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Comparison of Slovenian Traditional Plum Materials with Genetic Resources from the Slovenian Plant Gene Bank

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ABSTRACT

The Slovenian Plant Gene Bank (SPGB) of the Faculty of Agriculture and Life Sciences houses approximately 250 accessions of stone fruit, with most of the material belonging to the species *Prunus domestica* L. and *Prunus cerasifera* Ehrh. The main objectives of this study using a set of 11 SSR primers were: 1. to determine the genetic structure of the traditional Slovenian *in situ* plum material in comparison to the *ex situ* the SPGB collection; 2. to identify unique material among the *in situ* collected accessions; 3. to gain insight into the genetic relationship between the two studied species. The genetic structure of 60 plum samples was analyzed using Principal Coordinate Analysis (PCoA) and Bayesian model-based analysis. PCoA separated the *P. cerasifera* and *P. domestica* accessions, while Bayesian model-based analysis revealed that many accessions of *P. domestica* and *P. cerasifera* shared a common ancestral history. The *ex situ* material showed greater genetic diversity as it was distributed over more populations than the *in situ* material. Promising *in situ* genotypes, especially from the Prekmurje and Lower Styria, were identified as valuable additions to enrich the existing collection.

Keywords: plum, *Prunus domestica* L., *Prunus cerasifera* Ehrh., SSR markers, genetic structure

INTRODUCTION

Germplasm collections serve as an important reservoir of plant genetic resources as well as a source of diversity and are essential for successful crop improvement (Butac, 2020). As a result of careful collection, conservation and evaluation, these collections provide a valuable source of genes (e.g., wild genotypes, landraces, local populations, clones and lines bred from indigenous plant materials and ecotypes) that can be used in breeding programs or as cultivars with high tolerance to abiotic and biotic stresses, suitable for sustainable agricultural practices (Blazek, 2007; Butac et al., 2010; Dey et al., 2016).

The Slovenian Plant Gene Bank (SPGB) of the Faculty of Agriculture and Life Sciences is part of the Slovenian Plant Gene Bank Program (SRGB), which aims to preserve, evaluate, regenerate and conserve indigenous Slovenian germplasm, including local ecotypes, populations and landraces of agricultural, medicinal and aromatic plants, as well as forest trees and other woody plants from Slovenian forests (Šiško, 2016). It was established in 2007 on an area of approximately 3 ha and is located in an isolated location according to FAO standards next to the Botanical Garden of the University of Maribor (Pivola). It is a permanent collection plantation intended both for the storage of accessions and for evaluation of accessions. Part of the collection, including vines, is located in the Meranovo viticulture center (Limbuš). The germplasm collection includes accessions from the following genera: *Prunus*, *Rubus* and *Vitis*. After 17 years of work on the collection, there are currently approximately 250 accessions belonging to stone fruit (e.g., plums, sweet and sour cherries, apricots, peaches, and almonds). Of these, around 170 plum accessions belong to the *Prunus domestica* L. and *Prunus cerasifera* Ehrh. species (Šiško, 2018 and unpublished data from the SPGB collection).

The European plum, *P. domestica* (2n = 6x = 48), and the myrobalan plum, *P. cerasifera* (2n = 2x = 16) are species which belong to a group of European plums (Hartmann et al., 2009; Neumuller, 2011). The first species is used worldwide in fruit production due to the versatility of the fruit use (fresh, dried

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or processed) (Milošević et al., 2023). It is also known to form different pomological groups (based on fruit use and morphological characteristics such as fruit shapes and color), including large-fruited European plums, prunes, egg plums, greengages, mirabelles, damsons, bullaces and St. Julien plums (Zhebentyayeva et al., 2019; Gaši et al., 2020). The use of fresh and dried fruits of *P. cerasifera* is limited to traditional use in Western Asia (Hanelt, 2001; Okie and Hancock, 2008), while in Europe, myrobalan is often used for processing (e.g., jams and chutneys) and for the spirit production (Topp et al., 2012). In addition, *P. cerasifera* is resilient species and adapted to a wide range of rural and urban areas (forest edges, open woodlands, along roads and rivers, abandoned orchards, around farm buildings, gardens and parks) (Hartmann et al., 2009; Popescu and Caudullo, 2016). It is widely used as an ornamental tree and as an important rootstock for other *Prunus* species (e.g., plum, peach, apricot and almond) (Sancin, 1988; Sedaghathoor et al., 2009; Das, 2011).

The use of plant genetic resources, in particular when they are not present in dedicated conservation centers, is often limited by insufficient information, such as material identification, phenotypic and genetic diversity and pomological data (Milošević and Milošević, 2018). Comprehensive information on the phenotypic and genotypic characteristics of the material and various preservation strategies are crucial for the conservation process (Ramanatha and Hodgkin, 2002). Traditionally, plum material is characterized and identified based on morphological traits (Martínez-Gómez et al., 2005). However, this method of distinguishing genotypes has limitations, as some traits are unreliable (e.g., variations in growing conditions, plant age, and phenological stage) (Mehdi et al., 2012). To identify plant genetic relationships, structure and diversity, molecular marker technology has developed in recent decades to replace or complement morphological markers (Soriano, 2020).

As part of a broader research endeavor (Ternjak et al., 2023), the main objective of this study was to determine the genetic structure of the traditional Slovenian *in situ* plum material in comparison to the *ex situ* material of SPGB collection, using SSR molecular markers. The focus was on identifying unique material among the accessions collected *in situ* that could be used to enrich the collection. In addition, we wanted to gain insight into the genetic relationship between the two studied species (*P. domestica* and *P. cerasifera*).

MATERIALS AND METHODS

Plant material

The accessions examined in this research are part of a broader study investigating the genetic diversity and structure of three plum species: *P. domestica*, *P. cerasifera*, and *P. spinosa* (Ternjak et al., 2023). This paper focuses specifically on the analyzes and comparison of plum material belonging to *P. domestica* and *P. cerasifera* collected in Slovenia (*in situ* and *ex situ*).

From 2018 to 2019, young and healthy leaves were collected from 60 plum accessions. Roughly half of the material (29 accessions) belonged to *P. domestica* species and 31 accessions to *P. cerasifera*. Sixteen accessions were collected *ex situ* in SPGB, while 44 accessions were collected *in situ* from different regions in Slovenia (Lower Styria, Prekmurje, Upper Carniola and Istria). Each sample was documented, and for the *in situ* accessions, the exact location of the tree was determined using latitude and longitude coordinates (WGS84 system). For the *ex situ* accessions, the location of the collection site was recorded together with the data on the origin of the material. Table 1 lists the data of the collected material, including name, species, status, origin and other passport data.

Table 1: The data of the 60 genotypes (29 accessions of *Prunus domestica* L. and 31 accessions of *Prunus cerasifera* Ehrh.), including name, species, origin and other passport data as well as the membership values to the populations were analyzed with STRUCTURE (Pritchard et al., 2000)

Sample No.	Name	Species	Accession ID	Conservation type	Origin of the material	Structure analysis	
						K = 2	K = 7
37	Plum_green_37	*P. domestica*	/	*ex situ* SPGB*	Brdce, Vojnik, Lower Styria	K2	6
38	Plum_38	*P. cerasifera*	/	*in situ*	Črnc, Brežice, Lower Styria	K1	2
44	Bluish_plum_44	*P. domestica*	/	*in situ*	Lendavske gorice, Prekmurje	K1	2
45	Bluish_plum_45	*P. domestica*	/	*in situ*	Lendavske gorice, Prekmurje	K1	2
46	Bluish_plum_46	*P. domestica*	/	*in situ*	Lendavske gorice, Prekmurje	K1	2

Sample No.	Name	Species	Accession ID	Conservation type	Origin of the material	Structure analysis	
						K = 2	K = 7
51	Plum_51	*P. cerasifera*	6356	*ex situ* SPGB*	Črnc, Brežice, Lower Styria	K1	2
52	Plum_52	*P. domestica*	6085	*ex situ* SPGB*	Črnc, Brežice, Lower Styria	K1	2
55	Plum_55	*P. cerasifera*	6372	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	2
58	Plum_58	*P. cerasifera*	6370	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	Admixed
59	Plum_59	*P. cerasifera*	6391	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	4
60	Plum_60	*P. cerasifera*	6371	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	3
61	Plum_61	*P. cerasifera*	/	*ex situ* SPGB*	Brezina, Brežice, Lower Styria	K1	3
62	Plum_62	*P. cerasifera*	6392	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	3
63	Plum_63	*P. cerasifera*	6394	*ex situ* SPGB*	Trnje pri Brežicah, Lower Styria	K1	1
67	Bluish_plum_67	*P. domestica*	3576	*ex situ* SPGB*	Maribor, Lower Styria	K1	1
76	Plum_76	*P. cerasifera*	6350	*ex situ* SPGB*	Črnc, Brežice, Lower Styria	K1	1
79	Common_prune_Bistrica_79	*P. domestica*	6317	*ex situ* SPGB*	NA	K1	1
82	Plum_82	*P. cerasifera*	6347	*ex situ* SPGB*	Črnc, Brežice, Lower Styria	K1	1
84	Common_prune_Bistrica_84	*P. domestica*	6416	*ex situ* SPGB*	Sromlje, Brežice, Lower Styria	K1	1
92	Bluish_plum_92	*P. domestica*	/	*in situ*	Maribor, Lower Styria	K1	1
101	Bluish_plum_101	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
102	Bluish_plum_102	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
103	Bluish_plum_103	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
104	Bluish_plum_104	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
105	Bluish_plum_105	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
106	Bluish_plum_106	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
107	Common_prune_107	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	1
108	Common_prune_108	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K1	Admixed
109	Common_prune_109	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K2	5
110	Common_prune_110	*P. domestica*	/	*in situ*	Orehova vas, Lower Styria	K2	5
111	Plum_111	*P. cerasifera*	/	*in situ*	Orehova vas, Lower Styria	K2	5
112	Bluish_plum_112	*P. domestica*	/	*in situ*	Maribor, Lower Styria	K2	5
113	Bluish_plum_113	*P. domestica*	/	*in situ*	Limbuš, Lower Styria	K2	5
114	Bluish_plum_114	*P. domestica*	/	*in situ*	Limbuš, Lower Styria	K2	5
116	Bluish_plum_116	*P. domestica*		*in situ*	Lovrenc na Pohorju, Lower Styria	K2	5

Sample No.	Name	Species	Accession ID	Conservation type	Origin of the material	Structure analysis	
						K = 2	K = 7
117	Common_prune_117	*P. domestica*	/	*in situ*	Miklavž na Dravskem polju, Lower Styria	K2	5
118	Plum_118	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	K2	5
119	Plum_119	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	K2	Admixed
120	Plum_120	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	K2	5
121	Plum_121	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	K2	5
122	Plum_122	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	K2	5
129	Plum_yellow_129	*P. cerasifera*	/	*in situ*	Koper, Istria	K2	5
130	Plum_bluish_130	*P. cerasifera*	/	*in situ*	Koper, Istria	K2	5
131	Plum_red_131	*P. cerasifera*	/	*in situ*	Koper, Istria	K2	5
132	Plum_yellow_132	*P. cerasifera*	/	*in situ*	Koper, Istria	K2	5
133	Plum_yellow_133	*P. cerasifera*	/	*in situ*	Izola, Istria	K2	5
134	Plum_violet_134	*P. cerasifera*	/	*in situ*	Strunjan, Istria	K2	5
135	Plum_red_135	*P. cerasifera*	/	*in situ*	Vas Dragonja, Istria	K2	5
136	Plum_bluish_136	*P. cerasifera*	/	*in situ*	Krkavče, Istria	K2	5
137	Myrobalan_137	*P. cerasifera*	/	*in situ*	Kasaze, Lower Styria	K2	5
138	Myrobalan_138	*P. cerasifera*	/	*in situ*	Kasaze, Lower Styria	K2	5
139	Myrobalan_139	*P. cerasifera*	/	*in situ*	Kasaze, Lower Styria	K2	5
140	Mirabelle_140	*P. domestica*	/	*in situ*	Kasaze, Lower Styria	K2	5
145	Bluish_plum_145	*P. domestica*	/	*in situ*	Ruše, Lower Styria	2	5
146	Bluish_plum_146	*P. domestica*	/	*in situ*	Ruše, Lower Styria	2	5
147	Plum_147	*P. cerasifera*	/	*ex situ*	Črnc, Brežice, Lower Styria	2	5
153	Spindel_plum_153	*P. domestica*	/	*in situ*	Rašica, Upper Carniola	2	7
155	Plum_yellow_155	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	2	5
156	Plum_red_156	*P. cerasifera*	/	*in situ*	Maribor, Lower Styria	2	5
189	Bluish_plum_189	*P. domestica*	/	*in situ*	Zakot, Brežice, Lower Styria	2	5

* SPGB (Slovenian Plant Gene Bank)

Molecular analyzes

DNA isolation and molecular markers analyzes

Total genomic DNA was extracted from the young leaf material following the CTAB protocol described by Doyle and Doyle (1987), with some modifications. Two separate extractions per sample were performed. The DNA concentration of each sample was estimated using a fluorimeter (Hoefer DQ 300, California, USA). The quality was also checked on a 3% agarose gel by electrophoresis (Bio-Rad, California, USA), and the products were visualized under UV light.

All studied accessions were analyzed using a set of eleven 11 SSR primer pairs: UDP96-005, BPPCT034, EMPAS12, UCD-CH17, EMPAS06, EMPAS11, EMPAS14, BPPCT014, BPPCT025, CPSCT026 and CPPCT006, which were developed on different *Prunus* species. Information on marker selection, polymerase chain reaction (PCR) amplification procedures and fragment size analysis was previously published by Ternjak et al. (2023).

Data analyzes

The genetic structure among the studied accessions was analyzed using the Principal Coordinate Analysis (PCoA) and complemented with the Bayesian model-based clustering method. For the PCoA calculations, the microsatellite allele data were converted into a binary matrix. Dissimilarities were calculated with Sokal and Michener index and transformed into Euclidean distances using the 0.5 power transformation. Using DARwin 6.0.21 software (Perrier and Jacquemoud-Collet, 2006), each accession was assigned to a location in a two-dimensional space and the figure was constructed. The STRUCTURE V2.3.4 software package (Pritchard et al., 2000) was used to perform the Bayesian model-based clustering method. Ternjak et al. (2023) have already presented a detailed description of the settings and construction of the bar plots. The most relevant parameter K (number of populations) for the analyzed data was determined by calculating ΔK according to the method described by Evanno et al. (2005). This calculation was performed with the Structure Harvester V0.6.94 application (Earl and von Holdt, 2012). The individuals with a membership coefficient (ql) > 0.9 were assigned to a specific population, and those with a threshold value below the estimated membership were considered admixed. The STRUCTURE 2.3.4 software (Pritchard et al., 2000) was also used to compute the average distances (expected heterozygosity) between individuals within the same population.

RESULTS AND DISCUSSION

Principal Coordinate Analysis provided insight into the distribution of different plum groups and allowed us to obtain a global representation of diversity. In the analysis, the examined material was divided according to species and when considering the conservation type (*in situ* and *ex situ*) the material was distributed over the entire graph (Fig. 1).

The accessions of *P. cerasifera* were located on the left side of the figure and formed a denser arrangement in space, while the accessions of *P. domestica* were located on the right side of the figure. The only exception was the *ex situ* accession Plum 52 on the left side of the figure, which clustered slightly closer to the *P. cerasifera* species. Although the study by Ternjak et al. (2023) considered Plum 52 to be *P. domestica,* as the flow cytometry results confirmed that the accession was hexaploid, the analyzed SSR profiles also showed similar behavior to other *P. domestica* accessions. However, morphological observations also revealed a similarity with the *P. cerasifera* species. In addition, genetic diversity assessment using three universal cpDNA primers showed that Plum 52 belonged to haplotype H4, which was shared by *P. domestica* and *P. cerasifera* (Ternjak et al., 2023). Possibly, accession Plum 52 is an example of a putative hybrid origin, which is not surprising as *P. domestica* can easily hybridize with *P. cerasifera* (Topp et al., 2012). The *P. domestica* material was more scattered on the right side of the figure, forming different clusters. On the upper right side of the figure, two clusters of the landrace Bluish plum could be observed. The Bluish plum accessions mostly collected *in situ* (except Bluish plum 67) were positioned according to their origin, with a larger cluster of Bluish plums from the Styria region and a smaller cluster of only three Bluish plum accessions (44,45 and 46) from the Prekmurje region. On the lower left side of the figure, four accessions were highlighted, material slightly different from the other clusters: Bluish plum (189), which was also collected in the Styria region, but is genetically distinct and was separated from the two Bluish plum clusters; Plum green (37), a small-fruited landrace with green color, yellow-oval-fruited landrace Spindel plum (153) and the accession Mirabelle 140, which belongs to the mirabelle pomological group. Mirabelles are a specialty of the Lorraine region in France, sweet small-fruited plums that are predominantly yellow to orange, often with red spots (Gaši et al., 2020). The PCoA analysis also identified a pomological group of common prunes which included *in situ* and *ex situ* material and clustered in the lowest part of the figure.

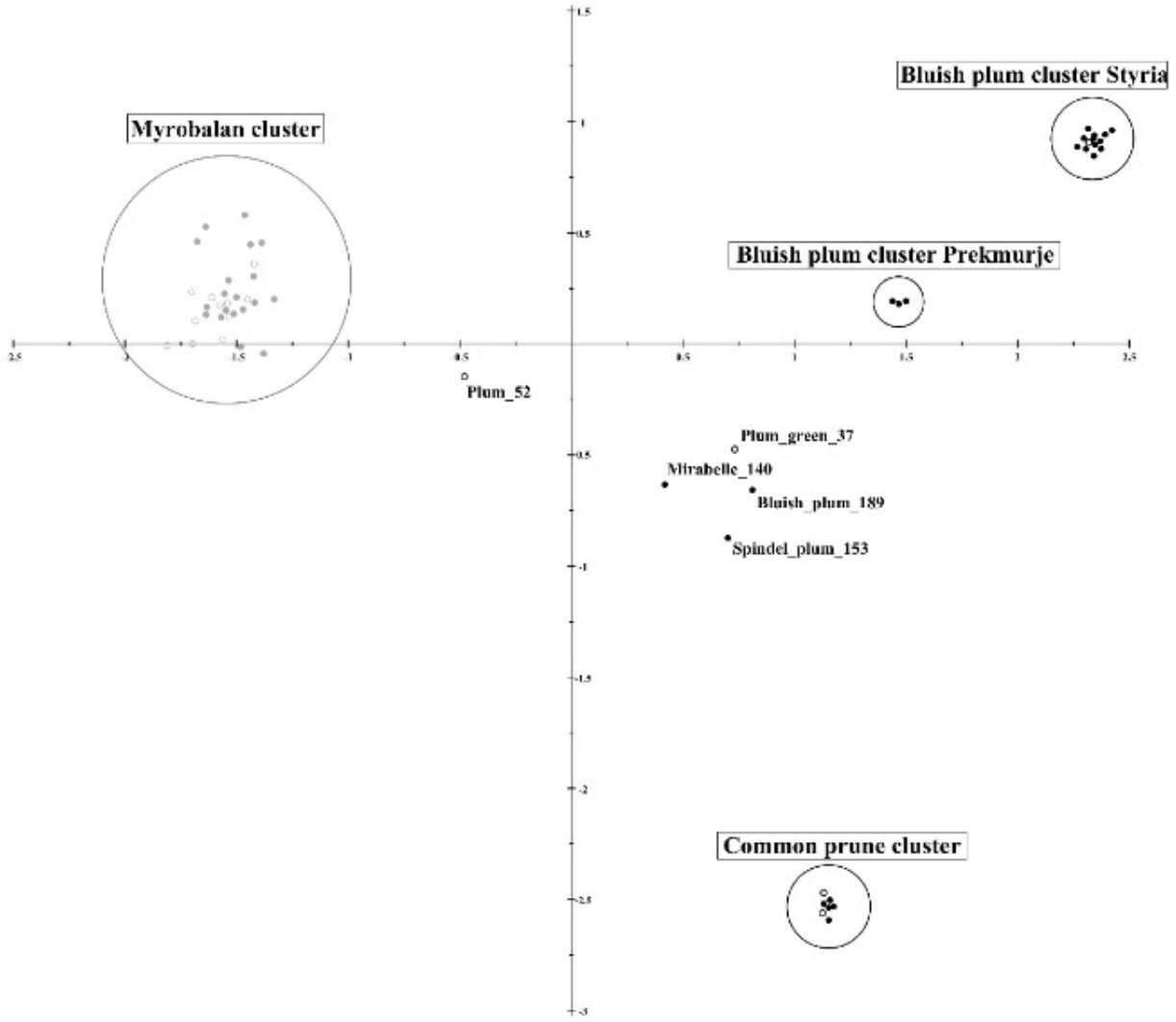

Figure 1: Principal Coordinate Analysis (PCoA) based on polymorphism at 11 SSR loci of the 60 plum genotypes. The accessions of *P. cerasifera* Ehrh. (Myrobalan cluster, grey color) formed a dense cluster, whereas the material of *P. domestica* L. (black color) is more scattered and consists of different clusters. The accessions marked with a full dot represent *in situ* material, while the accessions with an empty dot are *ex situ* material

Bayesian model-based clustering analysis revealed the structural patterns and divided the analyzed material into ancestral populations. The maximum value for ΔK was K = 2 (975.15), dividing the material into two populations (Fig. 2, Fig. 3 and Table 2). Population K1 (bar plots in black color) accounted for 27 genotypes and consisted of 13 *in situ* and 14 *ex situ* accessions (Table 1). Population K2 (bar plots in grey color) accounted for 33 genotypes, mostly belonging to the *in situ* material (31), with two *ex situ* accessions (Table 1). This collected material has the potential to increase and enrich the SPGB genetic resources collection. Both populations were comprised of various accessions belonging to both studied species and different pomological groups. For the K = 2, no material was considered admixed.

Table 2: Table summarizing the results using Evanno et al. (2005) method, output of Structure Harvester V0.6.94 application (Earl and von Holdt, 2012)

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-10143.72000	0.85739	NA	NA	NA
2	10	-7461.45000	2.21071	2682.27000	2155.77000	975.14990
3	10	-6934.95000	94.39210	526.50000	186.26000	1.97326
4	10	-6594.71000	223.41714	340.24000	36.47000	0.16324
5	10	-6290.94000	210.95657	303.77000	1477.68000	7.00466
6	10	-7464.85000	4418.45931	-1173.91000	2612.48000	0.59126
7	10	-6026.28000	4.16034	1438.57000	1386.09000	333.16733
8	10	-5973.80000	5.99574	52.48000	32.35000	5.39550
9	10	-5953.67000	18.74923	20.13000	125.85000	6.71228
10	10	-6059.39000	25.61642	-105.72000	NA	NA

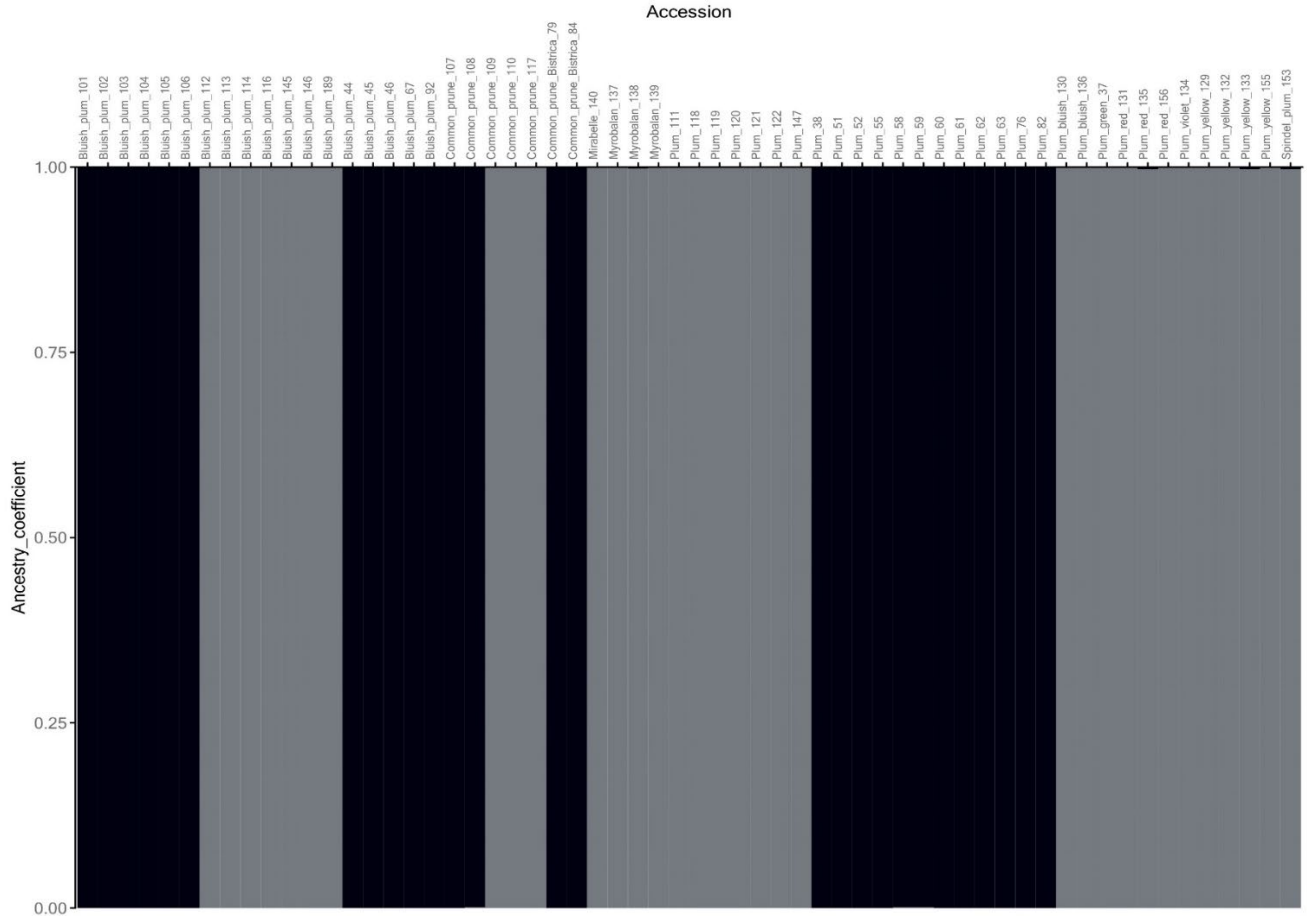

Figure 2: Bar plot of the results of the Bayesian model-based clustering (K = 2) for 60 plum genotypes. Population K1 accessions are shown in black and Population K2 accessions are shown in grey. No accessions were admixed

In terms of allelic variation within the two populations, STRUCTURE software revealed that the mean distances between individuals were the greatest in Population K1 (0.7410), while the distances were smaller in Population K2 (0.5213) (Table 3).

Table 3: Average distances (expected heterozygosity) between individuals in the same population

Population K1:	0.7410
Population K2:	0.5213

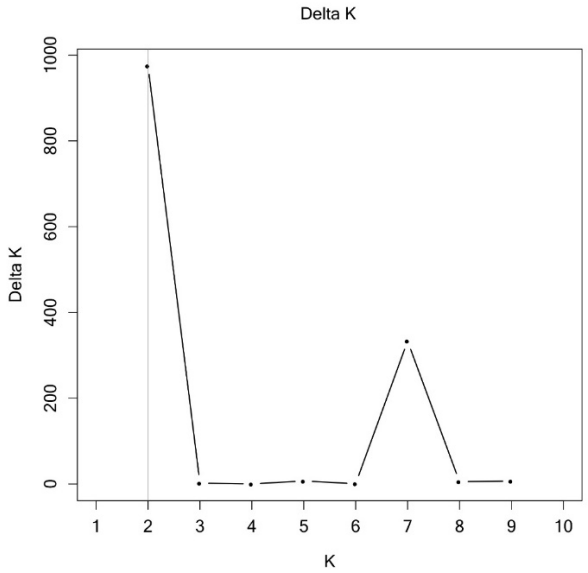

Figure 3: Graphical method, as in Evanno et al. (2005), allowing the detection of the number of groups K for the 60 plum genotypes using ΔK

The Evanno criterion, used to evaluate the genetic structure showed a weaker signal for K = 7 (333.17), dividing the material into seven populations (Fig. 4, Fig. 3 and Table 2). Population K1 split into four sub-clusters (Populations 1-4), while Population K2 split into three sub-clusters (Populations 5-7) (Table 1). Most accessions (14) belonging to Population 1 (black) maintained the same profile or remained unchanged from the original Population K1 (Plum: 63, 76, 82; Bluish plum: 67, 92, 101, 102, 103, 104, 105, 106; Common prune: 79, 84 and 107). Seven accessions, Bluish plums: 44, 45, 46 separated and formed a new Population 2 (light grey) that also included accessions Plum: 38, 51, 52 and 55, while Population 3 (grey with black dots) contained three accessions (Plum: 60, 61 and 62). Population 4 (white) consisted of a single accession (Plum 59). Most accessions (30) were assigned to Population 5 (dark grey) and had the same profile or remained unchanged from the original Population K2. The other two populations contained only one accession, namely Population 6 (white with black stripes), Plum green (37) and Population 7 (black with white dots), Spindel plum (153). In the remaining three admixed accessions, the largest part of their genome 0.7 for Plum 58, 0.87 for Plum 119 and 0.7 for Common prune 108, was associated with populations 3, 6 and 7, respectively.

The results of the STRUCTURE software for K = 7 have also highlighted some accessions or groups that stood out for their originality. For some of them, results were consistent with the PCoA analysis, e.g., Plum green (37) and Spindel plum (153). For the other accessions, Bayesian model-based clustering analysis showed that they belonged to a new ancestral population, that was not shown in previous analysis. For example, Plum 59 from Population 4, or accessions from the Population 3.

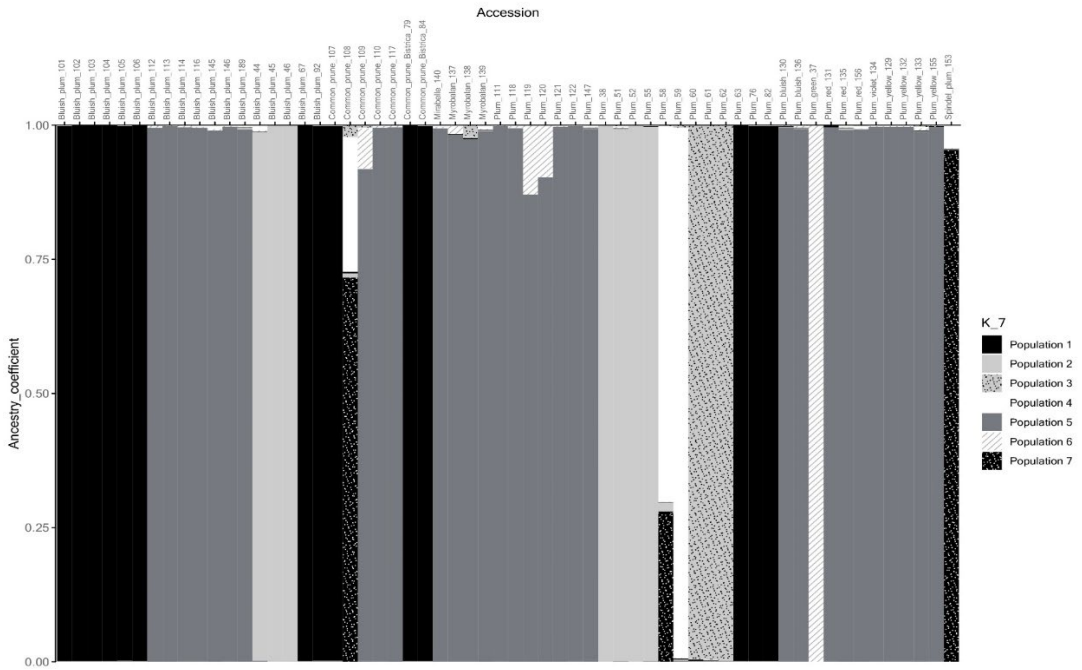

Figure 4: Bar plot of the results of the Bayesian model-based clustering analysis (K = 7) for 60 plum genotypes. Population 1 accessions are shown in black, Population 2 accessions are shown in light grey, Population 3 accessions are shown in grey with black dots, Population 4 accessions are shown in white, Population 5 accessions are shown in dark grey, Population 6 accessions are shown in white with black stripes and Population 7 accessions are shown in black with white dots. Three accessions were admixed.

When comparing *in situ* and *ex situ* material at K = 7, the first was distributed among four ancestral populations (1, 2, 5 and 7), while the second was distributed among six populations (1, 2, 3, 4, 5 and 6) and thus showed higher genetic diversity. This was expected, as the material from the SPGB collection had been selected for its high diversity. Nevertheless, we found promising material among the accessions collected *in situ* that could be included to enrich the existing collection. For example, Bluish plum genotypes originating from the Prekmurje region and belonging to Population 2 (accessions 44, 45 and 46), as well as Bluish plum genotypes belonging to Population 5 and originating from Lower Styria, but both different from Bluish plum material in the current SPGB collection. In addition, we also discovered common prune genotypes belonging to Population 5, which also differed from the existing collection. Another example was already mentioned, Spindel plum (153) which was pointed out as a unique material by the both analyzes.

When we studied the structure of *P. domestica* and *P. cerasifera* together with other wild relatives such as *P. spinosa*, the Bayesian model-based clustering analysis revealed *P. domestica* and *P. cerasifera* as independent groups (Ternjak et al., 2023). Interestingly, the present study, which focused exclusively on the analysis of *P. domestica* and *P. cerasifera* material, showed that many accessions of the two species belonged to the same population and thus share ancestral history. This is supported too by the results of genetic diversity assessment studies using chloroplast DNA markers reported by Bortiri et al. (2009), Reales et al. (2010), Horvath et al. (2011) and Ternjak et al. (2023), in which *P. domestica* and *P. cerasifera* clustered together, suggesting that *P. cerasifera* may have contributed to the maternal chloroplast DNA of *P. domestica*.

CONCLUSIONS

We aimed to determine the genetic structure of traditional Slovenian *in situ* plum material in comparison to the *ex situ* material of the SPGB collection, using SSR molecular markers. The combined results provided valuable insights into the genetic diversity and structure of the Slovenian plum genetic resources. PCoA clearly separated the *P. cerasifera* and *P. domestica* accessions, with *P. cerasifera* forming a dense cluster and the *P. domestica* accessions being more scattered and consisting of different clusters. An exception was accession Plum 52, which, although classified as *P. domestica*, genetic markers suggesting a hybrid origin with *P. cerasifera*. An exception was accession Plum 52, which, although classified as *P. domestica*, clustered near *P. cerasifera*. The combined results of the different analyzes based on genetic markers therefore suggest a hybrid origin with *P. cerasifera*. The *ex situ* material showed higher genetic diversity and was distributed among more populations compared to the *in situ* material, reflecting the selection for high diversity that was made when accessions were introduced into the SPGB collection. Nonetheless, promising *in situ* accessions, particularly from the Prekmurje and Lower Styria regions, were identified as valuable additions to enrich the existing collection. Certain accessions, such as Plum green (37) and Spindel plum (153), were highlighted as unique by both PCoA and Bayesian model-based clustering analysis. Bluish plum populations from different regions and common prune genotypes were identified as distinct from the ones in existing collections, suggesting potential new additions to the Slovenian germplasm collection. The analyzes revealed that many accessions of *P. domestica* and *P. cerasifera* share a common ancestral history. This finding is consistent with previous studies using chloroplast DNA markers, which also suggested that *P. cerasifera* may have contributed to the maternal chloroplast DNA of *P. domestica*. Overall, this study demonstrated the utility of integrating molecular markers and advanced clustering methods to uncover the complex genetic relationships and diversity within plum species to aid future breeding and conservation efforts.

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Primerjava slovenskih tradicionalnih genotipov sliv z genskimi viri iz Slovenske rastlinske genske banke

IZVLEČEK

Rastlinska genska banka Fakultete za kmetijstvo in biosistemske vede hrani poleg drugih vrst tudi okrog 250 akcesij koščičarjev, pri čemer večina materiala pripada vrstama *Prunus domestica* L. in *Prunus cerasifera* Ehrh. Z uporabo 11 mikrosatelitskih lokusov smo v raziskavi želeli: 1. oceniti genetsko strukturo slovenskih tradicionalnih genotipov sliv nabranih *in situ* v primerjavi z *ex situ* materialom iz kolekcije genske banke koščičarjev; 2. prepoznati edinstven material med *in situ* nabranimi akcesijami; 3. raziskati genetske odnose med dvema proučevanima vrstama. Genetska struktura 60 genotipov sliv je bila analizirana s pomočjo Principalne koordinatne analize (PCoA) ter Bayesove analize. PCoA analiza je razdelila akcesije glede na pripadnost preučevanima vrstama (*P. cerasifera* oz. *P. domestica*), medtem ko je Bayesova analiza pokazala, da si številne akcesije tako iz vrste *P. domestica*, kot *P. cerasifera* delijo pripadnost znotraj specifične populacije. Material nabran *ex situ* je pokazal večjo genetsko raznolikost, saj je bil razdeljen na več populacij v primerjavi z materialom nabranim *in situ*. Med slednjim smo identificirali unikatne genotipe sliv, predvsem iz Prekmurja in Štajerske, ki bi jih bilo smiselno vključiti v obstoječo kolekcijo rastlinske genske banke.

Ključne besede: sliva, *Prunus domestica* L., *Prunus cerasifera* Ehrh., mikrosatelitski markerji, genetska struktura