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POPULATION GENETIC CHARACTERISTICS OF HORSE CHESTNUT IN SERBIA

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Abstract – The general population genetic characteristics of cultivated horse chestnut trees excelling in growth, phenotype characteristics, type of inflorescence, productivity and resistance to the leafminer *Cameraria ohridella* Deschka and Dimić were analyzed in Serbia. The analyzed population genetic parameters point to fundamental differences in the genetic structure among the cultivated populations in Serbia. The study shows the variability in all properties among the populations and inter-individual variability within the populations. The variability and differential characteristics were assessed using statistical parameters, taking into account the satisfactory reflection of the hereditary potential. The assessed differences in the vitality and evolution potential of different populations can determine the methods of horse chestnut gene pool collection, reconstruction and improvement.

Key words: Aesculus hippocastanum L., cultivated species, gene pool, polymorphism, selection

INTRODUCTION

A population-genetic approach to the utilization and study of ornamental trees and shrubs is a direct contribution to the solution of a series of problems related to the microevolution of cultivated woody species (Stojičić et al., 2010). In addition, the implementation of the population approach is also reflected in other fields of tree and shrub biology, i.e. on the systematics, nomenclature, diversity enrichment, etc. (Stevanović, 1995). The history of cultivar synthesis includes examples of efficient selection, among which rose breeding is unsurpassed (with more than 25,000 cultivars created from 142 biological species (Saakov et al., 1973) where entire complexes of characteristics were modified compared to biological species in the genus *Rosa* L. and the family Rosaceae Juss.).

Ornamental trees and shrubs make up only a part of the total species gene pool. There are two categories of ornamental plants: (1) introduced species, subject to adaptation and domestication processes in which functional and natural selections are interrelated, and (2) improved species, whose ornamental properties are created by man and which demand special conditions for their cultivation (a technogenic environment). There are several types of selection at the level of biological organization of ornamental species: selection that leads to ecological isolation, selection which leads to reproductive isolation, and selection which leads to the replacement of autochthonous plants by introduced or improved plants.

Many species of ornamental trees and shrubs, including horse chestnut, are characterized by structure complexity, extraordinary diversity, adaptability and longevity. Thanks to its successful domestication, i.e. cultivation, the horse chestnut has a special role in forest plantations and urban environments in Serbia and worldwide (Ocokoljić et al., 2009).

MATERIALS AND METHODS

The study areas are the sites of four horse chestnut populations in Serbia (Belgrade, Zemun, Pančevo and Avala). The populations were cultivated on the following soil types (Nejgebauer, 1971): brown forest soil (Belgrade), calcareous chernozem on a loess plateau (Zemun), chernozem with signs of gleying in loess (Pančevo), and eutric brown soil on lacustrine sediments (Avala). However, taking into account that the populations, except that on Avala, are in urban environments, the soil properties are modified in all soil horizons due to building and utility activities. The study populations, based on the degree of pollution and the length of episodes with high SO_2 and NO_2 concentrations in the air (Vukmirović et al., 1987), can be ranked as follows: extremely polluted (Pančevo), polluted (Belgrade), slightly polluted (Zemun) and relatively unpolluted (Avala). In the surroundings of Belgrade and on Mt. Avala (Tomić, 1998), there is an oak hornbeam forest – an xerothermic subassociation with butcher's broom, *Ruscus aculeatus* (Subas. *Querceto-Carpinetum serbicum aculeatetosum* Jov., 1956). In Zemun and Pančevo, the climate-zonal potential vegetation is a forest in the alliance *Aceri tatarici-Quercion* Zolyomi et Jakucs 1957 (Tomić, 1998). Based on the annual data of the Republic Hydrometeorological Service of Serbia of the mean monthly air temperatures, the average was calculated for the first decade of the 21st century: in Belgrade, 12.65°C, in Zemun, 11.90°C and in Pančevo, 12.17°C. The average mean annual air temperature in the study years deviated from 0.3°C to 0.8°C compared to the calculated averages in the previous 100-year period. The average annual precipitation in the study ten-year period was relatively low at all three localities: Belgrade, 658.55 mm; Zemun, 624.10 mm, and Pančevo, 600.56 mm. The mean annual relative air humidity during the same study period in Belgrade was 68.45%, Zemun 71.23%, and Pančevo 70.25%.

All trees in the populations were subject to preliminary study and 100 test trees were selected (25 from each population) based on the crown shape, tree height, diameter at breast height, straightness of stem, type and color of dead bark, inflorescence structure, presence, type and quantity of tannin in the leave, and seed yield.

Tree height was measured using a Blume-Leiss altimeter; diameters at breast height by standard calipers, and bark thickness by micrometer to the nearest 0.1 mm. The abundance of seed yield was evaluated by the quantification of phenological observations, from 0-5 points, using whole numbers and not decimal numbers. The number of seeds per one kilogram and the mass of 100 seeds was determined for each selected test tree.

The morphological analysis of inflorescences was performed on inflorescences collected on May 10th. The inflorescences were taken from the lower third of the crown, from its southern aspect in the same phase of maturity, from which leaf samples were collected in July for the quantification of physiologically significant secondary metabolites – tannins. The seeds were collected in September of the same year. Flowers were determined visually: unmagnified and with a microscope, without control of their sexual functionality.

The collected leaves were dried naturally and powdered directly before analysis. The results (mean value of a minimum three measurements) were calculated for oven-dried material, since the moisture content was also always determined by a gravimetric method (PH.Jug.IV). The presence of tannin in 1% decoction of dried ground leaves was determined by sediment reactions with strychnine sulphate solution, gelatin solution and heavy metal salts. The group of tannins present was determined using a stain response to $FeCl_3$ solution and the tannoform test with HCl and HCOH in which the tannoform sediment is separated after cooking. The sediment was then neutralized with sodium acetate and $FeCl_3$ solution was added, which caused staining that confirmed the group (type) of present tannins. Tannin percentage was determined gravimetrically in 1% drug decoction. First, the tannins were precipitated with a solution of Zn (NH_3) acetate. Their quantity was determined from the difference in Zn-tannate and Zn-oxide mass obtained by tannate combustion and tannate annealing.

For the assessment of horse chestnut seed yield in Serbia, seeds were collected by the end of September, in the phase of complete (technical) maturity.

The quantitative data were processed using Statistica 9.0.

RESULTS

Horse chestnut populations in Serbia are characterized by polymorphism of several properties. The phenogroups (Table 1) in the four study populations were classified based on crown features (wide conical crown (A) with diameter below 12 m, conical crown (B) with diameter below 8 m and irregular crown (C) with diameter below 15 m); dead bark characteristics (smooth bark (D) without fissures, shallowly fissured (E) with fissures less than 2 mm deep, fissured (F) with fissures less than 4 mm; grey-brown bark (G) and dark brown bark (H) without grey nuances); and stem straightness and branching features (trees with monopodial growth (I) without forking and forked trees (J), low – below 2 m, medium – at the height of 2 to 5 m, and high – above 5 m).

There was also variability among the studied populations with regard to tree height and diameter at breast height. On Mt. Avala, the mean height was 13.12 m and the diameter at breast height was 21.10 cm; in Pančevo they were 10.88 m and 21.38 cm, in Belgrade 8.74 m and 20.59 cm, and in Zemun 8.12 m and 18.00 cm, respectively (Table 2).

The morphological analysis of inflorescences showed a specific arrangement of flowers in the inflorescences: the flowers were macrostylous at the bottom of the inflorescence, homostylous in the middle, and on the top of the inflorescence, the flowers were microstylous. The number of flowers per inflorescence within each category was uniform, i.e., in all studies populations the inflorescences showed approximately the same average numbers (Table 2): microstylous (Belgrade 31, Zemun 32, Pančevo, Avala 33), homostylous (Belgrade and Zemun 17, Pančevo 19, Avala 18) and macrostylous (Belgrade 13, Zemun and Avala 14, Pančevo 15).

The yield in the horse chestnut populations in Belgrade, Zemun, Pančevo and on Mt. Avala, during the studied vegetation period was abundant. The total yield was evaluated as follows: Belgrade – 4 points in 52% of trees, 5 points in 43% and 3 points in 5%; Zemun – 4 points in 41% of trees and 5 points in 59%; Pančevo – 4 points in 46% of trees and 5 points in 54%; Avala – 4 points in 63% of trees, 5 points in 24% and 3 points in 13% of the study trees.

The studied horse chestnut populations differentiated in the number of seeds per one kilogram and a mass of 100 seeds (Table 3). The number of seeds per kilogram was 61 in Belgrade, 52 in Zemun, 60 in Pančevo and 96 on Avala. The mass of 100 seeds per kilogram varied per population from 1.71 kg in Belgrade, 2.26 kg in Zemun, 1.56 kg in Pančevo to 1.53 kg on Avala.

The reactions performed on horse chestnut leaves showed that they did not contain pyrogallic tannins, only catechin tannins. There was a great difference in the quantity of tannins between the leaves of horse chestnut trees infested by the leafminer *Cameraria ohridella* Deschka and Dimić and the leaves of trees that were not infested. In the leaves of infested trees, the quantity of tannin was 17.70% in Belgrade, 23.55% in Zemun, 27.35% in Pančevo and 25.00% on Avala compared to 7.92% in Belgrade, 13.45% in Zemun and 12.70% in Pančevo of tannin in the leaves of trees of the same populations not infested by leafminers.

DISCUSSION

There are three phenogroups of habit aspect in the studied populations in Serbia: wide conical, conical, and irregular (Table 1). Considering that it has been reported (Ocokoljić et al., 2009; Ocokoljić, 2011) that an irregular wide globular habit is typical of the species (identified in the highest percentage in the four studied populations: 48 in Zemun, and 60 in the

Table 1. Segregation of phenogroups of horse chestnut trees in populations in Serbia

Properties / Locality	Crown form			Dead bark					Straightness	
	A	B	C	D	E	F	G	H	I	J
	Frequency									
Belgrade	6 :	4 :	15	12 :	7 :	6	15 :	10	8 :	17
Zemun	10 :	3 :	12	8 :	4 :	13	5 :	20	16 :	9
Pančevo	5 :	5 :	15	12 :	7 :	6	12 :	13	11 :	14
Avala	8 :	2 :	15	8 :	8 :	9	25 :	0	10 :	15

Table 2. Biometrical parameters of horse chestnut test trees in Serbia

Locality	Min. –Max.	$\bar{x} \pm S\bar{x}$	$S \pm S_S$	$V \pm S_V$
Tree height (m)				
Belgrade	5 – 12	8.74 ± 0.46	1.45 ± 0.32	16.56 ± 3.70
Zemun	6 – 11	8.12 ± 0.24	1.31 ± 0.30	14.51 ± 2.99
Pančevo	7 – 16	10.88 ± 0.58	2.18 ± 0.22	34.21 ± 5.16
Avala	11 – 15	13.12 ± 0.27	1.44 ± 0.17	10.50 ± 2.50
Diameter at 1.30 m (cm)				
Belgrade	10 – 34	20.59 ± 1.29	6.70 ± 1.24	32.38 ± 7.24
Zemun	12 – 27	18.00 ± 1.21	4.18 ± 1.25	22.24 ± 5.16
Pančevo	13 – 39	21.38 ± 1.44	7.20 ± 1.25	37.24 ± 7.21
Avala	12 – 42	21.10 ± 2.15	7.24 ± 1.54	44.44 ± 8.66
Number of functionally ♂ (microstylous) flowers in inflorescence				
Belgrade	30 – 32	31 ± 1.00	3.45 ± 1.12	4.96 ± 1.22
Zemun	29 – 35	32 ± 3.00	8.41 ± 2.25	10.24 ± 2.88
Pančevo	29 - 37	33 ± 4.00	11.14 ± 2.47	12.25 ± 3.14
Avala	31 - 35	33 ± 2.00	14.54 ± 3.24	17.14 ± 4.14
Number of functionally ♀♂ (homostylous) flowers in inflorescence				
Belgrade	15 – 19	17 ± 2.00	6.21 ± 1.41	11.84 ± 2.78
Zemun	16 – 18	17 ± 1.00	4.74 ± 1.56	13.24 ± 3.78
Pančevo	17 - 21	19 ± 2.00	6.24 ± 1.24	11.87 ± 3.53
Avala	17 - 19	18 ± 1.00	1.74 ± 0.54	6.21 ± 1.95
Number of functionally ♀ (macrostylous) flowers in inflorescence				
Belgrade	11 – 15	13 ± 2.00	3.56 ± 1.54	15.30 ± 2.35
Zemun	12 – 16	14 ± 2.00	2.71 ± 1.03	11.87 ± 2.40
Pančevo	14 - 16	15 ± 1.00	1.89 ± 0.57	5.34 ± 1.49
Avala	10 - 18	14 ± 4.00	7.98 ± 1.72	20.14 ± 6.00

Table 3. Statistical parameters of horse chestnut seed properties

Locality	$\bar{x} \pm S\bar{x}$	$S \pm S_S$	$V \pm S_V$
Mass of 100 seeds (kg)			
Belgrade	1.71 ± 0.07	0.19 ± 0.04	16.54 ± 3.12
Zemun	2.26 ± 0.14	0.36 ± 0.09	12.58 ± 1.45
Pančevo	1.56 ± 0.04	0.29 ± 0.05	12.98 ± 2.14
Avala	1.53 ± 0.10	0.47 ± 0.04	31.67 ± 5.24
Number of seeds per 1 kilogram			
Belgrade	61.00 ± 4.00	15.22 ± 2.20	26.56 ± 4.14
Zemun	52.00 ± 1.00	5.15 ± 1.24	10.54 ± 2.21
Pančevo	60.00 ± 2.00	10.18 ± 1.01	16.90 ± 2.51
Avala	96.00 ± 8.00	25.24 ± 5.84	27.15 ± 6.25

other three populations), the selection of genotypes with conical habit (16% Belgrade, 12% Zemun, 20% Pančevo and 8% Avala) and wide conical habit (24% Belgrade, 40% Zemun, 20% Pančevo and 32% Avala) should be pointed out.

Regarding dead bark structure, there are three phenogroups (Table 1). As it has been reported (Vilotić, 2000; Ocokoljić et al., 2003) that deeply fissured bark is typical of the species (24% Belgrade and Pančevo, 52% Zemun and 36% Avala), the genotypes with smooth bark (48% Belgrade and Pančevo; 32% Zemun and Avala) and shallowly fissured bark (28% Belgrade and Pančevo, 16% Zemun, and 32% Avala) were observed.

There were two phenogroups with dead bark color (Table 1). The phenogroup characterized by a grey-brown color (all trees on Avala, 60% of trees in Belgrade, 20% in Zemun and 48% in Pančevo) was reported (Vilotić, 2000; Ocokoljić, 2011). However, the phenogroup with dark brown bark without grey nuances, which has not been reported in the literature, was found in three populations (40% Belgrade, 80% Zemun and 52% Pančevo), but was not observed in the population on Mt. Avala.

The genotypes of the new selected phenogroups with a special visual appearance can be taken as the initial material for the synthesis of cultivars suitable for cultivation in urban environments.

There are two phenogroups of branching and stem straightness (Table 1). The phenogroup with monopodial growth accounted for 32% in Belgrade, 64% in Zemun, 44% in Pančevo and 40% on Avala. The phenogroup with forked trees, reported in the literature (Ocokoljić et al., 2003), accounted for 68% in Belgrade, 36% in Zemun, 56% in Pančevo and 60% on Avala. The genotypes selected in the phenogroup with monopodial growth can be taken as the initial material for the synthesis of cultivars suitable for cultivation in forest plantations.

The height of the horse-chestnut test trees varied from 5 m (Belgrade) to 16 m (Pančevo), and the diameter at breast height varied from 10 cm (Belgrade) to 42 cm (Avala) (Table 2). Based on comparative analysis of reference data (Richards, 1983), stating a height of 30 m and a diameter at breast height of 100 cm, the maximal mean value of height and diameter at breast height at the population level was calculated on Avala (13.12 m), and in Pančevo (21.38 cm). The result calculated for tree height confirmed the initial hypothesis that the studied population on Avala will achieve the best growth parameters because it is cultivated in conditions that are similar to environmental conditions in natural horse chestnut populations. On the other hand, the result calculated for the diameter at breast height does not correlate with published data (Richards, 1983; Venugopal et al., 2007) which suggests that the diameter at breast height increases proportionally with tree height and that trees cultivated in urban environments exhibit reduced growth parameters. The genotypes that were selected based on the achieved growth elements should be multiplied, because they are significant to landscape architecture, horticultural practice and forestry.

The genus *Aesculus* L. has a specific inflorescence structure (Dirr, 1990; Stojičić et al., 2010). The results of such studies are of special significance as the study of variability and sexuality is necessary for successful breeding using selection and hybridization. Our study showed a specific arrangement of flowers in the inflorescences, and the number of flowers within each category was more or less uniform (Table 2).

The studied horse chestnut populations differentiated in the variability of the number of seeds per one kilogram and the mass of 100 seeds (Table 3). The number of seeds per kilogram varied from 51 in Zemun to 104 on Avala, which is in direct correlation with the variation in the mass of 100 seeds from 2.40 kg in Zemun to 1.43 kg on Avala. The variability of seed characteristics was confirmed by the value of variation coefficients obtained by this biometric analysis. The mass of 100 seeds was higher than that reported as the species average value by 34% on Avala, 36% in Pančevo, 98% in Zemun and 50% in Belgrade (Ocokoljić, 2006).

In the first decade of the 21st century there was mass occurrence of horse-chestnut leafminers (*Cameraria ohridella* Deschka and Dimić), resulting in early defoliation and physiological weakness. This can retard growth and prevent tissue maturation, causing the freezing of one-year-old shoots during winter, and even the death of individual branches and entire trees. The occurrence of horse-chestnut leafminers was first detected on the territory of Ohrid in 1985 (Simova-Tošić et al., 1985; Dimić, 1995). Taking into account its sudden spread in Europe and throughout the world (Skurhavy, 1998; Tomiczek et al., 1998), and the consequences, it is important to select genotypes that were not infested by leafminers.

In the leaves of trees infested by leafminers, the quantity of tannin was from 1.75 (Zemun) to 2.23 (Belgrade) times higher compared to the leaves of uninfected trees. The direct correlation of the quantity of tannin in the leaves and the presence of pathogens was confirmed in the trees in all the populations.

CONCLUSIONS

The general characteristics of horse chestnut populations were studied. The population genetic diversity has some selection and technological advantages, but at the same time it reflects the specific genetic potential, having in mind the higher tendency to mutations, gene drift and subspontaneous hybridization.

The results of multiannual analyses at the population level contribute to the study of the genetic potential of horse chestnut populations cultivated in Serbia. The study of variability of several morphological-physiological characters of horse chestnut trees, leaves, inflorescences and seeds has resulted in information on the degree of variability and the existence of genotypes excelling in specific properties. Uniform ecological factors can be assumed in each study population. The proved individual variability can be ascribed to differences in the genetic constitution of the test trees. The differences at the interpopulation level, in addition to differences in the genetic constitution of the trees, are also conditioned by ecological factors.

As the secondary metabolites represent the mechanism of plant adaptation to ecological factors and prevent bacterial, fungal and viral infections, i.e. enable species survival, it is important to select resistant genotypes. Taking into account the presented facts, it can be concluded that the dependence between the quantity of tannin in the leaves and the degree of leafminer infestation is under strong genetic control. Horse chestnut varieties resistant to *Cameraria ohridella* Deschka and Dimić can be synthesized by the selection of superior genotypes and their multiplication.

The results of the study of horse chestnut variability are the basis for the selection of genotypes for application in landscape architecture, horticulture, forestry and for the establishment of plantations intended for the pharmaceutical industry and chemical processing. In addition, they are the basis for the conversion of horse chestnut potential variability into free variability. The research confirmed that a species is not a simple typological unit, but that it has a complex population structure.

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