Assessment of the phenotypic diversity of wild cherry (*Prunus avium* L.) populations and half-sib lines by multivariate statistical analyses

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Abstract

Wild cherry (*Prunus avium* L.) is a multi-purpose tree species with great ecological and economic importance for European forestry. Evaluating this species phenotypic diversity and quantitative traits characterization is of great importance to define its genetic resources conservation and breeding strategies. In this work, variations of physiological, biochemical, anatomical and morphological traits of one-year-old wild cherry seedlings were evaluated within and among populations to distinguish and characterize their phenotypic portfolio. We observed significant differences at the intra- and inter-population levels considering both biochemical and physiological leaf traits, whereas differences in morphological and anatomical traits were found to be significant only among half-sib lines within populations (i.e. intra-population level). With a multivariate approach, we explored the inter-population specificity and found out that the tiered approach spanning from organ morphology, across physiological scale, to the biochemical level gave out enough power to discriminate between different populations, and their acquisition and resource-use strategies. Moreover, stepwise discriminative analysis showed that radical scavenger capacity against 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS•+) and water-use efficiency contributed to discrimination of studied populations to the largest extend. Lastly, our study highlights the robustness of certain functional traits, such as ABTS•+, water-use efficiency, net photosynthesis, total flavonoid content, width of stomata guard cell, and stomatal aperture length, which could be considered as a proxy to discriminate between wild cherry populations and assess phenotypic diversity.

Keywords: *Prunus avium L., functional traits, variability, discrimination, common garden experiment.*

Introduction

Wild cherry (*Prunus avium* L.) is a broadleaf, self-incompatible tree species in the *Rosaceae* family, with multiple ecological and economic importance (Martinsson, 2001; Ganopoulos et al., 2011). Due to solid and dense wood, this species has been particularly valued in wood processing industry, for paneling and cabinet-making (Ducci et al., 2013). Likewise, due to its

decorative and medicinal properties, as well as edible fruits, this species found an important place in urban forestry and other branches of economy, such as food and pharmaceutical industry. Lastly, as a pioneer tree species, wild cherry may be successfully used in land reclamation programs (Stojnić et al., 2021). For these reasons a number of European countries have developed wild cherry conservation and breeding programs aimed at protection of the species genetic diversity and its genetic improvement, respectively (Kobliha, 2002; Vuksanović et al., 2020).

Intraspecies variability plays an important role for longevous organisms to withstand the climate change and environmental stress factors. It has been noted that high diversity contributes to better adaptation potential of species, which is of great importance for designing conservation programs (Possen et al., 2014; Avramidou et al., 2015). According to Garzón et al. (2011), genetic differentiation between populations could be decisive in prediction of species survival under the impact of global climate change. Assessment of populations genetic diversity and phenotypic plasticity might unravel putative functional traits, thus building and strengthening the core knowledge for wild cherry conservation programs. Among others, stomatal phenotype and gas-exchange quantifications have been proven very effective (Konôpková et al., 2020; Petrík et al., 2020). Stomata are small water-conserving apparatus that plays an important role in water and gases transport (Drake et al., 2013; Vastag et al., 2020). Due to this fact, their activity is closely connected with photosynthesis (Lawson and Vialet-Chabrand, 2019; Harrison et al., 2020). Typically, stomatal traits are dependent on genetic background (Gailing et al., 2008; Vastag et al. 2019), although various factors, including environmental conditions (Lin et al., 2021), extreme events (Petrík et al., 2022a) and position of the leaf within crown (Kardiman and Ræbild, 2018; Wakefield et al., 2021) may trigger a specific developmental change in stomatal density and phenotype. Similarly, due to physiological traits are tightly linked to plant functions (Ramírez-Valiente et al., 2020; Wang et al., 2021), gas exchange parameters have often been used as useful bio-indicators of the species' ability to withstand stressful conditions, as well as to obtain patterns of tolerance of individual species across the environmental gradients (Pšidova et al., 2018; Petrík et al., 2022b). Furthermore, a shift in secondary metabolites content and/or occurrence follows the changes in environment and might be considered as a functional trait. The importance of phenolic and flavonoid compounds in protection from oxidative stress, which follows after any disturbance in plant environmental conditions, has been pointed out in literature (Kebert et al., 2016; Khaleghi et al., 2019; Vuksanović et al., 2022).

According to Mataruga et al. (2013) the information on inter- and intra-population structure and genetic diversity may provide a valuable basis for further development of forest management strategies and conservation programs of forest genetic resources. Indeed, several studies have demonstrated high genetic diversity of wild cherry populations using nuclear microsatellite loci (Mariette et al., 1997; Jarni et al., 2012; De Rogatis et al., 2013), indicating a great evolutionary potential

of the species for adaptation to changed climatic conditions. Likewise, recent findings regarding phenotypic variability of wild cherry led to new insights on the genetic potential of this species for using in breeding programmes aimed at increased resistance to various stress factors (Temel, 2018; Miljković et al., 2019; Vuksanović et al., 2022). However, majority of these studies were limited to a single group of the phenotypic traits, primarily morphological (Rakonjac et al., 2014; Popović and Kerkez, 2016; Miljković et al., 2019), and few statistical methods, such as univariate statistical procedures and canonical discriminant analysis (Ballian et al., 2012; Orlović et al., 2014), thus failing to provide a comprehensive overview of the species diversity and functional traits association. Recent studies showed that multivariate statistical analyses can be efficiently used for evaluation of the phenotypic diversity, providing a valuable information on the species variability patterns and multi-traits relationships (Ganopoulos et al., 2015). In addition, these analyses proved particularly useful for characterization of extensive data sets consisting of large number of parameters (see Petruccelli et al., 2013; Moradi et al., 2020; Poljak et al., 2021). Hence, our research assessed the variability of populations and half-sib lines of wild cherry, originating from natural populations in Bosnia and Herzegovina, using a set of morphological, anatomical, physiological and biochemical traits. In addition, using multivariate statistical analyses we further investigated: (i) the relationship among studied functional traits, and (ii) the relevance of examined traits in discrimination of studied populations and half-sib lines, in order to identify those that mostly contributed to the total diversity (Ganopoulos et al., 2015). We have hypothesized that significant differences would be observed both at intra and inter-population levels, supporting previous results reporting high genetic variability in wild cherry, as well as that application of multivariate statistical methods would facilitate identification of functional traits that achieve the major contribution to differentiation of examined wild cherry populations.

Material and methods

Plant material and experimental design

The research is conducted on one-year-old seedlings of wild cherry (*Prunus avium* L.) cultivated in nursery progeny test and originating from four natural populations (Kalinovik, Milići, Prnjavor and Vlasenica) located in Bosnia and Herzegovina (Table 1). Climate data are derived from the FORESEE database (Dobor et al., 2014) and averaged for the period 1981-2010.

Seeds used for the establishment of progeny test were collected from 10 randomly selected vigorous mother trees per each population (40 dominant mother trees were selected in total) during July and August 2015. The experimental plot was founded at the nursery (44o34' N; 18o83' E; 740 m a.s.l.) managed by the "Center for seeds, nursery, and afforestation" in Doboj, Bosnia and Herzegovina. After pre-sowing treatment, the seeds were sown manually at seedbed to the depth of 1 cm below the soil surface. Sowing was done in November 2015.

Table 1

Geographic and climatic characteristics of studied Wild cherry (*Prunus avium* L.) populations origin sites.

Population	Kalinovik	Milici	Prnjavor	Vlasenica
Latitude (N)	43°31′	44°10′	44°54′	44°11′
Longitude (E)	18°29′	19°07′	17°39′	18°54′
Altitude (m a.s.l.)	1090	498	180	660
T (ºC)	9.0	10.2	9.6	9.1
T_{veg} (ºC)	14.8	15.0	15.9	15.2
P (mm)	1265	1005	1020	984
P_{veg} (mm)	537	549	516	532

Legend: T – mean annual air temperature, T_{veg} - mean annual air temperature during growing season (April - September), P – annual sum of precipitations, P_{veg} – sum of precipitations during growing season (April - September).

The spacing between sown seeds was approximately 10 × 10 cm. The nursery has a temperate-continental climate. The mean annual temperature in the nursery is approximately 9.5°C, while the annual sum of precipitation amounts to about 1111 mm. Details of pre-sowing treatment, the chosen natural populations and the description of the environmental conditions of the experimental plot were given by Stanković Neđić et al. (2018).

In the present study, 18 physiological, biochemical, anatomical, and morphological parameters were studied (Table 2). Measurements of leaf gas exchange and leaf chlorophyll content, as well as the collection of plant material for biochemical, anatomical, and morphological analyses, were performed between the 4th and 8th of August 2016. The height of seedlings (*H* [mm]), and the root collar diameter (*d* [mm]) were measured at the end of the first growing season. *H* was measured between the cotyledon scar and the base of the terminal bud with a precision of 1 mm, using a ruler. The diameter was measured in the zone of root collar with a digital caliper with a precision of 0.1 mm (Stanković Neđić et al., 2018).

All analyses were performed on five half-sib lines per population, on five plants within each half-sib line; i.e., the research was done on 100 plants in total.

Physiological analyses

Gas-exchange parameters were acquired with CIRAS - 3 Portable Photosynthesis System (PP Systems, Amesbury, MA, USA): net photosynthesis (*A* [$\mu mol\ m^{-2}\ s^{-1}$]), transpiration rate (*E* [$\mu mol\ m^{-2}\ s^{-1}$], intercellular CO_2 concentration (*Ci* [$\mu mol\ mol^{-1}$]) and stomatal conductance (*g_s* [$mol\ m^{-2}\ s^{-1}$]). The saturating photosynthetic active radiation (PAR) in the leaf chamber was set to 1000 $\mu mol\ m^{-2}\ s^{-1}$, while CO_2 concentration, air temperature, and relative humidity were taken ambient from the atmosphere. These parameters were measured on five plants within each line, with five replications taken per plant. They were measured on 5 fully developed leaves, oriented in the south-southwest direction, located in the upper third of the crown. Measurements were done on sunny days between 09:00 and 11:00 a.m. (Orlović et al., 2014). Water use efficiency (*WUE* [$\mu mol\ mol^{-1}$]) was calculated as a quotient based on measured photosynthesis parameter and transpiration intensity (*A/E*) (Bramley et al., 2013).

Table 2

List of studied parameters, their acronyms and units.

Parameter	Acronym	Unit
Physiological		
Intercellular CO2 concentration	Ci	µmol mol-1
Transpiration	E	mmol m-2 s-1
Stomatal conductance	gs	mol m-2 s-1
Net photosynthesis	A	µmol m-2 s-1
Water-use efficiency	WUE	µmol mol-1
Chlorophyll content	Chl	µg cm-2
Biochemical		
Total phenolic content	TPC	mg GA g-1
Total flavonoid content	TFC	mg QE g-1
Ferric reducing antioxidant power	FRAP	µmol ASA g-1
Radical scavenger capacity against ABTS•+	ABTS	%
Radical scavenger capacity against DPPH free radical	DPPH	%
Anatomical		
Stomatal density	SD	number per mm2
Length of stomata guard cell	LS	µm
Width of stomata guard cell	WS	µm
Stomatal aperture length	La	µm
Stomatal aperture width	Wb	µm
Morphological		
Height of seedling	H	mm
Root collar diameter	d	mm

Leaf chlorophyll content (*Chl* [$\mu g\ cm^{-2}$]) was measured on fully expanded leaves using a Chl meter SPAD-502Plus (Konica Minolta Optics, Inc., Osaka, Japan) (Bielinis et al., 2014). To reduce the impact of daily chloroplast movement on SPAD values, the measurements in each individual sampling time were conducted on the same day from 9:00 to 12:00 (Nauš et al., 2010). Three measurements were taken at the adaxial leaf surface and averaged per leaf in each seedling. The obtained *SPAD* values (Chl a+b) were converted to chlorophyll content (*$\mu g\ cm^{-2}$*) following Bielinis et al. (2014):

$$y = 0.0374x^2 + 0.5345x + 0.5137\ (mg\ m^{-2})\ (Eq.1)$$

Biochemical analyses

Harvested leaf samples were immersed in dry ice and transported to the laboratories of the Institute of Lowland Forestry and Environment (Novi Sad, Serbia), where they were stored in the freezer at -70ºC until laboratory testing began. About 200 mg of frozen leaf material (5 leaves in total) was macerated with 96% ethanol in ratio 1:10. The homogenate was centrifuged 10 minutes at 15.000 rpm/minute at 4ºC. The extracts obtained were diluted six times before starting the analysis. The resulting supernatant was used for the determination of the content of total phenols (*TPC* [mg GAE g⁻¹ FW]) and total flavonoids (*TFC* [mg QE g⁻¹]), determination of radical scavenger capacity (RSC) of ABTS [%] and DPPH [%] radicals, and determination of the ferric reducing antioxidant power (*FRAP* [µmol ASA g⁻¹]). All spectrophotometric measurements were performed on a MultiskanTM GO Microplate Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) according to the Stojnić et al. (2019).

The determination of *TPC* was done based on the reaction of phenol with the Folin-Ciocalteu reagent (Singleton et al., 1999; Lee et al., 2015), where *TPC* was calculated from a calibration curve obtained by measuring the absorbance of a series of different concentrations of standard gallic acid solution. *TFC* determination was performed spectrophotometrically according to the method of Chang et al. (2002). The ability to neutralize 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; short ABTS⁺) radicals were determined according to Arnao et al. (2001), which is an advanced version of the determination of the ability to neutralize ABTS⁺ radicals by Miller and Rice-Evans (1997). The ethanol extract was used for another test to determine capture activity, the so-called *DPPH* test (Soler-Rivas et al., 2000). The method is based on spectrophotometric monitoring of the stability assurance, nitrogen-centered DPPH radical (2,2'-diphenyl-1-picrylhydrazyl) in the reduced, conventional DPPH-H formula at 515 nm (Kebert, 2014). Lastly, the total antioxidant activity was tested by the Ferric reducing antioxidant power (*FRAP*) method, developed by Benzie and Strain (1999).

Leaf anatomical and morphometric analyses

Leaf epidermal peel for determining the stomatal density (*SD* [number per mm²]), length of stomata guard cell (*LS* [µm]), a width of stomata guard cell (*WS* [µm]), stomatal aperture length (*La* [µm]) and stomatal aperture width (*Wb* [µm]) were taken between the third and fourth veines from leaf adaxial surface (n=5). Due to the unequal openness of stomata during the day, the samples were collected between 09:00 and 11:00, on sunny and windless days. The imprints were taken from sun leaf, which was fully formed, located in the upper third of the canopy (Stojnić et al., 2015) and oriented south-southwest. Determination of the number and size of the stomata was performed in the laboratory of the ILFE, using an "Olympus Vanox" (Olympus, Tokyo, Japan) light microscope and the "Leica Application Suite - LAS EZ 3.1.1" (Leica Microsystems, Wetzlar, Germany) software to determine stomatal parameters. The software "tpsDig2.17" (Rohlf, 2013) was used for the determination of the number of stomata. Stomatal density was determined by 10×10 magnifications of the eyepieces and lenses, while the size of the stomata was determined by 40×10 magnification and lenses. Five "fields of view" were selected by random sampling method for each imprint. The density of the stomates was obtained by counting the number of stomata per area of 1 mm² in every selected "fields of view". In each "field of view", size of the stomata were determined on five stomates selected by random sampling method, which represents 25 stomates per imprint. The average value of stomata parameters per plant entered further statistical analysis.

Statistical analysis

Two-way hierarchical analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test were applied to test differences within and between studied populations of wild cherry (Eq.2).

$$Yijk = \mu \cdot \cdot + \alpha i + \beta j(i) + \epsilon ijk \text{ (Eq.2)}$$

where, μ is the overall mean response, αi is the effect due to the i-th population, βj is the effect due to the j-th half-sib line within the i-th population, while єijk is residual effect.

Both methods are univariate and performed in order to present the variability of each used parameter, as well as to relate these results with performance of examined parameters in applied multivariate methods. Beforehand ANOVA, the normality of distribution was assessed using Shapiro-Wilk's test. Within the analysis of variance, expected variance components of populations, half-sib lines within populations and residual were calculated. The Random model was used, as both "populations" and "half-sib lines within populations" were treated as random sources of variation. They were used to calculate coefficients of variation, contribution to the total expected variance and phenotypic divergence, according to Brommer (2011):

$$P_{ST} = \frac{\frac{c}{h^2}\sigma_B^2}{\frac{c}{h^2}\sigma_B^2 + 2\sigma_W^2} \text{ (Eq.3)}$$

where σ_B^2 and σ_W^2 stand for between and within population components of variance of the quantitative trait, respectively, h² for narrow-sense heritability and c is the proportion of the total variance which is due to additive genetic effects across populations. Estimates were done under the assumption that c=h²; i.e. $\frac{c}{h^2} = 1$ (Gömöry et al., 2015).

Principal Component Analysis (PCA) was applied to transform original variables into uncorrelated, derived variables in such a way that a majority of variance of original variables could be explained by few of derived ones (Rakonjac et al., 2010, Vastag et al., 2019). The variance of particular principal components was obtained by the calculation of its eigenvalue. According to Keiser's rule (Kaiser, 1960), the first four principal components that had their eigenvalues higher than 1 were used to present the relationship among the examined populations based on physiological, biochemical, anatomical and morphological parameters. According to that role, those four principal components that explained 84 % of the total variation (Table S1), are sufficient for the further analysis of relationship between original parameters. For orthogonal rotation, the

varimax method was applied on the first four principal components. By this method, selected principal components are rotated in order to maximize the sum of the variances of the squared loadings within rotated principal component. These results were used for the analysis of relationship between original parameters, as original parameters having their highest loading with the same principal component could be considered to be more closely related to each other than to other original parameters that have their highest loadings with some of other principal components. The relationship between populations, as well as between examined traits, was also described by canonical discrimination analysis (CDA), while forward stepwise discriminant analysis (SDA) was used as a way to additionally evaluate the discrimination power of the examined parameters. All statistical methods were performed by Statistica for Windows version 13 (TIBCO Software Inc., 2017).

Results and Discussion

Variability of natural wild cherry populations occur at both intra and inter-species level

Differences between populations were found to be statistically significant for the biochemical and morphological traits, as well as certain physiological characters (A, E and WUE), which is also confirmed by the P_{ST} values (Table 3). Considering within-populations variability, F and Tukey's HSD tests showed that most of the examined traits significantly varied between half-sib lines, excluding TFC, SD and gs (Table 3).

Similar variability pattern was noticed at intra- and inter-population levels in a study obtained by Ballian et al. (2012), who investigated 16 morphological traits in 22 natural populations in Bosnia and Herzegovina. Likewise, Orlović et al. (2014) reported significant differences of net photosynthesis, transpiration rate and water-use efficiency between wild cherry half-sib lines originating from Serbia. Due to homogeneity of climatic and edaphic factors in the nursery trial, the observed variation at the inter- and intra-population levels is probably the result of populations and half-sibs' genetic architecture, which is developed as a consequence of their local adaptation within the species natural range. Previous studies showed that high level of genetic variability within and between wild cherry populations could be influenced by the reproduction system of this species (e.g., gametophytic self-incompatibility, controlled by the presence of multi-allelic S locus) (Sharma et al., 2017), as well as by the fact that pollen and seed might be dispersed over large distances by the insects and birds/mammals, respectively (Ballian, 2012; Vekemans and Hardy, 2004).

According to the contribution of examined sources of variation to the total expected variance, there is a considerable difference between examined parameters. The contribution of the factor "Population" is dominant in the examined biochemical parameters, as well as A, E, gs and WUE, while the contribution of "Half-sib lines within-population" dominates in anatomical and morphological traits. It corresponds to the recent finding of Miljković et al. (2019), who reported larger intra-population variability of wild cherry leaf shape and size in comparison to inter-population variation. Similarly, studying the preference of *Myzus cerasi* (Fabricius 1775) to populations and half-sib lines of wild cherry, Poljaković-Pajnik et al. (2019) found that the contribution of half-sib lines (i.e. intra-population variability) was significantly higher than population in the total expected variance of the damaged leaves percentage. Finally, the contribution of "Residual" variation lower than 50% was observed in TPC, ABTS, DPPH, FRAP, E, and WUE, suggesting these traits as a considerable basis for selection between population and half-sib lines (i.e., parent trees) (Figure 1). These results are in agreement with Kebert et al. (2016) who noticed that biochemical parameters could serve as one of the main criteria for selecting species, clones and individuals having a higher ability to survive in drought conditions. Furthermore, Shahidi and Chandrasekara (2010) reported that biochemical parameters could be crucial for assessing the tolerance, plasticity and adaptability of species to changing environmental conditions.

Multivariate patterns support the genetic variability of wild cherry

According to the contribution of the examined sources, there were four components with eigenvalues higher than 1 (Kaiser, 1960), which accounted for 84.4 % of the total variance (Table S1). The highest contribution on PC1 corresponded to parameters of stomatal aperture size (LS, WS, La and Wb), and physiological traits indicative for photosynthetic capacity (Chl, A and Ci) (Table 4), indicating a key role of stomata in the CO_2 uptake for photosynthesis and its volume control in the intercellular air spaces (Lawson and Blatt, 2014; Yin et al., 2020).

The traits contributing differentiation along PC2 were those related to plants antioxidant capacity (TPC, ABTS, DPPH and FRAP). PCA plot shows that the half-sib lines originating from Vlasenica population are distributed close to the origin of coordinate system, while half-sib lines of other populations are distributed around this group (Figure 2). Also, cluster of half-sib lines from Prnjavor population is distinct from populations from Vlasenica, Kalinovik, and Milići, particularly by second principal component.

Indeed, population Prnjavor is characterized by the highest values of TPC, ABTS, DPPH and FRAP, as well as the lowest transpiration rate, which might be the consequence of local adaptation to more xeric conditions in this region. Namely, this population is geographically the most distinct population and originates from the lowest altitude (see Table 1). Likewise, the locality is characterized by the lowest precipitation sums and the highest mean air temperatures during growing season. Therefore, higher content of antioxidants and secondary metabolites in the seedlings of populations Prnjavor probably represents the drought-tolerance mechanism aimed at neutralization of reactive oxygen species, and, consequently, avoidance of the oxidative damages caused by water stress (Mechri et al., 2020; Poljak et al., 2021). For example, Visi-Rajczi et al. (2021) reported that European beech provenances originating from warmer climate were characterized by higher enzyme activity. Likewise, Varela et al. (2016) found that production of

Table 3

Variability of physiological, biochemical, morphological and anatomical traits in studied wild cherry populations, assessed by two-way hierarchical analysis of variance (F test) and phenotypic divergence index (P_{ST}). For each trait, mean (± standard deviation) values are presented. The letter codes denote homogeneous groups (Tukey's HSD test, $p<0.05$) among populations. The confidence interval for SD (in parentheses) was calculated by retransforming the confidence interval obtained from transformed data. The parameter acronyms are defined in Material and Methods.

Parameter	Population		Half-sib line within population		Coefficient of variation			PST	Mean values of populations with 95 % confidence intervals and Tukey's HSD test							
									Kalinovik		Milići		Prnjavor		Vlasenica	
	Fp	pp	Fhsl(p)	phsl(p)	CVp	CVhsl(p)	CVr									
TPC	11.10	0.000	2.54	0.003	22.01	12.03	21.45	0.626	2.26±0.36	c	2.87±0.64	b	3.71±0.41	a	2.47±0.31	bc
TFC	5.75	0.007	0.89	0.582	7.03	0.00	16.92	1.000	2.96±0.28	ab	2.63±0.12	b	2.78±0.23	ab	3.14±0.19	a
ABTS	14.88	0.000	2.46	0.005	18.85	8.70	15.94	0.702	49.23±5.9	b	55.41±9.73	b	75.35±4.85	a	55.04±5.13	b
DPPH	11.57	0.000	2.87	0.001	29.25	16.20	26.24	0.620	30.13±7.35	b	36.57±10.5	b	56.71±8.77	a	33.88±4.31	b
FRAP	9.79	0.001	2.79	0.001	29.81	17.98	29.69	0.579	4.78±1.01	b	5.69±1.99	b	9.12±1.66	a	5.44±0.63	b
Chl	3.66	0.035	3.65	0.000	18.30	21.32	28.94	0.269	79±24.48	a	48.98±10.08	b	56.74±10.99	b	57.7±8.77	b
LS	0.71	0.558	5.07	0.000	0.00	5.75	6.19	0.000	21.69±2.12	a	20.48±1.24	b	21.05±0.85	ab	21.23±0.69	ab
WS	2.97	0.064	2.17	0.013	3.38	3.94	7.92	0.269	14.91±1.26	a	13.73±0.46	b	14.44±0.31	ab	15.07±0.63	a
La	1.07	0.389	4.36	0.000	0.86	6.24	7.41	0.009	14.68±1.53	a	13.89±0.61	b	13.57±0.8	b	14.02±0.68	ab
Wb	1.07	0.389	4.36	0.000	1.82	3.52	4.09	0.117	5.21±0.81	a	4.41±0.32	b	4.55±0.49	b	4.86±0.46	ab
SD	1.49	0.256	1.65	0.075	2.22	4.46	12.07	0.110	* 251.810 (214.143-292.527)	a	262.226 (208.642-321.928)	a	293.938 (265.026-324.347)	a	250.982 (237.117-265.241)	a
A	6.02	0.006	2.89	0.001	19.45	15.66	25.19	0.436	16.52±3.47	a	9.77±1.15	c	12.37±3.51	b	13.63±1.41	b
Ci	2.17	0.132	2.03	0.021	1.94	2.86	6.24	0.187	288.1±18.74	b	306.82±7.55	a	298.89±9.77	ab	294.98±7.47	ab
E	8.01	0.002	2.68	0.002	15.19	10.14	17.29	0.529	7.46±1.13	a	6.14±0.72	b	5.11±0.74	c	5.54±0.46	bc
gs	3.14	0.055	1.64	0.078	13.90	13.27	36.68	0.354	0.51±0.09	a	0.37±0.11	b	0.34±0.09	b	0.39±0.06	b
WUE	8.20	0.002	2.80	0.001	16.73	11.16	18.38	0.529	2.22±0.3	b	1.6±0.23	a	2.35±0.44	a	2.44±0.17	a
H	1.29	0.311	4.20	0.000	7.00	25.29	31.64	0.037	292±24.5	ab	244.2±34.55	b	350.8±139.56	a	297±90.37	ab
d	0.21	0.892	2.91	0.001	0.00	13.14	21.26	0.000	5.11±0.49	a	4.83±0.61	a	5.02±1.11	a	4.77±0.84	a

phenolic compounds was the main strategy of Patagonian native shrubs to adapt to extreme environments.

Furthermore, morphological properties (d and H) were included in third component and closely related to SD and gs, suggesting significant influence of regulation of water status to growth and development of wild cherry seedlings. It corresponds to the previous studies that demonstrated tight relationship between increased SD and elevated stomatal conductance (Schlüter et al., 2003; Franks et al., 2015; Yin et al., 2020), as well as beneficial influence of higher SD to the plant biomass production (Sakoda et al., 2020). Lastly, the fourth component is represented with higher contribution of TFC and WUE, thus confirming positive effect of flavonoids on gas exchange, and therefore, overall plant drought-tolerance (Li et al., 2021).

According to the results of the CDA, there were three canonical variables (roots) extracted, where eigenvalue of the first canonical variable was the highest and described most of the total variance (98.1%), while the contribution of other two canonical variables were around 1% (Table S2). According to the first two canonical variables, the examined populations are clearly discriminated (Figure 3). Although the considerable discrimination provided the first canonical variable, the second one also clearly discriminated populations Prnjavor and Kalinovik from Milići and Vlasenica.

Loadings of examined traits with the first canonical variable, which described the most of total variance (98.1%) were considerably small, and amounted less than 0.1 for all the traits, except d (Table S3). This fact suggested that none of the examined parameters had considerable discrimination ability alone. In order to select the group of parameters that dominantly contribute to the discrimination between half-sib lines, the forward SDA was applied (Table S4). According to SDA, eight traits have been selected to contribute to discrimination of populations to the largest extend. The first three traits were ABTS, WUE, and A, representing different groups defined by loadings from rotated components. This finding suggests that besides high contribution of "Population" and "Half-sibs" to the total variance, the selection of a parameter depends on its correlation with previously selected parameters, thus emphasizing the importance of coping with multicollinearity within the process. The further evaluations of the discriminative power of
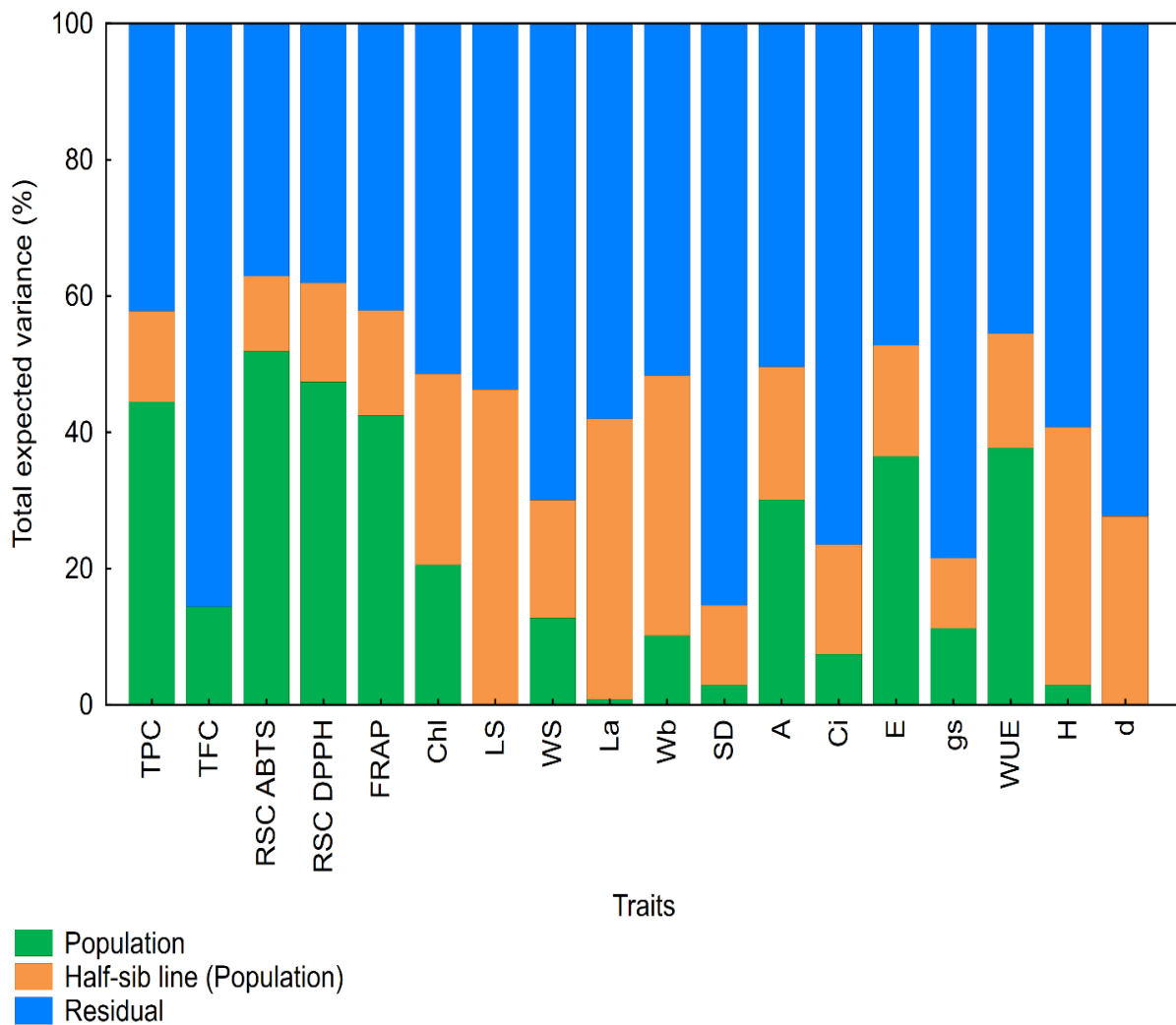
Figure 1

Contribution of examined sources of variation to the total variance for measured parameters within wild cheery populations.

selected traits were performed according to the correct allocation of successive models formed by forward stepwise discriminant analysis (Figure 4). According to these results, the second model with just two traits (ABTS and WUE) was able to achieve 90% of correct allocation, while the model with six selected traits (ABTS, WUE, A, TFC, WS and La) was sufficient to achieve a correct allocation of all half-sib lines.

Conclusions

As it is already presented and discussed throughout the paper, our aim was to explore in-depth the variability of wild cherry at intra- and inter-population levels, as well as to unravel the traits (variables) contributing mostly to differentiation of populations. Our study provides evidence on high genetic variation both within and among studied wild cherry populations. We showed that, according to investigated functional traits, there was a substantial difference between four analyzed populations, since both PCA and CDA approaches were able to resolve distinct groups based on the input dataset. Further exploratory analysis using SDA profiled ABTS, WUE, A, TFC, WS and La as a "core functional traits" group having enough discriminative power to resolve different populations by their half-sibs. Gathering all valuable information from applied multivariate analyses, we showed that a tiered approach in analysis from stomatal anatomy to the gas-exchange and biochemical traits might boost selection and breeding programs of this tree species. Moreover, such approach enabled the identification of populations (e.g. population Prnjavor) showing capacity to face drought, which is particularly important in the light of climate change impact on forest in the region of Southern Europe, and selection of site-adapted reproductive material for future reforestation programs, accordingly.

Table 4

Loadings of the first four principal components rotated by Varimax method. The bolded values represent the highest loading of particular examined trait.

Original parameter	Rotated principal components			
	RC1	RC2	RC3	RC4
TPC	-0.288	-0.921	0.002	-0.141
TFC	0.191	0.190	0.019	0.807
ABTS	-0.147	-0.961	-0.106	0.047
DPPH	-0.252	-0.945	0.053	-0.034
FRAP	-0.193	-0.951	0.006	-0.026
Chl	0.731	0.275	0.162	0.389
LS	0.889	0.171	-0.024	0.202
WS	0.830	0.146	0.010	0.334
La	0.856	0.354	0.219	0.041
Wb	0.837	0.249	0.212	0.108
SD	-0.351	-0.351	0.562	-0.392
A	0.596	0.190	0.452	0.558
Ci	-0.672	-0.146	-0.067	-0.618
E	0.479	0.536	0.500	-0.069
gs	0.456	0.367	0.666	0.054
WUE	0.314	-0.223	0.198	0.801
H	-0.021	-0.297	0.643	0.603
d	0.220	0.040	0.757	0.316
Eigenvalue	5.206	4.658	2.378	2.951
Proportion of total variance	0.289	0.259	0.132	0.164
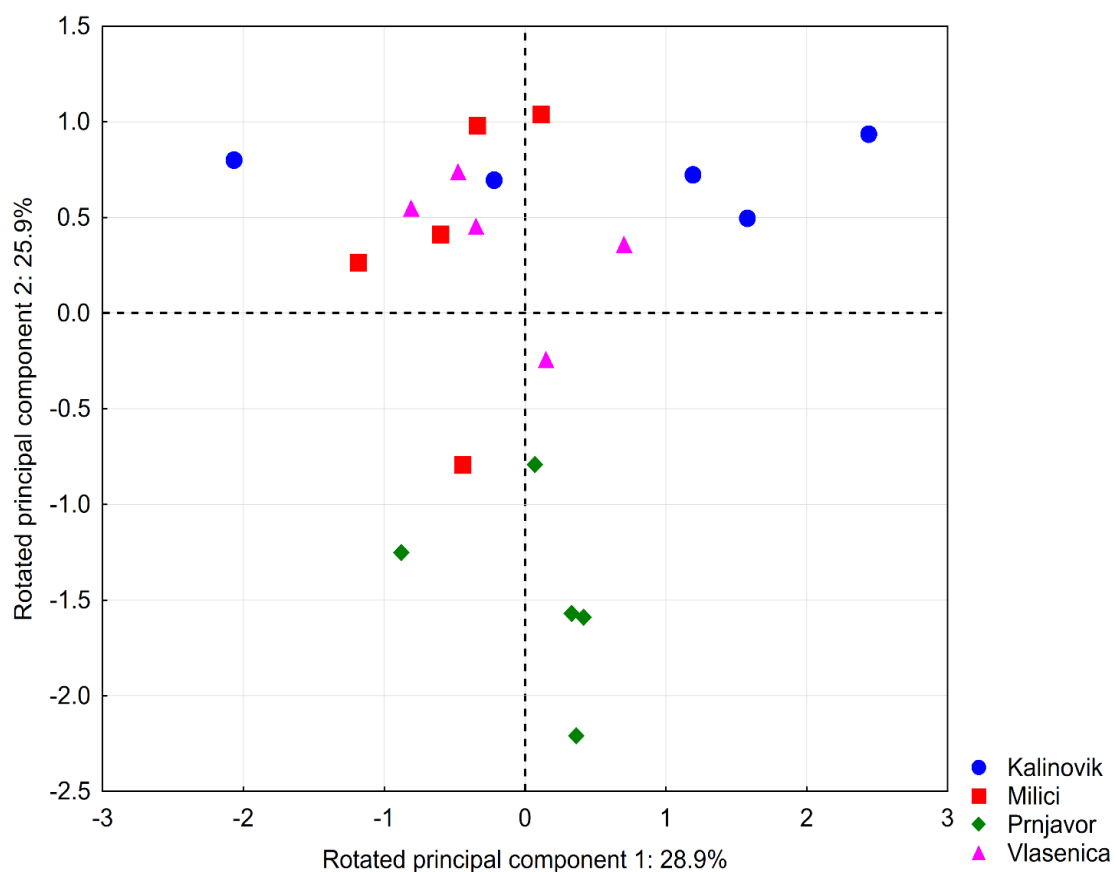
Figure 2

Relationship between examined populations based on the first two components (PCA).
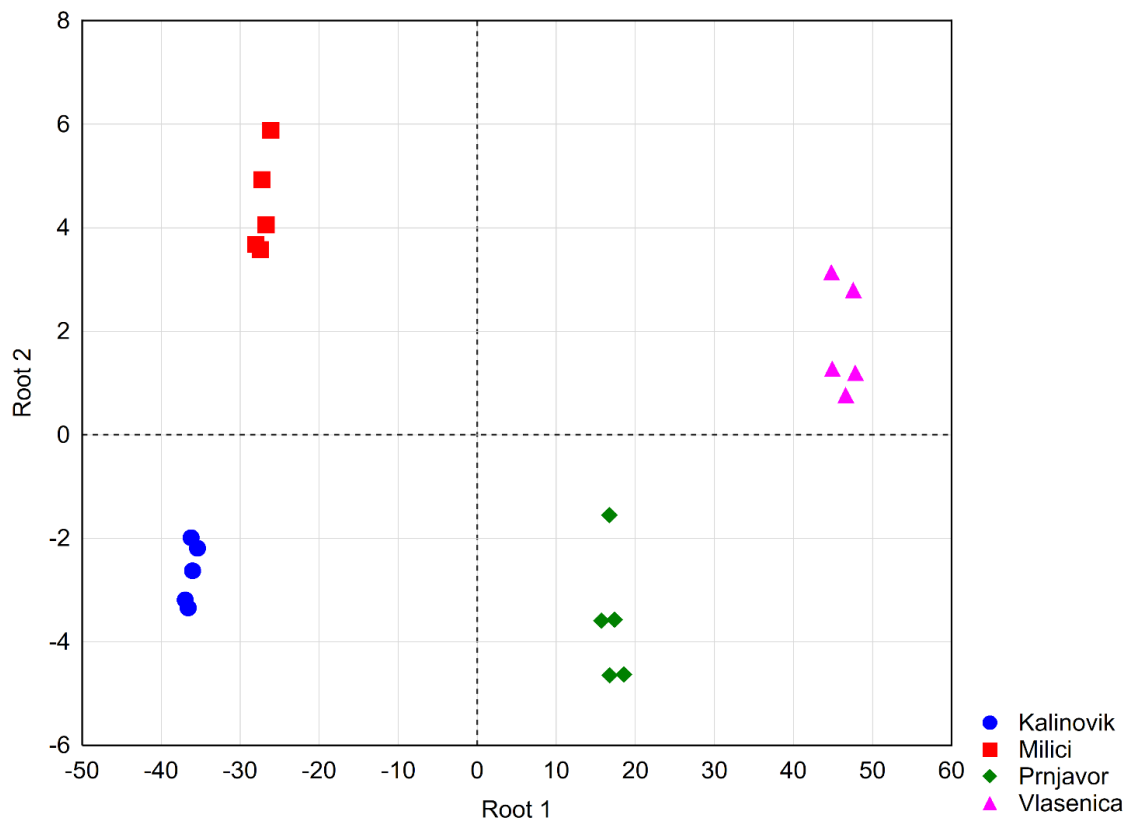
Figure 3

Relationship between examined populations of wild cheery according to the first two canonical variables of eight original characteristics selected by forward stepwise discriminant analysis.
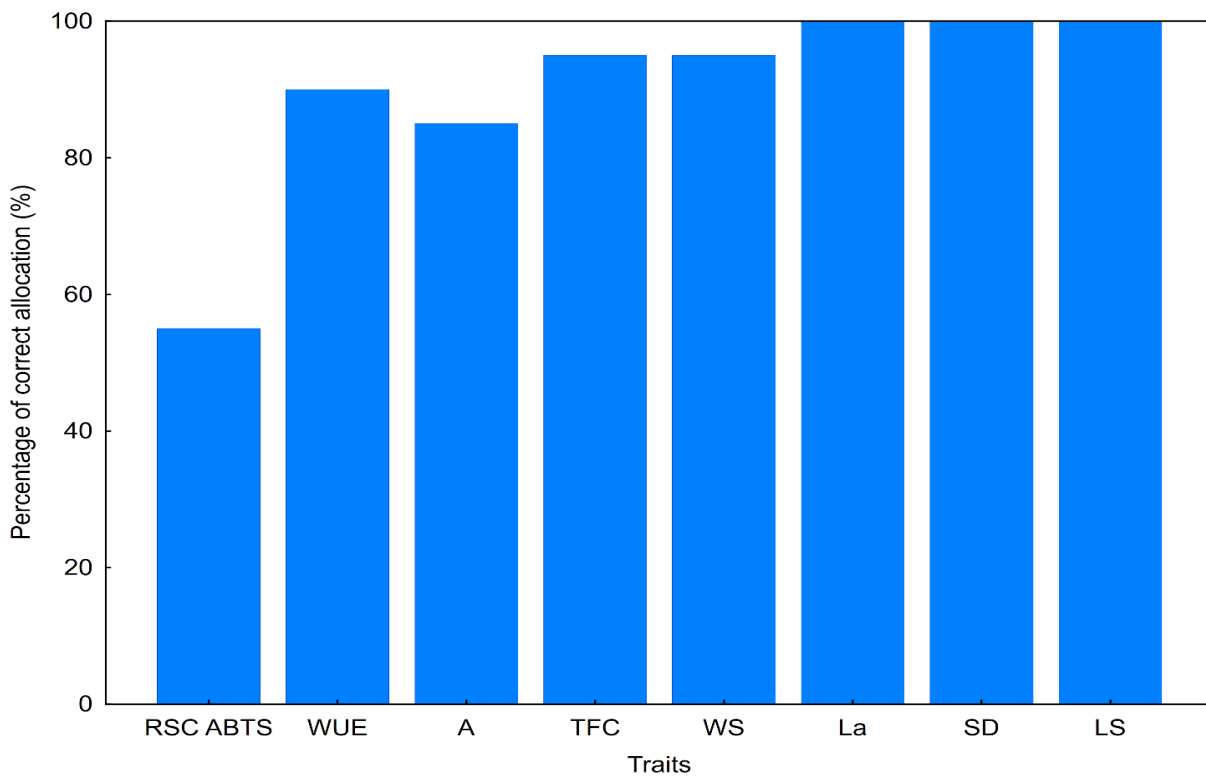

Figure 4

The percentage of correct allocation for models formed by forward discriminant analysis.

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References

Arnao MB, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73:239-244. https://doi.org/10.1016/S0308-8146(00)00324-1

Avramidou E, Ganopoulos I, Doulis A, Tsaftaris A, Aravanopoulos F (2015) Beyond population genetics: natural epigenetic variation in wild cherry (*Prunus avium*). Tree Genetics and Genomes 11:95. https://doi.org/10.1007/s11295-015-0921-7

Ballian D, Bogunić F, Čabaravdić A, Pekeč S, Franjić J (2012) Population differentiation in the wild cherry (*Prunus avium* L.) in Bosnia and Herzegovina. Periodicum Biologorum 114(1):43-54.

Benzie IFF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299: 15-27. https://doi.org/10.1016/S0076-6879(99)99005-5

Bielinis E, Jóźwiak W, Robakowski P (2014) Modelling of the relationship between the SPAD values and photosynthetic pigments content in *Quercus petraea* and *Prunus serotina* leaves. Dendrobiology 73:125-134. https://doi.org/10.12657/denbio.073.013

Bramley H, Turner CN, Siddique NMK (2013) Water use efficiency. In: Kole C (Ed.) Genomics and Breeding for Climate-Resilient Crops Vol. 2. Springer, Berlin, Heidelberg, pp. 225-268. ISBN: 978-3-642-37047-2

Brommer JE (2011) Whither P_{ST}? The approximation of Q_{ST} by P_{ST} in evolutionary and conservation biology. Journal of Evolutionary Biology 24:1160-1168. https://doi.org/10.1111/j.1420-9101.2011.02268.x

Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10:178-182. https://doi.org/10.38212/2224-6614.2748

De Rogatis A, Ferrazzini D, Ducci F, Guerri S, Carnevale S, Belletti P (2013) Genetic variation in Italian wild cherry (Prunus avium L.) as characterized by nSSR markers. Forestry: An International Journal of Forest Research 86(3):391-400. https://doi.org/10.1093/forestry/cpt009

Dobor L, Barcza Z, Hlásny T, Havasi A, Horváth F, Ittzés P, Bartholy J (2014) Bridging the gap between climate models and impact studies: The FORESEE Database. Geoscience Data Journal 2:1-11. https://doi.org/10.1002/gdj3.22

Drake PL, Froend RH, Franks PJ (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. Journal of Experimental Botany 64(2): 495–505. https://doi.org/10.1093/jxb/ers347

Ducci F, De Cuyper B, De Rogatis A, Dufour J, Santi F (2013) Wild cherry breeding (Prunus avium L.). In: Pâques LE (Ed) Forest tree breeding in Europe. Springer, Dordrecht, pp. 463-511. ISBN: 978-94-007-6145-2

Franks PJ, Doheny-Adams TW, Britton-Harper ZJ, Gray JE (2015) Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytologist 207:188-195. https://doi.org/10.1111/nph.13347

Gailing O, Langenfeld-Heyser R, Polle A, Finkeldey R (2008) Quantitative trait loci loci affecting stomatal density and growth in a Quercus robur progeny: implications for the adaptation to changing environments. Global Change Biology 14:1934-1946. https://doi.org/10.1111/j.1365-2486.2008.01621.x

Ganopoulos IV, Aravanopoulos F, Argiriou A, Kalivas A, Tsaftaris A (2011) Is the genetic diversity of small scattered forest tree populations at the southern limits of their range more prone to stochastic events? A wild cherry case study by microsatellite-based markers. Tree Genetics and Genomes 7:1299-1313. https://doi.org/10.1007/s11295-011-0414-2

Ganopoulos I, Moysiadis T, Xanthopoulou A, Ganopoulou M, Avramidou E, Aravanopoulos FA, Tani E, Madesis P, Tsaftaris A, Kazantzis K (2015) Diversity of morpho-physiological traits in worldwide sweet cherry cultivars of Gene-

Bank collection using multivariate analysis. Scientia Horticulturae 197:381-391. https://doi.org/10.1016/j.scienta.2015.09.061

Garzón M, Alía R, Robson M, Zavala MA (2011) Intra-specific variability and plasticity influence potential tree species distributions under climate change. Global Ecology and Biogeography 20:766-778. https://doi.org/10.1111/j.1466-8238.2010.00646.x

Gömöry D, Ditmarová L, Hrivnák M, Jamnická G, Kmeť J, Krajmerová D, Kurjak D (2015) Differentiation in phenological and physiological traits in European beech (Fagus sylvatica L.). European Journal of Forest Research 134: 1075-1085. https://doi.org/10.1007/s10342-015-0910-2

Harrison EL, Arce Cubas L, Gray JE, Hepworth C (2020) The influence of stomatal morphology and distribution on photosynthetic gas exchange. The Plant Journal 101(4):768-779. https://doi.org/10.1111/tpj.14560.

Jarni K, De Cuyper B, Brus R (2012) Genetic variability of wild cherry (Prunus avium L.) seed stands in Slovenia as revealed by nuclear microsatellite loci. PLoS One 7(7):e41231. https://doi.org/10.1371/journal.pone.0041231.

Kaiser HF (1960) The application of electronic computers to factor analysis. Educational and Psychological Measurement 20:141-151. https://doi.org/10.1177/001316446002000116

Kardiman R, Ræbild A (2018) Relationship between stomatal density, size and speed of opening in Sumatran rainforest species. Tree Physiology 38:696–705. https://doi.org/10.1093/treephys/tpx149

Khaleghi A, Naderi R, Brunetti C, Maserti BE, Salami SA, Babalar M (2019) Morphological, physiochemical and antioxidant responses of Maclura pomifera to drought stress. Scientific Reports 9:19250. https://doi.org/10.1038/s41598-019-55889-y

Kebert M (2014) Biohemijska i fiziološka karakterizacija klonova topole (Populus spp.) u procesu fitoekstrakcije bakra, nikla i kadmijuma. PhD thesis, University of Novi Sad, Faculty of Natural Sciences, Serbia, pp. 250.

Kebert M, Matović B, Orlović S, Trudić B, Vuksanović V, Katanić M, Galović V (2016) Biochemical screening assays as drought indicators in spruce (Picea abies Karst.) on mountain Kopaonik. Topola 197/198:65-80.

Kobliha J (2002) Wild cherry (Prunus avium L.) breeding program aimed at the use of this tree in Czech Forestry. Journal of Forest Science 48:202–218. https://doi.org/10.17221/11876-JFS

Konôpková A, Pšidová E, Kurjak D, Stojnić S, Petrík P, Fleischer P, Kučerová J, Ježík M, Petek A, Gömöry D, Kmeť J, Longauer R, Ditmarová L (2020) Photosynthetic performance of silver fir (Abies alba) of different origins under suboptimal growing conditions. Functional Plant Biology 47(11):1007-1018. https://doi.org/10.1071/FP20040

Lawson T, Blatt MR (2014) Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiology 164:1556-1570. https://doi.org/10.1104/pp.114.237107

Lawson T, Vialet-Chabrand S (2019) Speedy stomata, photosynthesis and plant water use efficiency. New Phytologist 221(1):93-98. https://doi.org/10.1111/nph.15330

Lee Y, Choo C, Watawana MI, Jayawardena N, Waisundara V (2015) An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. Journal of the Science of Food and Agriculture 95:2956-64. https://doi.org/10.1002/jsfa.7039

Li B, Fan R, Sun G, Sun T, Fan Y, Bai S, Guo S, Huang S, Liu J, Zhang H, Wang P, Zhu X, Song C (2021) Flavonoids improve drought tolerance of maize seedlings by regulating the homeostasis of reactive oxygen species. Plant and Soil 461:389-405. https://doi.org/10.1007/s11104-020-04814-8

Lin Y, Kuang L, Tang S, Mou Z, Phillips OL, Lambers H, Liu Z, Sardans J, Peñuelas J, Lai Y, Lin M, Chen D, Kuang Y (2021) Leaf traits from stomata to morphology are associated with climatic and edaphic variables for dominant tropical forest evergreen oaks. Journal of Plant Ecology 14(6): 1115-1127. https://doi.org/10.1093/jpe/rtab060

Mariette S, Lefranc M, Legrand P, Taneyhill D, Frascaria-Lacoste N, Machon N (1997) Genetic variability in wild cherry populations in France. Effects of colonizing processes. Theoretical and Applied Genetics 94:904-908. https://doi.org/10.1007/s001220050494

Martinsson O (2001) Wild Cherry (Prunus avium L.) for timber production: consequences for early growth from selection of open-pollinated single-tree progenies in Sweden. Scandinavian Journal of Forestry Research 16:117-126. https://doi.org/10.1080/028275801300088161

Mataruga M, Isajev V, Orlović S (2013) Forest Genetic Resources. University book, University of Banja Luka, Faculty of Forestry, Republic of Srpska, pp. 399.

Mechri B, Tekaya M, Hammami M, Chehab H (2020) Effects of drought stress on phenolic accumulation in greenhouse-grown olive trees (Olea europaea). Biochemical Systematics and Ecology 92:104112. https://doi.org/10.1016/j.bse.2020.104112

Miljković D, Stefanović M, Orlović S, Stanković-Neđić M, Kesić L, Stojnić S (2019) Wild cherry (Prunus avium L.) leaf shape and size variations in natural populations at different elevations. Alpine Botany 129:163–174. https://doi.org/10.1007/s00035-019-00227-1

Miller N, Rice-Evans C (1997) Factors influencing the antioxidant activity determined by the ABTS radical cation assay. Free Radical Research 26:195-199. https://doi.org/10.3109/10715769709097799

Moradi Y, Khadivi A, Mirheidari F, Paryan S (2020) Morphological variability of naturally grown Prunus scoparia Spach accessions. Scientia Horticulturae 267:109331. https://doi.org/10.1016/j.scienta.2020.109331

Nauš J, Prokopová J, Řebíček J, Špundová M (2010) SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. Photosynthesis Research 105:265–271. https://doi.org/10.1007/s11120-010-9587-z

Orlović S, Stojnić S, Pilipović A, Pekeč S, Mataruga M, Cvjetković B, Miljković D (2014) Variation in leaf photosynthetic traits of wild cherry (Prunus avium L.) families in a nursery trial. Šumarski list 138:381-385.

Petrík P, Petek A, Konôpková A, Bosela M, Fleischer P, Frýdl J, Kurjak D (2020) Stomatal and leaf morphology response of European beech (Fagus sylvatica L.) provenances transferred to contrasting climatic conditions. Forests 11(12):1359. https://doi.org/10.3390/f11121359

Petrik P, Petek-Petrik A, Kurjak D, Mukarram M, Klein T, Gömöry D, Střelcová K, Frýdl J, Konôpková A (2022a) Interannual adjustments in stomatal and leaf morphological traits of European beech (Fagus sylvatica L.) demonstrate its climate change acclimation potential. Plant Biology. https://doi.org/10.1111/plb.13401

Petrik P, Petek-Petrik A, Konôpková A, Fleischer P, Stojnic S, Zavadilova I, Kurjak D (2022b) Seasonality of PSII thermostability and water use efficiency of in situ mountainous Norway spruce (Picea abies). Journal of Forestry Research. https://doi.org/10.1007/s11676-022-01476-3

Petruccelli R, Ganino T, Ciaccheri L, Maselli F, Mariotti P (2013) Phenotypic diversity of traditional cherry accessions present in the Tuscan region. Scientia Horticulturae 150:334-347. https://doi.org/10.1016/j.scienta.2012.11.034

Poljak I, Vahčić N, Liber Z, Tumpa K, Pintar V, Zegnal I, Vidaković A, Valković B, Kajba D, Idžojtić M (2021) Morphological and chemical diversity and antioxidant capacity of the service tree (Sorbus domestica L.) fruits from two eco-geographical regions. Plants 10:1691. https://doi.org/10.3390/plants10081691

Poljaković-Pajnik L, Drekić M, Kovačević B, Stanković-Neđic M, Stojnic Ś, Orlović S (2019) Host preference of Myzus cerasi (Fabricius, 1775) to half-sib lines of Prunus avium L. from six populations assessed in the nursery trial. Topola 203:87-94.

Popović V, Kerkez I (2016) Population variability of wild cherry (Prunus avium L.) in Serbia according to the leaf morphology. Šumarski list 140:347-355.

Possen BJ, Anttonen MJ, Oksanen E, Rousi M, Heinonen J, Kostiainen K, Vapaavuori EM (2014) Variation in 13 leaf morphological and physiological traits within a silver birch (Betula pendula) stand and their relation to growth. Canadian Journal of Forest Research 44:657-665. https://doi.org/10.1139/cjfr-2013-0493

Pšidová E, Živčák M, Stojnić S, Orlović S, Gömöry D, Kučerová J, Ditmarová L, Střelcová K, Brestič M, Kalaji HM (2018) Altitude of origin influences the responses of PSII photochemistry to heat waves in European beech (Fagus sylvatica L.). Environmental and Experimental Botany 152:97-106. https://doi.org/10.1016/j.envexpbot.2017.12.001

Rakonjac V, Fotirić Akšić M, Nikolić D, Milatović D, Čolić S (2010) Morphological characterization of 'Oblačinska' sour cherry by multivariate analysis. Scientia Horticulturae 125:679-684. https://doi.org/10.1016/j.scienta.2010.05.029

Rakonjac V, Mratinić E, Jovković R, Fotirić-Akšić M (2014) Analysis of morphological variability in Wild cherry (Prunus avium L.) genetic resources from Central Serbia. Journal of Agricultural Science and Technology 16:151-162.

Ramírez-Valiente JA, López R, Hipp AL, Aranda I (2020) Correlated evolution of morphology, gas exchange, growth rates and hydraulics as a response to

precipitation and temperature regimes in oaks (Quercus). New Phytologist 227(3):794-809. https://doi.org/10.1111/nph.16320

Rohlf FJ (2013) TpsDig2 ver 2.1.7 Ecology and evolution. SUNY, Stony Brook, USA.

Sakoda K, W Yamori, T Shimada, SS Sugano, I Hara-Nishimura and Y Tanaka (2020) Higher stomatal density improves photosynthetic induction and biomass production in Arabidopsis under fluctuating light. Frontiers in Plant Sciences 11:589603. https://doi.org/10.3389/fpls.2020.589603

Schlüter U, Muschak M, Berger D, Altmann T (2003) Photosynthetic performance of an Arabidopsis mutant with elevated stomatal density (sdd1-1) under different light regimes. Journal of Experimental Botany 54:867-874. https://doi.org/10.1093/jxb/erg087

Shahidi F, Chandrasekara A (2010) Hydroxycinnamates and their in vitro and in vivo antioxidant activities. Phytochemistry Reviews 9:147-170. https://doi.org/10.1007/s11101-009-9142-8

Sharma K, Korecký J, Soldateschi EDP, Sedlák P (2017) S-genotype diversity in wild cherry populations in the Czech Republic. Scientia Agriculturae Bohemica 48(1):92–97. https://doi.org/10.1515/sab-2017-0015

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299:152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Soler-Rivas C, Espín JC, Wichers H (2000) An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochemical Analysis 11:330-338. https://doi.org/10.1002/1099-1565(200009/10)11:5<330::AID-PCA534>3.0.CO;2-G

Stanković Neđić M, Stojnić S, Orlović S, Čolić H, Petrović D, Isajev V (2018) Varijabilnost klijavosti sjemena i morfoloških osobina sadnica divlje trešnje porijeklom iz Republike Srpske (BiH). Topola 201/202: 213-225.

Stojnić S, Orlović S, Trudić B, Živković U, Von Wuehlisch G, Miljković D (2015) Phenotypic plasticity of European beech (Fagus sylvatica L.) stomatal features under water deficit assessed in provenance trial. Dendrobiology 73:163-173. http://dx.doi.org/10.12657/denbio.073.017

Stojnić S, Kovačević B, Kebert M, Vaštag E, Bojović M, Stanković-Neđić M, Orlović S (2019) The use of physiological, biochemical and morpho-anatomical traits in tree breeding for improved water-use efficiency of Quercus robur L. Forest Systems 28:017. https://doi.org/10.5424/fs/2019283-15233

Stojnić S, Bojović M, Pilipović A, Orlović S (2021) Selecting tree species for reclamation of coal mine tailings based on physiological parameters. Topola 208:27-38. https://doi.org/10.5937/topola2108027S

Temel F (2018) Leaf size variation in natural wild cherry (Prunus avium) populations in Turkey. International Journal of Agriculture and Biology 20(9):2005-2011. https://doi.org/10.17957/IJAB/15.0722

TIBCO Software Inc (2017) Statistica (data analysis software system), version 13. URL: http://statistica.io.

Varela MC, Arslan I, Reginato MA, Cenzano AM, Luna MV (2016) Phenolic compounds as indicators of drought resistance in shrubs from Patagonian shrublands (Argentina). Plant Physiology and Biochemistry 104:81-91. http://dx.doi.org/10.1016/j.plaphy.2016.03.014

Vastag E, Kovačević B, Orlović S, Kesić L, Bojović M, Stojnić S (2019) Leaf stomatal traits variation within and among fourteen European beech (Fagus sylvatica L.) provenances. Genetika 51:937-959. https://doi.org/10.2298/GENSR1903937V

Vastag E, Cocozza C, Orlović S, Kesić L, Kresoja M, Stojnić S (2020) Half-sib lines of pedunculate oak (Quercus robur L.) respond differently to drought through biometrical, anatomical and physiological traits. Forests 11:153. https://doi.org/10.3390/f11020153

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology 13:921-935. https://doi.org/10.1046/j.1365-294X.2004.02076.x

Visi-Rajczi E, Hofmann T, Albert L, Mátyás C (2021) Tracing the acclimation of European beech (Fagus sylvatica L.) populations to climatic stress by analyzing the antioxidant system. iForest-Biogesciences and Forestry 14:95-103. https://doi.org/10.3832/ifor3542-013

Vuksanović V, Kovačevic B, Kesic L, Pavlovic L, Vaštag E, Kebert M (2020) Effect of IBA and TIBA on rhizogenesis of Wild cherry in vitro. Topola 206:5-11. https://doi.org/10.5937/topola2006005V

Vuksanović V, Kovačević B, Stojnić S, Kebert M, Kesić L, Galović V, Orlović S (2022) Variability of tolerance of Wild cherry clones to PEG-induced osmotic stress in vitro. iForest 15:265-272. http://doi.org/10.3832/ifor4033-015

Wakefield S, Topp B, Alam M (2021) Crown Position and rootstock genotype influence leaf stomatal density in Macadamia sp. Biology and Life Science Forum 11:9. https://doi.org/10.3390/

Wang J, Ma J, OuYang F, Wang J, Song L, Kong L, Zhang H (2021) Instrinsic relationship among needle morphology, anatomy, gas exchanges and tree growth across 17 Picea species. New Forests 52:509-535. https://doi.org/10.1007/s11056-020-09808-z

Yin Q, Tian T, Kou M, Liu P, Wang L, Hao Z, Yue M (2020) The relationships between photosynthesis and stomatal traits on the Loess Plateau. Global Ecology and Conservation 23:e01146. https://doi.org/10.1016/j.gecco.2020.e01146

Yang Z-Y, Ran J-H, Wang Z-Q (2012) Three genome-based phylogeny of Curessaceae s.l.: further evidence for the evolution of gymnosperms and southern Hemisphere biogeography. Mollecular Phylogenetis and Evolution 64:452-470. https://doi.org/10.1016/j.ympev.2012.05.004

Zhang J-W, D'Rozario A, Adans JM, Li Y, Liang X-Q, Jaques FM, Su T, Zhou ZK (2015) Sequoia maguanensis, a new Miocene relataive of the cost redwood, Sequoia sempervirens, from China: implications for paleography and paleoclimate. Am J. Bot 102:103-118. https://doi.org/10.3732/ajb.1400347

Zhang L, Wu S, Chang X, Wang X, Zhao Y, Xia Y, Trigiano RN, Jiao Y, Chen F (2020) The ancient wave of polyploidization events in flowering plants and their adaptation to environmental stress. Plant Cell Environ 2020:1-10. https://doi.org/10.1111/pce.13898