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Population differentiation in the wild cherry (*Prunus avium* L.) in Bosnia and Herzegovina

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Abstract

Background and Purpose: The wild cherry (Prunus avium) has great and multiple importance. The fruits it produces are used for several purposes (as food for people, birds and other animals, as well as in phytotherapy). As many birds and mammals feed on the fruit of the wild cherry, it has the ability of dispersion over large areas in a very short time. It is present in from river deposits up to 1900 m/alt, while it is quite rare in the Submediterranean. Wild cherry grows as a solitary tree or in small groups, usually at the edge of the forest or within the forest in areas with more sunlight. The significance of the wild cherry is reflected in the high economic value of its wood, which makes it much demanded and popular, and thus endangered.

Materials and Methods: The plant material was collected from 22 natural populations in Bosnia and Herzegovina. The fruit and leaves were collected from marginal or solitary trees, usually from the south-facing, outer sun-exposed parts of the tree crown. We measured the following fruit characteristics: fruit length (FL), fruit width (FW), fruit thickness (FT), seed length (SL), seed width (SW), seed thickness (ST), length of the stalk (LOS) and width of the stalk (WOS), and leaf characteristics: length of the petiole (LP), length of the leaf blade (LB), distance from the blade base to the blade's widest part (BBW), width of the leaf blade (WB), insertion angle of the leaf venation (AV), number of leaf teeth on a 2-cm length (NT), blade width at 1 cm from the blade apex (WBA) and blade width at 1 cm from the blade's base (WBB). All statistical analyses of the data were made using the SPSS 15.0 package for Windows.

Results: The results obtained show the presence of a high level of intrapopulational, as well as interpopulational, morphological variability in the natural populations of the wild cherry which have been investigated. Analyses of population differentiation have not confirmed our expectations. Our results only indicate differentiation in fruit size characteristics, but the indicators are very weak. The resulting high values of the regression coefficient in this research can serve to estimate the values of some features and characteristics without their measurement.

Conclusions: The analyses of 16 morphological characteristics in 22 natural populations of the wild cherry in Bosnia and Herzegovina showed statistically significant differences between investigated populations. Differentiation in natural populations of the wild cherry was very low and identified only in fruit dimension characteristics.

INTRODUCTION

The wild cherry (Prunus avium) has great and multi-L ple importance. The fruit of the wild cherry is used for several purposes (as food for people, birds and other animals, as well as in phytotherapy). As many birds and mammals feed on the fruit of the wild cherry, it has the ability of dispersion over large areas in a very short time. The wild cherry grows on nutrient-rich soils of neutral reaction which are moderately moist, but is also often found on sun-baked and dry habitats. It is found in deposits up to 1900 m/alt (1, 2, 3, 4), while it is quite rare in the Sub-Mediterranean. It is a fast-growing species with a production period of 40-60 years (5). It grows as a solitary tree or in small groups, usually at the edge of the forest or within the forest in areas with more sunlight. The significance of the wild cherry is reflected in the high economic value of its wood, which makes it much demanded and popular, and thus endangered. Literature data about the morphological variability of the wild cherry are scarce and only general information for particular characteristics can be found, usually for seed and less for flower characteristics (6, 7), as well as some data about fruit size which serve for the taxonomic determination of the wild cherry (8).

The long-standing growth of local populations in different ecological conditions has resulted in a high number of ecotypes and forms of the wild cherry. For example, five ecotypes were defined based on morphology (9), two forms according to fruit color (10), four forms according to several other morphological characteristics of leaves (11), and three forms of the wild cherry (12).

It is considered that the natural wild cherry populations are distributed from West Asia to North Africa and North Europe (4). There are several different pieces of information available about the origin of the wild cherry. It is generally assumed that it originated in west Asia and Caucasus (13), while others believe its origin is in the Mediterranean (14). However, some authors (15) state that the wild cherry originated in Asia Minor, as well as South and Mid Europe, whereas others point out the Pontic area (the east coast of Asia Minor) (16). Nevertheless, existing archeological and fossil evidence suggest North-West Europe as the homeland of the wild cherry.

The aim of our research was to identify the level of ecological and genetic differences between the investigated natural wild cherry populations in Bosnia and Herzegovina, based on the application of comparative statistical analysis on fruit, leaves, seeds and fruit stalk characteristics.

With this research, we wanted to evaluate the level of variability of several morphological characteristics by using descriptive statistics of parameters, variance analysis, canonical and regression analysis. Efficient conservation measures and programmes, as well as conservation activities using ex situ and in situ methods depend on the knowledge about the differentiation level of wild cherry populations.

MATERIAL AND METHODS

The plant material was collected from 22 natural wild cherry populations in Bosnia and Herzegovina from three main phytogeographical provinces (Table 1, Figure 1). We used the same sampling methodology as with the *Quercus robur* samples (17, 18, 19, 20, 21, 22, 23, 24). We used only leaves from short shoots because their cha-



Figure 1. Studied population:1 Žepče, 2 Olovo, 3 Majevica, 4 Posušje, 5 Sarajevo, 6 Rogatica, 7 Livno, 8 Kalinovik, 9 Višegrad, 10 Drvar, 11 Kongora, 12 Sanski Most, 13 Kupres, 14 Bosansko Grahovo, 15 Turbe, 16 Ključ, 17 Trnovo, 18 Sokolac, 19 Konjic, 20 Cazin, 21 Kakanj, 22 Ustikolina.

Pop.	Population name	Longitude	Latitude	Altitude (m)	Phytogeographic Provenance
1	Ženče	44°26' 09''	17°57' 58''	609	Zavidovici – Teslic area (3.4)
2	Olovo	44°10' 32"	18°33' 06"	563	$\Omega_{\text{zrep}} = \Omega_{\text{truglica region}} (3.5.1)$
2	Majovica	44°21' 27"	18°51' 43''	510	North Bospian area (1.1)
3	De ere Xie	42°22' 20"	10 J1 TJ	1076	Sech Meditermanes and a (1.1)
4	Posusje	45 52 50	17 27 24	1076	Sub-Mediterranean mountain area (4.5)
5	Sarajevo	43°50′ 09″	18°23' 04''	560	Sarajevo – Zenica region (3.3.3)
6	Rogatica	43°49' 26''	18°57' 44''	685	Rogatica region (2.2.2)
7	Livno	43°46' 40''	16°57' 24''	805	Region without evergreen elements (4.3.1)
8	Kalinovik	43°30' 07"	18°28' 41''	1054	Igman – Zelengora region (3.6.1)
9	Višegrad	43°48' 04"	19°18' 46''	587	Višegrad region (2.2.1)
10	Drvar	44°22' 16''	16°22' 22''	499	Sub-Mediterranean mountain area (4.3)
11	Kongora	43°38' 28''	17°21' 42''	958	Sub-Mediterranean mountain area (4.3)
12	Sanski Most	44°46' 19"	16°40' 20''	155	North-west Bosnian area (1.1)
13	Kupres	43°59' 56"	17°16' 16''	1226	Glamoč – Kupres region
14	Bosansko Grahovo	44°10' 46''	16°21' 38''	873	Sub-Mediterranean mountain area (4.3)
15	Turbe	44°12' 28''	17°30' 39''	778	Vranica region (3.3.2)
16	Ključ	44°31' 52"	16°46' 43''	286	Kljuc – Petrovac region (3.2.1)
17	Trnovo	43°41' 11''	18°20' 41''	929	Igman – Zelengora region (3.6.1)
18	Sokolac	43°56' 25"	18°46' 37''	954	Romanija region (3.5.2)
19	Konjic	43°34' 37"	18°00' 44''	696	Sub-Mediterranean mountain area (4.3)
20	Cazin	44°56' 44''	15°55' 44''	379	The area of Cazinska Krajina (3.1.)
21	Kakanj	44°06' 49''	18°04' 57"	558	Sarajevo – Zenica region (3.3.3)
22	Ustikolina	43°36' 10"	18°44' 43''	820	Gorazde – Foca region (2.2.3)

TABLE 1

General information about investigated populations.

racteristics reflect a recent condition of the species and are suitable for precise morphological research, as well as for fruit research. The leaves were collected from marginal or solitary trees, usually from the south-facing, outer sun-exposed parts of the tree crown, because only such trees can entirely express its phenotype and genotype (22, 23).

Following collection of the material, additional selection was made and we removed all fruits and leaves that were not fully or properly developed, those that were damaged or of irregular phenotype appearance. Apart from collection, it was necessary to herbarize all the leaf material to make it suitable for later measurements, so an additional selection of the leaf material was done during the herbarization process.

We measured the following fruit characteristics: fruit length (FL), fruit width (FW), fruit thickness (FT), seed length (SL), seed width (SW), seed thickness (ST), length of the stalk (LOS) and width of the stalk (WOS), and leaf characteristics: length of the petiole (LP), length of the leaf blade (LB), distance from the blade base to the blade's widest part (BBW), width of the leaf blade (WB), insertion angle of the leaf venation (AV), number of leaf teeth on a 2-cm length (NT), blade width at 1 cm from the blade apex (WBA) and blade width at 1 cm from the blade base (WBB).

All measurements were made with 0,1-mm precision. Overall, we analyzed 22 populations, 204 trees, 2926 fruits, seeds and stalks and 3069–3087 leaves. Altogether we have statistically analyzed 55.720 measurement data items. All measurements were entered into an Excel spreadsheet and statistical analyses of the data were made using the SPSS 15.0 package for Windows. We performed the following statistical analysis: descriptive statistics, variance analysis, regression analysis and canonical discriminant analyses.

RESULTS

The results obtained show the presence of a high level of intrapopulation, as well as interpopulation, morphological variability in the natural populations of the wild cherry which were investigated (Table 2, 3a and 3b). The analyses of population differentiation did not confirm our expectations. Our results only suggest differentiation in fruit size characteristics, but the indicators are very weak.

As we can observe in Tables 2, 3a and 3b, the obtained mean value for fruit length (FL) is 11.76 mm (Kongora

Descriptive statistical data describing fruit, seed, fruit stalk and leaf - summary

TABLE 2

		FRUIT			SEED		FRUIT S	TALK				LEA	H			
	FL (mm)	FW (mm)	FT (mm)	SL (mm)	SW (mm)	ST (mm)	(mm)	(mm)	LP (mm)	LB (mm)	BBW (mm)	WB (mm)	AV	LN	WBA (mm)	WBB (mm)
Z	2926	2926	2926	2926	2926	2926	2925	2925	3057	3087	3087	3087	3087	3087	3079	3069
Minimum	7.83	8.18	7.48	6.05	5.01	2.32	20.24	0.38	10.82	49.53	24.22	15.40	26.00	6.00	1.58	9.36
Maximum	21.12	21.88	18.62	11.16	9.13	7.43	68.57	0.88	48.54	112.90	66.62	65.51	78.00	16.00	32.69	41.57
Mean	11.76	12.13	11.05	8.11	7.03	5.48	37.28	0.60	29.51	80.03	44.45	42.94	45.20	10.16	14.79	22.24
Std. Deviation	1.47	1.70	1.43	0.72	0.55	0.51	6.72	0.07	4.96	9.16	6.67	5.84	6.44	1.57	5.06	4.66
CV (%)	12.53	13.99	12.89	8.94	7.88	9.35	18.01	11.79	16.80	11.44	15.01	10.94	14.25	15.46	34.21	20.95

= 9.61 mm, Drvar = 13.23 mm) with the highest variation in the Drvar population (19.21 %) and minimum variation in the Trnovo population (5.08 %). The mean value for fruit width (FW) is 12.13 mm (Kongora = 9.50 mm, Sarajevo = 13.70 mm) with maximum variation observed in the Drvar population (20.00 %), and minimum variation in the Posušje population (5.42 %). For fruit thickness (FT) the characteristic mean value is 11.05 mm (Kongora = 9.01 mm, Sarajevo = 12.63 mm) with maximum and minimum variation in the Drvar (17.71 %) and the Kongora (6.51 %) populations respectively.

The obtained mean value for seed length (SL) was 8.11 mm (Kongora = 7.31 mm, Bosansko Grahovo = 8.76 mm) and the highest variation was displayed within the Cazin population (11.39 %), while the minimum variation was observed in the Kongora population (5.35 %).

The mean value for seed width (SW) is 7.03 mm (Kongora = 6.61 mm, Olovo = 7.37 mm), with maximum variation in the Ustikolina (10.38 %) and minimum variation in the Kupres (4.29 %) population. The mean value for seed thickness (ST) is 5.48 mm (Konjic = 4.86 mm, Drvar = 5.88 mm) with maximum and minimum variation in the Konjic (14.95 %) and the Posušje (4.44 %) populations, respectively. The mean value for length of the fruit stalk (LOS) is 37.28 mm (Sokolac = 32.10, Olovo = 43.39 mm), with maximum variation observed in the Bosansko Grahovo population (19.60 %) and minimum variation in the Drvar population (11.87 %). The mean value for width of the fruit stalk (WOS) is 0.60 mm (Kakanj = 0.56 mm, Turbe i Trnovo = 0.63mm) with maximum and minimum variation in the Majevica (13.53 %) and the Kongora (9.47 %) populations, respectively.

Moreover, as presented in Tables 2 and 3b the mean values for investigated leaf characteristics were as follows: the mean value for length of the leaf blade (LB) was 80.03 mm (Kongora = 72.69 mm, Sarajevo = 85.65 mm) with maximum variation in the Kalinovik population (18.61 %) and minimum variation in the Majevica population (6.63 %), the mean value for length of the petiole (LP) was 29.51 mm (Kalinovik = 24.18 mm, Olovo = 33.15 mm) with maximum and minimum variation in the Sanski Most (19.19 %) and the Kongora (10.31 %) populations, respectively, the mean value for distance from the blade base to the blade's widest part (BBW) was 44.45 mm (Turbe = 39.62 mm, Sarajevo = 49.18 mm) with maximum and minimum variation in the Kalinovnik (21.77 %) and the Majevica (8.60 %) population, the mean value for width of the leaf blade (WB) was 42.94 mm (Kalinovik = 37.81 mm, Kakanj = 49.95 mm) with maximum and minimum variation observed in the Žepče (13.55 %) and the Rogatica (7.03 %) populations, respectively, the mean value for insertion angle of the leaf venation characteristic (AV) was 45.20° (Sanski Most = 48.68° , Žepče = 42.37°) with maximum and minimum variation recorded in the Konjic (16.38%) and the Majevica (9.79 %) population, the mean value for the number of teeth on a 2-cm leaf length (NT) was 10.16 (Olovo i Sarajevo = 9.41, Kakanj = 11.44) with

				FRU	UIT					SE	ED				FRUIT	STALK	
Population	Population	FL (mm)	FW ((mm)	FT (mm)	SL (1	nm)	SW (mm)	ST (I	nm)	LOS	(mm)		WOS
number	name	Mean (mm)	CV (%)	$\overline{}$	fean mm)												
1	Žepče	11.86	8.16	12.43	10.98	11.55	10.18	8.15	6.72	7.19	8.25	5.55	13.20	39.22	17.58).59
2	Olovo	12.70	8.97	12.91	11.21	11.94	10.24	8.70	7.53	7.37	6.61	5.67	8.19	43.39	13.86).62
3	Majevica	10.33	9.12	10.66	13.28	9.86	12.81	7.89	6.17	6.73	8.43	5.38	9.54	33.52	18.15	0).62
4	Posušje	11.04	5.49	10.43	5.42	9.81	6.58	8.33	7.36	6.88	4.99	5.44	4.44	38.92	16.27	0	.57
J	Sarajevo	12.94	14.26	13.70	13.86	12.63	13.65	8.03	8.03	7.12	5.90	5.50	6.96	39.77	13.93	0	.60
9	Rogatica	11.54	9.50	12.35	10.34	11.34	10.12	7.89	7.47	7.29	6.73	5.57	7.09	37.49	18.15	0	.61
7	Livno	12.09	7.34	11.25	6.76	10.54	7.40	8.64	6.69	7.12	5.03	5.43	6.14	42.61	12.86	0	.60
8	Kalinovik	10.38	8.22	10.45	10.14	9.63	10.00	7.89	8.04	6.93	9.83	5.33	13.29	36.56	15.42	0.	60
9	Visegrad	10.41	7.07	10.83	8.69	9.98	8.02	7.66	7.02	6.73	6.30	5.26	5.89	36.02	14.84	0.	57
10	Drvar	13.23	19.21	13.45	20.00	12.27	17.71	8.63	9.39	7.37	5.98	5.88	6.93	34.37	11.87	0.0	51
11	Kongora	9.61	6.32	9.50	6.96	9.01	6.51	7.31	5.35	6.61	5.54	5.27	4.98	41.10	13.63	0.5	7
12	Sanski Most	11.88	10.09	12.83	12.33	11.29	10.81	7.97	7.52	6.75	7.12	5.44	7.37	42.25	16.46	0.5	8
13	Kupres	11.07	7.33	11.17	7.91	10.26	7.13	7.98	7.14	6.97	4.29	5.46	4.65	36.34	12.51	0.5	57
14	Bosansko Grahovo	12.09	7.70	11.93	10.55	10.81	8.96	8.76	7.94	7.11	5.35	5.59	6.67	40.83	19.60	0.6	51
15	Turbe	12.99	12.37	13.29	12.52	11.84	11.06	8.70	10.20	7.38	7.61	5.79	7.47	34.37	18.42	0.6	5
16	Ključ	12.20	6.73	12.63	9.10	11.27	7.07	8.14	6.13	7.09	9.79	5.46	6.99	32.53	17.00	0.6	0
17	Trnovo	11.78	5.08	12.52	7.74	11.35	7.62	8.18	5.89	7.31	5.27	5.72	7.17	38.72	14.78	0.6	53
18	Sokolac	11.80	6.47	12.50	6.70	11.21	6.15	7.94	5.68	6.99	6.22	5.55	6.72	32.10	13.03	0.6	51
19	Konjic	12.16	6.93	12.44	8.17	11.14	8.41	8.05	7.83	6.63	8.08	4.86	14.95	33.04	13.22	0.0	51
20	Cazin	11.25	5.77	12.26	7.66	10.71	5.12	7.93	11.39	7.06	7.56	5.58	6.61	36.21	17.47	0.6	00
21	Kakanj	11.31	10.04	12.15	8.93	10.91	7.85	7.68	8.17	6.68	6.22	5.17	6.81	33.99	16.05	0.5	6
22	Ustikolina	12.47	6.88	12.89	9.99	11.87	9.94	8.00	6.22	7.09	10.38	5.54	11.34	34.50	17.36	0.5	69
Average		11.76	12.53	12.13	13.99	11.05	12.89	8.11	8.94	7.03	7.88	5.48	9.35	37.28	18.01	0.6	50

 TABLE 3a.

 Descriptive statistical data for investigated populations describing measured fruit, seed and fruit stalk characteristics.

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Descriptive statistical data for investigated populations describing measured leaf characteristics.

									LEA	F							
Pop.	Pop.	LP (n	(mn)	LB (n	(mn	BBW ((mm)	WB (n	nm)	AV		ĹŊ	τ.	WBA ((uu	WBB ((uu
	Itallic	Mean (mm)	CV (%)	Mean (mm)	CV (%)	Mean (mm)	CV (%)	Mean (mm)	CV (%)	Mean (°)	CV (%)	Mean	CV (%)	Mean (mm)	CV (%)	Mean (mm)	CV (%)
-	Žepče	82.77	10.76	29.86	18.53	44.89	14.90	47.03	13.55	42.37	14.26	9.77	16.15	13.71	41.68	24.16	18.33
2	Olovo	82.05	8.57	33.15	12.50	46.14	11.81	47.10	8.98	46.71	15.89	9.41	14.47	15.74	30.95	24.21	19.41
3	Majevica	77.81	6.63	25.72	12.91	42.98	8.60	41.63	8.07	43.89	9.79	10.42	9.91	13.10	19.29	22.12	14.51
4	Posušje	84.71	11.07	29.93	17.06	46.60	12.39	44.39	9.51	45.23	11.34	9.87	12.12	11.07	34.53	22.13	15.79
5	Sarajevo	85.65	9.52	33.82	16.28	49.18	13.05	44.13	8.33	44.20	10.67	9.41	11.75	14.30	33.59	20.45	14.66
9	Rogatica	76.91	8.66	27.65	13.14	41.50	13.45	44.99	7.03	47.70	13.18	9.99	11.72	15.35	26.89	26.10	19.44
7	Livno	79.08	8.68	32.66	12.47	45.24	12.62	45.56	7.80	44.21	11.57	10.63	17.01	15.30	29.37	22.66	16.04
8	Kalinovik	73.99	18.61	24.18	19.12	40.11	21.77	37.81	13.45	44.70	10.95	10.60	14.70	15.08	30.21	20.88	18.92
9	Višegrad	80.87	8.03	30.83	15.41	46.78	11.97	42.86	10.64	44.39	12.36	10.10	12.76	15.67	34.70	21.91	19.61
10	Drvar	78.56	10.02	29.71	13.22	44.05	13.32	39.17	9.99	43.27	12.54	10.60	12.60	13.41	33.75	20.30	14.98
11	Kongora	72.69	8.22	28.45	10.31	39.97	11.91	46.84	11.19	43.88	14.83	9.68	10.18	20.15	18.77	25.03	17.01
12	Sanski Most	84.05	13.27	30.90	19.19	45.75	16.45	45.74	13.00	48.68	14.02	10.34	13.01	15.83	27.23	24.08	19.78
13	Kupres	80.76	9.86	29.54	17.49	47.50	11.94	40.12	11.44	42.52	15.29	9.76	13.36	15.02	31.05	17.67	17.31
14	Bosansko Grahovo	83.95	8.59	30.00	13.82	46.81	11.60	39.03	12.93	45.27	15.08	9.59	13.25	12.81	34.00	19.94	22.29
15	Turbe	75.26	11.12	29.42	18.51	39.62	14.98	41.24	13.35	48.55	14.10	11.04	16.75	16.65	26.66	22.12	16.42
16	Ključ	77.58	14.22	28.15	16.15	42.01	17.89	37.90	12.08	45.23	14.31	11.26	16.92	12.46	36.16	20.82	20.09
17	Trnovo	81.73	12.71	28.40	16.20	45.77	14.13	42.96	11.52	46.09	14.18	9.80	17.04	15.67	36.70	21.54	17.66
18	Sokolac	76.76	10.54	29.36	15.52	41.84	14.09	43.07	11.88	46.95	12.31	10.11	15.47	17.20	26.34	23.47	20.52
19	Konjic	81.24	8.72	29.83	11.85	46.40	10.87	40.29	13.54	43.05	16.38	10.13	14.08	10.94	45.26	20.15	19.82
20	Cazin	8518	8.72	28.04	14.53	46.62	12.43	41.61	9.57	44.98	13.68	9.65	12.83	11.72	32.46	22.18	18.76
21	Kakanj	83.35	7.45	30.30	12.24	45.85	11.84	49.95	10.26	45.10	15.16	11.44	13.21	14.74	25.67	25.29	18.14
22	Ustikolina	77.57	10.80	27.03	12.59	42.79	14.91	40.73	12.65	46.63	13.26	9.82	17.70	15.31	37.30	21.45	24.28
Average		80.03	11.44	29.51	16.80	44.45	15.01	42.94	10.94	45.20	14.25	10.16	15.46	14.79	34.21	22.24	20.95

								INTRA	APOI	PUL	ATI	ON												
Ustikolina	Kakanj	Cazin	Konjic	Sokolac	Trnovo	Ključ	Turbe	Bosansko Grahovo	Kupres	Sanski Most	Kongora	Drvar	Višegrad	Kalinovik	Livno	Rogatica	Sarajevo	Posušje	Majevica	Olovo	Žepče	Pop. name		
24.81	43.88	16.62	43.71	27.47	7.40	28.20	102.70	21.18	36.43	50.30	15.34	314.86	12.13	63.96	26.97	39.38	120.35	8.52	13.61	37.05	15.23	FL (mm)		
51.89	25.25	34.79	35.80	20.51	23.64	37.91	91.61	20.16	30.40	63.77	9.41	287.12	31.97	75.66	10.52	29.19	97.68	0.62	28.72	36.81	27.65	FW (mm)	FRUIT	
45.48	22.05	29.27	62.10	24.62	33.62	25.50	90.25	18.43	30.71	41.45	6.52	259.40	19.60	77.63	8.34	23.34	92.69	1.36	25.23	43.18	20.17	FT (mm)		
29.03	40.60	170.87	56.89	20.78	23.18	30.89	66.76	26.16	39.10	44.98	6.69	147.20	16.82	53.71	27.07	21.51	54.35	37.66	21.00	14.16	27.86	SL (mm)		
111.80	15.74	101.64	25.76	33.43	30.37	44.01	67.37	10.09	9.27	38.27	6.28	44.86	22.02	98.75	13.39	9.75	18.50	1.34	54.33	16.27	23.71	SW (mm)	SEED	
119.84	29.60	76.10	115.56	29.52	33.32	25.27	56.04	24.97	12.51	34.52	7.65	47.24	24.54	80.55	16.61	12.09	33.94	2.56	83.20	21.47	77.19	ST (mm)		\triangleright
31.59	26.62	33.01	10.42	5.98	17.91	17.08	17.70	14.14	6.88	39.43	16.91	8.38	32.91	17.15	12.56	17.96	9.60	3.61	60.08	13.47	23.66	LOS (mm)	FRUIT	TABLE
6.57	5.47	5.38	6.87	4.85	13.16	3.03	11.24	6.50	8.32	7.00	12.11	18.63	15.83	30.38	4.64	7.14	16.82	9.95	14.42	10.34	10.15	WOS (mm)	STALK	4 values.
25.96	2.08	4.90	5.87	9.64	24.23	34.01	21.10	10.84	13.52	27.70	10.19	20.51	15.80	70.12	10.22	12.45	27.15	4.08	2.77	9.40	41.17	LP (mm)		
18.62	6.04	11.74	5.85	25.89	16.63	21.38	43.74	15.04	24.96	48.20	4.89	9.74	31.65	26.95	17.85	8.62	21.05	8.78	25.34	10.55	54.65	LB (mm)		
21.04	1.22	8.08	7.35	11.88	10.72	25.96	9.75	10.93	13.42	21.22	8.97	12.22	11.76	65.46	9.56	9.18	16.27	15.14	0.54	5.32	22.65	BBW (mm)		
53.24	3.60	6.49	39.83	15.10	20.70	24.45	25.60	44.93	10.12	13.51	23.27	15.84	17.67	19.01	8.27	5.10	18.04	9.99	36.53	20.53	73.69	WB (mm)	LEA	
10.40	6.48	7.35	13.02	4.02	5.24	9.00	4.02	6.69	14.16	12.38	5.41	9.57	5.33	7.54	5.84	4.84	8.19	22.49	1.11	15.89	10.66	AV	ΛF	
32.01	5.75	4.68	18.67	13.48	18.09	20.44	20.01	6.68	8.05	3.29	4.32	6.95	6.40	14.53	24.46	3.76	4.22	0.00	0.63	8.19	17.93	NT		
68.13	1.43	4.98	26.29	6.25	18.10	32.35	8.58	36.08	13.42	8.93	6.33	18.86	50.16	18.75	41.71	9.45	34.89	0.34	4.61	23.53	37.10	WBA (mm)		

BETWEEN POPULATIONS

90.69

90.26

89.85

53.14

35.85

33.10

48.42

13.04

27.48

33.98

28.24

71.20

12.32

23.26

27.32

D. Ballian

24.08 35.75 10.88 19.97

14.95

2.63

11.52

1.60

8.98

20.92

18.71 10.94

9.86 3.26

16.51 12.97 8.08 13.38

9.66 15.38

7.13 6.57 13.06

WBB (mm)

Canonical Discriminant Functions



Figure 2. Discriminant analysis of fruit.



Figure 4. Discriminant analysis of fruit stalk.

maximum variation in the Trnovo population (17.04 %) and minimum variation in the Majevica population (9.91 %), the mean value for characteristic blade width at 1 cm from the blade apex (WBA) was 14.79 mm (Konjic = 10.94 mm, Kongora = 20.15 mm) with maximum and

Canonical Discriminant Functions



Figure 3. Discriminant analysis of seeds.



Figure 5. Discriminant analysis of seed.

minimum variation in the Konjic (45.26 %) and the Kongora (18.77 %) population and, finally, the mean value for characteristic blade width at 1 cm from the blade's base (WBB) was 22.24 mm (Grahovo = 19.94 mm, Rogatica = 26.10 mm) with maximum and mi-

Regression analysis of fruit. seed. fruit stalk and leaf (linear regression parameters correlation coefficient and coefficient of determination).

TABLE 5

Variable	Dependent	Independent	Intercept	b1	b2	R	R ²
EDLUT	FL	EW	2.87	0.73		0.84	0.71
FKUII	FT	F' W	1.57	0.78		0.93	0.87
	SL		3.90	0.60		0.46	0.21
SEED	ST	SW	0.33	0.73		0.79	0.63
	LOS		23.39	0.16		0.22	0.05
FRUIT STALK	WOS	LOS	0.628	-0.001		0.08	0.01



Figure 6. Regression model of relation between seed thickness (PLOD DEB) and seed width (PLOD SIR).

nimum variation observed in the Ustikolina (24.28 %) and the Majevica (14.41 %) populations, respectively.

For all measured characteristics, statistically highly significant interpopulation variability was obtained at the 99% significance level, as well as for the tree-population interaction, as shown in Table 4 where linear regression parameters, the correlation coefficient and coefficient of determination were used.

Statistical analysis at the individual level by the ANOVA F-test showed statistically significant differences for almost all characteristics and in all populations (Table 4). Such results should have a significant influence on succeeding analyses of population differentiation.

In canonical discriminant analysis, where we the grouped measured characteristics (fruit, seed, fruit stalk and leaf) of investigated populations from the central Dinarides, no differentiated groups were obtained by use of analyzed morphological markers (Table 5 and Figure



Figure 7. Regression model of overall length of the leaf blade (*LIST_DU_PLO*) in relation to blade width (*LIST_OD-STOJANJE*) at 1 cm from the blade apex and width of the leaf blade(*LIST_SIR_PLOJ*).

2–5). Therefore, populations did not form groups according to the Ecological Vegetation Classes, altitude above sea level, soil type, aspect of a slope, etc. Only weak differentiation was noticeable for fruit characteristics which can be associated with altitude above sea level.

For specific characteristics, high regression values were obtained by regression analysis, as, for example, for fruit thickness (FT) ($R^2 = 0.87$) (Table 5), while other characteristics displayed very low values, as in the case of fruit stalk characteristic relationship ($R^2 = 0.01$). As an example of high regression value $(R^2 = 0.71)$, the relation between seed thickness and seed width is shown in Figure 6. A similar three- dimensional regression model was obtained by analysis of three different characteristics (length of the leaf blade, blade width at 1 cm from the blade apex and width of the leaf blade) where high regression value was obtained but without significant influence on population differentiation (shown in Figure 7). Regarding soil reaction (pH), wild cherry populations in acid soil did not show any difference with respect to populations in limestone soil.

DISCUSSION

Based on our results from this research, we can see that the wild cherry represents a very specific species, characterized by high variability at the individual as well as interpopulational level, with a lack of differentiation between populations. From available literature data about the wild cherry, we were able to observe certain variability at the morphological level (5, 6, 7, 8, 25, 26, 27, 28, 29, 30, 31) and less variation at the molecular and genetic level (32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42), which provided a certain specificity to this species, that is the topic of much research. In earlier studies of the wild cherry (7, 43) with smaller number of populations, negligible differences were recorded between populations. This is the reason why this research included a higher number of populations from different habitats and investigated a higher number of characteristics.

For some characteristics, we confirmed differences at the individual and population level using an F-test, but with very weak or, in most cases, absent population differentiation. Earlier studies, due to their small scope, could not explain the obtained weak interpopulational differentiation of the wild cherry. Analyses of obtained results, along with those from other researchers, indicate that phylogenetic youth of the species could be one of the reasons for such results. This is reflected in the late and relatively fast colonization of these areas, although some researchers based on the wild cherry fossil records, point out that it was spread over Europe in the ancient past (15).

As the usage of particular morphological markers enables detection of differences within or between populations, as demonstrated with results for Pedunculate Oak (17, 18, 19, 20, 21, 22, 23, 24), we expected the same results in this research, but they were not confirmed. Possible causes for these differences, if we exclude natural selection and anthropogenic effects, are developmental factors, that is, the process of adaptation to given ecological conditions in which the individual appears, as well as very complex fertilization mechanism and fast gene flow among populations (40). From all investigated characteristics, results imply that we can indicate very low differentiation only in fruit dimension characteristics, which can be explained by specific ecological conditions. In this way, we can conditionally group populations with small, middle-size and large fruits but with a high risk and precaution because the values are too small to speculate about strong and clear differentiation between populations.

The reproduction system of the wild cherry is very complex and genetic analyses indicate that gametophytic self-incompatibility is controlled by the multi-allelic S--locus $(S_1, S_2, S_3, ..., S_n)$ (15, 43). Some authors report six S alleles (44), while others believe that they produce certain proteins in the pollen, which control the incompatibility phenomenon by the enzymatic degradation of the growth hormone in the pollen tube when identical male and female allele appear both in the pollen grain and the style (45). In addition, there are explanations for the gametophytic self-incompatibility mechanism provided by hereditary regulatory mechanism theory (46, 47). New data (4) obtained by the analysis of amino-acids and DNA of the sweet cherry confirm the presence of 12 S alleles, whereas 25-30 alleles controlling the incompatibility were identified in the wild cherry. The self-incompatibility phenomena have to be taken into account when analyzing intrapopulation and interpopulation variation because the presence of S alleles decreases differentiation, along with very fast gene flow by seed and pollen dispersal (entomophily and ornithochory).

From all investigated characteristics, results imply that we can indicate very low differentiation only in fruit dimension characteristics, which can be explained by specific ecological conditions. In this way, we can conditionally group populations with small, middle-size and large fruits but with a high risk and precaution because the values are too small to speculate about strong and clear differentiation between populations.

For other analyzed characteristics (seed, fruit stalk and leaf characteristics) we did not find such or similar results. When drawing conclusions based on the analysis of obtained results, we must not overlook the presence of adjacent glacial refugia where the wild cherry probably survived the glacial period. One of these refugia is situated in the South, in the area of Greece (48, 49), while other secondary refugia, mentioned in the research of European oaks (50, 51), is situated in the vicinity of middle Dalmatia and could have influenced the low differentiation. Usually species close to their glacial refugia do not lose much of their genetic potential and are therefore less differentiated, as opposed to species quite distant from their refugia. However, in this research it was not possible to answer in a satisfactory way whether this had any influence on the low differentiation of investigated wild cherry populations.

Taking into account that the wild cherry grows in areas where morphological characteristics depend on climatic, edaphic and orographic factors which can have direct influence on morphological characteristics and adaptation of the wild cherry, we expected the formation of matching ecotypes, which, however, could not be confirmed by this research. Unlike in this study, several researchers working with other species successfully correlated obtained statistical differences between investigated characteristics with climatic conditions in investigated areas (52, 53, 54).

To explain the weak differentiation, we can refer to several studies carried out at a molecular level (55, 56, 57, 58, 59, 60), and dealing with gene flow analyses in tree species where seed dispersal by birds was present. In the research of Frangula alnus (61), it was pointed out that birds are the crucial factor for fast gene flow, which also happens to be the case with the wild cherry. According to the same author, species migration is influenced by bird migration routes, as well as the movement routes of other animals that feed on fruits of this scrub species. As we have the same situation with the wild cherry which serves as food for numerous birds and animals, this statement is very logical. Nevertheless, we must not forget the complex reproduction system present in the wild cherry, regulated by a series of multiple S-alleles mentioned by many researchers (4, 15, 40, 43, 44, 45, 46, 47). The aforementioned multiple alleles regulate incompatibility during fertilization, functioning independently one from another (4, 15, 44, 45, 46, 47). The number of discovered multiple alleles ranges from 25 to 30, depending on the applied method of research. Based on these findings, the gene flow from one population to another is quite fast and, along with good ecological adaptation of the wild cherry, it does not allow significant population differentiation.

When we cannot exclude the effects of applied methodology (the number of sampled individuals or selection of analyzed characteristics) or developmental and anthropogenic factors in the wild cherry, the existing differences can sometimes indicate that different adaptation processes are taking effect in investigated populations, and can drive the populations from divergent ecological conditions toward the same evolutionary direction, that is, toward the establishment of genetic equilibrium between populations. Based on our results and the arguments given in the discussion, we can present certain points of view about the wild cherry:

- The obtained variability in the intrapopulational, and individual level can be associated with very complex fertilization mechanisms in the wild cherry, above all with the activity of a large number of multiple S-alleles which are the key factor for the differentiation decrease between populations.
- Pollination by insects indicates very rapid gene flow between populations, which additionally contributes to the overall low level of differentiation.
- Fast long-distance seed dispersal by birds contributes to fast gene exchange which has a significant effect on low differentiation level.

- Based on analyses of investigated morphological features, we could not identify the differentiation of wild cherry populations in given ecological conditions on any basis. Apart from complex and fast gene flow, such results could be a consequence of the late colonization of these areas, phylogenetic youth and fast expansion of the species, and finally strong anthropogenic and zoogenic impact.
- Regarding soil reaction (pH), wild cherry populations in acid soil did not show any difference with respect to populations in limestone soil. Furthermore, according to geology and soil type, we could not find any strong differences between investigated populations.
- Relatively small, isolated populations from higher altitudes (in our case we are referring to the populations of Kupres, Kalinovik and Sokolac) do not show any differences at the morphological level from the populations from lower altitudes of the central Dinarides and the Alps, which have a longer vegetation period and grow in soils of higher quality. These three populations show a relatively high level of degradation due to very pronounced negative anthropogenic and zoogenic impact.

Based on our results and our obtained knowledge about the variability of the wild cherry in Bosnia and Herzegovina, we can develop quality regeneration plans for this species, as well as in situ and ex situ genetic diversity conservation plans.

As we have at our disposal small-size populations or only individual trees, often with low quality and scarce arrangement of trees in the stand, it is necessary to adopt a number of breeding regulations in order to improve the condition of the trees in the field. This is specifically meaningful for some of the investigated populations (Kupres, Kalinovik, Sokolac). For the adaptation and maintenance of a certain population in situ we have to consider the fact that survival depends on basic life factors, as well as on the individual itself which carries the genetic material, that is, on its ability to transfer the genetic inheritance to the next generation (through vitality, resistance, fructification...). Therefore, it is necessary, apart from knowing the variability obtained by the applied markers, to know and understand the ecological conditions in which the species develops. In general, we can conclude that research of the wild cherry and similar species, which are bird-dispersed (ornithochory) and have a specific fertilization mechanism, is very complex and has to be based on individual-level research, because individuals can grow one next to the other and originate in very distant areas at the same time.

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