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VARIATION IN LEAF PHOTOSYNTHETIC TRAITS OF WILD CHERRY (*Prunus avium* L.) FAMILIES IN A NURSERY TRIAL

VARIJABILNOST FOTOSINTETIČKIH OSOBINA LISTA PORODICA DIVLJE TREŠNJE (*Prunus avium* L.) U RASADNIČKOM POKUSU

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Summary:

The paper presents the results of investigation of variability of net photosynthesis (A), transpiration (E), stomatal conductance (gs) and water use efficiency (WUE) in one year old seedlings of wild cherry (*Prunus avium* L.), assessed in a nursery trial. Study involved 5 families of half-sib progenies which originate from the Northern part of Serbia (Vojvodina Province). The results showed significant differences among families in regards of stomatal conductance (0.163–0.256 mol m^{-2} s^{-1}), transpiration (3.27–5.28 mmol m^{-2} s^{-1}) and water use efficiency (1.98–3.80 µmol $mmol^{-1}$) ($p \leq 0.001$), while differences regarding net photosynthesis (10.49–12.44 µmol m^{-2} s^{-1}) were not statistically significant ($p \leq 0.124$). Canonical discriminant analysis (CDA) was performed in order to estimate multivariate relations among analyzed leaf photosyntetical traits. Families were separated by the first canonical axis (CD1), which described 82% of variability. Presence of differences in regards of *E*, *gs* and *WUE* indicate the possibility of choosing the best families for a breeding program.

KEY WORDS: Wild cherry, leaf photosynthetic traits, half-sib progenies

INTRODUCTION
Uvod

Wild cherry (*Prunus avium* L.) is the most important European tree species in the family *Rosaceae* (Russell 2003). It is a fast growing tree, occurring generally as isolated trees, clumps, rows at edges of forest, and only rarely in denser mixed stands (Santi et al. 1998). Wild cherry is highly interesting for both ecological and economic reasons. Due to its valuable wood, the species is now increasingly planted in Europe, both in afforestation of abandoned farm land and enrichment of forests. Also, the species is important in terms of increasing biodiversity and sustainability of forest ecosystems (Stojecová & Kupka 2009).

Wild cherry breeding programs have a long tradition in Europe. According to Kobliha (2002) the greatest wild cherry programs are realized in France and Germany. Except of these countries, breeding programs are also successfully implemented in Italy, Belgium, Netherlands, Slovakia, Spain, Czech Republic, etc. Wild cherry breeding program in Serbia has been established in 2005, when inventarisation of

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wild cherry plus trees has been started according to natural distribution of the species and information about seed sources. On this occasion, totally 58 genotypes, from five localities were described. Geographical coordinates of plus trees were determined and stored in the database together with information about stem straightness, branching, diameter, height and vitality of trees (Pilipović et al. 2011). Similar activities were continued in the following years, as well. During 2011, seeds of 61 genotypes from 12 localities in Serbia, were collected and sown in seedbeds for the purposes of establishing of progeny test. These tests has an important role in plant breeding, because it provides useful information, both, about the properties of the progeny tested and parent trees.

Variations in net photosynthesis and related gas exchange parameters have been reported as determinants of plant productivity. Researches of numerous authors showed that research of these parameters can provide useful information about growth potential of genotype and plant productivity (Kundu & Tigerstedt 1998; Orlović et al. 2001; Orlović et al. 2006). For example, investigating the relationships between growth and leaf-scale physiological parameters in five WildstarTM cherry clones, Stokes and Kerr (2006) reported that varieties with high net photosynthesis tended to have good height increment and relative growth rate, while those with low net photosynthesis, transpiration and stomatal conductance had poor height increment. Similarly, discussing about possibility of use of physiological traits in tree breeding for improved yield in drought-prone environments, Pita et al. (2005) highlighted the relevance of stomatal conductance and water use efficiency, as well as hydraulic traits.

This paper presents study on the variability of leaf photosyntetic traits of five Serbian wild cherry families, assessed in a nursery trial. The objectives of the study were to: 1) estimate the magnitude of the family variability in gas exchange parameters and 2) to determine the physiological parameters which has the highest discriminatory capacity.

MATERIALS AND METHODS
Materijali i metode

The trial is situated in the nursery of the Institute of Lowland Forestry and Environment, which is located at the territory of Experimental Estate "Kaćka šuma" in Kać (N 45°17'; E 19°53'). The site is at 76 m altitude with temperate continental climate. Mean annual temperature is 11.1°C and annual precipitation sum of 624 mm. During the vegetation period (April-September) mean air temperature is 17.8°C and the sum of precipitations amounts 369 mm. The climate records are from the weather station Rimski Sancevi (N 45°20', E 19°51'; 84 m a.s.l). Temperature and precipitation have been averaged for the time period between 1966–2004.

Study involved five families of wild cherry (*Prunus avium* L.) originating from Northern part of Serbia (Figure 1). Details of the families used in trial are given in Table 1.

Seed used for the establishing of progeny trial was collected separately from each plus tree during the vegetation period of 2011 and sown in seedbeds during October of the same year. Measurements were carried out in August 2012 at the one year old seedlings.

Net photosynthesis (A, μmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}) and stomatal conductance (gs, mol m^{-2} s^{-1}) were recorded by using *ADC Bioscientific Ltd. LCPro+* portable gas analysis system, on 10 plants from each family, in five replications per single plant. Measurements were made on fully expanded leaves, between 09:00 and 11:00 hours a.m., on days with clear and sunny weather. Photosynthetic active radiation (PAR) has been set to volume of 1000 μmol m^{-2} s^{-1}, while the temperature, humidity and the concentration of CO_2 in the device's chamber were set to ambient. The ratio of net photosynthesis (A) to transpiration (E) was calculated to determine water use efficiency (WUE, μmol $mmol^{-1}$) (Zhang et al. 2004).

Statistical analyses were conducted by using software Statistica 10 (StatSoft, Inc.). Descriptive statistics and one-way

Table 1. General data about wild cherry half-sib progenies involved in study.
Tablica 1. Opći podaci o half-sib potomstvu divlje trešnje obuhvaćenom istraživanjem.

Acronym *Akronim*	Locality *Lokalitet*	Altitude (m) *Nadmorska visina (m)*	Annual mean air temperature (t °C) *Prosječna godišnja temperatura zraka (t °C)*	Annual sum of precipitation (mm) *Ukupna godišnja količina padalina (mm)*	Mean air temperature – vegetative period (t °C) *Prosječna temperatura zraka tijekom vegetacijskog perioda (t °C)*	Sum of precipitation – vegetative period (mm) *Ukupna količina padalina tijekom vegetacijskog perioda (mm)*
1	Čortanovci	87	11.2	782	18.0	351
2	Čortanovci	89	11.2	782	18.0	351
7	Molovin	143	10.9	570	17.6	343
11	Jamena	82	10.9	579	17.6	344
13	Jamena	82	10.9	579	17.6	344

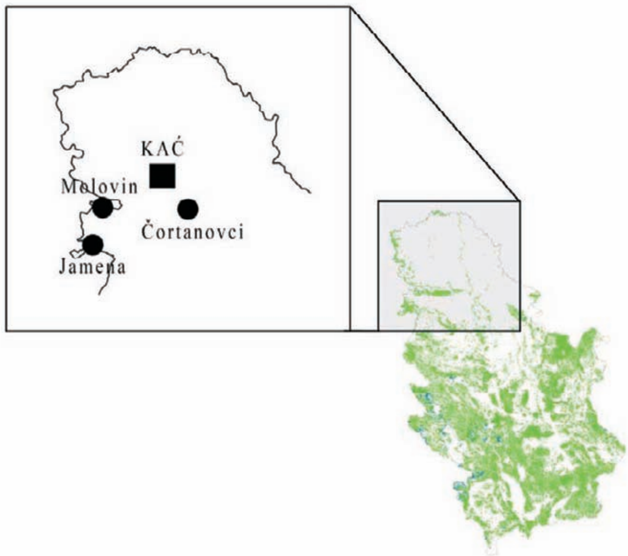

Figure 1. Geographic distribution of Wild cherry families involved in study (●) and location of nursery trial (■).

Slika 1. Geografska rasprostranjenost familija divlje trešnje obuhvaćenih istraživanjem (●) i lokacija rasadničkog pokusa (■).

analysis of variance (ANOVA) with Fisher's LSD test (post hoc analysis) were provided for each parameter in order to verify the significance of differences among provenances. In order to define the differences among the analyzed families, as well as to determine which of investigated parameters discriminate families in the highest level, canonical discriminant analysis (CDA) was performed.

RESULTS AND DISCUSSION
Rezultati i rasprava

results of our study showed that families differ significantly in regards of intensity of transpiration ($p \leq 0.001$), stomatal conductance ($p \leq 0.001$) and water use efficiency ($p \leq 0.001$), while differences, among families, regarding net photosynthesis were not statistically significant ($p \leq 0.124$) (Table 2).

As the environmental variability in nursery trials is minimized, differences in more uniform environments are likely to be the result of genetic differentiation (Meier & Leuschner 2008). Also, presence of significant differences in regards of *E*, *gs* and *WUE* indicate the possibility of choosing the best families for a breeding program in the climate conditions of Vojvodina province. Similar findings have been reported by Orlović et al. (1998) for poplar and by Orlović et al. (2006) for white willow, eventhough their results were based on the clone level.

The range of assimilation values recorded by our study is similar to those observed by other authors. For example, Niederleitner and Knoppik (1997) recorded net photosynthesis values of 9–17 μmol m^{-2} s^{-1} for five wild cherry clones in Germany. Transpiration rates recorded in our study were also similar to those of 4.5–5.5 mmol m^{-2} s^{-1} recorded by above mentioned authors. Also, stomatal conductance values were similar to those of 50–650 mmol m^{-2} s^{-1} reported for *P. davidiana* by Quilot et al. (2004).

According to Flexas et al. (2013), a key objective for sustainable forestry is to breed plants with both high carbon gain and water-use efficiency. Among the investigated families, *Family 11*, from the locality Jamena, was characterized by the highest rates of *A*, *gs* and *WUE*, and the lowest rate of *E*. Generally, families from lowland (1, 2, 11 and 13) showed more adaptive physiological response to prevailing climate conditions comparing to *Family 7*, which originates from a higher altitude (143 m a.s.l.). This is visible through the higher mean values of *A* and *WUE* in these families, as well as lower transpiration rate (Table 2). Chandra (2003) stated that the ability of plants to acclimate to different environments is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and, therefore, the growth of the whole plant.

inouanonical discriminant analysis (CDA) was applied in order to estimate multivariate relationship among analyzed

Table 2. Results of One-way ANOVA. Means followed by the same letters within a variable are not significantly different ($p \leq 0.05$).

Tablica 2. Rezultati jednofaktorijalnog ANOVA testa. Srednje vrijednosti praćene istim slovom ne razlikuju se značajno za razinu značajnosti od $p \leq 0.05$.

Family *Familija*	A (μmol m^{-2} s^{-1})		E (mmol m^{-2} s^{-1})		gs (mol m^{-2} s^{-1})		WUE (μmol $mmol^{-1}$)	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
1	11.16 ^{ab}	1.36	4.74 ^{b}	0.96	0.163 ^{c}	0.042	2.42 ^{c}	0.47
2	11.39 ^{ab}	2.22	4.36 ^{b}	0.41	0.227 ^{ab}	0.033	2.62 ^{c}	0.47
7	10.49 ^{b}	1.62	5.28 ^{a}	0.38	0.212 ^{b}	0.041	1.98 ^{d}	0.24
11	12.44 ^{a}	1.83	3.27 ^{c}	0.35	0.256 ^{a}	0.064	3.80 ^{a}	0.32
13	11.92 ^{ab}	1.33	3.57 ^{c}	0.23	0.246 ^{ab}	0.042	3.35 ^{b}	0.44
F value	F=1.91 $p \leq 0.1244$		F=24.18 $p \leq 0.0001$		F=6.40 $p \leq 0.0001$		F=34.03 $p \leq 0.0001$	

Table 3. Chi-Square test of significance for observed physiological parameters in wild cherry families.
Tablica 3. Hi-kvadrat test značajnosti dobivenih kanonijskih osi za ispitivane fiziološke parametre kod familija divlje trešnje.

	Eigen-value *Svojstvena vrijednost*	Canonicl R *Kanonička R*	Wilks' Lambda *Wilks' Lambda*	Chi-Square *Hi-kvadrat test*	df *Stepeni slobode*	p-level *Signifi-kantnost*
0	6.01	0.93	0.05	130.04	16	0.0000
1	1.05	0.72	0.38	43.39	9	0.0000
2	0.29	0.48	0.77	11.51	4	0.0214
3	0.01	0.05	0.99	0.11	1	0.7369

physiological parameters in wild cherry families. Families are separated by the first canonical axis (CD1) which describes 82% of variability (Table 4). According to this axis, there is a clear separation of *Family 11* and *Family 13* (locality Jamena) from the rest of families, mainly based on the values of water use efficiency (Table 4; Figure 2). *WUE* is important parameter in identification of families and genotypes adapted to water stress, as the optimization of carbon assimilation and minimization of water losses has been described as an adaptive trait (Ehleringer 1993). Plants could achieve high *WUE* through lower transpiration or high net photosynthesis, or both (Rouhi et al. 2007). In the present study, the highest *WUE* was registered in families 11 and 13, which had the highest net photosynthesis rate, as well as the lowest intensity of transpiration. Maintaining a high level of *WUE* plants show a specific water-saving strategy that allows them avoidance of larger water losses and moderates water absorption, which is the advantage in prolonged drought periods (Mészáros et al. 2007). Performance of the *Family 7* for water use efficiency was poor in comparison to other families in this study (Table 2). This family characterized by the lowest rate of net photosynthesis and the highest transpiration rate. This kind of water-use strategy permits absorption of water from the soil at the expense of a larger loss of water through transpiration. However, such strategy cannot be sufficient in long-lasting drought periods (Stojnić et al. 2012). Second axis (CD2) describes another 14% of variability. Separation of families by the second axis (CD2) is mostly based on the absolute values of standardized coefficient for net photosynthesis (–1.656) and stomatal conductance (1.248). *A* and *gs* are

Table 4. Standardized coefficients for canonical variables.
Tablica 4. Standardizirani koeficijenti kanonskih varijabli.

Variable *Varijabla*	CD1 *Diskr. funkcija 1*	CD2 *Diskr. funkcija 2*	CD3 *Diskr. funkcija 3*	CD4 *Diskr. funkcija 4*
E	1.29	0.68	–2.61	0.16
gs	0.36	1.25	0.45	0.03
A	–2.73	–1.66	2.72	0.97
WUE	3.06	0.69	–2.84	–0.10
Eigenvalue	6.01	1.05	0.29	0.00
Cum.prop.	0.82	0.96	0.99	1.00

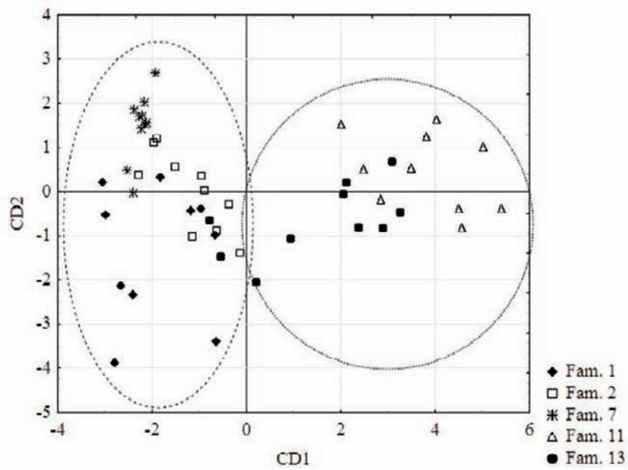

Figure 2. Scatterplot of the canonical scores of researched wild cherry families.
Slika 2. Projekcija kanonskih vrijednosti istraživanih familija divlje trešnje u prostoru.

frequently used in selection, as the growth and biomass production of plants are related to gas exchange parameters. Also, these parameters could be efficiently used in breeding, as the relationship between water stress, stomatal conductance and photosynthesis is important aspect of drought stress tolerance (Dickson & Tomlinson, 1996).

CONCLUSION
Zaključak

presence of differences in regards of *E*, *gs* and *WUE* indicate the possibility of choosing the best wild cherry (*Prunus avium* L.) families for a breeding program in the climate conditions of Vojvodina province. Due to the negligible environmental differences within the nursery trial, observed variations in stomatal conductance, transpiration and water use efficiency (WUE) could be linked to genetic differences among the investigated families. Canonical discriminant analysis revealed that parameter with the highest discriminatory capacity was water use efficiency. Based on these results it seems that *Family 11* and *Family 13* have the highest potential due to the highest rates of net photosynthesis, stomatal conductance and water use efficiency. In order to get more precise information about growth potential and adaptability of wild cherry families in Serbia it is necessary to analyze other parameters such as chemical parameters of oxidative stress, leaf morphological structure and wood anatomical properties. Based on this results, as well as, results of measurements of diameter, height, stem straightness and branching, it would be possible to determine families and genotypes with desirable properties.

ACKNOWLEDGEMENT

Zahvala

this paper was realized as a part of the project "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the period 2011–2014.

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Sažetak

Divlja trešnja je brzorastuća vrsta drveća, koja se u prirodi javlja uglavnom stablimično u obliku pojedinačnih stabala ili grupa stabala, rijetko formirajući prirodne populacije. S obzirom na ekonomsko i ekološko značenje koji divlja trešnja ima za šumarstvo, programi oplemenjivanja ove vrste imaju dugu tradiciju u Europi. Oplemenjivanje divlje trešnje u Srbiji započelo je 2005. godine kada je izvršena prva inventarizacija plus stabala na teritoriju čitave države. Tijekom 2011. godine izvršena je dodatna inventarizacija plus stabala divlje trešnje, kada je prikupljeno i sjeme od kojega je osnovan rani test potomstva.

Fiziološki parametri danas su često korišteni u oplemenjivanju glede činjenice da su istraživanja brojnih autora pokazala da oni mogu pružiti korisne informacije o potencijalu rasta i produktivnosti ispitivanih biljaka.

Pokus obuhvaćen ovom studijom osnovan je na pokusnom dobru Instituta za nizijsko šumarstvo i životnu sredinu "Kaćka šuma", u mjestu Kać, nedaleko Novog Sada. Istraživanje je obuhvatilo pet familija divlje trešnje, porijeklom iz sjevernog dijela Republike Srbije (AP Vojvodina) (Slika 1; Tablica 1). Mjerenja su provedena na jednogodišnjim sadnicama tijekom kolovoza 2012. godine i obavljena pomoću prijenosnog uređaja *ADC Bioscientific Ltd. LCPro+*. Statistička obrada podataka izvršena je u programskom paketu Statistica 10.

Rezultati su pokazali postojanje statistički značajnih razlika između familija u pogledu transpiracije ($p \leq 0.001$), stomatalne provodljivosti ($p \leq 0.001$) i učinkovitosti korištenja vode ($p \leq 0.001$), dok razlike u pogledu neto fotosinteze nisu bile statistički značajne ($p \leq 0.124$) (Tablica 2). Familija 11, s lokaliteta Jamena, okarakterizirala se najvećim vrijednostima neto fotosinteze, stomatalne provodljivosti, učinkovitosti korištenja vode, kao i najmanjom vrijednošću transpiracije. Generalno promatrano, porodice iz ravničarskog dijela (1, 2, 11 i 13) pokazale su bolju adaptiranost na klimatske uvjete u rasadniku, u usporedbi s porodicom 7, koja potječe s veće nadmorske visine. Ovo je posebno vidljivo kroz više vrijednosti neto fotosinteze i učinkovitosti korištenja vode, kao i niže vrijednosti transpiracije. Definiranje razlika između analiziranih provenijencija, kao i procjena doprinosa pojedinačnih karakteristika dobivena je iz rezultata primijenjene kanoničke diskriminantne analize (CDA). Chi-Square testom je utvrđeno statistički značajno odvajanje familija po tri kanoničke diskriminantne osi (Tablica 3). Prva kanonijska os (CD1) opisivala je 82 % svih razlika između analiziranih familija, dok su druga (CD2) i treća os (CD3) opisivale 14 %, odnosno 4 % razlika (Tablica 4). Razdvajanje familija 11 i 13 od ostalih familija, po prvoj kanonijskoj osi, u najvećoj mjeri je rezultat razlika u pogledu učinkovitosti korištenja vode (Tablica 4; Slika 2).

Kako su ekološki uvjeti u rasadnicima ujednačeni, utvrđene razlike između familija su vjerojatno posljedica njihove genetičke konstitucije. Postojanje spomenutih razlika upućuje na mogućnost izbora najboljih familija za programe oplemenjivanja u klimatskim uvjetima koji vladaju u Vojvodini. U daljnjem radu, posebnu pozornost trebalo bi obratiti na porodice 11 i 13, s obzirom da su one posjedovale najveće vrijednosti neto fotosinteze i učinkovitosti korištenja vode koje se smatraju adaptivnim svojstvima i pokazateljima tolerancije biljaka na stres od suše. Također, kako bi se dobili precizniji podaci vezano za potencijal različitih familija divlje trešnje u Srbiji, neophodna su višegodišnja istraživanja u koja bi, osim fizioloških, bili uključeni i parametri koji upućuju na oksidativni stres, odnosno morfološku i anatomsku građu biljaka.

KLJUČNE RIJEČI: divlja trešnja, fotosintetičke osobine lista, rasadnici, half-sib potomstvo, porodice.