

Article

SSR-Based Genotyping of Korean Native Pear Germplasm for Accession Identity Assessment and Germplasm Management

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Abstract

Korean native pear germplasm is valuable for conservation and breeding, but long-term ex situ maintenance may result in accession redundancy and identity uncertainty. This study established simple sequence repeat (SSR) fingerprints, identified putative duplicate accessions, assessed phenotypic consistency within duplicate groups, and characterized genetic relationships and regional differentiation in the collection. A total of 644 accessions, including 626 Korean native pear accessions and 18 additional *Pyrus* accessions, were genotyped using 18 SSR markers from the European Cooperative Programme for Plant Genetic Resources and U.S. *Pyrus* Genetic Resources sets. The markers produced 4–13 alleles per locus, with mean gene diversity, observed heterozygosity, and polymorphic information content values of 0.669, 0.448, and 0.628, respectively. Forty-three putative duplicate groups comprising 142 accessions were identified, with no discrepancies observed in the available flower and young leaf traits. A non-redundant set of 545 accessions was established and used for subsequent genetic analyses. Genetic relationship and population structure analyses revealed diverse genetic backgrounds and broad sharing of genetic components across regions. Regional differentiation was weak but significant, with 92.72% of variation occurring within regions and 7.28% among regions; Ulleungdo accessions showed the most distinctive regional pattern. This study demonstrates the utility of SSR fingerprinting for accession verification, redundancy management, and germplasm curation and provides a non-redundant resource set for subsequent phenotypic evaluation and potential use in pear breeding.

Keywords: *Pyrus*; SSR fingerprinting; accession identity; duplicate accessions; germplasm management



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1. Introduction

The genus *Pyrus* L. comprises diverse pear species distributed across Europe, Asia, and North Africa [1], and cultivated pears are generally classified into European and Asian pears [2]. Recent single nucleotide polymorphism (SNP)-based studies have also shown genetic differentiation between European and Asian pears and substantial population structure within Asian pear germplasm [3,4]. Asian pears, including species such as *Pyrus pyrifolia* (Burm.f.) Nakai, *Pyrus ussuriensis* Maxim., and *Pyrus bretschneideri* Rehder, are closely associated with East Asia, where they have been cultivated and maintained as important fruit genetic resources [2]. Within East Asia, Korea harbors diverse native pear genetic resources that form part of the broader East Asian pear gene pool [5,6]. These

resources are important for conserving East Asian pear diversity and provide a genetic foundation for future pear breeding.

Korean native pear resources are important for conserving genetic diversity and broadening the genetic base of pear breeding [6]. To secure these resources, germplasm collection surveys were conducted throughout Korea during the 1980s and again in the early 2000s. As a result, naturally growing native pear trees were collected from diverse geographic regions, including Ulleungdo, Gangwon, Gyeongsang, and Jeolla provinces in Korea. This collection constitutes an important ex situ living collection of Korean pear genetic resources. Accurate accession identification and efficient collection management are therefore essential for its long-term conservation and subsequent utilization.

Although these accessions were collected from diverse geographic regions, some were obtained from neighboring or geographically proximate locations, raising questions regarding their genetic distinctness. It is therefore important to determine whether individual accessions represent distinct genetic resources or whether identical genotypes have been maintained under different accession names or at multiple collection sites. Pear accessions in ex situ germplasm collections are commonly maintained clonally by grafting, which preserves selected genotypes over time but may also facilitate the long-term maintenance and dissemination of identical clones under different accession names [7–9]. Such issues are common in long-term ex situ collections, where synonyms, homonyms, and redundant accessions may accumulate over time [7,8]. Uncertainty in accession identity can result in redundant maintenance, bias estimates of genetic diversity and relationships, and reduce the efficiency of core collection development and subsequent germplasm evaluation. Reliable verification of accession identity is therefore fundamental to the effective curation, conservation, and utilization of pear genetic resources.

Morphological and pomological descriptors have been widely used to characterize phenotypic diversity in both cultivated and wild *Pyrus* germplasm [10–14]. Morphological traits provide useful information for germplasm characterization, but their expression may be influenced by developmental stage and environmental conditions, which can limit their use for accession identification when considered alone [6,8]. Genetic diversity and accession identity in pear germplasm have also been evaluated using various molecular marker systems, including Simple sequence repeat (SSR), inter-simple sequence repeat, and SNP markers [15–17]. SSR markers are codominant, multiallelic, highly polymorphic, and reproducible, and have been widely used for molecular fingerprinting and germplasm characterization [8,18]. In pear, standardized SSR marker sets established by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the U.S. *Pyrus* Genetic Resources program (USPGR) have been used for accession identification and the management of germplasm collections [7,8,19]. The USPGR marker set performed similarly to the ECPGR set and the 70 K pear single-nucleotide polymorphism array in the assessment of accession identity, pedigree, genetic diversity, and population structure [8], supporting the continued use of SSR markers for accession identification and duplicate detection in large pear germplasm collections.

Although previous studies have examined the genetic diversity and population structure of Korean pear germplasm, accession identity and redundancy within Korean native pear collections have not been evaluated in the context of germplasm management [5,6]. This study aimed to establish SSR fingerprints for Korean pear germplasm, identify putative duplicate accessions, assess morphological consistency within putative duplicate groups, and establish a non-redundant set of accessions for more efficient germplasm management. Genetic relationships, population structure, and regional differentiation were also analyzed to characterize the genetic composition of the non-redundant set. The non-redundant set provides a basis for improved germplasm management and future

standardized phenotypic evaluation and high-density genomic analyses to support the use of Korean pear germplasm in breeding.

2. Materials and Methods

2.1. Plant Materials and DNA Extraction

A total of 644 pear accessions were used in this study (Tables 1 and S1). The plant materials consisted of 626 Korean native pear accessions collected from Korea and 18 additional *Pyrus* accessions, including 3 primary species, 9 Asian pear cultivars and related accessions, 4 European pear cultivars, and 2 hybrid pears. The 626 Korean native pear accessions originated from naturally growing trees collected during nationwide germplasm surveys. Collection records were available for 574 accessions, comprising Gangwon-do ($n = 159$), Gyeongsangbuk-do ($n = 95$), Gyeongsangnam-do ($n = 162$), Gyeonggi-do ($n = 12$), Chungcheongbuk-do ($n = 30$), Chungcheongnam-do ($n = 20$), Jeollabuk-do ($n = 35$), Jeollanam-do ($n = 31$), and Ulleungdo Island ($n = 30$); collection-region information was unavailable for 52 accessions. The collection regions listed in Table S1 were standardized to the city or county level based on the original collection records. The full set of 644 accessions was used for SSR fingerprinting and identification of putative duplicate accessions.

Table 1. Plant materials used in this study.

Category	No. of Accessions	Description
Korean native pear accessions	626	Accessions collected from Korea
Primary species	3	<i>Pyrus fauriei</i> C.K.Schneid, <i>Pyrus calleryana</i> Decne., <i>Pyrus betulifolia</i> Bunge
Asian pear cultivars	9	‘Yali’, ‘Cili’, <i>P. bretschneideri</i> , ‘Dongjieri’, ‘Soohyangri’, ‘Hongjieri’, ‘Whangkeumbae’, ‘Qiuzi pear’, ‘Shichiho’
European pear cultivars	4	‘Bartlett’, ‘Max Red Bartlett’, ‘Abate Fetel’, ‘Conference’
Hybrid pears	2	‘Greensis’, ‘Oharabeni’
Total	644	

Two trees were maintained for each accession in the collection. Genomic DNA was extracted from young leaves collected from one tree per accession using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

2.2. SSR Amplification and Genotyping

A total of 20 SSR markers selected from the ECPGR and USPGR marker sets were initially tested for amplification and genotyping (Table 2) [8,19]. Polymerase chain reaction (PCR) amplification was performed in a total volume of 10 μ L containing 5 μ L of Prime Taq Premix (Genet Bio, Daejeon, Republic of Korea), 2 μ L of genomic DNA (10 ng/ μ L), 1 μ L each of forward and reverse primers (10 pmol/ μ L), and 1 μ L of distilled water. PCR amplification was conducted under the following conditions: initial denaturation at 95 $^{\circ}$ C for 5 min; 35 cycles of denaturation at 95 $^{\circ}$ C for 30 s, annealing at the marker-specific temperature for 30 s, and extension at 72 $^{\circ}$ C for 60 s; followed by a final extension at 72 $^{\circ}$ C for 5 min.

Table 2. Information on SSR markers used for genotyping.

Marker Set	Marker Name	Primer Sequences (5′–3′)	Motif	T _m (°C)	Final Use
ECPGR	CH01d09	F: GCCATCTGAACCAGAATGTGC R: CCCTTCATTCACATTTCAG	(GA) _n	60	Used
ECPGR	CH03d12	F: GCCCAGAAGCAATAAGTAAACC R: ATTGCTCCATGCATAAAGGG	(CA) _n TT(CT) _n	50	Used
ECPGR	CH05c06	F: ATTGGAAGTCTCCGTATTGTGC R: ATCAACAGTAGTGGTAGCCGGT	(TC) _n A(CT) _n	64	Used
ECPGR	CH03g07	F: AATAAGCATTCAAAGCAATCCG R: TTTTCCAAATCGAGTTTCGTT	(TC) _n	56	Used
ECPGR	EMPc11	F: GCGATTAAAGATCAATAAACCAT R: AAGCAGCTGGTTGGTGAAAT	(AC) _n	64	Used
ECPGR	GD96	F: CGGCGGAAAGCAATCACCT R: GCCAGCCCTCTATGTTCCAGA	(CT) _n	55	Excluded
ECPGR	GD147	F: TCCCGCCATTTCTCTGC R: GTTTAAACCGCTGCTGCTGAAC	(AG) _n	60	Used
ECPGR	CH01f07a	F: CCCTACACGTTTCTCAACCC R: CGTTTGGAGCGTAGGAAC	(CT) _n	60	Used
ECPGR	CH02b10	F: CAAGGAAATCATCAAAGATTCAAG R: CAAGTGGCTTCGGATAGTTG	(GA) _n	60	Used
ECPGR	EMPc117	F: GTTCTATCTACCAGCCACGCT R: CGTTTGTGTGTTTACGTGTTG	(CT) _n	60	Used
ECPGR/USPGR	CH01d08	F: CTCCGCCGCTATAACACTTC R: TACTCTGGAGGGTATGTCAAAG	(GA) _n	60	Used
ECPGR/USPGR	CH04e03	F: TTGAAGATGTTTGGCTGTGC R: TGCATGTCTGTCTCCTCCAT	(GA) _n	60	Used
USPGR	NAU _{py} 40d	F: ATCCCAGCATTCCCAAGATTTT R: CCGTCCGTGATACCTCTGTC	(GATCAT) _n	60	Used
USPGR	TsuENH046	F: GGTCATCACCCACTTAAAAACCA R: GTTCTTGTGCCCTGAAGTAATTGATGG	(TTC) _n	60	Used
USPGR	TsuENH076	F: CATTAATACGCTGCTGTTTCTGC R: GTTCT-TACTTGAATTGGGGTAGGGATTGT	(ACG) _n	60	Used
USPGR	TsuENH080_354	F: CTACCACAAACAAGCTCAACAG R: GTTTATGTCATCCTGCTGATGTCC	(CCA) _n , (CCT) _n	60	Used
USPGR	TsuENH083	F: ACTCTCCGCAAAACAATGTGTA R: GTTCTTTGTGAGAGTTTGAGGAGGA-GAGC	(ACC) _n	60	Used
USPGR	TsuENH089	F: TTCCTGCCCCTTTTACGTATGC R: GTTCTTCCCGACAATCTGTAGA-GAATCA	(CCACCG) _n , (CCA) _n	60	Used
USPGR	TXY144_347	F: GTTTCCTGAACTTGCCAATTGAAAC R: GTTCTGCTACTTTCAACCCCATCT	(GAG) _n	60	Excluded
USPGR	TXY276	F: CCCTACAGAGTCATGCATCC R: GTTTGATGCTGGAGACGAGAAA	(CAGCT) _n	60	Used

PCR products were separated using a 5300 Fragment Analyzer with the dsDNA 1–500 bp method (Agilent Technologies, Santa Clara, CA, USA). Allele sizes were scored using ProSize software v6.0.0 (Agilent Technologies, Santa Clara, CA, USA), and multilocus SSR profiles were constructed for all 644 accessions.

2.3. Duplicate Accession Identification

Putative duplicate accessions were identified by comparing multilocus SSR profiles across all 18 SSR markers retained after excluding GD96 and TXY144_347. Accessions with identical multilocus SSR profiles were classified as putative duplicate accessions and assigned to the same duplicate group. To avoid overrepresentation of identical SSR genotypes in subsequent analyses, one representative accession from each duplicate group was selected to construct a non-redundant set. This non-redundant accession set was used for unweighted pair group method with arithmetic mean (UPGMA) dendrogram construction and STRUCTURE analysis. UPGMA was used to visualize genetic relationships among accessions based on SSR genetic distances. Non-redundant Korean native pear accessions with available collection-region information were used for analysis of molecular variance (AMOVA), pairwise fixation index (F_{ST}) analyses, and regional summaries of STRUCTURE membership coefficients.

2.4. Morphological Consistency Within SSR-Based Duplicate Groups

Morphological consistency was assessed among accessions within each SSR-based duplicate group. Phenotypic traits were evaluated on two trees per accession. The evaluated traits included petal color at the balloon stage, anther color, the presence or absence of trichomes on young leaves, and young leaf color (Table 3 and Figure 1A). These traits were recorded using discrete scoring classes. Because the analysis was intended to assess phenotypic concordance among accessions sharing identical multilocus SSR profiles, comparisons were restricted to SSR-based duplicate groups rather than the entire collection.

(A) Phenotypic scoring criteria

Petal color at the balloon stage (scores 1–3)



Anther color (scores 1–5)



Presence or absence of young leaf trichome



Presence (1)

Absence (0)

Young leaf color (scores 1–5)



(B) Flower traits

Geoje 03

Geojedo 03



(C) Young leaf traits

Taebaek 34

Taebaek 36



Figure 1. Phenotypic scoring criteria and representative phenotypes of SSR-identical accessions. (A) Representative images showing the scoring criteria for petal color at the balloon stage, anther color, presence or absence of young leaf trichomes, and young leaf color. (B) Flower phenotypes of the SSR-identical accessions Geoje 03 and Geojedo 03. (C) Young leaf phenotypes of the SSR-identical accessions Taebaek 34 and Taebaek 36.

Table 3. Phenotypic scoring criteria used for comparison of SSR-based duplicate groups.

Trait Category	Trait	Evaluation Stage	Scoring Scale	Description
Flower	Petal color	Balloon stage	1–3	1 = white, 2 = pale pink, 3 = pink
Flower	Anther color	Flowering stage	1–5	1 = cream/yellow, 2 = pale pink, 3 = pink, 4 = dark pink, 5 = red
Leaf	Young leaf trichome	Young leaf stage	0/1	0 = absence, 1 = presence
Leaf	Young leaf color	Young leaf stage	1–5	1 = green, 2 = yellow-green, 3 = bronze-green, 4 = reddish bronze, 5 = red

2.5. Genetic Diversity Parameters and Regional Differentiation

Genetic diversity parameters for each SSR marker were calculated using PowerMarker v3.25 [20]. The number of alleles, gene diversity/expected heterozygosity (H_e), observed heterozygosity (H_o), and polymorphic information content (PIC) were estimated for each marker using the following formulas:

$$H_o = \frac{N_{het}}{N}$$

$$H_e = 1 - \sum_{i=1}^k p_i^2$$

$$PIC = 1 - \sum_{i=1}^k p_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2p_i^2 p_j^2$$

where N_{het} is the number of heterozygous individuals, N is the number of genotyped individuals, p_i and p_j are the frequencies of the i th and j th alleles, respectively, and k is the number of alleles at the locus.

Genetic differentiation among collection regions was evaluated using AMOVA and pairwise F_{ST} [21,22]. For regional analyses, accessions collected in Ulsan and Incheon were assigned to Gyeongsangnam-do and Gyeonggi-do, respectively, whereas accessions from Ulleungdo Island were treated as a separate regional group. AMOVA was performed using the R package poppr v2.9.8 [23,24], and the significance of AMOVA variance components was assessed using 9999 permutations. Pairwise F_{ST} values among collection regions were calculated using the R package hierfstat v0.5-11 [25].

2.6. Genetic Relationship and Population Structure Analyses

Genetic relationship and population structure analyses were performed using the non-redundant accession set. Genetic relationships among accessions were inferred using a UPGMA dendrogram. Nei's genetic distance [26] was calculated from SSR genotype data using PowerMarker v3.25 [20], and the UPGMA dendrogram was constructed in MEGA11 [27].

Population structure was analyzed using STRUCTURE v2.3.4 [28]. The number of genetic clusters was evaluated from $K = 1$ to $K = 10$, with 10 independent runs for each K . Each run was performed with a burn-in period of 100,000 iterations followed by 100,000 Markov chain Monte Carlo iterations. Replicate runs for each K were aligned and averaged using the CLUMPP algorithm implemented in CLUMPAK (<http://clumpak.tau.ac.il>, accessed on 28 September 2026) [29,30]. The optimal K was determined using the Evanno ΔK method implemented in STRUCTURE HARVESTER [31,32],

which is also available as a stand-alone program from its public GitHub repository (<https://github.com/dentearl/structureHarvester>, accessed on 28 September 2026). Higher-K solutions were additionally examined descriptively to evaluate finer-scale subdivision of the genetic structure.

3. Results

3.1. SSR Marker Informativeness

Of the 20 SSR markers tested, two markers, GD96 and TXY144_347, were excluded because PCR amplification was not detected. The remaining 18 SSR markers were successfully used to genotype the pear germplasm collection. Among the 11,592 possible genotype calls, 62 (0.53%) were missing and were treated as missing data without imputation. The number of alleles per marker ranged from 4 to 13, with an average of 7.39 alleles per marker (Table 4). The highest number of alleles was observed for CH03g07, whereas TsuENH046 showed the lowest number of alleles. Five unique alleles, each detected in a single accession, were identified at CH05c06, CH03g07, NAUpy40d, TsuENH083, and TXY276.

Table 4. Genetic diversity parameters of SSR markers used in this study.

Marker Set	Marker	No. of Alleles	<i>He</i> *	<i>Ho</i>	PIC
ECPGR	CH01d09	7	0.807	0.603	0.780
ECPGR	CH03d12	7	0.682	0.404	0.649
ECPGR	CH05c06	10	0.765	0.409	0.735
ECPGR	CH03g07	13	0.812	0.653	0.789
ECPGR	EMPc11	7	0.778	0.415	0.743
ECPGR	GD147	7	0.802	0.686	0.776
ECPGR	CH01f07a	6	0.790	0.531	0.758
ECPGR	CH02b10	9	0.695	0.588	0.645
ECPGR	EMPc117	6	0.758	0.475	0.721
ECPGR/USPGR	CH01d08	11	0.771	0.341	0.748
ECPGR/USPGR	CH04e03	7	0.773	0.436	0.740
USPGR	NAUpy40d	7	0.605	0.388	0.538
USPGR	TsuENH046	4	0.520	0.312	0.406
USPGR	TsuENH076	7	0.619	0.569	0.585
USPGR	TsuENH080_354	7	0.737	0.433	0.699
USPGR	TsuENH083	6	0.300	0.269	0.280
USPGR	TsuENH089	6	0.282	0.259	0.268
USPGR	TXY276	6	0.545	0.301	0.443
Mean		7.39	0.669	0.448	0.628

* *He*, expected heterozygosity; *Ho*, observed heterozygosity; PIC, polymorphic information content.

He values ranged from 0.282 to 0.812, with a mean value of 0.669. *Ho* values ranged from 0.259 to 0.686, with an average of 0.448. PIC values ranged from 0.268 to 0.789, with an average of 0.628. Among the markers, CH03g07 showed the highest *He* and PIC values, whereas TsuENH089 showed the lowest values for both parameters. Overall, the SSR markers showed substantial polymorphism across the pear germplasm collection.

3.2. SSR-Based Duplicate Identification

To detect putative duplicate accessions, SSR multilocus profiles were compared across all 644 pear accessions. Accessions with identical multilocus SSR profiles across all 18 markers were classified as putative duplicates and assigned to the same duplicate group.

A total of 43 putative duplicate groups comprising 142 accessions were identified (Table S3). Duplicate group size ranged from 2 to 20 accessions. The largest group, DG001, contained 20 accessions, followed by DG024 with 15 accessions and DG013 with 11 accessions.

After retaining one representative accession from each duplicate group, a non-redundant set of 545 accessions, comprising 528 Korean native pear accessions and 17 additional *Pyrus* accessions, was established. Among these, 479 non-redundant Korean native pear accessions had available collection-region information and were used for regional analyses. A minimum of 11 SSR markers was sufficient to distinguish all 545 unique multilocus genotypes identified using the full set of 18 markers. The non-redundant set of 545 accessions was used for subsequent genetic relationship and population structure analyses.

3.3. Phenotypic Consistency of Duplicate Groups

Morphological traits were compared among accessions within each SSR-based duplicate group. The evaluated traits included petal color, anther color, the presence or absence of trichomes on young leaves, and young leaf color (Tables 3 and S3).

No phenotypic discrepancies were observed among accessions within the same SSR-based duplicate group for the available flower and young leaf traits. These comparisons were used to support, rather than define, SSR-based duplicate groups (Figure 1B,C and Table S3).

3.4. Genetic Relationships of Non-Redundant Accessions

Genetic relationships among the 545 non-redundant pear accessions were examined using a UPGMA dendrogram based on Nei's genetic distance (Figure 2). The UPGMA dendrogram separated the accessions into two major groups, designated Group I and Group II.

Group I contained seven accessions, including six Korean native pear accessions and one *P. fauriei* accession. The remaining 538 accessions were assigned to Group II, which was further divided into nine subgroups, designated II-1 to II-9 (Table S1).

Among the subgroups in Group II, II-2, II-6, and II-4 contained the largest numbers of accessions, with 192, 162, and 91 accessions, respectively. Subgroup II-2 consisted entirely of Korean native pear accessions, including accessions from Gangwon-do and the Gyeongsang regions. Subgroups II-4 and II-6 were also composed mainly of Korean native pear accessions but included several Asian pear accessions or related *Pyrus* species, such as *P. pyrifolia*, *P. ussuriensis*, and *P. bretschneideri*.

Subgroup II-9 contained 17 accessions, including ten Korean native pear accessions, all four European pear cultivars, two hybrid pear accessions, and *P. betulifolia*. Korean native pear accessions were distributed across multiple subgroups within Group II.

3.5. Regional Genetic Differentiation

Regional genetic differentiation was evaluated using non-redundant Korean native pear accessions with available collection region information. AMOVA revealed significant genetic differentiation among collection regions ($p = 0.0001$; Table 5). Variation among collection regions accounted for 7.28% of the total genetic variation, whereas variation within collection regions accounted for 92.72%.

Pairwise F_{ST} estimates varied among collection-region comparisons (Table S4). The highest pairwise F_{ST} values were observed in comparisons involving Gangwon-do or Ulle-

ungdo Island, including Gangwon-do–Ulleungdo Island ($F_{ST} = 0.1727$), Gyeongsangnam-do–Ulleungdo Island ($F_{ST} = 0.1329$), and Gangwon-do–Jeollanam-do ($F_{ST} = 0.1152$). In contrast, the lowest estimates were observed for Chungcheongbuk-do–Jeollabuk-do ($F_{ST} = -0.0074$) and Chungcheongnam-do–Jeollabuk-do ($F_{ST} = -0.0027$).

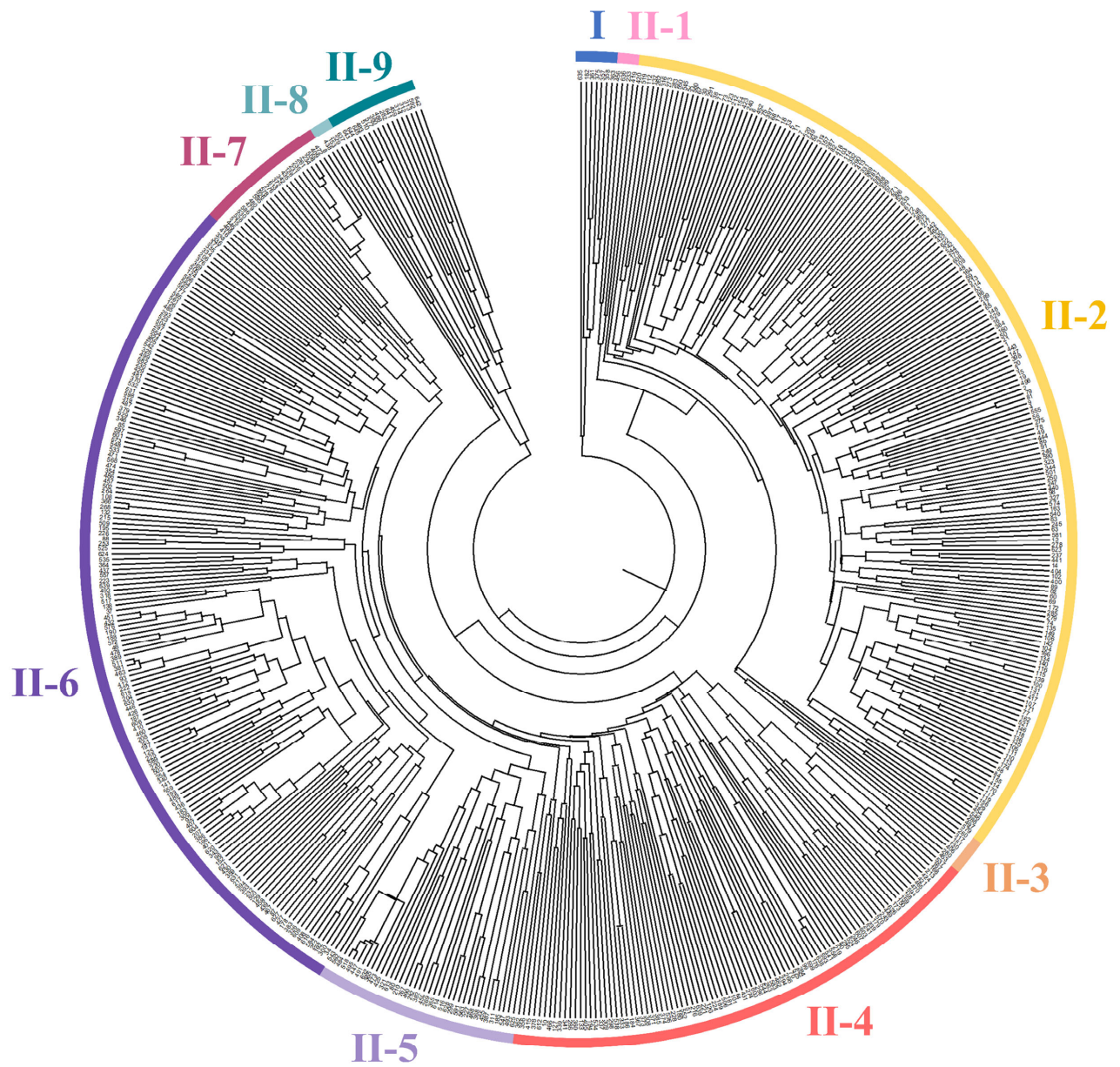


Figure 2. UPGMA dendrogram of 545 non-redundant pear accessions based on Nei’s genetic distance calculated from 18 SSR markers. Colored outer bars indicate Group I and subgroups II-1 to II-9. Accession information and subgroup assignments are provided in Table S1.

Table 5. AMOVA of genetic differentiation among collection regions.

Source of Variation	<i>df</i>	Sum of Squares	Variance Component	Percentage of Variation	<i>p</i> -Value
Among collection regions	8	286.0054	0.5891	7.28	0.0001
Within collection regions	470	3526.7034	7.5036	92.72	
Total	478	3812.7088	8.0927	100.00	

3.6. Population Structure and Regional Membership Patterns

STRUCTURE analysis was performed using the 545 non-redundant accessions. The Evanno ΔK method showed the highest value at $K = 2$ (Figure 3A). At $K = 2$, one genetic component predominated in UPGMA subgroup II-2, whereas the other predominated in most of the remaining subgroups. Among the higher- K solutions examined, $K = 6$ showed a local increase in ΔK and revealed finer subdivisions within the broad genetic components observed at $K = 2$ (Figure 3B).

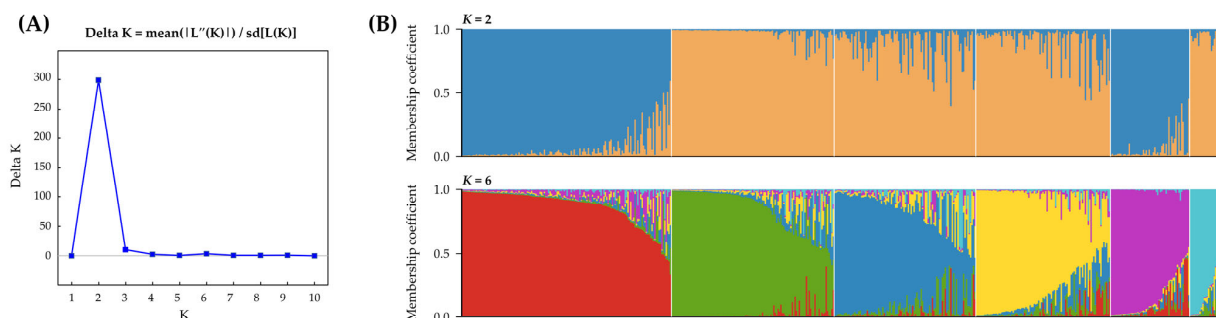


Figure 3. STRUCTURE analysis of 545 non-redundant pear accessions. (A) ΔK values for $K = 1$ – 10 calculated using the Evanno method. (B) Individual STRUCTURE membership coefficients at $K = 2$ (upper) and $K = 6$ (lower). Each vertical bar represents one accession, and colors indicate proportional membership in the inferred genetic clusters. Accessions were ordered according to their predominant membership at $K = 6$, and the same accession order was used at $K = 2$.

At $K = 6$, the relative proportions of the six membership components differed among collection regions (Figure 4 and Table S5). Cluster 5 was the predominant component in Gangwon-do, Gyeongsangbuk-do, and Gyeongsangnam-do, with Gangwon-do showing the largest proportion of this cluster among the collection regions.

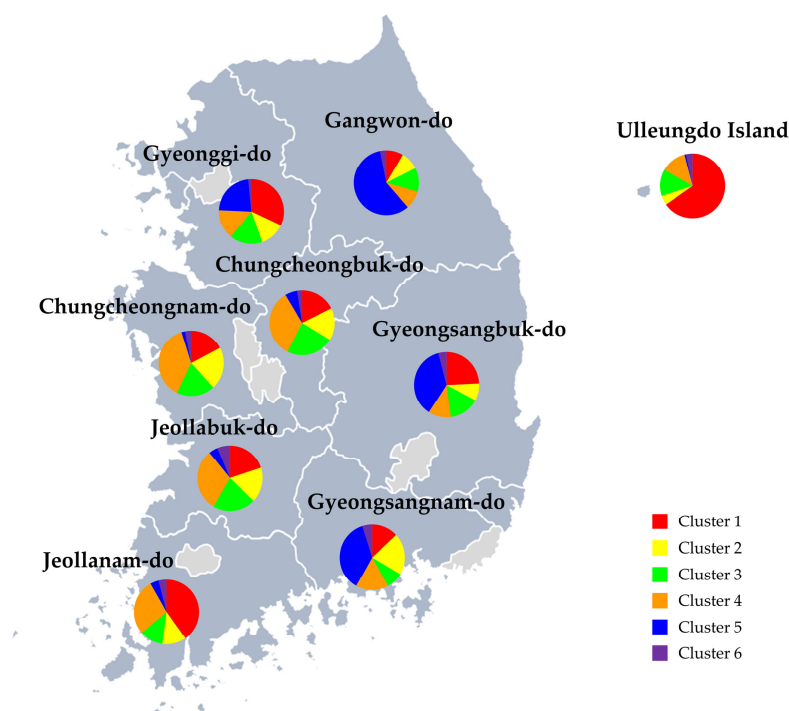


Figure 4. Regional mean STRUCTURE membership coefficients at $K = 6$ for 479 non-redundant Korean native pear accessions. Each pie chart represents the mean membership coefficients of accessions assigned to a collection region. Colors indicate Clusters 1–6 as shown in the legend, and regional sample sizes and numerical membership coefficients are provided in Table S5.

Cluster 4 was the major component in Chungcheongbuk-do, Chungcheongnam-do, and Jeollabuk-do. In Ulleungdo Island, Cluster 1 accounted for the largest proportion of the regional membership. Jeollanam-do also showed a relatively high proportion of Cluster 1, whereas Gyeonggi-do showed a mixed regional membership profile, with Cluster 1 as the largest component.

4. Discussion

4.1. SSR Fingerprinting and Duplicate Identification in Ex Situ Pear Germplasm

The mean PIC across the 18 SSR loci was 0.628, corresponding to the highly informative category proposed by Botstein et al. [33]. The mean PIC in this study was slightly higher than the value of 0.59 reported for Portuguese *Pyrus communis* L. germplasm [34]. PIC values ranging from 0.485 to 0.870 were also reported for nine SSR loci in a collection comprising cultivated pears and 13 *Pyrus* species [35]. Overall, the PIC values indicate that the SSR markers used in this study were sufficiently informative for accession fingerprinting and duplicate identification.

The identification of 43 SSR-identical groups comprising 142 accessions indicates that multiple accessions with identical multilocus SSR profiles have been maintained within the collection. The consistency of the available flower and young leaf traits provided additional support for their classification as putative duplicate accessions. SSR-identical accessions maintained under different names may represent candidate synonyms. Similar findings have been reported in pear collections from Bosnia and Herzegovina, Friuli Venezia Giulia, and the Canary Islands, where SSR fingerprinting identified duplicate accessions and synonyms, demonstrating that different accession names or collection localities do not necessarily represent distinct genotypes [36–38]. More recently, SSR-based characterization of 1251 accessions from Spanish pear collections resolved 760 unique multilocus genotypes, further illustrating the substantial genetic redundancy that may occur in long-term germplasm collections [7].

The occurrence of identical multilocus genotypes among accessions bearing different names or collected from different locations may reflect the long-term clonal propagation and historical exchange of pear planting materials. Grafting is widely used for vegetative propagation in pear and allows selected genotypes to be maintained clonally over successive generations [9]. Accordingly, the SSR-identical groups detected in the present study may reflect, at least in part, the clonal propagation and dissemination of selected genotypes, together with repeated collection and local naming conventions.

The inclusion of multiple accessions with identical multilocus genotypes can lead to the overrepresentation of particular clones in genetic relationship and population structure analyses. Therefore, one representative accession was retained from each SSR-identical group, resulting in a non-redundant set of 545 accessions for subsequent analyses. The removal of redundant genotypes reduces clonal bias and provides a more representative basis for genetic relationship and population structure analyses, as well as subsequent core collection development [7,39]. These results demonstrate the utility of SSR fingerprinting for accession verification, duplicate detection, and curation of the Korean pear germplasm collection.

4.2. Genetic Relationships and Diverse Backgrounds of Korean Native Pear Accessions

The UPGMA tree revealed a distinct Group I comprising six Korean native pear accessions and *P. fauriei*. These Korean accessions showed a closer SSR-based genetic affinity to *P. fauriei* than to the predominant genetic background represented by Group II. Genome-wide analyses have shown that wild *Pyrus* taxa, including *P. fauriei*, may possess complex genetic backgrounds shaped by historical hybridization and admixture [2]. Thus,

Group I may represent a differentiated wild pear-related genetic component within the Korean pear collection. However, this grouping does not establish direct ancestry or taxonomic identity.

Most Korean native pear accessions were distributed across multiple subgroups within Group II, several of which also included Asian pear cultivars and accessions representing *P. pyrifolia*, *P. ussuriensis*, and *P. bretschneideri*. This pattern indicates that the Korean native pear collection includes accessions with diverse genetic backgrounds within the broader East Asian pear gene pool. Previous SSR-based analyses revealed admixture between Japanese pear and continental *P. ussuriensis*-related gene pools in Chinese and Korean cultivated pears [5], while a broader East Asian survey found extensive genetic exchange among sand pears and geographically overlapping clusters [40]. Genome-wide studies have likewise shown that historical hybridization, gene flow, and introgression have contributed to the complex genetic structure of East Asian *Pyrus* taxa [2,41,42]. The clustering observed in the present study is consistent with these previously reported genetic relationships among East Asian pear lineages. The long-term maintenance and local selection of wild, locally selected, and cultivated pear materials in Korea may also have contributed to the genetic diversity represented in the collection.

The assignment of all four European pear cultivars to subgroup II-9 further indicates that the UPGMA analysis captured the broad genetic differentiation between European and Asian pears. This differentiation is consistent with previous genome-wide SNP and haplotype-resolved comparative genomic analyses showing substantial genetic divergence between Asian and European pears [6,41]. However, subgroup II-9 also contained ten Korean native pear accessions, two hybrid pear accessions, and *P. betulifolia* and therefore should not be regarded as a taxonomically homogeneous European pear group. Rather, it represents a genetically differentiated subgroup in which the European cultivars clustered together with Korean native and other *Pyrus* accessions. Taken together, the distinct placement of Group I and subgroup II-9 indicates that the UPGMA analysis differentiated major genetic backgrounds within the collection, although the resulting groups did not correspond strictly to formal taxonomic classifications.

Overall, accession names and collection origins did not correspond consistently with the SSR-based groupings of Korean native pear accessions. The broad genetic diversity represented in the non-redundant set supports its use in core collection development and the conservation of major components of genetic variation in Korean pear germplasm [39].

4.3. Regional Genetic Differentiation and Population Structure

AMOVA indicated that 92.72% of the total genetic variation occurred within collection regions, whereas 7.28% was attributable to differences among regions. Although the among-region component was statistically significant, its relatively small contribution indicates that geographic differentiation accounted for only a limited proportion of the overall genetic variation. A similar predominance of within-region variation has been reported in Chinese sand pear landraces, where most genetic variation occurred among accessions within geographic populations despite detectable geographic structure [43]. In local pear germplasm from mountainous areas of Aragón, northeastern Spain, UPGMA clustering showed no clear correspondence with geographic origin; this pattern was attributed to the historical exchange of grafting materials among regions [44]. These findings collectively indicate that collection region may account for part of the genetic structure of pear germplasm without defining genetically homogeneous regional groups.

The predominance of within-region variation may be associated with both the reproductive biology of pear and the historical maintenance of local germplasm. Gametophytic self-incompatibility promotes outcrossing between genetically different individuals and

contributes to the maintenance of genetic diversity in pear populations [45]. Natural hybridization and the local selection and maintenance of seedling-derived trees may further increase genetic variation within a collection region. Conversely, the exchange of clonally propagated materials can introduce identical or closely related genotypes into different regions and thereby reduce regional differentiation. Village pear populations in northeastern Türkiye similarly exhibited high genetic diversity and broad sharing of genetic components across regions associated with farmer selection and the widespread dispersal of clonal materials [46]. The regional STRUCTURE profiles were consistent with this interpretation: although the predominant cluster differed among regions at $K = 6$, all regions contained contributions from multiple genetic clusters. Regional structure was therefore expressed primarily as differences in the relative proportions of shared genetic components rather than as the complete separation of regional populations.

Within this broadly shared regional membership structure, Ulleungdo Island exhibited the most distinctive regional pattern. Pairwise F_{ST} values were relatively high in comparisons between Ulleungdo and several mainland regions, and Cluster 1 constituted the largest component of the Ulleungdo membership profile. The concordance between the pairwise F_{ST} and STRUCTURE results supports the recognition of the Ulleungdo accessions as a differentiated regional component of the Korean native pear collection. Geographic isolation may have restricted the exchange of pear planting materials between Ulleungdo and the mainland, while prolonged local propagation and selection may have reinforced differences in allele frequencies. A population genetic study of the Ulleungdo endemic *Acer takesimense* identified clear differentiation from its continental progenitor and a pattern consistent with strong genetic drift during colonization and speciation [47], illustrating how the island setting can influence genetic structure. A second spatial pattern was observed in eastern Korea, where Cluster 5 predominated in Gangwon-do, Gyeongsangbuk-do, and Gyeongsangnam-do. This distribution broadly corresponds to the eastern mountainous corridor that includes the Baekdu-daegan, the principal mountain system extending along the Korean Peninsula [48]. Although the present analysis did not test a direct barrier effect, this geographic correspondence provides a spatial context for the shared membership pattern observed across the eastern regions.

4.4. Germplasm Conservation and Utilization

Because most genetic variation occurred within regions, conservation and core collection development should include multiple genetically differentiated accessions from each region rather than relying solely on geographic representation. Accessions representing relatively differentiated regional components, particularly those from Ulleungdo, should also be retained to ensure that the full range of genetic variation in Korean native pear germplasm is conserved.

Beyond its value for conservation and core collection development, the non-redundant set provides a basis for the systematic evaluation of horticulturally important traits. In the present study, phenotypic observations were used only to assess concordance within SSR-based duplicate groups, and collection-wide phenotypic characterization was beyond the scope of the study. Consequently, phenotypic variation among the 545 non-redundant accessions remains to be characterized. Broader standardized evaluation of fruit and other horticulturally important traits would complement the SSR-based genetic characterization and further support germplasm management and the identification of potentially useful breeding materials.

5. Conclusions

This study established SSR fingerprints for 644 pear accessions and identified 43 putative duplicate groups comprising 142 accessions, with no discrepancies observed in the available flower and young leaf traits. The resulting non-redundant set of 545 accessions reduced redundancy and provided a more representative basis for characterizing genetic relationships, population structure, and regional differentiation within the collection. Korean native pear germplasm showed diverse genetic backgrounds, extensive variation within regions, and limited but significant regional differentiation, with Ulleungdo showing the most distinctive regional pattern. These findings demonstrate the value of SSR fingerprinting for accession verification, germplasm curation, and core collection development and provide a practical basis for standardized phenotypic evaluation and the identification of potential breeding materials.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12101225/s1>, Table S1: Accession metadata and UPGMA group information of pear germplasm used in this study; Table S2: SSR genotype matrix of pear accessions used in this study; Table S3: Putative duplicate accession groups and phenotypic consistency identified by SSR fingerprinting; Table S4: Pairwise F_{ST} values among collection regions; Table S5: Mean STRUCTURE membership coefficients by collection region at $K = 6$.

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