two-year-old seedlings at the level of 20 Bald cypress half-sib lines.

Based on the obtained data (Table 5), it can be concluded that the seedling root collar diameter is in the range of 3.31 to 8.25 mm. The highest mean value of root collar diameter has the half-sib line number 1 (6.29 mm), while the smallest mean value of root collar diameter has the half-sib line number 15 (4.64 mm). Seedling height ranges from 29.7 to 82.5 cm. The highest mean value for seedling height was measured in the half-sib line number 1 (64 cm) while the smallest mean value for seedling height has the half-sib line number 19 (44.5 cm). Comparing the obtained data it can be concluded that seedlings of half-sib line number 1 show high mean values for both the root collar diameter and height, while half-sib line number19 stands out with the smallest mean values.

By analyzing the calculated coefficient of variation (Table 5), as the relative indicator of statistical set homogeneity, we can conclude that for the character root collar diameter the most homogeneous is the half-sib line number 7 (10.42%) and the most heterogeneous is the half-sib line number 4 (19.15%). For the character seedling height, the most homogeneous is the half-sib line number 2 (10.15%), and the most heterogeneous is the half-sib line number 9 (20.74%).

Regarding to values of coefficient of variation for all characters it can be concluded that they are below 30% which indicates that the sample used for the research is homogeneous.

Table 5. Variability of morphometric characters of two-year-old seedlings at the level of 20 Bald cypress half-sib lines

Half-sib lines	Parameter	Average (mm)	Min	Max	Standard deviation	Coefficient of variation
1	diameter	6.29	4.69	8.09	0.92	14.67
	height	64.0	40.6	80.5	11.48	17.93
2	diameter	5.78	4.26	7.26	0.69	11.92
	height	58.9	45.2	71.3	5.98	10.14
3	diameter	5.90	4.55	7.22	0.68	11.53
	height	61.3	48.1	73.2	7.34	11.97
4	diameter	5.43	3.61	7.11	1.04	19.15
	height	50.0	33.1	70.6	9.18	18.36
5	diameter	5.94	4.21	7.34	0.92	15.41
	height	59.5	36.5	81.1	10.80	18.16
6	diameter	5.37	4.21	7.21	0.76	14.19
	height	58.2	44.9	72.4	6.94	11.93
7	diameter	5.33	4.56	6.51	0.56	10.42
	height	58.9	47.2	69.1	7.05	11.97
8	diameter	5.87	4.08	8.16	1.00	17.03
	height	52.2	35.6	73.4	9.39	17.98
9	diameter	4.78	3.31	6.81	0.80	16.67
	height	45.2	31.5	73.3	9.37	20.74
10	diameter	4.77	3.64	6.44	0.72	15.06
	height	48.7	37.0	69.6	7.85	16.13
11	diameter	5.08	3.81	6.22	0.67	13.17
	height	58.2	45.5	69.3	7.11	12.21
12	diameter	5.08	3.78	6.36	0.72	14.20
	height	55.3	37.9	68.3	6.72	12.15
13	diameter	5.14	3.88	6.29	0.67	12.97
	height	56.3	42.2	68.3	5.96	10.58
14	diameter	5.09	3.92	6.27	0.66	12.91
	height	55.8	39.8	68.2	5.84	10.47
15	diameter	4.64	3.60	646	0.67	14.45
	height	49.7	34.7	69.5	8.93	17.96
16	diameter	5.04	3.71	6.47	0.73	14.42
	height	57.0	42.7	71.3	7.43	13.12
17	diameter	5.48	4.00	7.38	0.93	16.94
	height	57.0	36.3	82.5	10.96	19.23
18	diameter	5.39	3.81	6.33	0.69	12.75
	height	56.8	42.8	67.1	6.50	11.45
19	diameter	5.17	3.33	8.25	0.95	18.43
	height	44.5	29.7	54.7	5.92	13.30
20	diameter	5.21	3.58	7.11	0.78	14.94
	height	58.3	39.3	69.8	7.90	13.55

By analyzing the calculated coefficient of variation (Table 5), as the relative indicator of statistical set homogeneity, we can conclude that for the character root collar diameter the most homogeneous is the half-sib line number 7 (10.42%) and the most heterogeneous is the half-sib line number 4 (19.15%). For the character seedling height, the most homogeneous is the half-sib line number 2 (10.15%), and the most heterogeneous is the half-sib line number 9

(20.74%). Regarding to values of coefficient of variation for all characters it can be concluded that they are below 30% which indicates that the sample used for the research is homogeneous.

Table 6. Analysis of variance for morphometric characters of two-year-old seedlings

Root collar diameter	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
Between groups	107.40	19	5.6526	9.10	0.0000
Within groups	360.14	580	0.62093		
Total	467.54	599			
Height of seedlings	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
Between groups	15658.21	19	824.1161	12.50	0.0000
Within groups	38235.37	580	65.92305		
Total	53893.58	599			

Table 7. Tukey HSD test for root collar diameter

Half-sib lines	Mean	**1**	**2**	**3**	**4**	**5**	**6**	**7**
15	4.64	****						
10	4.77	****	****					
9	4.78	****	****					
16	5.04	****	****					
12	5.08	****	****	****				
11	5.08	****	****	****				
14	5.09	****	****	****				
13	5.14	****	****	****				
19	5.17	****	****	****	****			
20	5.21	****	****	****	****	****		
7	5.33	****	****	****	****	****	****	
6	5.37		****	****	****	****	****	
18	5.39		****	****	****	****	****	
4	5.43		****	****	****	****	****	
17	5.48		****	****	****	****	****	
2	5.78			****	****	****	****	****
8	5.87				****	****	****	****
3	5.90					****	****	****
5	5.94						****	****
1	6.29							****

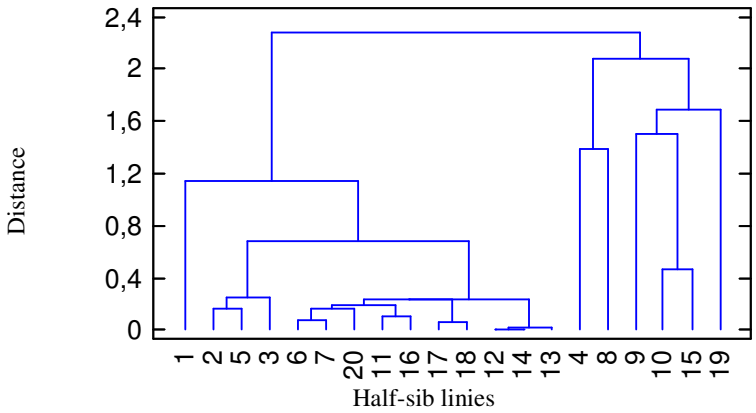
Table 8. Tukey HSD test for height of seedlings

Half-sib lines	Mean	1	2	3	4	5	6	7
19	44.53	****						
9	45.17	****	****					
10	48.67	****	****	****				
15	49.70	****	****	****	****			
4	50.02	****	****	****	****			
8	52.16		****	****	****	****		
12	55.34			****	****	****	****	
14	55.79			****	****	****	****	
13	56.31				****	****	****	
18	56.76				****	****	****	****
16	56.97				****	****	****	****
17	57.00				****	****	****	****
6	58.16					****	****	****
11	58.22					****	****	****
20	58.30					****	****	****
2	58.90					****	****	****
7	58.90					****	****	****
5	59.52					****	****	****
3	61.31						****	****
1	63.99							****

The analysis of variance for observed characters of two-year-old Bald cypress seedlings was performed in order to determine the existence of statistically significant differences (Table 6). The results of the analysis of variance showed the statistically significant differences at level $p < 0.05$ between the root collar diameter and seedling height of 20 Bald cypress half-sib lines.

Based on Tukey HSD test of homogeneity it can be concluded that when we observe the root collar diameter of seedlings, the half-sib lines are grouped into 7 homogeneous groups (Table 7). Also when observe seedling height, the half-sib lines are grouped into 7 homogeneous groups (Table 8). For the parameters of root collar diameter and seedling height the half-sib line number 15 is in a homogeneous group with the smallest values, while the half-sib lines 1, 3 and 5 are in a homogenous group with the highest values.

The cluster analysis was performed in order to determine similarity or distance of half-sib lines, based on the root collar diameter, seedling height and ratio seedling height/root collar diameter (Graph 2). The dendrogram of cluster analysis shows that the half-sib lines number 6, 7, 20, 11 and 16 are grouped at the greater distance than other half-sib lines. At the shortest distance are mutually connected half-sib lines number 12 and 14. At the greatest distance are connected half-sib lines number 1 and 19.



Graph 2. Dendrogram of cluster analysis for measured characters of two-year-old seedlings

By comparative analysis of the obtained results in the study of variability of morphometric characters of cones, seeds and seedlings of Bald cypress can be concluded that there is considerable intra-population variability which is directly dependent on the type of the analyzed character. This suggests that the obtained results need to be supplemented and verified using genetic methods which are based on the use of other types of markers (POPOVIĆ *et al.,* 2013).

Researches of inter-population and interline variability performed on Scots pine (LUČIĆ *et al.,* 2012), Spruce (ŠIJAČIĆ-NIKOLIĆ *et al.,* 2010) and Austrian pine (MATARUGA *et al.,* 2003) show that the intensity of variability is directly dependent on the type of the analyzed trait. Morphometric markers can be used to determine the degree of variability but for the more accurate conclusions it is necessary to expand the research by using molecular markers.

CONCLUSIONS

The results obtained from the analysis of variability of morphometric characteristics of one-year-old and two-year-old seedlings of 20 Bald cypress half-sib lines originated from seed stand near Bačka Palanka, contribute to better understanding of relationship of analyzed characters, as well as the influence of analyzed characters on differentiation of half-sib lines. Based on these results differences in the values of the observed characters were noticed for each half-sib line individually. When observe characters of one-year-old seedlings the highest value of root collar diameter has half-sib line number 1, while the smallest value have seedlings of half-sib line number 3. The greatest values for character seedling height have seedlings of half-sib line number 6, while the smallest values have seedlings of half-sib line number 2. When observe characters of two-year-old seedlings the highest values of root collar diameter and height have seedlings of half-sib line number 1, while the smallest value of root collar diameter have seedlings of half-sib line number 15, and the smallest value of height have seedlings of half-sib line number 19.

By the comparative analysis of the obtained results in the study of variability of morphometric characters of one- and two-year-old seedlings of Bald cypress of different half-sib lines, it can be concluded that there is a considerable inter-line variability which is directly dependent on the type of the analyzed character. This suggests that the obtained results need to be supplemented and verified using genetic methods which are based on the use of other types of markers (biochemical and molecular markers).

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ANALIZA MEĐULINIJSKE VARIJABILNOSTI JUVENILNIH SADNICA TAKSODIJUMA (*Taxodium distichum* L. Rich.) UPOTREBOM MORFOMETRIJSKIH MARKERA

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Izvod

U radu su prikazani rezultati ispitivanja međulinijske varijabilnosti morfometrijskih svojstava juvenilnih sadnica taksodijuma na nivou 20 linija polusrodnika. Dobijeni rezultati daju doprinos boljem upoznavanju veza analiziranih svojstava, kao i uticaju analiziranih svojstava na diferencijaciju linija polusrodnika. Na osnovu dobijenih rezultata uočene su razlike u vrednostima posmatranih svojstava za svaku liniju polusrodnika ponaosob. Utvrđene vrednosti dimenzija sadnica ukazuju na dobar genetski i adaptivni potencijal ove vrste, što se može smatrati polaznom osnovom za masovnu proizvodnju kvalitetnog sadnog materijala i njegovu upotrebu u Srbiji.

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