

GENETIC DIVERSITY AND RELATEDNESS OF LATVIAN *PYRUS* GERMPLASM ASSESSED BY A SET OF SSR MARKERS

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Pears (Pyrus communis L.) are not native plant species in Latvia but have a long history of introduction and growing, using various sources of plant material over time. Therefore, the existing collection of genetic resources combines accessions of different origin. To plan the further development of this crop and to ensure its successful preservation, this study aimed to characterise the Latvian Pyrus germplasm collection using SSR molecular markers and to estimate the level of genetic diversity within the pear germplasm collection. Six SSR markers were used to genotype 206 pear accessions of different origin, such as cultivars and hybrids bred in Latvia, landraces collected during expeditions throughout the country, and introduced foreign cultivars. The marker set identified 197 accessions with unique genotypes, and enabled a comparison of different origin groups, and an assessment of genetic diversity. Allele numbers ranged from 15 to 28 for the markers used, whereas observed heterozygosity ranged from 0.519 to 0.956. Grouping of accessions according to geographical origin was not observed. However, the genetic background of Latvian samples was entirely in alignment with international germplasm.

Keywords: pear, microsatellites, cultivars, landraces, crop development.

INTRODUCTION

The origin of domesticated European pear (*Pyrus communis* L.) forms is the Caucasus Mountains and Asia Minor, later spreading to the west (Ozturk *et al.*, 2009). It is the most widely cultivated pear species in Europe, North America, South America, Australia, and Africa (Bassil and Postman, 2010). Currently, pears are the third most economically important fruit crop in the temperate region (Erfani *et al.*, 2012; Silva *et al.*, 2014), commercially cultivated in more than fifty countries (Zarei *et al.*, 2019), and reaching up to 24 million tons in annual production with a growing area of over one million hectares (FAO, 2021).

The crop should constantly evolve to develop as it faces new challenges such as climate change, the spread of diseases and pests, and consumer expectations. An important precondition for the development of each crop is the availability of raw material or genetic resources (GR). Pear

germplasm is used in breeding programmes to introduce novel and desirable alleles into the existing populations (Asanidze *et al.*, 2014) and fulfil the demands for improved yield, quality, and adaptability to changing climate conditions and new and recurring plant diseases and pests. Proper conservation and utilisation of genetic resources also meet specific issues like misnaming and duplication (Sehic *et al.*, 2012), as maintaining duplicates is a waste of resources and can lead to a possible loss of genetic diversity. Another issue important for genetic resources can be inbreeding — such problems have been observed with Japanese cultivars (Nishio *et al.*, 2016), where a proposed solution was the introduction of foreign germplasm from China. Similarly, a problem of genetic erosion has been observed in Portugal (Queiroz *et al.*, 2015), which points to the importance of genetic resource collection evaluation and characterisation to maintain genetic diversity, identify gaps and needs for the introduction of new material.

The introduction of molecular markers made the germplasm characterisation more targeted, less dependent on the environment and more informative. Molecular research allows the study of the evolution, origin, ploidy of different species and cultivars, evaluation of genetic diversity, and understanding the origin of local and introduced germplasm (Nadeem *et al.*, 2018). In the studies of pear germplasm, different types of markers have been used: RAPD (e.g., Saba *et al.*, 2017), ISSR (e.g., Khorshidi *et al.*, 2017), AFLP (Ali *et al.*, 2018; Cao *et al.*, 2019), and SNP (Nadeem *et al.*, 2018). One of the most common molecular markers in the evaluation of genetic resources are simple sequence repeats (SSR). They have become popular as a molecular marker of choice due to many valuable properties — high mutation rate, locus specificity, high polymorphism and reproducibility, they are multiallelic, and use across related taxa (Taheri *et al.*, 2018). Molecular studies to evaluate the genetic diversity of pear germplasm have been done in a variety of countries in different climates, including but not limited to Belarus (Urbanovich *et al.*, 2011), Bosnia and Herzegovina (Gasi *et al.*, 2013; Zeljković *et al.*, 2021), Georgia (Asanidze *et al.*, 2014), Germany (Reim *et al.*, 2016), Italy (Ferradini *et al.*, 2017), Japan (Nishio *et al.*, 2016), Poland (Wolko *et al.*, 2015), Portugal (Queiroz *et al.*, 2015; 2019), Spain (Ferreira dos Santos *et al.*, 2011; Alonso Segura *et al.*, 2021), and Tunisia (Brini *et al.*, 2008; Ouni *et al.*, 2020). These studies focus on different subjects such as evaluating local cultivars, comparison of wild relatives, advanced cultivars and other *Pyrus* species and assessing the issues of the genetic diversity of each country's germplasm.

Pear (*P. communis*) is not a native plant species in Latvia, and the current germplasm results from introduction and breeding. The beginnings of pear cultivation in Latvia can be attributed to the crop's introduction through the orchards of German nobility, who planted material brought from West Europe already in the Middle Ages. Most of these cultivars were not suitable for the local climate; and from these introductions, only the most winter-hardy may have survived. An increase in pear introduction occurred during the 19th century when the Wagner and Shoch nurseries were opened in Riga, bringing new pear material from abroad. However, many of these introduced cultivars were either unsuited for Latvian winters or become susceptible to diseases. Therefore, the demand for local pear breeding was updated, and the first targeted local pear breeding was started in the 1930s (Kārkliņš, 2004). The most significant contributions to pear breeding were made by P. Upītis, R. Āboliņš, A. Maizītis, and R. Dumbravs in the 20th century. P. Upītis collected and evaluated landraces and made crosses with advanced cultivars, which led to selection of the most prospective hybrids, such as the cultivars 'Jumurda', 'Dzintra', and 'Paulina' (Blukmanis *et al.*, 1997). R. Āboliņš and A. Maizītis carried out targeted breeding using adapted Western European cultivars, local landraces and high fruit quality sources as raw material. This resulted in Latvian pear germplasm cultivars 'Dina', 'Rūsa', 'Dace', and 'Vidzeme'. In addition, R. Dumbravs contributed to Latvian pear germplasm by breeding the cultivars 'Talis-

mans' and 'Suvenīrs'. 'Suvenīrs' is still among the most popular cultivars in modern-day Latvia (Ikase *et al.*, 2015).

According to the Central Statistics Bureau of Latvia, the area of pear growing reached about 200 ha in 2020 (CSB, 2021), pears being important fruit crops grown in Latvia. However, despite their popularity, pears are mainly sold within the internal market and can still meet local demand. The most popular cultivars commercially grown in Latvia are 'Belorusskaya Pozdnaya' and 'Suvenīrs', which are acclimated to local conditions (Ikase *et al.*, 2015); direct introduction of new high-quality Western European cultivars is not possible due to climate requirements. Therefore, further crop development still depends on the availability of suitable local germplasm. Given Latvia's pear GR development history, it is vital to understand how genetically diverse the current collection is and how it fits into the world's pear germplasm. Therefore, to plan the further development of the pear crop, to ensure the successful preservation of the existing collection, this study aimed to characterise Latvia's *Pyrus* GR collection using molecular markers and estimate the level of genetic diversity within germplasm.

MATERIAL AND METHODS

Plant material and isolation of genomic DNA. Young leaves of 206 *Pyrus* accessions were collected at the genetic resources collection of the Institute of Horticulture, Dobeles, Latvia (56°37'0"N, 23°16'0"E) (Table 1). Total DNA isolation was done using the Genomic DNA Purification Kit (Thermo Scientific™, Lithuania) according to the manufacturer's methodology.

SSR genotyping. Approximately 50 ng of genomic DNA was used for PCR amplification in a 25 µl reaction containing 1× PCR reaction buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 2.5 pmol of each primer and 0.6 U Taq DNA polymerase (Thermo Scientific™, Lithuania). According to the published protocols for each SSR marker, PCR reactions were run in a Mastercycler egradient thermal cycler (Eppendorf, Germany). The following SSR markers were used in the study: MS14h03, CH04e05, CH03g12, CH03g07, CH02b10, and CH02b11 (Liebhard *et al.*, 2002). PCR products were first checked on a 1% agarose gel in 1× TAE buffer and visualised by staining with ethidium bromide to test for the presence of PCR products. The same PCR products were then analysed on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA) and genotyped using GeneMapper® Software v4.0 (Applied Biosystems, USA).

Data analysis. In order to characterise the applicability of the utilised markers, the following parameters were calculated: observed heterozygosity (Weir, 1990), the polymorphic information content (PIC) (Röder *et al.*, 1995), the probability of matching genotypes and their discriminating power (Jones, 1972; Kloosterman *et al.*, 1993). SSR fragments were coded as present (1) or absent (0) in a binary matrix, which was analysed by software GenAlEx 6.5

Table 1. Description of the 206 analysed *Pyrus* accessions

No.	Genotype	Origin*	Pedigree
1	Abbe Fetel	FRA	Unknown
2	Alexandrine Dovillard	FRA	Unknown
3	Almas Tūrmās	LVA	Unknown
4	Altmaņa bumbiere	LVA	Unknown
5	AM 1-1-7	LVA	-
6	AMD 24-15-12	LVA	-
7	AMD 35-76-1	LVA	AM-5-3-19 × Clapp's Favourite
8	AMD 39-76-3	LVA	AM-5-3-19 × Beurre Dumont
9	AMD 41-10-21	LVA	Dina × Talsu Skaistule
10	AMD 41-17-14	LVA	Malnavas bumbiere seedling No. 7 × Clapp's Favourite
11	AMD 41-5-16	LVA	AM-5-7-21 × Princesse Marianne
12	AMD 41-5-22	LVA	AM-5-7-20 (Guna) × AM-5-5-11
13	AMD 41-5-3	LVA	Fondante de Bois o. p.
14	AMD 42-2-36	LVA	Malnavas bumbiere seedling No. 1 F1 × Beurre Dumont
15	AMD 42-3-16	LVA	Rūsa × Beurre Dumont
16	AMD 42-4-31	LVA	AM-5-4-19 × Viktorija
17	AMD 42-5-28	LVA	AM-5-3-9 (Kurzemes Sviesta seedling)=(Dina) × Viktoria
18	AMD 48-17-14	LVA	Kursa × Lira
19	AMD 51-5-18	LVA	-
20	AMD 51-6-21	LVA	-
21	AMD 53-7-19	LVA	-
22	AMD 55-78-3	LVA	Kurzemes Sviesta × Clapp's Favourite
23	AMD 61-76-3	LVA	AM-5-7-21 × Clapp's Favourite
24	AMD 80-76-1(3)	LVA	Clapp's Favourite o. p.
25	AMD 82-76-1	LVA	Clapp's Favourite o. p.
26	Anda no Bauskas	LVA	Basu Ziemas o.p.
27	Apškalniete No. 215	LVA	Unknown
28	Arion	—	Unknown
29	Armida	DEU	Jules Guyot × Comice
30	Bārtas Bergamote	LVA	Unknown
31	Basu Ziemas	LVA	Unknown
32	Bauskas Sviesta	LVA	Unknown
33	Beloruskaya Pozdnjaya	BLR	Bonne Louise d'Avranches o. p.
34	Bergamot Esperen	BEL	Unknown
35	Besemyanka	RUS	Unknown
37	Beurre Alexandr Lucas	FRA	Seedling
38	Beurre Bosc	FRA	o.p. seedling
40	Beurre D'Anjon	BEL	Unknown
36	Beurre des Charneuses	BEL	Unknown
41	Beurre Lebrun	FRA	Unknown
39	Beurre D'Amanlis	FRA	Unknown
42	Bonne Louise Lavrauches	FRA	Unknown
43	BP-30	—	Unknown

44	Briežu Neistā Sviesta	LVA	Unknown
45	Burakivka	UKR	Unknown
46	Carpica	ROU	Napoca × Beurre Precoc Morettini
47	Caunas meža bumbiere	LVA	Unknown
48	Cisiņu bumbiere	LVA	Unknown
49	Citrine	LTU	Unknown
50	Chizhovskaya	RUS	Olga × Fondante de Bois
51	Clapp's Favorite	USA	Fondante de Bois o. p.
52	Coloree de Juillet	FRA	Unknown
53	Comtesse de Paris	FRA	Saint-Germain Blanche × Cure
54	Concorde	GBR	Doyenne du Comice × Conference
55	Conference	GBR	Leon Leclerc de Laval seedling
56	Coscia	ITA	Seedling
57	D-10-2	LVA	Dina × Souvenirs
58	D-4-6	LVA	Conference × AM-5-7-21
59	Dace	LVA	Fondante des Bois × Kurzemes Sviesta
60	Devoe	USA	USA
61	Dina	LVA	Fondante de Bois o.p.
62	Doria	POL	<i>Pyrus communis</i> var. <i>caucasica</i> fed. seedling
63	Doyenne De Juillet	BEL	Seedling
64	Doyenne D'Hiver	BEL	Seedling
65	Dzintra	LVA	Unknown
66	Elia	POL	<i>Pyrus communis</i> var. <i>caucasica</i> fed. seedling
67	Epargne	FRA	Unknown
68	Epine Du Mas	FRA	Seedling
69	Ešnapūres Dadzītis	LVA	Unknown
70	Garokalna bumbiere	LVA	Unknown
71	General Leclerc	FRA	Doyenne du Comice o. p.
72	Green Windsor	NLD	Unknown
73	Grosdemange	—	Unknown
74	Grumnower Butterbirne	DEU	Unknown
75	Guna	LVA	Precocose de Trevoux o.p.
76	Howell	USA	Seedling of Jonah
77	Janitēna No. 8	LVA	Unknown
78	Jaunlagzdīņa bumbiere	LVA	Unknown
79	Josephine De Malines	BEL	Major Esperen chance seedling
80	Jumurda	LVA	Unknown
81	Junsko Zlato	SRB	'Precocose de Trevoux' × 'Doyenne de Juillet'
82	Justina	LVA	Unknown
83	Kadi	EST	Unknown
84	Kalētu bumbiere	LVA	Unknown
85	Karamelnaya	RUS	Unknown
86	Katrīna	LVA	Livija o.p.
87	Kazraušu bumbiere	LVA	Unknown
88	Kazraušu bumbiere No.13	LVA	Unknown
89	Kazraušu bumbiere No.18	LVA	Unknown
90	Kiras bumbiere	LVA	Unknown

Continuation of Table 1. Description of the 206 analysed *Pyrus* accessions

No.	Genotype	Origin*	Pedigree
91	Kosmicheskaya	RUS	Doč Blankovoi × Russkaja Malgoržatka
92	Krol Sobieski	POL	Unknown
93	Kursa	LVA	Kurzemes Sviesta seedling
94	Latgale	LVA	Kurzemes Sviesta × Clapp's Favourite
95	Lauriņa (syn. BP 8965)	SWE/LVA	Clapp's Favourite × Beurre Blumenbach
96	Leimaņa Bumbiere	LVA	Unknown
97	Liepājas Sviesta	LVA	Unknown
98	Madam Favre	FRA	Beurre d'Hardenpont seedling
99	Madam Verte	BEL	Unknown
100	Madonas Bergamote	LVA	Unknown
101	Malnavas Bumbiere	LVA	Unknown
102	Marsa	LVA	Mičurina Ziemas Sviesta bumbieres seedling
103	Mellina	ITA	Beurre Clairgeau × Williams Bon Cretien
104	Miku	EST	Bere Zimnyaya Michurina × Lutsu Voipirn
105	Muižas No.1	LVA	Unknown
106	Muižas No.2	LVA	Unknown
107	Muižas No.4	LVA	Unknown
111	No. 217	—	Unknown
112	No. 8	EST	Lutsu Voipirn × Saaremaa Punane
108	Norma	ITA	Dr.J. Guyot × Bella di Giugno
109	Noyabrskaya	MDA	Triomphe de Vienne × Krier
113	Oliver De Serres	FRA	Bergomote Fortunee o.p.
114	Orlovskaya Letnyaya	RUS	Bergamot Novik × Clapp's Favourite
115	Osennyyaya Zholtaya	RUS	Unknown
116	P.Petrilas No. 49	LTU	Unknown
117	P.Petrilas No. 11	LTU	Unknown
118	P.Petrilas No. 22	LTU	Unknown
119	P.Petrilas No. 31	LTU	Unknown
120	P.Petrilas No. 71	LTU	Unknown
121	P.Petrilas No. 97	LTU	Unknown
122	Pamatj Yakovlevu	RUS	Tema × Olivier de Serres
123	Pamatj Zhegalova	RUS	Olga × Fondante de Bois
124	Paulina	LVA	Unknown
125	Pierre Corneille	FRA	Beurré Diel × Doyenné du Comice
126	Pitmaston Duchessed Angouleme	GBR	Duchesse d'Angouleme × Beurre d'Hardenpont
127	Plaužu kompaktais	LVA	Unknown
128	Popes	LVA	Unknown
129	Potapovskaya	RUS	Olga × Fondante de Bois + Clapp's Favourite + Koshkarnok
130	PU-11-5-32	LVA	Unknown
131	PU-15142	LVA	Unknown

132	PU-15147	LVA	Unknown
133	PU-15-18-31	LVA	Unknown
134	PU-16758	LVA	Unknown
135	PU-17785	LVA	Unknown
136	PU-20378	LVA	Unknown
137	PU-20493	LVA	Unknown
138	PU-20597	LVA	Unknown
139	PU-20598	LVA	Unknown
140	PU-20903	LVA	Unknown
141	<i>Pyrus (Cydonia oblonga)</i>	—	Unknown
146	<i>Pyrus bolleriana</i>	CZE	<i>Pyrus</i> × <i>Sorbus</i>
142	<i>Pyrus eleagnifolia</i>	—	Unknown
147	<i>Pyrus</i> PU-1	LVA	Unknown
143	<i>Pyrus pyrastra</i>	—	Unknown
144	<i>Pyrus salicifolia</i>	—	Unknown
145	<i>Pyrus ussuriensis</i>	—	Unknown
148	Rībeļa Dārzniecības bumbiere	LVA	Unknown
149	Rīgas autoostas bumbiere	LVA	Unknown
150	Rīgas Kanālmalas bumbiere	LVA	Unknown
151	Rižas	LVA	Unknown
152	Rumpi No. 8	LVA	Unknown
153	Rūsa	LVA	Fondante de Bois × Princesse Marianne
154	Rūta (syn. BP-10529)	SWE/LVA	Clapp's Favourite × Beurre Blumenbach
155	Saaremaa Punane	EST	Unknown
157	Saldus Apaļā No.1	LVA	Unknown
158	Saldus Apaļā No.2	LVA	Unknown
159	Saldus Apaļā No.3	LVA	Unknown
156	Saldus bumbiere	LVA	Unknown
160	Seces No.7	LVA	Unknown
161	Seces No.8	LVA	Unknown
162	Selanka	RUS	Fondante de Bois o.p.
163	Serenāde	LVA	Fondante de Bois o.p.
164	Sibiryachka	RUS	Bere Kozlovskaya × <i>P. ussuriensis</i>
165	Sierra	CAN	Bartlet (Williams) × Marguerite Marillat
166	Snēpeles Ziedīņu bumbiere	LVA	Unknown
167	Štaras No. 31	LTU	Unknown
168	Stark Bros New	USA	Unknown
169	Starkrimson	USA	bud mutation of Clapp's Favourite
110	Šterka Ābolveida (No. 20527)	LVA	Unknown
170	Summercrisp	USA	Unknown
171	Suvenirs	LVA	Malnavas bumbiere seedling No.1 F1 × Beurre Dumont
172	Talgarskaya Krasavitsa	KAZ	Fondante de Bois o.p.
173	Talismans	LVA	Malnavas bumbiere seedling No. F1 × Beurre Dumont/Clapp's Favourite
174	Talsu Skaistule	LVA	Unknown

Continuation of Table 1. Description of the 206 analysed *Pyrus* accessions

No.	Genotype	Origin*	Pedigree
175	Tihij Don	RUS	Rossošankaja Krasivaja × Mramornaja
176	Timaņa Agrā	LVA	Unknown
177	Tipa	EST	Lutsu Voipirn × Karmila
178	Tjomas	RUS	Finlandskaja Zoltaja × <i>P. ussuriensis</i>
179	Tongre	BEL	Chance seedling
180	Trapezitsa	BGR	Beurre Giffard × Cerovka
181	Triumf	FRA	Unknown
182	U-2568	LVA	Unknown
183	U-3981	LVA	Unknown
184	U-678	LVA	Unknown
185	Uzala	LVA	Bere Zimnyaya Michurina × Comtesse de Paris
186	Valdgales	LVA	Unknown
187	Valdgales No.1	LVA	Unknown
188	Valdgales No.2	LVA	Unknown
189	Valtera No.27	LVA	Unknown
190	Valtera No.94	LVA	Unknown
191	Vasaras bumbiere Kripene	LVA	Unknown

(Peakall and Smouse, 2012). Principal coordinate analysis (PCoA) based on genetic distance was applied to identify the genetic structure of *Pyrus* germplasm and to evaluate relationships among genotypes.

The model-based clustering method of STRUCTURE 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was applied to discover the possible genetic structure of the 206 *Pyrus* samples and define the most likely number of clusters (K value). Ten independent runs of Structure were performed for each K value from 2 to 11. Each run consisted of a burn-in period of 100 000 steps, followed by 100 000 Monte Carlo Markov Chain replicates, assuming an admixture model and correlated allele frequencies. No prior information was used for cluster definition. The most likely K was chosen to compare the average estimates of the likelihood of the data, $\ln[\Pr(X|K)]$, for each value of K (Pritchard *et al.*, 2000), as well as calculating the *ad hoc* statistics ΔK , based on the rate of change in the $\ln$ -probability of data between successive K values (Evanno *et al.*, 2005). The proportion of membership (*q*) of each individual in each gene pool was estimated. The cluster matching and permutation was calculated by software CLUMPP 1.1 (Jakobsson and Rosenberg, 2007) and visualised by Structure Plot V2.0 (Ramasamy *et al.*, 2014).

RESULTS

Characterisation of SSR molecular marker polymorphism in *Pyrus* collection. All of the tested SSR markers gave a successful polymorphic amplification in the tested pear material (Table 2). The primer pairs used provided

192	Veisberga bumbiere	LVA	Unknown
193	Vēvera bumbiere	LVA	Unknown
194	Vidzeme	LVA	Fondante de Bois o.p.
195	Viestura bumbiere	LVA	Unknown
196	Viksna No.2	LVA	Unknown
197	Viļānu bumbiere	LVA	Unknown
198	Vilma	LVA	Malnavas bumbiere seedling No.31 F1 × Clapp's Favourite
199	Volopska	RUS	Unknown
200	Voskovka	RUS	<i>P. communis</i> seedling
201	Wilard	USA	Unknown
02	Williams Bon Cretien	—	Unknown
203	Zādzēnes bumbiere	LVA	Unknown
204	Zarizak	—	Unknown
205	Zemgale	LVA	AM-5-5-6 × Princesse Marianne
206	Zundes bumbiere	LVA	Unknown

* BLR – Belarus, BEL – Belgium, BGR – Bulgaria, CAN – Canada, CZE – Czech Republic, EST – Estonia, FRA – France, GBR – United Kingdom, DEU – Germany, ITA – Italy, KAZ – Kazakhstan, LVA – Latvia, LTU – Lithuania, MDA – Moldova, NLD – Netherlands, POL – Poland, ROU – Romania, RUS – Russia, SRB – Serbia, SWE/LVA – Sweden, Latvia, UKR – Ukraine, USA – the United States of America, — – the exact country of cultivars origin was unidentifiable

identification of unique fingerprints for 197 *Pyrus* accessions. The set of markers used failed to distinguish nine accessions: identical pattern of amplification patterns were observed for accessions AMD 42-3-16, Muižas No. 4, *Pyrus* PU-1; accession pairs PU 15-18-31 and 'Beurre D'Anjou', Muižas No. 2 and *Pyrus ussuriensis*; 'Apškalniete' and 'Saldus Bumbiere'. Allele numbers varied from 15 (MS14h03) to 28 (CH02b11), with an average of 21 per marker. In terms of allele range (in base pairs), the marker CH02b11 stood out with a range of 145 bp (49-194 bp), while the rest of the markers were within a range from 38 bp (MS14h03) to 56 bp (CH03g12). Observed heterozygosity (*H*_o) ranged from 0.519 (CH03g12) to 0.956 (CH02b10), averaging 0.709 per marker. The polymorphic information content (PIC) for the tested markers ranged from 0.712 (MS14h03) to 0.914 (CH02b10) with an average of 0.819. Discrimination power ranged from 0.855 (MS14h03) to 0.955 (CH03g07) with an average value of 0.927.

In order to assess the diversity of Latvian pear germplasm developed throughout the history of cultivation and its connection with global plant material, three accession groups were defined: advanced cultivars, developed in Latvia by targeted breeding (LVA-advanced); landraces and locally grown clones collected throughout Latvia with unknown origin (LVA-local), and introduced foreign pear cultivars and hybrids (Introduced). When comparing between groups, specific patterns were discovered. The number of alleles for introduced accessions was highest for five of six markers (MS14h03, CH04e05, CH03g12, CH02b10, CH02b11) (Table 2). However, for marker CH03g07, introduced and LVA-local accessions had the same number of alleles — 19. The highest values of *H*_o for markers MS14h03 and

Table 2. Genotyping results for 206 *Pyrus* accessions using six SSR loci

Locus	Group*	Number of alleles	Allele range, bp	Observed heterozygosity	PIC**	Discrimination power at the locus in the population
MS14h03	Total	15	104–142	0.515	0.712	0.855
	1	11	108–142	0.509	0.665	0.819
	2	11	108–142	0.557	0.746	0.874
	3	14	104–134	0.489	0.702	0.837
CH04e05	Total	18	169–219	0.757	0.790	0.926
	1	15	169–219	0.745	0.747	0.908
	2	15	169–207	0.705	0.795	0.917
	3	18	169–209	0.800	0.801	0.909
CH03g12	Total	19	160–216	0.519	0.834	0.928
	1	11	164–206	0.527	0.833	0.924
	2	13	160–200	0.459	0.847	0.925
	3	18	164–216	0.556	0.809	0.909
CH03g07	Total	20	217–263	0.820	0.850	0.955
	1	16	219–261	0.764	0.819	0.932
	2	19	217–263	0.902	0.852	0.943
	3	19	219–263	0.800	0.862	0.956
CH02b10	Total	22	120–167	0.956	0.914	0.981
	1	16	124–167	0.964	0.897	0.961
	2	18	120–167	0.934	0.914	0.971
	3	19	120–165	0.967	0.912	0.975
CH02b11	Total	26	49–127	0.686	0.813	0.916
	1	18	49–127	0.673	0.843	0.922
	2	17	49–127	0.600	0.813	0.906
	3	23	49–127	0.750	0.780	0.905

* 1, advanced cultivars, developed in Latvia by targeted breeding; 2, landraces and locally grown clones collected throughout Latvia with unknown origin; 3, introduced foreign pear cultivars and hybrids

** PIC, polymorphic information content

CH03g07 were observed for LVA-local and introduced accessions, respectively. In comparison, the group of introduced accessions had the highest value of H_o for the remaining markers. The highest polymorphic information content (PIC) values were observed mainly in LVA-local and introduced accession groups, for markers MS14h03, CH03g12, CH02b10 and CH04e05, CH03g07, respectively. Only the marker CH02b11 had the highest PIC value for the LVA-advanced accession group.

Genetic structure of *Pyrus* collection. The defined three accession groups were compared using AMOVA, which did not show statistically significant differences between these groups. Complete overlap of the pear accession groups was also shown by PCoA (Fig. 1) without distinguishing genetically distinct groups. Therefore, the Structure software (Pritchard *et al.*, 2000) model-based approach was used to analyse the genetic structure of the pear material. Evaluation of the most likely number of clusters was done with the ΔK method, and the highest peak was stated for $K = 9$ (Fig. 2), showing nine clusters with 27, 25, 29, 20, 20, 15, 15, 30,

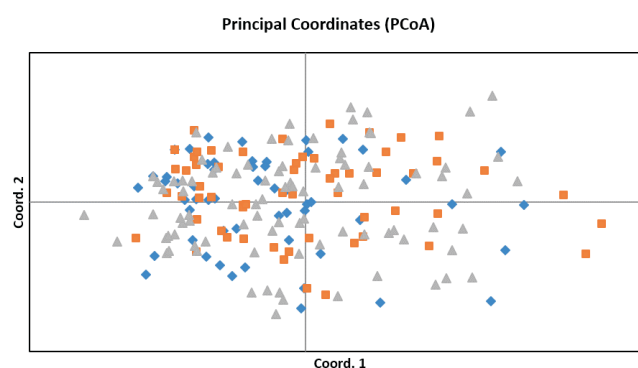


Fig. 1. Relationships among 206 *Pyrus* accessions shown in the two-dimensional scatter plot of the first two principal coordinates, based on six SSR molecular markers.

◆ advanced cultivars, developed in Latvia by targeted breeding; ■ landraces and locally grown clones collected throughout Latvia with the unknown origin; ▲ introduced foreign pear cultivars and hybrids.

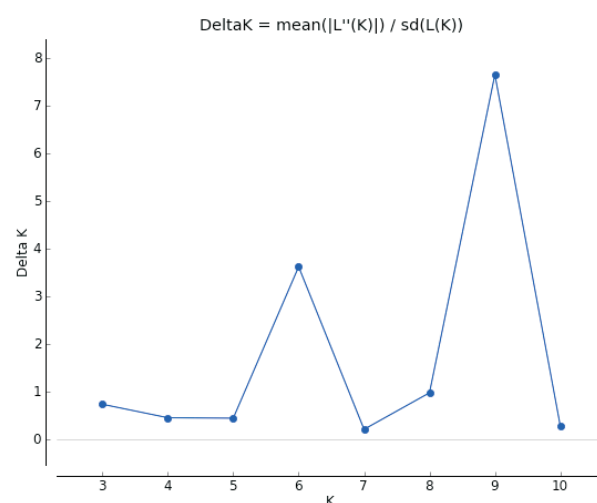


Fig. 2. DeltaK plot for K values 2–11 calculated from six SSR marker data of 206 *Pyrus* accessions.

and 25 accessions in each, respectively. The proportions of membership (q) of each *Pyrus* accession are shown in Figure 3. The six SSR markers grouped the pear accessions into nine distinct groups (Fig. 3). However, the admixture between groups was high, as membership values above 80% were found for only 27.7% of the accessions. Group 3 had the highest level of admixture (44.8%), whereas Group 2 had the lowest (11.5%). Accessions of the same country of origin appeared equally in multiple groups. Only accessions of Latvian origin were found in all groups, followed by French and Russian accessions, represented in seven groups. Accessions from other countries are distributed relatively evenly across all groups without definable regularity. The accessions of different *Pyrus* species included in the study were only in two groups defined by Structure: *P. pyraeaster* (L.) Burgsd. – Group 1 and *P. eleagnifolia* Pall., *P. salicifolia* Pall., *P. ussuriensis* Maxim. – Group 4. In terms of linkage between pedigree and defined groups – no prevalence of any parental cultivar was observed in certain

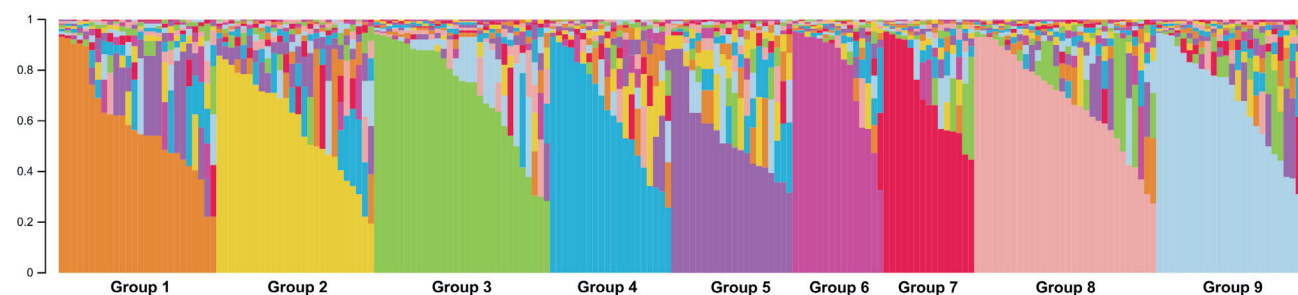


Fig. 3. Graphical representation of the results of the Structure analysis based on 206 *Pyrus* accessions and six SSR markers, inferred at $K = 9$. Each accession is represented by a vertical line segmented into a number of colours identical to the K number.

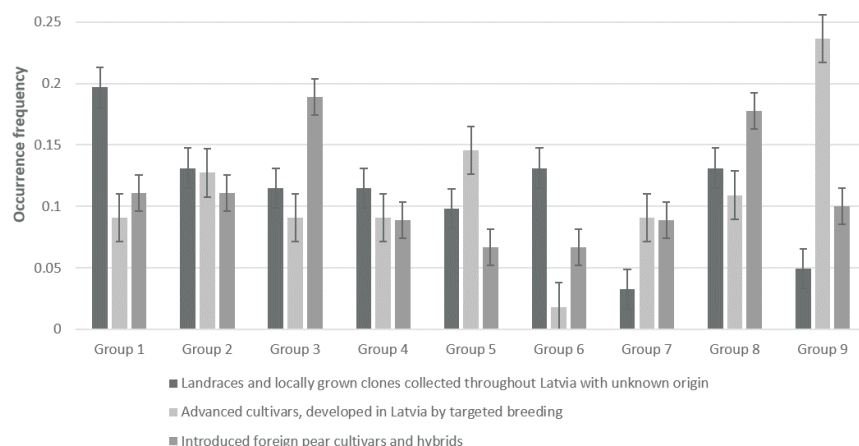


Fig. 4. Relationship of the accession groups identified by the Structure software to the *Pyrus* germplasm accession groups based on their origin. Groups 1 to 9 correspond to the accession groups identified in the Structure software (Fig. 3). Error bars represent the standard error values.

groups, while one parent – ‘Fondante de Bois’, showed up as an ancestor for at least one accession in each group, except Group 3. However, some association with pedigree can be observed, for example, accessions with the same pedigree as ‘Rūta’ and ‘Lauriņa’ (‘Clapp’s Favorite’ × ‘Beurre Blumenbach’) and rootstocks ‘Doria’ and ‘Elia’ (*Pyrus communis* var. *caucasica* fed. seedling) are grouped in one group (groups 5 and 6, respectively).

The representation of Latvian *Pyrus* germplasm groups (advanced Latvian cultivars, landraces and introduced cultivars) in the groups defined by Structure showed certain characteristics (Fig. 4). Advanced Latvian cultivars and hybrids dominate in groups 5 and 9, landraces and local clones collected during genetic resources collection expeditions dominate groups 1 and 6, while foreign *Pyrus* germplasm was predominant in groups 3 and 8. Equivalent representation was observed in the other Structure accession groups.

DISCUSSION

Overall, the marker characterisation data obtained in this study showed similarity to previously published reports and was favourable in terms of high values for diversity (number of alleles, observed heterozygosity) and marker informativeness (PIC, discrimination power). Several studies that were used for comparison included common cultivars — ‘Conference’ (Ferreira dos Santos *et al.*, 2011; Urbanovich *et al.*, 2011; Gasi *et al.*, 2013; Liu *et al.*, 2015; Urrestarazu *et al.*, 2015; Ouni *et al.*, 2020), ‘Clapp’s Favorite’ (Liu

et al., 2015; Ouni *et al.*, 2020), ‘General Leclerc’ (Ferreira dos Santos *et al.*, 2011; Urrestarazu *et al.*, 2015), and ‘Williams’ (Ferreira dos Santos *et al.*, 2011; Kocsisne *et al.*, 2020). Therefore, it can be concluded that the acquired data is representative, can be compared to other collections and can be used to describe the genetic diversity of the analysed material.

The number of pear accessions in different studies varies significantly, starting with less than 100 accessions (Urbanovich *et al.*, 2011; Gasi *et al.*, 2013; Ouni *et al.*, 2019) to over 200 accessions (Ferreira dos Santos *et al.*, 2011; Liu *et al.*, 2015; Urrestarazu *et al.*, 2015; Nishio *et al.*, 2016; Reim *et al.*, 2016). The size of the analysed collection affected the number of identified alleles. However, other genetic studies of pears had a comparable number of alleles for selected markers: CH02b10 (Ferreira dos Santos *et al.*, 2011; Gasi *et al.*, 2013; Urrestarazu *et al.*, 2015; Alonso Segura *et al.*, 2021), CH03g07 (Ferreira dos Santos *et al.*, 2011; Urrestarazu *et al.*, 2015; Zeljković *et al.*, 2021), and CH03g12 (Urbanovich *et al.*, 2011). The overall higher number of alleles in our study can be explained by a more extensive number of samples and their potential diversity. The allele ranges found in the study (Table 2) was comparable to those obtained in other studies (Pierantoni *et al.*, 2004; Ferreira dos Santos *et al.*, 2011; Urbanovich *et al.*, 2011; Liu *et al.*, 2015; Kocsisne *et al.*, 2020; Ouni *et al.*, 2020), the slight shifts being related to the difference in the studied plant material. Allele length similarities confirm that marker transferability has been successful and can be used to study different collections.

Observed heterozygosity (Ho) was variable among markers. In the case of marker CH02b10, the Ho values were similar to data published by Quieroz *et al.* (2019) but higher than in other studies (Ferreira dos Santos *et al.*, 2011; Erfani *et al.*, 2012; Reim *et al.*, 2016; Baccichet *et al.*, 2019; Sau *et al.*, 2020). Marker CH03g07 had similar Ho values to several publications (Ferreira dos Santos *et al.*, 2011; Sehic *et al.*, 2012; Baccichet *et al.*, 2019; Quieroz *et al.*, 2019). Marker CH03g12 with an average Ho of 0.519 was higher than the data of Urbanovich *et al.* (2011) but lower than the data provided by Liu *et al.* 2015. These results demonstrated the effect of the sample size — 43 accessions (Urbanovich *et al.*, 2011), 385 accessions (Liu *et al.*, 2015) and 206 accessions in our study. The PIC values represent informativeness of the marker, and PIC values of over 0.7 are considered highly informative (Hildebrand *et al.*, 1994). The PIC values of all markers included in the current study were above this value (Table 2) and can be considered highly informative and well suited for describing the pear germplasm. The suitability of the markers is also supported by high values of discrimination power, the lowest value in this study was 0.855. This supports the use of a limited number of markers (six SSR markers in the current study) for germplasm fingerprinting — it ensured unique genotypes for 197 out of 206 accessions. However, the possibility of misnaming and other identification errors must be considered, especially in the case of local germplasm.

Although the markers used provided good resolution of genotypes, genotyping data in the studied *Pyrus* collection did not distinguish distinct accession groups (Fig. 1). The origin groups (LVA-advanced, LVA-local and Introduced) completely overlap; no association with origin was observed. A similar phenomenon was also observed in other studies (Ferreira dos Santos *et al.*, 2011) — when minor differences were observed between groups of different geographic origins. Distinct grouping was identified in comparisons between wild and domesticated pears (Nishio *et al.*, 2016) or region-specific accessions and international reference cultivars (Gasi *et al.*, 2013). This indicates the disconnection of genetic diversity and relatedness with geographical origin. In the case of Latvian *Pyrus* germplasm (both landraces and advanced cultivars), this indicates the significant association of plant material with the world, especially Western European cultivars, through the direct introduction and later adaptation and use of parent plants in breeding.

The Structure grouping into nine distinct categories leads to the assumption about the genetic diversity of analysed material. Latvian accessions, being represented in all groups, show the different ways of incorporating foreign material into the local gene pool and solves the previous problem with the three initial groups — simply grouping the material by origin masks the mechanisms by which the selected material was acquired. The fact that foreign material was evenly distributed between groups also confirms this — introduced material was evenly incorporated into local material to introduce valuable traits. Some studies concluded

that the groups formed could indicate the introgression of foreign cultivars into local populations (Ferreira dos Santos *et al.*, 2011; Zeljković *et al.*, 2021). Structure grouping discovered the noticeable pattern — accessions with the same pedigree as 'Rūta' and 'Laurina' were found to be in the same group, and the same was observed in rootstocks 'Doria' and 'Elia' with *P. communis* var. *caucasica* seedlings in pedigree. The grouping of accessions revealed possible breeding trends — two groups dominated cultivars obtained with targeted breeding, two groups included landraces, whereas foreign material grouped separately in two groups.

Latvian *Pyrus* germplasm possesses a diverse genetic background and can be used as valuable raw material for breeding and selecting cultivars suitable for the region. From the beginning of crop introduction, plant material from Western Europe and other world regions was introduced to select the highest quality. During later development of the crop through targeted breeding, initially introduced and adapted pear germplasm was involved in hybridisation with high-quality cultivars. The diversity and structure of the Latvian *Pyrus* genetic resources collection revealed by SSR molecular markers confirm the historical path of crop development.

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LATVIJAS *PYRUS* ĢENĒTISKO RESURSU DAUDZVEIDĪBAS UN RADNIECĪBAS NOVĒRTĒŠANA, IZMANTOJOT SSR MARĶIERU KOPU

Bumbieres (*Pyrus communis* L.) nav vietējā Latvijas augu suga, taču tām ir sena introducēšanas un audzēšanas vēsture, laika gaitā izmantojot dažādus augu avotus. Tāpēc esošajā ģenētisko resursu kolekcijā apvienoti dažādas izcelsmes paraugi. Lai plānotu šī kultūrauga turpmāku attīstīšanu un nodrošinātu tā veiksmīgu saglabāšanu, šī pētījuma mērķis bija raksturot Latvijas *Pyrus* ģenētisko resursu kolekciju un novērtēt tās ģenētiskās daudzveidības līmeni, izmantojot SSR molekulāros marķierus. Lietojot sešus SSR marķierus, tika genotipēti 206 dažādas izcelsmes bumbieru paraugi, piemēram, Latvijā audzētas šķirnes un hibrīdi, ekspedīcijās ievāktie tautas selekcijas un introducēto ārzemju šķirņu paraugi. Marķieru kopa ļāva identificēt 197 paraugus ar unikāliem genotipiem un salīdzināt dažādas izcelsmes grupas un novērtēt ģenētisko daudzveidību. Konstatētais marķieru alēļu skaits svārstījās no 15 līdz 28, savukārt novērotā heterozigotāte variēja no 0,519 līdz 0,956. Paraugu grupēšanās pēc ģeogrāfiskās izcelsmes netika novērota. Pētījumā konstatēts, ka Latvijas bumbieru paraugi pilnībā iekļaujas pasaules ģenētiskajos resursos.