Agricultura 19: No 1: 25-32(2022)
https://doi.org/10.18690/agricultura.19.1.25-32.2022

University of Maribor Press

Intra and between population genetic relationships among wild cherries [*Prunus avium* (L.) L.] based on molecular markers

Tina TERNJAK*, Metka ŠIŠKO
University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, 2311 Hoče, Slovenia

ABSTRACT

Wild cherries [*Prunus avium* (L.) L.] are considered typical trees of the Slovenian landscape and often form vigorous natural populations. The determination of genetic relationships among cultivated and wild cherry tree genotypes is important for scientific and economic reasons. Both old trees and young plants were included in our genetic analysis. Sampling took place at two different locations near Maribor, NE Slovenia. The aim of the study was to determine the genetic relationships and variability among plants belonging to the studied populations. We focused on young trees and seedlings growing around old trees, which could be their hypothetical parents. We also analysed genetic uniformity within populations and identified genetically distant plants that had likely migrated as seeds from other populations. Three microsatellite loci were taken into account, 38 genotypes were examined, and 19 polymorphic alleles were detected, corresponding to an average of 6.3 per locus. Genetic distances between the studied genotypes were calculated using the Jaccard's coefficient and a dendrogram was constructed. Genetic structure was analysed using both Principal Coordinate Analysis (PCoA) and Bayesian model-based analysis. Our study showed that there were differences between the two populations, however, there was some "communication" between them. We observed genetic relatedness as well as a relatively high level of uniformity within each of the wild cherry populations.

Key words: wild cherry**,** *Prunus avium* (L.) L., population, SSR molecular markers, genetic relatedness

INTRODUCTION

Wild cherries, which often form natural populations, are among the most important good quality timber species of the Rosaceae family and are very typical in some areas of the Slovenian countryside. Their natural (wild) populations are usually large and well adapted to the existing environments. They can cover several square kilometers and appear to have limited phenotypic variability and relatively high genetic stability (Ivančič, 2002b). Wild cherries inhabit deciduous forests, especially on sunny slopes throughout Slovenia, flowering before the canopies turn green. In some regions, the cherry tree traditionally announces spring (Smole, 2000). The name 'sweet cherry' is widely used for commercial forms, while the term 'wild cherry' is associated with a 'natural' or 'self-grown' form of this species (Vaughan et al., 2007).

Wild cherries are an important source of genetic material used for breeding new cultivars and rootstocks. They are also important ornamental plants in urban areas, city avenues, public green spaces, parks, and large home gardens. The flowers attract bees, the fruits and seeds serve as food for various animals, and the canopies harbor numerous organisms (butterflies, birds, etc.). In this way, they make an important contribution to the preservation of animal diversity in rural areas.

Most cherries are diploid (with 2n = 16); there are also genotypes with triploid (3n = 24) and tetraploid (2n = 4x = 32) number of chromosomes (Ivančič, 2002a). In addition to sexual reproduction via insect pollination, wild cherries can also spread vegetatively by root suckers that form clonal

**Correspondence to:*
E-mail: tina.ternjak1@um.si

groups. They are allogamous, meaning that self-fertilisation is prevented by the mechanism of self-incompatibility, controlled by a multi-allellic locus S, with gametophytic expression (Crane and Lawrence, 1928). According to Schueler et al. (2003), in natural populations, these factors have a major impact on genetic diversity and gene transfer between populations of a particular species through migration, which involves the transfer of pollen, seeds, and suckers. In the case of cherries, our observations suggest that bird-dispersed seeds are probably the most important.

The ability to distinguish between individual genotypes of cherries, both cultivated and wild, is important for sustainability, conservation and maintenance of genetic resources as well as for food production and economic reasons (Jarni et al., 2012; Antić et al., 2020). Traditional methods for determining the identity of individual cultivars (varieties) in fruit species are based on phenotypic observations, systematically presented as descriptors. Due to the large amount of data, which is often difficult to access and usually not completely reliable, as the environment, especially cultivation methods, and the age of the plants have a very strong impact (Kazija et al., 2014). Consequently, the new methods are based on a molecular approach. The use of microsatellite molecular (SSR) markers in genome mapping and DNA fingerprinting has been widely used among the cherries (Hormaza, 2002; Aka Kacar et al., 2005; De Rogatis et al., 2013) and in other economically important members of the genus *Prunus* such as: almonds, apricots, peaches, plums, etc. (Mnejja et al., 2004; Chin et al., 2014; Horvath et al., 2011).

The main objective of this study was to determine the genetic similarity within each of the two studied wild cherry populations and diversity between the two populations using SSR molecular markers. Genetic analysis included old trees and young plants growing nearby. We hypothesised that there would be differences between the two studied populations due to distance associated with altitude and soil characteristics. We also tried to determine if there was a genetic relationship between these populations that might be the result of seed transfer by birds or pollen transfer by insects. In addition, we were also interested in genetic uniformity within each population, genetic purity, and the number of strongly deviating genotypes that might be migrants from other populations.

MATERIALS AND METHODS

Plant material

In spring 2010, young and healthy leaf tissue material was collected from 38 wild cherry trees from two locations considered as different populations. The air distance between the two populations was approximately 1 km. Twenty-six samples were collected from three subpopulations (upper, lower, and lateral) located next to the Slovene Plant Gene Bank (SPGB) and compared with 12 samples from two subpopulations (upper, lower) from the location named Vila, situated above the Faculty of Agriculture and Life Sciences, Upper Pivola near Hoče, Slovenia. Five samples were taken from old trees (5 genotypes) and the rest (33 samples, i.e., 33 genotypes) were taken from young plants growing nearby. Table 1 lists the data of the collected material, including the information about the location (GPS coordinates), the type of tree and the abbreviations used in the figures.

Molecular analysis

DNA isolation and molecular markers analyses

DNA extractions from the fresh, young leaf material were performed using the CTAB protocol described by Doyle and Doyle (1987) The DNA concentration for each sample was estimated using a DNA fluorimeter TKO 100 (Hoefer, Holliston, MA). Two separate extractions per tree were performed.

A polymerase chain reaction (PCR) was used to amplify DNA sections. The total of 15 µl reaction volume contained: genomic DNA (5 µl), deionized water (4,51 µl), 10x PCR buffer (1,5 µl), MgCl2 (1,2 µl), dNTP (1,2 µl) (Invitrogen, California, USA), 0.75 µl of each primer (forward and reverse) (Sigma-Aldrich (St. Louis, USA) and enzyme Taq polymerase (0,09 µl) (Invitrogen, California, USA). All studied accessions were analysed using three SSR-markers developed in cherry: EMPA003, EMPA004 and EMPA005 (Clarke and Tobutt, 2003). Markers were selected based on previously published results and amplification quality. PCR was performed according to the originally published protocol (Clarke and Tobutt, 2003) in a thermocycler (Biometra TProfessional, Germany). PCR products were analysed with the CEQ™8000 Genetic Analysis System (Beckman Coulter Inc., USA), according to manufacturers' instructions, using a fluorescently labeled size marker (Beckman Coulter DNA Size Standard Kit 400 bp, Brea, California, USA).

Data analysis

For each SSR locus, the following measures of polymorphism were estimated: number of alleles per locus (n), number of effective alleles per locus (n_e), observed heterozygosity (H_o) and expected heterozygosity (H_e). Variability was calculated using the Identity 1.0 programme (Wagner & Sefc, 1999).

Genetic relatedness among the studied accessions was evaluated using the Unweighted neighbour-joining method, and structure was analysed with the Principal Coordinate Analysis (PCoA) and the Bayesian method.

Microsatellite allele data were converted to a binary matrix. Dissimilarities were calculated using the Jaccard's coefficient and the Unweighted Neighbor-Joining dendrogram was created. The PCoA calculations were based on the Euclidean distance that was used to assign each accession to a location in a two-dimensional space. DARwin 6.0.21 software (Perrier and Jacquemoud-Collet, 2006) was used to analyse the data and construct the figures for both methods. The Bayesian model-based analysis was performed using the STRUCTURE V2.3.4 software package (Pritchard et al., 2000) and admixture model with the correlated allele

Table 1: Thirty-eight wild cherry genotypes included in the study

Sample No.	*Location of the material*			Note	Abbreviation
	Name	*Latitude (°N)*	*Longitude (°E)*		
1	SPGB Upper part	46° 30' 29"	15° 38' 13"	Old tree	1_SPGB_UP_OLD
2	SPGB Upper part	46° 30' 29"	15° 38' 13"	Young tree	2_SPGB_UP
3	SPGB Upper part	/	/	Young tree	3_SPGB_UP
4	SPGB Upper part	46° 30' 29"	15° 38' 13"	Young tree	4_SPGB_UP
5	SPGB Upper part	/	/	Young tree	5_SPGB_UP
6	SPGB Upper part	46° 30' 29"	15° 38' 13"	Young tree	6_SPGB_UP
7	SPGB Upper part	46° 30' 29"	15° 38' 13"	Young tree	7_SPGB_UP
8	SPGB Upper part	46° 30' 29"	15° 38' 13"	Young tree	8_SPGB_UP
9	SPGB Upper part	/	/	Young tree	9_SPGB_UP
10	SPGB Lower part	46° 30' 29"	15° 38' 12"	Young tree	10_SPGB_LOW
11	SPGB Lower part	46° 30' 29"	15° 38' 13"	Young tree	11_SPGB_LOW
12	SPGB Lower part	46° 30' 29"	15° 38' 13"	Young tree	12_SPGB_LOW
13	SPGB Lower part	46° 30' 29"	15° 38' 13"	Old tree	13_SPGB_LOW _OLD
14	SPGB Lower part	46° 30' 29"	15° 38' 13"	Young tree	14_SPGB_LOW
15	SPGB Lower part	/	/	Young tree	15_SPGB_LOW
16	SPGB Lower part	46° 30' 29"	15° 38' 13"	Young tree	16_SPGB_LOW
17	SPGB Lower part	/	/	Young tree	17_SPGB_LOW
18	SPGB Lower part	/	/	Young tree	18_SPGB_LOW
19	SPGB Lower part	/	/	Young tree	19_SPGB_LOW
20	SPGB Lateral part	46° 30' 29"	15° 38' 11"	Old tree	20_SPGB_LAT _OLD
21	SPGB Lateral part	46° 30' 29"	15° 38' 11"	Young tree	21_SPGB_LAT
22	SPGB Lateral part	/	/	Young tree	22_SPGB_LAT
23	SPGB Lateral part	46° 30' 29"	15° 38' 11"	Young tree	23_SPGB_LAT
24	SPGB Lateral part	/	/	Young tree	24_SPGB_LAT
25	SPGB Lateral part	/	/	Young tree	25_SPGB_LAT
26	SPGB Lateral part	46° 30' 29"	15° 38' 11"	Young tree	26_SPGB_LAT
27	Villa Upper part	46° 30' 61"	15° 38' 24"	Young tree	27_Villa_ UP
28	Villa Upper part	46° 30' 61"	15° 38' 24"	Young tree	28_Villa_ UP
29	Villa Upper part	46° 30' 60"	15° 38' 25"	Young tree	29_Villa_ UP
30	Villa Upper part	46° 30' 61"	15° 38' 24"	Old tree	30_Villa_ UP _OLD
31	Villa Upper part	/	/	Old tree	31_Villa_ UP_OLD
32	Villa Upper part	46° 30' 60"	15° 38' 25"	Young tree	32_Villa_ UP
33	Villa Upper part	46° 30' 61"	15° 38' 24"	Young tree	33_Villa_ UP
34	Villa Upper part	46° 30' 61"	15° 38' 25"	Young tree	34_Villa_ UP
35	Villa Lower part	46° 30' 60"	15° 38' 25"	Young tree	35_Villa_ LOW
36	Villa Lower part	46° 30' 60"	15° 38' 27"	Young tree	36_Villa_ LOW
37	Villa Lower part	46° 30' 60"	15° 38' 27"	Young tree	37_Villa_ LOW
38	Villa Lower part	46° 30' 60"	15° 38' 27"	Young tree	38_Villa_ LOW

frequencies was chosen. The parameter K was set from 1-10 inferred clusters, with 20 independent iterations for each simulation. For each run, 100,000 burn-in periods followed by 750,000 MCMC (Markov Chain Monte Carlo) replicates were applied. The most relevant parameter K for the analysed data was estimated, by calculating ΔK based on the method of Evanno (Evanno et al., 2005), implemented using the Structure Harvester V0.6.94 application (Earl and vonHoldt, 2012).

RESULTS AND DISCUSSION

Allele length determination

The microsatellite fragments amplified with three combinations of oligonucleotides enabled discrimination between the analysed genotypes. Examination of electrophoretic curves identified unique combinations of amplified microsatellite markers for each individual plant.

The statistical calculations associated with the SSR-marker system of 38 wild cherry genotypes are presented in Tables 2 and 3. When comparing allele lengths with those published by (Clarke and Tobutt, 2003), base pairs (bp) discrepancies were observed for all three markers: EMPA003, EMPA005, EMPA004 with up to 4, 35 and 36 bp, respectively. Discrepancies in allele length were expected, as these were due to the use of different electrophoretic conditions and systems. Subjective assessment of the lengths of the PCR products may also have a partial effect.

Evaluation of SSR polymorphism

Three primer pairs produced a total of 19 alleles and an average of 6.3 alleles per locus (Table 2). Because the information value of the locus also depends on the frequency of alleles, we additionally calculated the average number of effective alleles (1.398). The lowest number of alleles was detected on EMPA003 microsatellite locus (3), on EMPA004 locus (7), and the most polymorphic locus was the EMPA005 with 9 alleles. The observed heterozygosity (Ho) values ranged between 0.205 for EMPA003 and 0.718 for EMPA005. Expected heterozygosity (He) ranged from 0.093 for the EMPA003 to 0.412 for the EMPA005.The observed and expected heterozygosity differed for each locus. The highest difference was observed for EMPA004 (0.335) and the lowest for EMPA003 (0.112). On average, heterozygosity at all loci was high (0.684), indicating relatively high variability in wild cherries. Cherries as a botanical species include numerous "wild" genotypes and cultivated varieties that differ in their morphological characteristics, productivity, and adaptation to a certain environment, thus showing high genetic variability (Ivančič, 2002a). The mean value of observed heterozygosity (0.684) differed greatly from the expected heterozygosity (0.262).

Table 2: Parameters of genetic variability calculated for 38 *P. avium* genotypes: number of alleles (n), effective number of alleles (ne), observed (Ho) and expected (He) heterozygosity

Locus	n	n_e	H_o	H_e
EMPA003	3	1.102	0.205	0.093
EMPA004	7	1.389	0.615	0.28
EMPA005	9	1.702	0.718	0.412
Average	6.3	1.398	0.684	0.262

To facilitate genotyping at each locus, letters were assigned to alleles: the shortest allele at the locus belongs to letter A, the next to B, and so on. At each locus, the number of letters corresponds to the number of different alleles. Genotypes in which only one allele was replicated were assumed to be homozygous for that allele. The highest number of homozygotes was observed at EMPA003 locus, 30 (heterozygotes 8), following by EMPA004 with 14 (heterozygotes 24) and 10 (heterozygotes 28) found at EMPA005 locus (data not shown in the table).

The calculated frequencies of the individual alleles were relatively low, with the exception of some alleles on EMPA004 and EMPA005 loci, which resulted in lower values of calculated effective alleles. The EMPA003 locus has the most commonly represented allele C (175 bp), the EMPA004 locus the E allele (187 bp), and the EMPA005 locus the H allele (255 bp). The frequencies of the individual alleles are shown in Table 3.

Table 3: Allele size (bp) and allele frequencies (in parenthesis) of the 38 *P. avium* genotypes at three microsatellite loci

	Locus		
Alleles	EMPA003	EMPA004	EMPA005
A	166 (0.05)	145 (0.05)	209 (0.05)
B	171 (0.231)	155 (0.026)	219 (0.05)
C	175 (0.923)	159 (0.026)	239 (0.026)
D	/	181 (0.436)	241 (0.154)
E	/	187 (0.667)	245 (0.256)
F	/	189 (0.256)	247 (0.103)
G	/	191 (0.016)	253 (0.462)
H	/	/	255 (0.513)
I	/	/	257 (0.077)

Evaluation of genetic relatedness

Neighbor-Joining tree based on SSR data underlines three main clusters that divide the two analysed populations, SPGB and Vila, into several groups (Figure 1). The SPGB UP subpopulation is quite scattered according to its position in the dendrogram. A group of three genotypes with an interesting connection can be observed in the Cluster I. Samples 1, 6, and 4, of which 1 represents an old tree, are genetically very close to genotype 6, suggesting the possibility that it originated from a seed produced by self-fertilization or hybridization with a genetically highly related genotype, while sample 4 probably represents a hybrid with a genetically distant genotype. Within the cluster II, the attention is drawn to sample pairs 2 and 3 and 7 and 9, where the bootstrap value

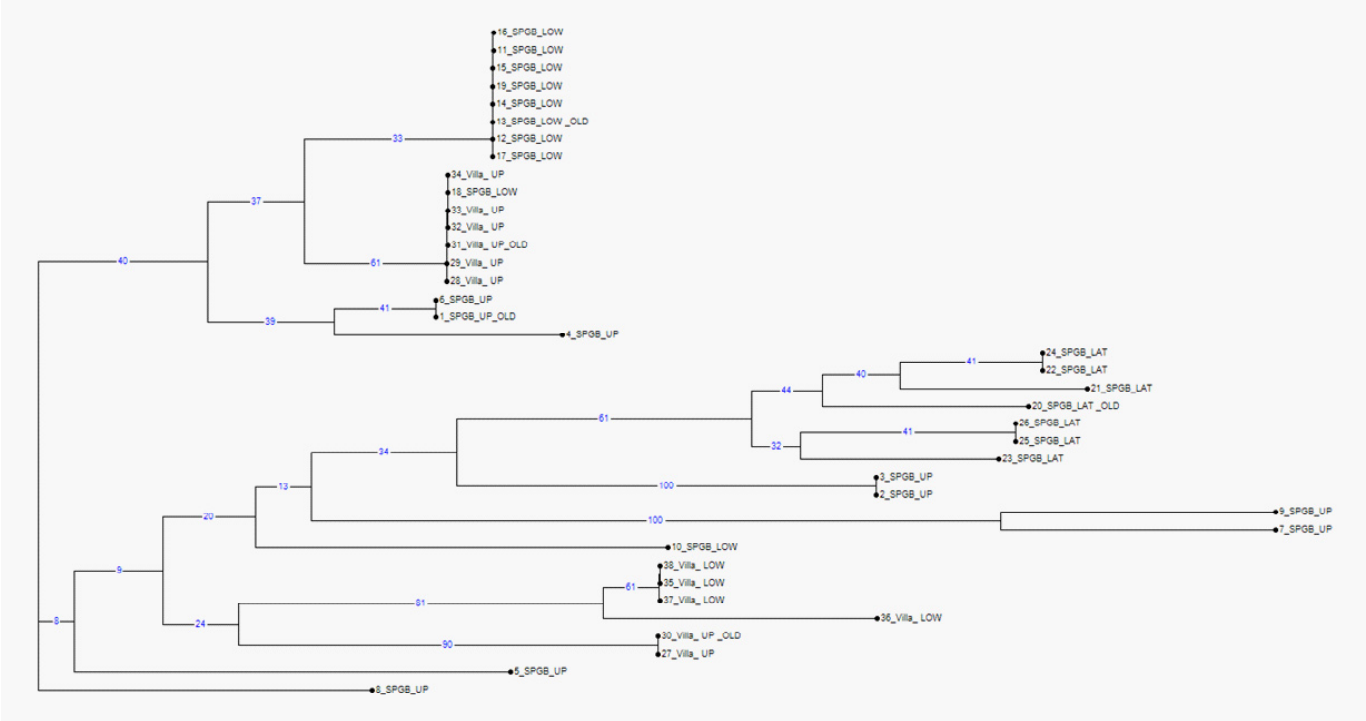

Figure 1: Unweighted Neighbor-Joining tree based on dissimilarity matrix calculated from dataset of three SSR loci across 38 *P. avium* genotypes

is 100, indicating that the probability for this distribution is very high. The last genotype (8) from the SPGB UP group can be found in cluster III, which consists of only one sample.

The SPGB LOW subpopulation is uniform as most of the trees (11, 12, 14, 15, 16, 17, 19) are located around sample 13 (old tree). Thus, we can assume that all are descendants of the old tree and originate from self-fertilization or hybridization with a genetically closely related tree. However, further details could not be detected by the used molecular markers. An exception in this group is genotype 18, which seems to be genetically close to the Villa UP subpopulation.

Due to the distance between these two populations, it could be possible that this genotype developed from a seed brought by a bird from the Villa UP location. The SPGB LAT subpopulation is also uniform: the samples are arranged around genotype 20 – an old tree. This can be explained by the specificity of the location. All the genotypes were collected on a large Celtic mound, which is an isolated islet in the middle of the cultivated area. Genetically close to this group, there are also two genotype pairs from the SPGB UP subpopulation. Villa UP subpopulation is divided into two groups. The first group consists of two genotype pairs: sample 30 (probably represents the parental tree) and 27. This group shows to be genetically closer to the Villa LOW. The second group of the Villa UP subpopulation, located in cluster I, is uniform, with the exception of the previously mentioned sample 18 SPGB LOW. The differences between the two groups can be attributed to the probability that two smaller, independent subpopulations were formed within the existing larger population. The Villa LOW subpopulation can be found in cluster II. All four genotypes appear to be genetically close and form a uniform group with no discrepancies.

Genetic structure

After studying genetic relationships between cherry genotypes from both populations, we examined the structure based on the PCoA (Figure 2). The analyzed SSR profiles revealed results that were consistent with the dendrogram. The Evanno method was implemented to estimate the most relevant parameter K for the Bayesian analysis in order to reveal the strongest level of genetic structure. The maximum value for ΔK was calculated for K = 2 (57.37), dividing the material into two groups (Figure 3). The first group (bar plots in white colour) consisted of 19 genotypes and corresponded to the distribution of samples in cluster I. The second group (bar plots in grey colour) comprised 15 genotypes, belonging to the cluster II. Four samples were considered admixed (5 SPGB UP, 10 SPGB LOW, 27 and 30 Villa UP)

CONCLUSIONS

We tried to determine whether there were differences between the two studied wild cherry populations and if there was a genetic link between them that would reflect genetic similarity among individual genotypes. The genetic analysis included old trees that were presumed to be parental plants, and young plants near these trees that were presumed to be their descendants. Our study showed, that there were differences between the two studied populations except for genotype 18 (SPGB LOW). Although collected from the population in SPGB, the results indicated a relatively close genetic relationship with the Villa population. Within each of the two populations of wild cherry, we noted obvious genetic relatedness, indicating that the individuals are

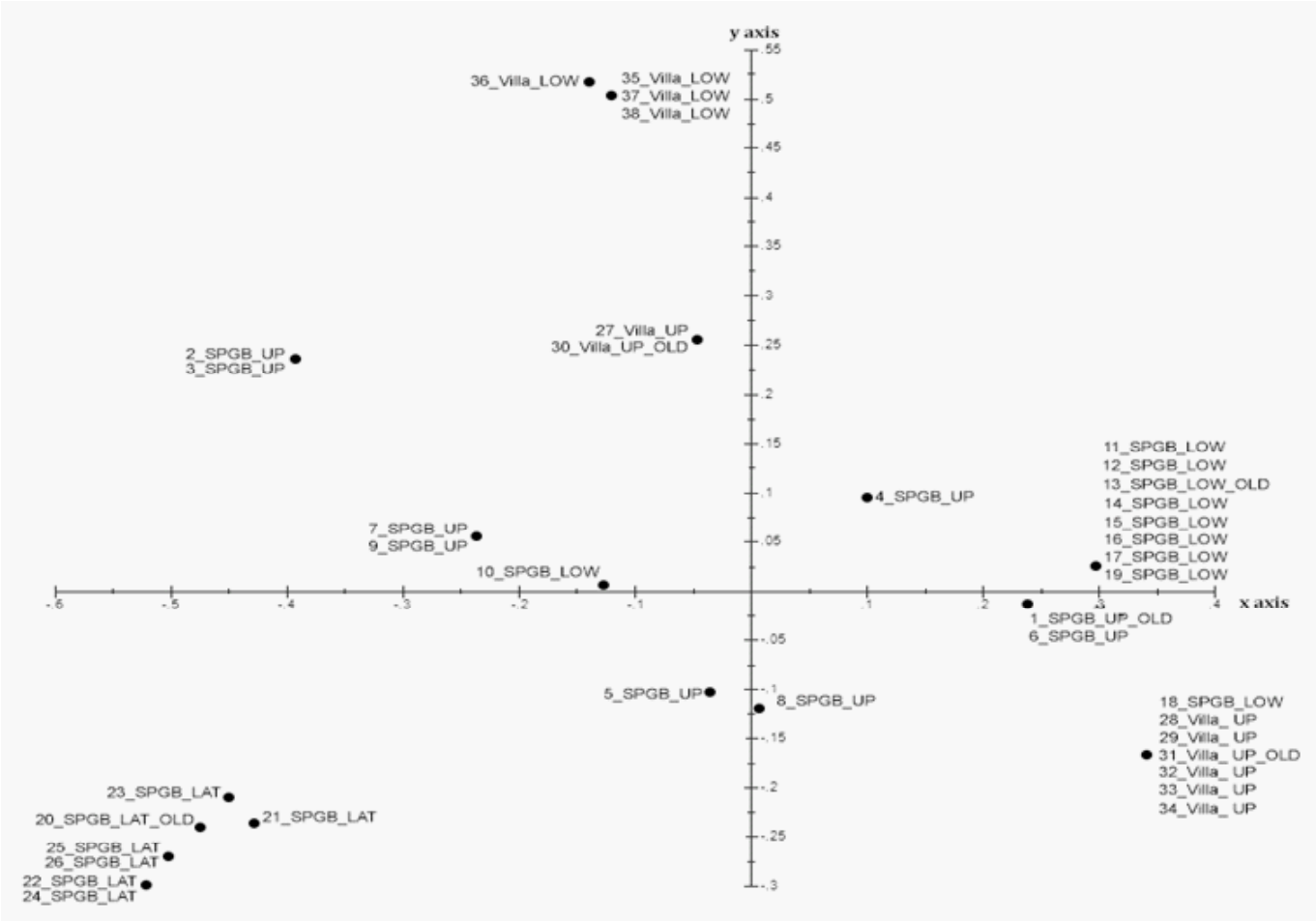

Figure 2: Distribution of wild cherry genotypes on the first two PCoA axes determined using three SSR loci

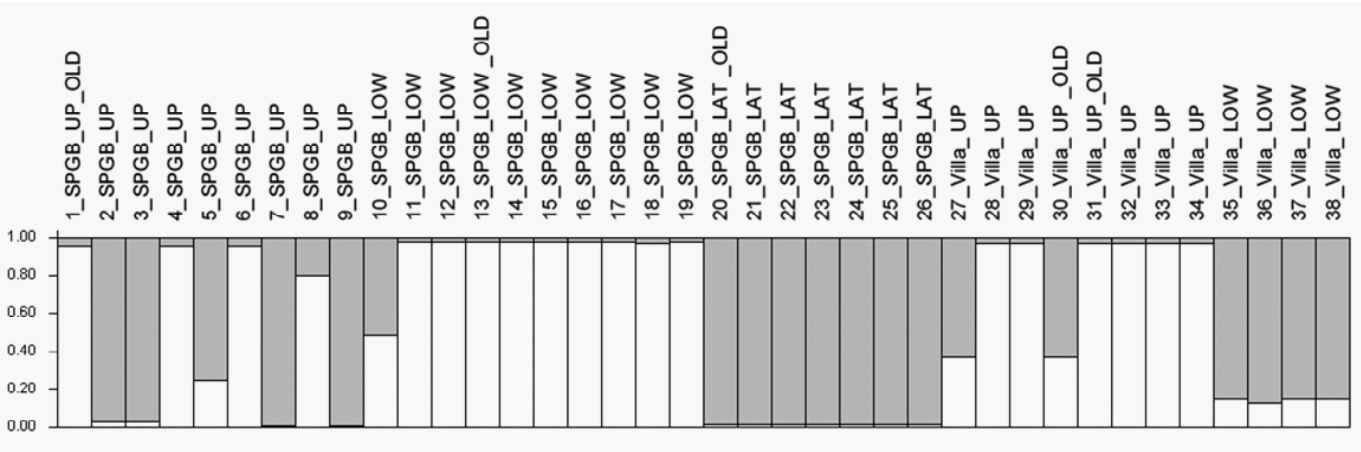

Figure 3: Bar plot of the results from the Bayesian analysis on wild cherry genotypes

genetically similar. We also observed a relatively high level of genetic uniformity within the studied populations. Based on the analysis, we can conclude that some of our groups were uniform, which is especially evident in the three subpopulations: SPGB LOW, SPGB LAT and Villa LOW. Wild cherry populations contain many different genotypes, which enables more efficient random mating and consequently high vitality. Populations are not reproductively isolated. The study shows that there is some "communication" between them (the example is genotype 18 (SPGB LOW). Due to a relatively short distance between our two studied populations, it could be concluded that they are just subpopulations of a larger population that covers an area of several square kilometers.

ACKNOWLEDGEMENTS

We would like to express our gratitude to prof. dr. Anton

Ivančič for monitoring the work, sampling assistance and expert advice with the experiments.

REFERENCES

1. Aka Kacar, Y., Iezzoni, A., & Çetiner, S. (2005). Sweet cherry cultivar identification by using SSR markers. *Journal of Biological Sciences*, *5*, 616-619.
2. Antić, M., Kajkut Zeljković, M., & Đurić, G. (2020). Diversity assessment of wild cherry germplasm by using RAPD markers. *Bulgarian Journal of Agricultural Science*, *26*, 404-408.
3. Chin, S. W., Shaw, J., Haberle, R., Wen, J., & Potter, D. (2014). Diversification of almonds, peaches, plums and cherries - molecular systematics and biogeographic history of *Prunus* (*Rosaceae*). *Molecular Phylogenetics andEvolution*, *76*, 34-48. Retrieved from: https://doi.org/10.1016/j.ympev.2014.02.024
4. Clarke, J. B., & Tobutt, K. R. (2003). Development and characterization of polymorphic microsatellites from Prunus avium 'Napoleon'. *Molecular Ecology Notes*, *3*(4), 578-580. Retrieved from: https://doi.org/https://doi.org/10.1046/j.1471-8286.2003.00517.x
5. Crane, M. B., & Lawrence, W. J. C. (1928). Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. *Journal of Pomology and Horticultural Science*, *7*(3), 276-301. Retrieved from: https://doi.org/10.1080/03683621.1928.11513345
6. 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. Retrieved from: https://doi.org/10.1093/forestry/cpt009
7. Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin,*19 (1), 11-15.
8. Earl, D. A., & von Holdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359-361. Retrieved from: https://doi.org/10.1007/s12686-011-9548-7
9. Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611-2620. Retrieved from: https://doi.org/10.1111/j.1365-294X.2005.02553.x
10. Hormaza, J. I. (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theoretical and Applied Genetics, 104*(2-3), 321-328. Retrieved from: https://doi.org/10.1007/s00122010068
11. Horvath, A., Balsemin, E., Barbot, J.-C., Christmann, H., Manzano, G., Reynet, P., Laigret, F., & Mariette, S. (2011). Phenotypic variability and genetic structure in plum (*Prunus domestica* L.), cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.). *Scientia Horticulturae*, *129*(2), 283-293. Retrieved from: https://doi.org/10.1016/j.scienta.2011.03.049
12. Ivančič, A. (2002a). *Hibridizacija pomembnejših rastlinskih vrst*. Faculty of Agriculture and Life Sciences.
13. Ivančič, A. (2002b). *Osnove rastlinske hibridizacije*. Faculty of Agriculture and Life Sciences.
14. 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-e41231. Retrieved from: https://doi.org/10.1371/journal.pone.0041231
15. Kazija, D. H., Jelačić, T., Vujević, P., Milinović, B., Čiček, D., Biško, A., Pejić, I., Šimon, S., Žulj Mihaljević, M., Pecina, M., Nikolić, D., Grahić, J., Drkenda, P., & Gaši, F. (2014). Plum germplasm in Croatia and neighboring countries assessed by microsatellites and DUS descriptors. *Tree Genetics & Genomes*, *10*(3), 761-778. Retrieved from: https://doi.org/10.1007/s11295-014-0721-5
16. Mnejja, M., Garcia-Mas, J., Howad, W., Badenes, M. L., & Arús, P. (2004). Simple-sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. *Molecular Ecology Notes*, *4*(2), 163-166. Retrieved from: https://doi.org/10.1111/j.1471-8286.2004.00603.x
17. Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software. Cirad, France.
18. Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945. DOI: 10.1093/genetics/155.2.945
19. Schueler, S., Tusch, A., Schuster, M., & Ziegenhagen, B. (2003). Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.) - Markers for individual identification and reproductive processes. *Genome*, *46*, 95-102. Retrieved from: https://doi.org/10.1139/g02-107
20. Smole, J. (2000). *Češnje in višnje, pridelovanje in uporaba*. Kmečki glas.
21. Vaughan, S. P., Cottrell, J. E., Moodley, D. J., Connolly, T., & Russell, K. (2007). Distribution and fine-scale spatial-genetic structure in British wild cherry (*Prunus avium* L.). *Heredity*, *98*(5), 274-283. Retrieved from: https://doi.org/10.1038/sj.hdy.6800935
22. Wagner, H. W., & Sefc, K. M. (1999). *IDENTITY 1.0.*, University of Agricultural Sciences, Vienna, Austria.

Določanje genetske sorodnosti med populacijami divjih češenj [*Prunus avium* (L.) L.] z uporabo mikrosatelitskih markerjev

IZVLEČEK

Divje češnje so značilna drevesa slovenske pokrajine, ki tvorijo naravne populacije. Zmožnost razlikovanja med posameznimi genotipi češnje [*Prunus avium* (L.) L.], tako gojenimi kot divje raslimi, je pomembno iz znanstvenih kot tudi iz ekonomskih razlogov. V genetsko analizo smo vključili stara drevesa in mlade rastline v okolici teh dreves. Vzorčenje je potekalo na dveh različnih lokacijah blizu Maribora, SV Slovenija. Namen raziskave je bil ugotoviti, kakšna je genetska sorodnost ter raznolikost med predstavniki obeh populacij. Osredotočili smo se na mlada drevesa, ki rastejo okoli starih dreves in bi lahko bila njihovi potomci. Ugotavljali smo še genetsko uniformnost znotraj populacije, število odstopajočih genotipov znotraj populacije, ki so bili verjetno preko semena preneseni iz neke druge populacije. Na treh mikrosatelitskih lokusih smo pri 38 preučevanih genotipih divje češnje skupaj namnožili 19 polimorfnih alelov, v povprečju 6,3 na lokus. Genetske razdalje med preučevanimi genotipi smo izračunali z Jaccardovim koeficientom in izdelali dendrogram. Za analizo genetske strukture smo uporabili PCoA metodo (analiza glavnih koordinat ali 'principal coordinate analysis') ter analizo na podlagi Bayesovega modela. Raziskava je pokazala, da se obe populaciji med seboj razlikujeta, vendar med njima obstaja tudi določena »komunikacija«. Znotraj vsake od populacij divjih češenj smo opazili relativno visoko stopnjo genetske sorodnosti kot tudi uniformnosti.

Ključne besede: divja češnja, *Prunus avium* (L.) L., populacija, mikrosatelitski markerji, genetska sorodnost