Plant Genetic Resources: Characterization and Utilization

cambridge.org/pgr

Genetic analysis of village pear (*Pyrus communis* L.) cultivar populations in northeastern Türkiye

Abdulbaki Çoban[1,2], Funda Ö. Değirmenci[3], Asiye Uluğ[4], Mevlüde Alev Ateş[5], Emrah Yüksel[6], Özgür Eminağaoğlu[6] and Zeki Kaya[1]

[1]Department of Biological Sciences, Middle East Technical University, Ankara, Türkiye; [2]The Institute for Evolutionary Biology, WWU Münster, Münster, Germany; [3]Department of Field Crops, Kırşehir Ahi Evran University, Kırşehir, Türkiye; [4]Department of Biology, Kafkas University, Kars, Türkiye; [5]Department of Agricultural Biotechnology, Kırşehir Ahi Evran University, Kırşehir, Türkiye and [6]Department of Forest Engineering, Artvin Çoruh University, Artvin, Türkiye

Research Article

Cite this article: Çoban A, Değirmenci FÖ, Uluğ A, Ateş MA, Yüksel E, Eminağaoğlu Ö, Kaya Z (2024). Genetic analysis of village pear (*Pyrus communis* L.) cultivar populations in northeastern Türkiye. *Plant Genetic Resources: Characterization and Utilization* **22**, 408–416. https://doi.org/10.1017/S1479262124000455

Received: 10 January 2024
Revised: 7 June 2024
Accepted: 10 June 2024
First published online: 23 September 2024

Keywords:
ex situ conservation; genetic structure; *in situ* conservation; land races; *Pyrus communis* L.; village pear cultivars

Corresponding author:
Zeki Kaya;
Email: kayaz@metu.edu.tr

Abstract

Pear (*Pyrus communis* L.) stands out as a prominent fruit species in temperate regions worldwide. The Çoruh River basin, nestled in the lower Caucasus in Türkiye, serves as a valuable repository of pear germplasm. To elucidate the genetic structure of pear populations in this region, 84 village pear cultivar genotypes (land races) from six villages, sample garden collections (SCC), and wild Panta root stock populations were analysed using eleven microsatellite markers. Genetic diversity and structure analyses indicated that village pear cultivar populations exhibit substantial genetic diversity and admixture. This diversity is attributed to local farming practices such as phenotypic selection and widespread dispersal of clonal materials. The genetic structure analysis, combined with the identification of private alleles, indicates that the pear genetic resources in the Çoruh river basin likely has originated from two gene pool sources, specifically the Meydancık and Camili village pear traditional cultivar populations. The Camili village pear cultivar population as a new *in situ* genetic reserve site has been proposed. Despite the existence an *ex situ* conservation site, the study suggests inadequateness of SCC as an *ex situ* site in capturing the full extent of genetic diversity of village pear cultivar genetic resources. Thus, enriching the genetic diversity in the SCC *ex situ* site is essential for effective pear genetic resource conservation in the Çoruh river basin. These findings contribute valuable insights for the development of targeted conservation strategies, ensuring the preservation of pear genetic resources in this region.

Introduction

The genus *Pyrus* (pears), belonging to the Rosaceae family, represents one of the most widespread fruits with significant economic and health values. Pear species have been used worldwide for more than two millennia as nutritious food, folk medicine and ornamental plants in landscaping (Hong *et al*., 2021; Simionca Mărcășan *et al*., 2023). The genus *Pyrus*, comprising deciduous species, demonstrates relatively an easy adaptation to environmental stresses, including drought and salinity (Tatari *et al*., 2020; Dbara *et al*., 2021). Pears are broadly categorized into two main types: The European and Western pears, represented by *P. communis* L., and *P. pyrifolia* (Burm.) Nak., respectively. The genus exhibits a rich diversity, consisting of a minimum of 22 primary species and 10 naturally occurring interspecific hybrid taxa. It is also known for hosting over 5000 subspecies or accessions worldwide (Li *et al*., 2016; Hong *et al*., 2021). However, due to inclination of the species towards hybridization, establishing a precise count of pear species remains challenging (Wolko *et al*., 2010).

Widely distributed across temperate regions of Europe, Asia, and North Africa, the genus *Pyrus* boasts a cultivation history spanning over 3000 years. Many species are believed to have originated in East Asia and have been cultivated in regions such as China, Japan and Korea. Notable centres of diversity extend beyond East Asia to include the Mediterranean, Georgia and Central Asia. The cultivation of pears in Europe dates back to at least 1000 BC (Aldasoro *et al*., 1996; Bell *et al*., 1996).

According to the Food and Agriculture Organization of the United Nations, *P. communis* is the second most produced and consumed pome fruit around the world (FAOSTAT, 2018). Although cultivars of the species are economically valuable throughout the World, the wild populations of cultivated pears are primarily distributed in Europe and the Caucasus. In support, fossils of *P. communis* leaves have been discovered in eastern Georgia, Azerbaijan and Türkiye during the Cretaceous or Palaeocene, predating the Tertiary period (Rubzov, 1944; Zeven and Zhukovsky, 1975).

Türkiye is one of the world's leading pear-producing countries, along with China, the European Union, the United States of America and Argentina (USDA Foreign Agricultural Service, 2023). There is a diverse array of pear genetic resources, encompassing several taxa, with some being endemic, including *P. anatolica* Browicz, *P. serikensis* Güner & Duman and *P. yaltirikii* Browicz. The rich tapestry of local pear cultivars encompasses approximately 500 varieties, displaying considerable diversity in fruit size, shape, colour and texture. Notably, some of these cultivars exhibit resistance to fire blight, contributing to the overall resilience and adaptability of pear cultivation in the region (Muminjanov and Karagöz, 2018). The country, particularly the lower Caucasus, stands out as a unique region where pear species have been cultivated for over 2000 years, harbouring wild genetic resources of cultivated pears (Kaya *et al.*, 1997). Specifically, the Çoruh River basin in the Artvin province, situated in northeastern Türkiye and part of the lower Caucasus Mountain regions, has wild populations of cultivated pears. The rugged terrains and deep canyons contribute to the isolation of many villages or settlements, compelling them to be agriculturally self-sufficient (Çakmakçı *et al.*, 2017). Wild resources of *Pyrus* exhibit notable characteristics such as cold resistance, drought resistance, disease resistance and saline–alkali tolerance (Li *et al.*, 2016; Hong *et al.*, 2021). These attributes make them valuable resources for screening high-quality rootstocks and molecular breeding purposes (Liu *et al.*, 2015). The diverse and complex nature of the genus *Pyrus* sets the stage for a comprehensive exploration of its genetic resources and conservation implications. The genetic resources of pear are generally conserved in clonally established or seedling-based gene banks around the world (Kocsisne *et al.*, 2020). The dispersion of the cultivars is facilitated mainly by the agricultural cultivation, mostly via seedlings and grafting on traditional varieties bred locally by farmers (Davarynejad and Davarynejad, 2004). These *ex situ* conservation programmes are important to maintain the existing genetic diversity in cultivated pears, but they do not allow evolutionary process to be continued. Assessing the magnitude and pattern of genetic diversity is crucial for the future utilization and maintenance of village pear cultivars (hereafter referred to as cultivars) which are kinds of landraces that are discovered, propagated and maintained by village farmers.

Population structure and genetic diversity have been assessed by using several different marker techniques as biochemical, morphological and DNA based ones (Kajiura *et al.*, 1985; Chevreau *et al.*, 1997; Iketani *et al.*, 1998; Kim *et al.*, 2000; Monte-Corvo *et al.*, 2000; Yamamoto *et al.*, 2001, 2002a, 2002b; Kimura *et al.*, 2002; Teng *et al.*, 2002; Paganova, 2003; Elshihy *et al.*, 2004; Wolko *et al.*, 2010; Bao *et al.*, 2007; Brini *et al.*, 2008). Simple sequence repeat (SSR) markers, among the other marker techniques, have been used to characterize pear germplasm in Türkiye (Akcay *et al.*, 2014; Öztürk and Demirsoy, 2015; Bozhüyük and Aslantaş, 2020; Kaymak and Pinar, 2020). As of now, no study has been conducted to assess the genetic structure of village pear cultivar genotypes in Türkiye. Several studies, which focused on sampling country-wide popular pear cultivars, reported that pear germplasm displays an intermixed population structure from different regions of Türkiye (Akçay *et al.*, 2014; Öztürk and Demirsoy, 2015; Bozhüyük and Aslantaş, 2020; Kaymak and Pinar, 2020). However, there is a lack of information regarding the genetic diversity of village pear cultivar populations (hereafter referred to as populations). Additionally, there is a need to understand how the practices of local farmers impact the genetic diversity and structure of the pear gene pool, particularly among and within village pear cultivar populations located in village territories.

Selected pear cultivars from wild populations of *P. communis* are extensively distributed clonally by grafting on compatible rootstocks throughout Türkiye to maintain genotypes with economically valuable fruit characteristics. Thus, the genetic information obtained from the populations in the Artvin province, situated in the lower Caucasus, would be highly valuable for future pear breeding programmes. We employed eleven microsatellite markers to screen the cultivars preserved by local village farmers in the Çoruh River basin in order to evaluate their genetic composition for conservation. The acquired information regarding the genetic diversity and structure of village pear cultivar populations could provide valuable insights for both *in situ* and *ex situ* pear conservation programmes. This research endeavours to fill the current knowledge gap by presenting a comprehensive analysis of the genetic diversity of the populations. Thus, findings advance our comprehension of the genetic structuring in traditional pear cultivars within the specified region.

Material and methods

Plant material and DNA extraction

Leaf tissues from 63 village pear (*P. communis*) cultivars (pear landraces) used in this study were collected from six villages in Artvin Province: Camili, Meydancık, Veliköy, Meşeli, Aşağı Koyunlu and Kirazlı. These pear cultivars were discovered in the wild pear populations in the past, propagated and maintained clonally by the village farmers to date. These cultivars could be considered as landraces being highly heterozygous, harbouring adaptive gene complexes, and adapting to the local climate. The villages, in which cultivars were sampled, were selected due to their remote locations and presence of traditional farming practices for maintaining pear cultivars. Especially, the Camili village located in the Camili Biosphere Reserve (with a 27,152-hectare area) where introduction of non-native plant and animal species are forbidden. Sample size ranged from 6 cultivars in Meşeli to 14 in Camili villages. Additionally, leaf samples from 15 unique genotypes (these are also village pear cultivars) were obtained from a special pear clone collection, referred to as the Sample Collection Garden (SCC). The SCC was a collection of cultivars and set up as *ex situ* conservation site by a family from the Dalkırmaz village under the guidance of the Artvin Directorate of Food, Agriculture and Livestock (Fig. 1). Since the Panta genotypes are used as a compatible root stock in grafting practices by villagers, six wild *P. communis* genotypes named by locals as 'Panta', with one genotype sampled from a forest land near each village, were also included in the study. Here after, this will be referred as wild Panta root stock population. The locations of the villages are depicted in Fig. 1, and geographic information about the sample locations can be found in online Supplementary Table S1. Except for the Panta genotypes, the remaining 78 genotypes are cultivated clonally and maintained as village pear cultivars by local village farmers. The Panta genotypes serve as rootstocks for grafting scions of village pear cultivars. The sampled cultivars were grouped based on village locations and treated as village pear cultivar populations in the analysis of population genetic diversity parameters.

During sampling, green, fresh leaves of village pear cultivars were collected and stored in silica gel-filled bags until DNA

https://doi.org/10.1017/S1479262124000455

Figure 1. The locations of the eight sampled village pear (*Pyrus communis* L.) cultivar populations from the Çoruh river basin (Artvin province) in northeastern Türkiye.

extraction. Initially, dried leaves in silica gel bags were crushed in a mortar with liquid nitrogen to obtain tissue powder. The tissue powder from all samples was stored at −80°C. For DNA extraction, an altered version of the CTAB (cetyltrimethylammonium bromide) protocol was utilized (Doyle and Doyle, 1987) (see online Supplementary methods). The purity of the extracted

https://doi.org/10.1017/S1479262124000455

DNA was quantified by measuring their OD values at 230, 260 and 280 nm using a NanoDrop Spectrophotometer (NanoDrop 2000, Thermo Scientific, USA).

PCR amplification of microsatellites

Out of 19 microsatellite loci which were previously developed for *Pyrus* and *Malus* species (Gianfranceschi *et al.*, 1998; Yamamoto *et al.*, 2002a, 2002b; Nishitani *et al.*, 2009), eleven of them were selected based on their polymorphism rates to assess the genetic diversity of the populations. Eight of the 19 microsatellite loci could not be amplified successfully in polymerase chain reaction (PCR) conditions despite of subjecting to extensive optimization experiments. Among the amplified loci, KU10, Bgt23b, NH013a, NB113a, TsuEnh008, NH007b and NH008b were primarily developed for *Pyrus* species (Yamamoto *et al.*, 2002a, 2002b, 2002c; Nishitani *et al.*, 2009), while CH03G06, CH02B10, CH02F06 and CH01F02 were specific to *Malus* species (Gianfranceschi *et al.*, 1998; Liebhard *et al.*, 2002) (online Supplementary Table S2).

PCRs were carried out using 5× HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) as a PCR reaction mixture. The PCR mix included 5 µl of PCR mix, 0.5 µl fluorescently labelled forward primer, 0.5 µl reverse primer, and 5 µl template DNA (20 ng/µl). The PCR cycles were set for 4 min at 94°C for denaturation, followed by 30 cycles of 94°C for 40 s, 30 s at the annealing temperature (Ta), and 2 min at 72°C, with a final extension at 72°C for 10 min. The PCR products were run on 3% agarose gels at 100 V for 45 min in electrophoresis. The bands were visualized under UV light (Vilber Lourmat, France), considering bands of a low-range DNA ladder (Fermentas, Generuler, EU).

Data collection and analysis

Fluorescently labelled PCR product analysis was conducted by BM Labosis Company (Çankaya, Ankara). The resulting electropherograms were manually checked, and allele sizes were scored using Peak Scanner Software 2.0 (Applied Biosystems Inc., Foster City, CA, USA). The collected data were utilized for investigating genetic diversity and characterization by *Genepop* (Raymond and Rousset, 1995; Rousset, 2008), and the *poppr* package in R (Kamwar *et al.*, 2014). Population genetics parameters were calculated using *GenAlEx* (Peakall and Smouse, 2012), null alleles were checked using *Micro-Checker* (Van Oosterhout *et al.*, 2004), and population structure was revealed by STRUCTURE software (Pritchard *et al.*, 2000). More detailed information on the written scripts and software parameters can be found in Coban (2019).

Results

Microsatellite marker selection

Eleven microsatellite loci were found to be suitable for further analysis based on their high level of polymorphism rate (online Supplementary Table S2). These loci are informative markers for genetic diversity analysis as they exhibit a large proportion of individuals within the population with different alleles at these SSR loci. No null alleles were identified. The linkage disequilibrium (LD) analysis, conducted using the *poppr* package (Kamwar *et al.*, 2014), revealed no significant LD between the studied loci (online Supplementary Fig. S1). Two out of the ten cultivars from the Meydancık population were found to be identical clones. Therefore, one of the clones was removed from the data used in further analysis.

Genetic diversity analysis

The average allele number per locus was found to be 12. While KU10 had the highest mean number of different alleles (Na) and effective alleles (Ne) values (9.38 and 6.65, respectively), the CH02B10 loci had the lowest Na and Ne values (5.00 and 2.68, respectively). Observed (Ho) and expected heterozygosity (He) ranged from 0.55 in TsuEnh008 to 0.97 in CH01F02 and from 0.62 in CH02B10 to 0.84 in KU10, respectively. An excess of heterozygosity was observed in NB113a, CH03G06, CH02B10 and CH01F02 loci. The remaining of the eleven loci had positive fixation indices. The high number of positive indices indicates that the studied populations included a high number of homozygote genotypes. Polymorphic information content (PIC) values of the loci ranged between 0.62 and 0.88 with an average of 0.75, indicating that the microsatellite loci used in the study are highly informative. The inbreeding coefficient (F_{IS}), genetic differentiation (F_{ST}), and number of migrants (N_m) were estimated as 0.03, 0.06 and 4.17, respectively (Table 1).

Regarding population-wise descriptive statistics, Na ranged between 8.09 and 5.36. The Veliköy population had the highest Na (8.09), and the wild Panta root stock population had the lowest Na (5.36). Ne varied between 4.03 and 5.57 with a mean of 4.74. Similarly, Veliköy had the highest Ne value (5.57), and the wild Panta root stock population had the lowest Ne value (4.04). The number of private alleles for the studied populations varied between 1 in Meşeli, Meydancık, and Veliköy to 4 in Camili population. The SCC had two private alleles (Table 2).

The average observed heterozygosity was calculated as 0.74, and the expected heterozygosity was 0.76. Observed heterozygosity ranged between 0.77 (Veliköy population) and 0.64 (wild Panta root stock population), while expected heterozygosity varied from 0.81 (Veliköy) to 0.71 (wild Panta root stock population). As expected, the SCC, serving as an *ex situ* conservation site, had the second-highest observed heterozygosity. Out of the eight populations, observed heterozygosity was only slightly higher than expected heterozygosity in the A. Koyunlu population, with an F_{IS} value of −0.02. The highest inbreeding was observed in the wild Panta root stock population with an F_{IS} value of 0.10 (Table 2).

Genetic structure and differentiation

The pairwise number of migrants ranged from 3.42 to 10.65. Therefore, F_{ST} values varied between populations. It was the lowest between Camili and Kirazlı (0.02), while it was the highest between the wild Panta root stock population and Meşeli population (0.07, Table 3). A principal coordinate analysis was performed based on the pairwise F_{ST} values (Fig. 2) and found that 95% of the total variation is explained by the first three axes, with 48, 36 and 11%.

Pairwise F_{ST} values and the principal coordinate analysis showed that wild Panta root stock population and Meydancık population are the most distinct ones among the pairwise village pear cultivar population comparisons. Two Structure analyses were performed, with and without locality information of the populations. However, in both cases, delta K was estimated as

https://doi.org/10.1017/S1479262124000455

Table 1. Descriptive statistics for the studied microsatellite loci

SSR Locus	*N*	Na	Ne	Ar	PIC	Ho	He	F_{IS}	F_{IT}	F_{ST}	Nm
NB113a	10.50 ± 1.16	7.50 ± 0.57	5.31 ± 0.34	5.23	0.78	0.81 ± 0.06	0.80 ± 0.01	−0.01	0.05	0.06	3.95
NH013a	10.50 ± 1.16	7.62 ± 0.68	5.74 ± 0.53	5.32	0.83	0.57 ± 0.08	0.81 ± 0.03	0.29	0.35	0.08	2.94
KU10	10.38 ± 1.24	9.38 ± 0.78	6.65 ± 0.53	5.99	0.82	0.80 ± 0.03	0.84 ± 0.02	0.04	0.09	0.04	5.56
NH008b	10.12 ± 1.01	6.62 ± 0.38	4.07 ± 0.26	4.63	0.70	0.71 ± 0.06	0.75 ± 0.02	0.05	0.13	0.08	2.99
CH03G06	10.38 ± 1.24	6.88 ± 0.64	4.24 ± 0.48	4.67	0.63	0.78 ± 0.04	0.74 ± 0.02	−0.04	0.02	0.06	3.75
NH007b	10.38 ± 1.24	6.88 ± 0.72	4.90 ± 0.50	4.9	0.80	0.70 ± 0.04	0.78 ± 0.03	0.09	0.16	0.07	3.27
TsuEnh008	10.50 ± 1.65	5.38 ± 0.32	3.83 ± 0.34	4.23	0.67	0.55 ± 0.06	0.72 ± 0.03	0.24	0.29	0.07	3.33
Bgt23b	10.00 ± 1.20	7.75 ± 0.70	5.13 ± 0.53	5.25	0.88	0.65 ± 0.08	0.79 ± 0.02	0.17	0.25	0.09	2.47
CH02B10	10.50 ± 1.16	5.00 ± 0.46	2.68 ± 0.17	3.63	0.76	0.84 ± 0.03	0.62 ± 0.02	−0.35	−0.32	0.02	9.62
CH01F02	10.38 ± 1.16	7.50 ± 0.33	5.49 ± 0.40	5.39	0.77	0.97 ± 0.02	0.81 ± 0.02	−0.20	−0.14	0.05	4.80
CH02F06	10.50 ± 1.16	7.12 ± 0.30	4.07 ± 0.16	4.74	0.62	0.72 ± 0.03	0.75 ± 0.01	0.04	0.11	0.07	3.15
Mean	**10.38 ± 0.33**	**7.06 ± 0.20**	**4.74 ± 0.16**	**4.91 ± 0.6**	**0.75**	**0.74 ± 0.02**	**0.76 ± 0.01**	**0.03**	**0.09**	**0.06**	**4.17**

N, Mean number of individuals with amplification; Na, Mean number of different alleles; Ne, Mean number of effective alleles; Ar, Allelic richness; PIC, Polymorphic information content; Ho, Observed heterozygosity; He, Expected heterozygosity; F_{IS}, Inbreeding coefficient within individuals; F_{IT}, Inbreeding coefficient within total population; F_{ST}, Genetic differentiation within total population.

Table 2. Genetic diversity parameters for the village pear cultivar populations

Locations (populations)	*N*	Na	Ne	Pa	Ho	He	*F*
A.Koyunlu	12	7.27 ± 0.54	4.56 ± 0.42	2	0.76 ± 0.06	0.74 ± 0.03	−0.02 ± 0.08
Camili	14	7.64 ± 0.62	4.69 ± 0.53	4	0.73 ± 0.06	0.76 ± 0.03	0.03 ± 0.09
Kirazlı	11	8.00 ± 0.38	4.92 ± 0.49	2	0.75 ± 0.05	0.78 ± 0.02	0.03 ± 0.06
Meşeli	6	5.82 ± 0.35	4.43 ± 0.40	1	0.75 ± 0.06	0.75 ± 0.02	0.01 ± 0.09
Meydancık	10	6.36 ± 0.31	4.54 ± 0.33	1	0.73 ± 0.04	0.76 ± 0.02	0.04 ± 0.07
Veliköy	10	8.09 ± 0.64	5.57 ± 0.48	1	0.77 ± 0.05	0.81 ± 0.02	0.03 ± 0.07
Sample Collection Garden	15	7.91 ± 0.44	5.13 ± 0.41	2	0.76 ± 0.04	0.79 ± 0.02	0.04 ± 0.04
Panta	6	5.36 ± 0.58	4.03 ± 0.54	3	0.64 ± 0.07	0.71 ± 0.03	0.10 ± 0.10
Mean	**10.38 ± 0.33**	**7.06 ± 0.20**	**4.74 ± 0.16**		**0.74 ± 0.02**	**0.76 ± 0.01**	**0.03 ± 0.03**

N, Number of individuals; Na, Mean number of different alleles; Ne, Mean number of effective alleles; Pa, Number of private alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; F, Fixation index.

Table 3. Pairwise F_{ST} values (below diagonal) and number of migrants (above diagonal) between the studied village pear cultivar populations

	A.Koyunlu	Camili	Kirazlı	Meşeli	Meydancık	SCC	Panta	Veliköy
A.Koyunlu	–	8.74	7.25	6.36	4.43	7.79	4.13	10.27
Camili	0.03	–	10.64	8.46	5.10	9.68	4.79	10.31
Kirazlı	0.03	0.02	–	7.23	7.67	9.61	4.46	10.31
Meşeli	0.04	0.03	0.03	–	6.71	8.17	3.42	10.60
Meydancık	0.06	0.05	0.03	0.04	–	8.73	3.76	7.26
SCC	0.03	0.02	0.02	0.03	0.03	–	3.69	9.26
Panta	0.06	0.05	0.05	0.07	0.06	0.06	–	4.86
Veliköy	0.02	0.02	0.02	0.02	0.03	0.03	0.05	–

https://doi.org/10.1017/S1479262124000455

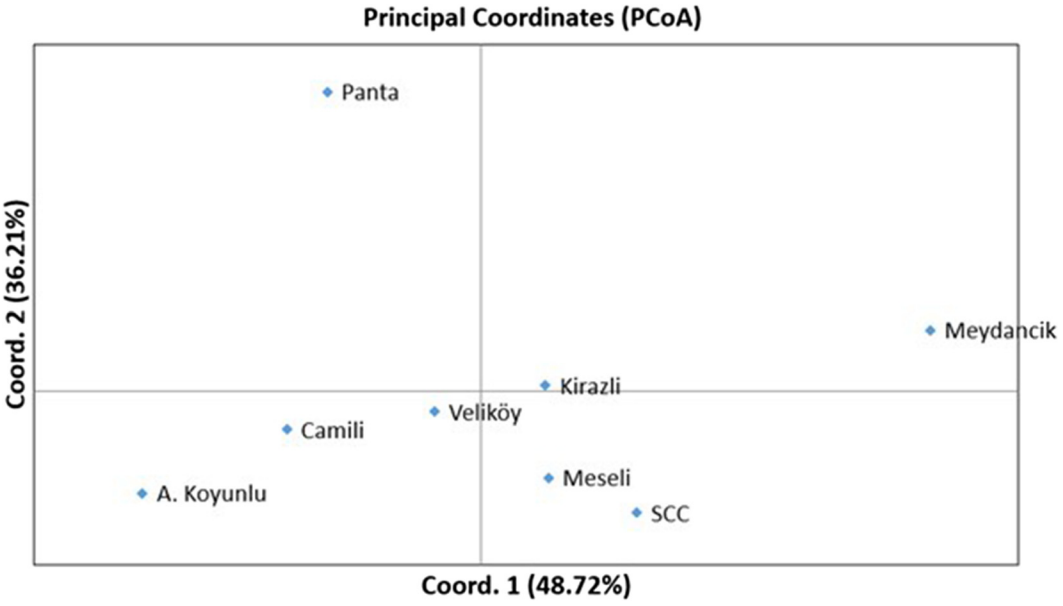

Figure 2. Principal coordinate analysis based on Nei's distance of studied village pear (*Pyrus communis* L.) cultivar populations.

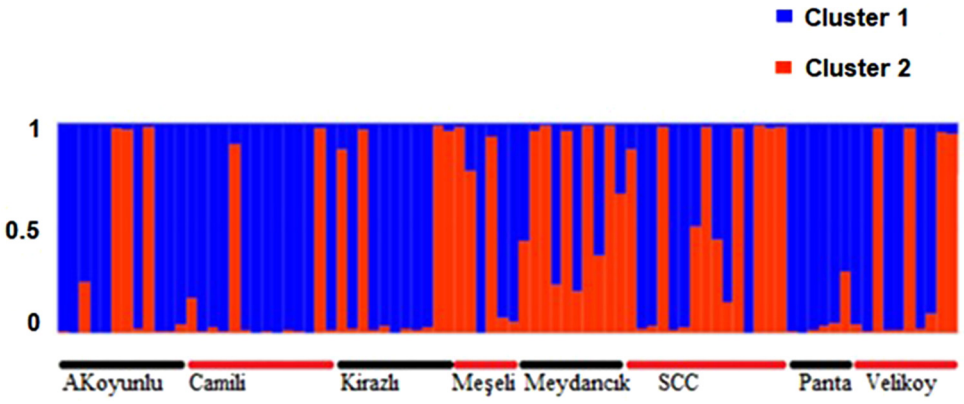

Figure 3. STRUCTURE clustering of village pear (*Pyrus communis* L.) cultivar populations sampled from the Çoruh river basin (Artvin province) in northeastern Türkiye. Each colour represents a different genetic cluster.

2. Akkoyunlu, Camili, Kirazlı, Meşeli, Meydancık, Veliköy populations and SCC were clustered into two groups with changing membership values, not related to their original locations of the populations. The members of the wild Panta root stock population were only found in Cluster 1, which also included several members of Camili and Kirazlı populations. The members of the populations clustered into the Cluster 1 with membership values ranging between 40% and 85.7%. The Cluster 2 included cultivars mainly from SCC, Meşeli and Meydancık populations, though there were also some cultivars from other populations (Fig. 3).

Discussion

This study has yielded valuable insights into the magnitude and structure of genetic diversity within village pear cultivar populations in Türkiye, including wild Panta root stock population. The outcomes of genetic diversity and structure analyses highlight the substantial genetic diversity and high admixture present in the populations, indicative of the practices employed by local farmers. These findings underscore the dynamic interplay between human cultivation practices (such as clonal material exchanges among village farmers and clonal propagation of pear cultivars) and the genetic composition of pear populations in the region, shedding light on the intricate patterns of diversity and gene flow that shape the landscape of pear cultivation in this geographic area.

The loci utilized in this study are informative and well-suited for conducting genetic diversity analysis in pear genetic resources. Regarding the suitability of microsatellite loci for the genetic characterization of cultivars, both the Ar and PIC values of the loci were observed to be higher than the critical values of 0.3 and 0.5, respectively. Consistent with previous studies (Yamamoto *et al.*, 2001; Bao *et al.*, 2007; Brini *et al.*, 2008; Sharifani *et al.*, 2017; Erfani-Moghadam and Zarei, 2018), high expected heterozygosities were identified for the studied loci, ranging between 0.62 and 0.84 for CH02B10 and KU10 loci, respectively. Notably, NH007b, TsuEnh008, NH013a, NH008b, Bgt23b and CH02F06 loci exhibited relatively higher F_{ST} values. As a result,

https://doi.org/10.1017/S1479262124000455

the differentiation of populations is mainly influenced by these markers, which identify unique cultivars and rare alleles that vary significantly in frequency between different populations. This variability allows populations to be differentiated on the basis of their genetic profiles. Consequently, these markers can be effectively used as valuable tools for establishing *in situ* or *ex situ* conservation programmes. Conservation efforts can prioritize the preservation of the genetic diversity captured by these markers, ensuring the maintenance of distinct populations within each village.

All populations exhibited high observed and expected heterozygosity values, with these values being of comparable magnitude within each population. Generally, He values were found to be greater than Ho values. The plausible explanation for the elevated observed heterozygosity values lies in the admixture of populations over the years. This admixture was driven by considerations of horticultural factors such as fruit size, colour, taste, softness and growth performance, as such selections practiced by village farmers. It appears that cultivars with heterozygote superiorities, selected for their desirable cultivation traits and dispersal, have been propagated and consistently distributed in the villages where the populations originated in the Artvin province of north-eastern Türkiye.

The overall estimated F_{ST} values suggest that genetic differences among the studied populations are low, indicating that *P. communis* is likely cultivated and dispersed through human activities. The extensive years of human involvement in pear propagation and dispersal through seeds or clonal materials are expected to contribute to the decrease in genetic differentiation among pear genetic resources, primarily due to the high level of gene flow. This is substantiated by estimated low pairwise F_{ST} values, ranging between 0.02 and 0.06 among the populations. Additionally, the presence of a high number of migrants in the populations further supports the influence of gene flow in minimizing genetic differentiation among them. Vegetatively growing pear species, facilitated by practices like grafting as well as pollination occurring over long distances could also play a role in gene flow between populations (Culley and Hardiman, 2009; Hardiman and Culley, 2010; Zheng *et al.*, 2014; Reim *et al.*, 2017).

The difference between pairwise F_{ST} values suggests that wild Panta root stock population is the genetically most distant group. Within the wild Panta root stock population, a high level of inbreeding was observed. The high level of inbreeding in the wild Panta root stock population may be attributed to the utilization of a limited number of Panta genotypes as rootstock among the six villages and consequences of mating among genetically similar genotypes within the population in the past. The observed level of inbreeding could be influenced by genetic isolation, population structure, mating patterns and demographic history. These factors may result in a decrease in genetic diversity and an increase in homozygosity within the population. This could potentially compromise the population's long-term viability and adaptability. Similar to the F_{ST} results, despite its lower sample size, the wild Panta root stock population exhibited a high number of private alleles that set it apart from the populations. These exclusive alleles hold significant value in identifying genotypes suitable for graft-compatible root stock, making them valuable candidates for future pear breeding programmes. Therefore, it is crucial to conserve these graft-compatible wild Panta root stock genotypes through a clonal orchard as part of an *ex situ* programme. This conservation effort can be easily achieved by transferring the graft-compatible wild Panta root stock genotypes to the SCC which already exists in the region as an *ex situ* programme. Furthermore, the SCC *ex situ* programme could be enhanced further by screening wild *P. Communis* trees located in the forests of the villages for compatible rootstocks using the private alleles identified in the wild Panta root stock population.

Both Structure analyses, conducted with and without prior information on population locations, reveal that genotypes of the populations in the Artvin Çoruh River Basin appear to have originated from two distinct gene pools. One gene pool is associated with Meydancık village, while the other is linked to Camili village, located in the Camili Biosphere Reserve. With the exception of the members of the wild Panta root stock population, cultivars from the populations did not consistently cluster into the same group. Some members grouped into the Cluster 1 alongside the wild Panta root stock population, while others were assigned to the Cluster 2. The population structure analysis indicates that the studied populations are highly admixed. This outcome is not unexpected, given that valuable pear cultivars within the region could be easily disseminated through clonal means, considering the remoteness of the region. Similar results have been reported by others (Liu *et al.*, 2015; Zurn *et al.*, 2020). Hybridization is a common phenomenon in *Pyrus*, even with other species such as *Malus*. Intergeneric hybridization has been facilitated between *Pyrus* and *Malus*, allowing for the incorporation of valuable traits from diverse gene pools. Given the high hybridization potential of pears combined with efficient human assisted dispersal mechanism, it is expected to observe high admixture among populations (Morimoto *et al.*, 2024).

The results of the population structure analysis and the population genetics parameters indicate that the studied populations are highly admixed. Artificial selection during pear cultivation has contributed to an increased genetic distance between the wild Panta root stock population and cultivar populations in the region. These findings shed light on the intricate dynamics of genetic diversity of the populations through gene flow, hybridization and village farmer practices, providing valuable insights into the evolutionary processes shaping the genetic landscape of *P. communis* in the region. The geography of the Çoruh River basin in the Artvin province, combined with traditional cultivation practices, significantly facilitates the exchange of genes among various village pear cultivars. This dynamic gene exchange makes this area be with a promising potential of pear genetic resources for establishing *in situ* conservation site. To preserve village pear cultivars in north-eastern Türkiye, *in situ* conservation on-farm by using the Camili population could be practiced since it exhibits the highest number of private alleles. Furthermore, the Camili village located in the Camili Biosphere Reserve is convenient place for *in situ* conservation due to its high protection status. The reserve includes two nature conservation sites where strict regulations are in practice for preventing the introduction of non-native plant and animal species to the reserve area (UNESCO, 2023). Thus, the Camili Biosphere Reserve in which Camili village located is the right place for active management and safeguarding of the village pear cultivar genetic resources as *in situ* in the Çoruh river basin.

Conclusions

This study has furnished crucial insights into the magnitude and structure of genetic diversity within the village pear cultivar populations, including the wild Panta root stock population. The findings from genetic diversity and structure analyses underscore that

https://doi.org/10.1017/S1479262124000455

populations maintain elevated genetic diversity and significant admixture due to the practices employed by local farmers. The pear genetic resources in the Çoruh River basin appear to have originated from two distinct gene pools, specifically the Meydancık and Camili populations. Furthermore, genetic diversity statistics and structure analysis indicate that the Sample Collection Garden did not fully capture the existing genetic diversity in the village pear cultivar populations in the Çoruh river basin. To enhance the Sample Collection Garden's representation of genetic diversity, it is recommended to incorporate new pear cultivars from the populations and graft-compatible genotypes from the wild Panta root stock population. This inclusion should consider the presence of private alleles and amount of heterozygosities. *In situ* genetic conservation of cultivars of the species emerges as crucial for sustaining genetic diversity and facilitating breeding efforts, especially considering the likelihood of increasing anthropogenic pressures (such as the replacement of traditional varieties with genetically improved ones) and climatic factors in the future.

To maintain the genetic diversity and allow evolution to progress which are necessary to provide genetic resources for future pear breeding programmes, the Camili village population should be considered as an *in situ* genetic reserve. The Camili village population is located within the Camili UNESCO Biosphere Reserve where plant and animal genetic resource uses in the reserve are already strictly regulated, but special efforts for design and management plan need to be devoted to conservation of the pear genetic resources in the area.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262124000455

Acknowledgements. The authors are grateful to the farmers and Artvin Directorate of Food, Agriculture and Livestock in Dalkırmaz village for their collaboration during field sampling. The manuscript is based on the study which was the master thesis of Abdulbaki Çoban (<https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp>, thesis number:548099). Also, we thank the editor, associate editor and anonymous reviewers of the journal for their constructive comments and critics.

Author contributions. Abdulbaki Çoban carried out the laboratory work and wrote the first draft of the manuscript. Funda Ö. Değirmenci and Asiye Uluğ helped to Mr. Çoban in the lab works, data analysis of the study. Mevlüde Alev Ateş, Emrah Yüksel and Özgür Eminağaoğlu involved in sampling and cultivar identifications. Zeki Kaya designed the experiment, provided the lab facilities, reviewed and edited the manuscript.

Competing interests. The authors declare that they have no conflicts of interest.

References

Akçay ME, Burak M, Kazan K, Yüksel C, Mutaf F, Bakir M, Ayanoglu H and Ergül A (2014) Genetic analysis of Anatolian pear germplasm by simple sequence repeats. *Annals of Applied Biology* **164**, 441–452.

Aldasoro JJ, Aedo C and Muñoz Garmendia F (1996) The genus *Pyrus* L. (Rosaceae) in south-west Europe and North Africa. *Botanical Journal of the Linnean Society* **121**, 143–158.

Bao L, Chen K, Zhang D, Cao Y, Yamamoto T and Teng Y (2007) Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. *Genetic Resources and Crop Evolution* **54**, 959.

Bell RL, Quamme HA, Layne REC and Skirvin RM (1996) Pears. In Janick J and Moore JN (eds), *Fruit Breeding, Vol I: Tree and Tropical Fruits*. New York, NY: John Wiley and Sons Inc, pp. 441–514.

Bozhüyük MR and Aslantaş R (2020) Molecular characterization of Türkiye's Çoruh Valley pears by SSR. *Acta Horticulture* **1297**, 367–376.

Brini W, Mars M and Hormaza JI (2008) Genetic diversity in local Tunisian pears (*Pyrus communis* L.) studied with SSR markers. *Scientia Horticulturae* **115**, 337–341.

Çakmakçı R, Aslantaş R, Erdoğan Y and Erdoğan Ü (2017) Flowers of Çoruh Valley. *Turkish Journal of Agriculture – Food Science and Technology* **5**, 1785–1791.

Chevreau E, Leuliette S and Gallet M (1997) Inheritance and linkage of isozyme loci in pear (*Pyrus communis* L.). *Theoretical Applied Genetics* **94**, 498–506.

Çoban A (2019) Genetic characterization of pear cultivars (*Pyrus communis*) in Çoruh river basin (Master of Science Thesis). Middle East Technical University, Department of Biological Sciences, 111p, Ankara, Türkiye. Available at https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp. thesis number: 54809.

Culley TM and Hardiman NA (2009) The role of intraspecific hybridization in the evolution of invasiveness: a case study of the ornamental pear tree *Pyrus calleryana*. *Biological Invasions* **11**, 1107–1119.

Davarynejad GH and Davarynejad E (2004) Comparative performance of graft incompatibility in pear/quince (*Pyrus communis*/Cydonia oblonga) combinations. In VIII International Symposium on Canopy, Rootstocks and Environmental Physiology in Orchard Systems 732, pp. 221–225.

Dbara S, Boussetta W, Hafi M and Mars M (2021) Performance assessment of three old pear cultivars (*Pyrus communis* L.) to cope drought caused by climate change. *Journal of Horticulture and Postharvest Research* **4**, 351–366.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**, 11–15.

Elshihy OM, Sharaf AN and Muzher BM (2004) Morphological, anatomical and biochemical characterization of Syrian pear (*Pyrus syriaca* Boiss) genotypes. *Arabian Journal of Biotechnology* **7**, 209–218.

Erfani-Moghadam J and Zarei A (2018) Assessment of genetic structure among different pear species (*Pyrus* spp.) using apple-derived SSR and evidence of duplications in the pear genome. *Biotechnology & Biotechnological Equipment* **32**, 591–601.

FAOSTAT (2018) FAO statistics database on the World Wide Web. Available at http://www.fao.org

Gianfranceschi L, Seglias N, Tarchini R, Komjanc M and Gessler C (1998) Simple sequence repeats for the genetic analysis of apple. *Theoretical and Applied Genetics* **96**, 1069–1076.

Hardiman NA and Culley TM (2010) Reproductive success of cultivated *Pyrus calleryana* (Rosaceae) and establishment ability of invasive, hybrid progeny. *American Journal of Botany* **97**, 1698–1706.

Hong SY, Lansky E, Kang SS and Yang M (2021) A review of pears (*Pyrus* spp.), ancient functional food for modern times. *BMC Complementary Medicine and Therapies* **21**, 219.

Iketani H, Manabe T, Matsuta N, Akihama T and Hayashi T (1998) Incongruence between RFLPs of chloroplast DNA and morphological classification in east Asian pear (*Pyrus* spp.). *Genetic Resources and Crop Evolution* **45**, 533–539.

Kajiura I, Nakajima M, Sakai Y, Kotani H and Oogaki C (1985) Identification of Japanese pear cultivars (*Pyrus serotina* Redh var *culta*) by phenolic compounds in leaves. *Bulletin of the Fruit Tree Research Station, Japan, A Yatabe* **12**, 1–27.

Kamwar ZN, Tabima JF and Grünwald N (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2**, e281.

Kaya Z, Kun E and Güner A (1997) National Plan for *in situ* Conservation of plant genetic diversity in Türkiye. *Milli Egitim Basimevi*, Istanbul, 125p.

Kaymak S and Pinar H (2020) Genetic diversity of pear cultivars using SSR markers and their reactions to pear rust (*Gymnosporangium fuscum*). *Plant Protection Bulletin* **60**, 15–24.

Kim C, Lee G, Han D, Ryu K and Lee C (2000) Classification and identification of *Pyrus pyrifolia* using RAPD. *Journal of the Korean Society for Horticultural Science* **41**, 119–124.

Kimura T, Shi YZ, Shoda M, Kotobuki K, Matsuda N, Hayashi T, Ban Y, Yoshiyuki B and Yamamoto T (2002) Identification of Asian pear varieties by SSR analysis. *Breeding Sciences* **52**, 115–121.

Kocsisne GM, Bolla D, Anhalt-Bruderl UCM, Forneck A, Taller J and Kocsis L (2020) Genetic diversity and similarity of pear (*Pyrus communis* L.) cultivars in central Europe revealed by SSR markers. *Genetic Resources and Crop Evolution* **67**, 1755–1763.

Li X, Li X, Wang T and Gao W (2016) Chapter 24 – nutritional composition of pear cultivars (Pyrus spp.). In Simmonds MSJ and Preedy VR (eds), *Nutritional Composition of Fruit Cultivars*. London: Academic Press, pp. 573–608.

Liebhard R, Gianfranceschi L, Koller B, Ryder CD, Tarchini R, Van de Weg E and Gessler C (2002) Development and characterization of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). *Molecular Breeding* **10**, 217–241.

Liu Q, Song Y, Liu L, Zhang M, Sun J, Zhang S and Wu J (2015) Genetic diversity and population structure of pear (*Pyrus* spp.) collections revealed by a set of core genome-wide SSR markers. *Tree Genetics and Genomes* **11**, 1–22.

Monte-Corvo L, Cabrita L, Oliveira C and Leitão J (2000) Assessment of genetic relationships among *Pyrus* species and cultivars using AFLP and RAPD markers. *Genetic Resources and Crop Evolution* **47**, 257–265.

Morimoto T, Narazaki R, Okabe H, Zhang L, Nishumura K and Itai A (2024) Introduction of a diverse genetic background of *Pyrus* into *Malus* through intergeneric hybridization. *Molecular Genetics and Genomics* **299**, 21. https://doi.org/10.1007/s00438-024-02131-8

Muminjanov H and Karagöz A (2018) Biodiversity of Türkiye: Contribution of Genetic Resources to Sustainable Agriculture and Food Systems Food and Agriculture Organization of the United Nations, Ankara. 222 p.

Nishitani C, Terakami S, Sawamura Y, Takada N and Yamamoto T (2009) Development of novel EST-SSR markers derived from Japanese pear (*Pyrus pyrifolia*). *Breeding Science* **59**, 391–400.

Öztürk A and Demirsoy L (2015) Evaluation of relationships between pear genotypes from North Anatolia, Türkiye using SSR markers. In III Balkan Symposium on Fruit Growing 1139, pp. 49–56.

Paganova V (2003) Taxonomic reliability of leaf and fruit morphological characteristics of the *Pyrus* L. taxa in Slovakia. *Horticultural Science* **30**, 98–107.

Peakall R and Smouse PE (2012) Genalex 6.5: genetic analysis in excel. population genetic software for teaching and research-an update. *Bioinformatics (Oxford, England)* **28**, 2537–2539.

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Raymond M and Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248–249.

Reim S, Proft A, Heinz S, Lochschmidt F, Höfer M, Tröber U and Wolf H (2017) Pollen movement in a *Malus sylvestris* population and conclusions for conservation measures. *Plant Genetic Resources* **15**, 12–20. doi: 10.1017/S1479262115000301

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103–106.

Rubzov GA (1944) Geographical distribution of the genus *Pyrus* and trends and factors in its evolution. *American Naturalist* **78**, 358–366.

Sharifani MM, Kimura T, Yamamoto T and Nishtani C (2017) Genetic diversity of pear (*Pyrus* spp) germplasm assessed by simple sequence repeat (SSR) and morphological traits. *International Journal of Horticultural Science and Technology* **4**, 145–155.

Simionca Mărcășan LI, Pop R, Somsai PA, Oltean I, Popa S, Sestras AF, Militaru M, Botu M and Sestras RE (2023) Comparative evaluation of *Pyrus* Species to identify possible resources of interest in pear breeding. *Agronomy* **13**, 1264.

Tatari M, Jafari A, Shirmardi M and Mohamadi M (2020) Using morphological and physiological traits to evaluate drought tolerance of pear populations (*Pyrus* spp.). *International Journal of Fruit Science* **20**, 837–854.

Teng Y, Tanabe K, Tamura F and Itai A (2002) Genetic relationships of Pyrus species and cultivars native to East Asia revealed by randomly amplified polymorphic DNA markers. *Journal of the American Society for Horticultural Science* **127**, 262–270.

USDA Foreign Agricultural Service (2023) Global leading pear producing countries in 2022/2023 (in 1,000 metric tons) [Graph]. In Statista. Retrieved March 28, 2024, from https://www.statista.com/statistics/739168/global-top-pear-producing-countries/

Van Oosterhout C, Hutchinson WF, Wills DP and Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538.

Web site of UNESCO (2023) Biosphere Reserves of Türkiye. Available at https://en.unesco.org/biosphere/eu-na/camili (Accessed at 17.11.2023).

Wolko L, Antkowiak W, Lenartowicz E and Bocianowski J (2010) Genetic diversity of European pear cultivars (*Pyrus communis* L.) and wild pear (*Pyrus pyraster* (L.) Burgsd.) inferred from microsatellite markers analysis. *Genetic Resources and Crop Evolution* **57**, 801–806.

Yamamoto T, Kimura T, Sawamura Y, Kotobuki K, Ban Y, Hayashi T and Matsuta N (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. *Theoretical Applied Genetics* **102**, 865–870.

Yamamoto T, Kimura T, Shoda M, Band Y, Hayashi T and Matsuta N (2002a) Development of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). *Molecular Ecology Notes* **2**, 4–16.

Yamamoto T, Kimura T, Sawamura Y, Manabe T, Kotobuki K, Hayashi T, Ban Y and Matsuta N (2002b) Simple sequence repeats for the genetic analysis in pear. *Euphytica* **124**, 129–137.

Yamamoto T, Kimura T, Shoda M, Imai T, Saito T, Sawamura Y, Kotobuki K, Hayashi T and Matsuta N (2002c) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theoretical and Applied Genetics* **106**, 9–18.

Zeven AC and Zhukovsky PM (1975) *Dictionary of Cultivated Plants and Their Centres of Diversity*, vol. 2. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation.

Zheng X, Cai D, Potter D, Postman J, Liu J and Teng Y (2014) Phylogeny and evolutionary histories of *Pyrus* L. revealed by phylogenetic trees and networks based on data from multiple DNA sequences. *Molecular Phylogenetics and Evolution* **80**, 54–65.

Zurn JD, Nyberg A, Montanari S, Postman J, Neale D and Bassil N (2020) A new SSR fingerprinting set and its comparison to existing SSR-and SNP-based genotyping platforms to manage *Pyrus* germplasm resources. *Tree Genetics and Genomes* **16**, 1–10.