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Genetic Characterization of European Plum (*Prunus domestica* L.) Accessions from Norway Using ECPGR-Selected SSR Markers

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Abstract: In order to ensure the long-term sustainability of the conservation process of Norwegian plum germplasm, as well as to enhance the possibility of its utilization, a central plum heritage cultivar collection was established in 2020. In this study, 40 plum accessions maintained at the Ullensvang plum heritage cultivar collection were genetically characterized using a set of nine microsatellite markers recently approved by the ECPGR *Prunus* working group. The obtained molecular data were used to investigate the genetic identity, diversity, and structure among the analyzed accessions. No redundancies were detected among the plum accessions, which is in stark contrast to the previous molecular study on plum samples collected through an on-farm inventory of Southern Norway. Furthermore, the obtained data indicate that the Ullensvang collection contains a significant genetic diversity of Norwegian plum germplasm, previously held in decentralized sites. With that in mind, this collection can certainly be considered for the role of the National Clonal Plum Germplasm Repository. The nine microsatellite markers, recommended by ECPGR, revealed a genetic structure not entirely tied to previously proposed pomological groups, possibly indicating a history of hybridization among members of the various groups.

Keywords: hexaploid plum; microsatellites; genetic diversity and structure

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1. Introduction

The European plum (*Prunus domestica* L.) is a hexaploid (2n = 6x = 48) fruit crop with a long tradition of cultivation in Norway, dating back to the period of the West European Middle Ages [1]. At that time, fruit cultivation was mainly conducted within monasteries, from where the practice then spread [2]. Plum production is currently located in the most suitable climatic regions, along the fjord sides in Western Norway and around lakes in Eastern Norway [3], where the short and relatively cool growing season limits the choices of plum cultivars that can be grown [4].

European plums represent a very phenotypically heterogeneous fruit crop, most recently classified into six pomological groups as follows: egg plums, two different prune types, greengages, mirabelles, and bullaces or damsons [5]. This heterogeneity is further compounded through the hybridization of genotypes belonging to different pomological groups.

In the last decades, plum production in Norway has relied mostly on cultivating two self-fertile plum cultivars, 'Opal´ and 'Victoria' [1]. More recently, other cultivars such as 'Edda', 'Mallard', 'Reeves', 'Jubileum', and 'Valor' have been introduced in commercial

plum orchards [6] and are now the main commercial plum cultivars in addition to 'Opal'. They are all grafted on the semidwarf rootstock, St. Julien A [7]. The introduction of novel plum cultivars has the potential to fully replace older and traditional cultivars, thus reducing the diversity of the Norwegian plum germplasm. In order to combat this process, the Norwegian Institute of Bioeconomy Research (NIBIO) and the Nordic Gene Bank (NGB) have led an effort to establish several germplasm collections. In a chapter within the 2006 report by the ECPGR (European Cooperative Programme for Plant Genetic Resources) *Prunus* working group, Sekse [8] identified a total of nine collections containing accessions of fruit trees in Norway. Furthermore, the report stated that these collections contain, among other fruit crops, 49 different plum cultivars (mainly *P. domestica* as well as some *P. salicina*) with different pomological and qualitative characteristics, as proven by Fotirić Akšić et al. [9]. This program has recently expanded and currently, there are 12 collections containing a total of 79 accessions in Norway [10].

It is important to note that old traditional plum cultivars and landraces represent a rich reservoir of genetic traits, especially genetic resistance, useful for the further genetic improvement of this crop [11]. In order to ensure long-term sustainability of the conservation process as well as to enhance the possibility of utilization, a central plum heritage cultivar collection was established in Ullensvang, Norway, in 2020. This approach was previously undertaken for the Norwegian apple germplasm [12].

Avoiding redundancies and mislabeling through the identification of synonyms and homonyms represents the main measure that ensures the long-term sustainability of a germplasm collection. Furthermore, determining the diversity and genetic relationships among the accessions enhances the possibility for future utilization. DNA markers represent an efficient tool for conducting all the mentioned activities. This is especially true for vegetatively propagated crops since there is no genetic variation between trees of the same cultivar but a significant variation between that of different cultivars [13].

Due to their multiallelic and highly polymorphic nature, microsatellites, or simple sequence repeats (SSRs) are one of the most suitable markers for estimating *Prunus* diversity [14]. In fact, this marker system has previously been used in numerous studies on local plum germplasm throughout the following European countries: France [15]; Croatia; Bosnia and Herzegovina; Serbia [16]; Italy [17,18]; Hungary [19]; Spain [20]; Romania [21]; and Slovenia [22]. More recently, SSRs were employed for a genetic assessment of the pomological classification of plum accessions sampled across Europe [5]. A molecular study conducted on the Nordic plum germplasm using microsatellites included samples from Norway [23]. However, these samples were collected from on-farm sites and revealed a significant presence of duplicates and mislabeling. In addition, SSR markers have recently been used to identify the male parent (successful pollinizer) in Norwegian commercial plum orchards [3]. Although new genomic approaches have enabled sequence-based genotyping [24], microsatellites remain an effective and cost-efficient marker [25].

An additional asset of SSR markers is their reproducibility [26], which enables adding new SSR-based data to an existing database. However, this asset can only be used if a standard set of SSR markers exists for a particular crop. In other fruit tree crops, such as the European pear (*Pyrus communis*) and sweet cherry (*Prunus avium*), sets of recommended SSR loci have been chosen by ECPGR and published 15 years ago [27,28]. The first such standard set was proposed by the ECPGR *Prunus* working group relatively recently [29] and has already been accepted by the most recent molecular study on plum germplasm [30]. It is, however, important to note that the reproducibility of SSR analyses between the laboratories is significantly complicated by plums hexaploidy, making scoring microsatellite alleles a somewhat subjective process.

In this study, 40 plum accessions maintained at the Ullensvang plum heritage cultivar collection were genetically characterized using a set of nine SSR loci approved by the ECPGR *Prunus* working group. The obtained molecular data were used to investigate the genetic identity, diversity, and structure of the analyzed plum collection.

2. Materials and Methods

2.1. Plant Material and Collection Management

The plum collection was established in 2020 at the experimental fields of NIBIO, Ullensvang (60.318655N, 6.652948E). The soil was a sandy loam with approximately 4% organic matter, very uniform in morphological and physical characteristics (color and structure). All accessions were grafted on St. Julien A rootstocks spaced 2.0 × 4 m apart, three trees of each accession, and all trees were trained as spindle trees and pruned to a maximum height of about 2.5 m. The selected trees were homogeneous in terms of fruit set, vigor, and health status. The weeds under the trees were removed by a meter-wide herbicide strip using glyphosat (trade name Roundup with 360 g/L of glyphosat, Monsanto Crop Sciences, Cambridge UK), and maintained each season together with frequent grass mowing in the interrow. Pest management was conducted according to integrated protocols, spraying against major pests (insects and diseases) when needed. When water deficits occurred, trees were irrigated by drip irrigation. All trees received the same amount of fertilizers based on soil and leaf analysis.

2.2. Genetic Characterization

During the summer of 2023, fresh and healthy leaves were collected from 40 plum accessions maintained at the heritage cultivar collection in Ullensvang, Norway (Table 1). The leaf tissue (approximately 20 mg/sample) was silica-dried before being reduced to a fine powder using a Qiagen Tissue Lyser device (Hilden, Germany). Genomic DNA from the leaves was isolated using the Qiagen Dneasy™ Plant Mini Kit (Qiagen AB, Sollentuna, Sweden), following the manufacturer's instructions. Genetic characterization was conducted using a set of nine SSR loci approved by the ECPGR *Prunus* working group. Pac A 33, representing an EST–SSR developed in apricots [31], was amplified according to [31]. Amplifications using primers for BPPCT 07, BPPCT 14, BPPCT 34, BPPCT 39, BPPCT 40, UDP 96-005, and UDP 98-407 SSRs, developed from genomic DNA in peaches [32,33], were analyzed according to [32]. The final marker, CPSCT026, was amplified according to Mnejja et al. [34]. Diluted PCR products were mixed with Hi-Di Formamide (Applied Biosystems, Warrington, UK) and GeneScan 500 LIZ size standard, after which the amplified fragments were analyzed on ABI 3500 Genetic Analyzer (Applied Biosystems). The software package GeneMapper 4.0 (Applied Biosystems) was used for the data analysis.

Table 1. A total of 40 plum accessions conserved in Ullensvang, Western Norway, and analyzed using 9 ECPGR-selected SSR markers, their abbreviation as well as assignment of each genotype to a Genetic Cluster (GC) (K = 3) defined by Structure [35] (probability of membership *qI* > 80%).

Number	Plum Accession	Abbreviation	GC
1	'Hestaplomme'	HSP	admixed
2	'Admiral'	ADM	2
3	'Plomme 19:6'	P19	3
4	'Sandeplomme'	SND	1
5	'Stokkarudplomme'	STK	3
6	'Rødplomme' ('Eikerplomme')	RDP	1
7	'Katarina'	KAT	1
8	'Jefferson'	JEF	2
9	'Gul eggeplomme'	GEP	1
10	'Early Laxton'	ELA	3
11	'Washington'	WAS	2
12	'Sviskeplomme'	SVP	2
13	'Tråneplomme'	TRP	Admixed

Table 1. *Cont.*

Number	Plum Accession	Abbreviation	GC
14	'Søgne'	SGN	3
15	'Sviske fra Tveit'	SVT	Admixed
16	'Sukkerplomme'	SKP	Admixed
17	'Sinikka'	SNK	1
18	'Reine Claude Noir'	RCN	Admixed
19	'Reine Claude Grøn'	RCG	1
20	'Reine Claude Precoce'	PRE	3
21	'Mount Royal'	MTR	1
22	'Italiensk sviske'	ITA	Admixed
23	'Experimentalfältets sviske'	EXP	2
24	'Emil'	EML	Admixed
25	'Edwards'	EDW	2
26	'Diamond'	DIA	Admixed
27	'Bonne de Bry'	BDB	1
28	'Laura'	LAU	2
29	'Victoria UMB'	VIC	2
30	'Rød Victoria Ruud'	RVR	Admixed
31	'Victoria EMLA'	VEM	2
32	'Blue Rock'	BLR	Admixed
33	'Edda'	EDD	3
34	'Reine Claude Souffriau'	RCS	3
35	'Ive'	IVE	3
36	'Czar'	CZR	Admixed
37	'Reine Claude d'Oullins'	RDO	Admixed
38	'Reine Claude d'Althanns'	ALT	3
39	'Rivers Early Prolific'	REP	3
40	'Henrik'	HNK	3

2.3. Biostatistical Analysis

Genetic diversity parameters, including allele frequency, number of rare and effective alleles, as well as gene diversity [36], were calculated in SPAGeDI 1.5 [37], a population genetics software, for all nine loci.

Population structure was assessed using a Bayesian model-based clustering algorithm within Structure ver. 2.3.4 [35]. Accessions with less than six allelic variants per locus were assigned with the missing data mark (-9). K (unknown) RPPs (reconstructed panmictic populations) were computed on individuals, testing K (log-likelihood) = 1–10 for all accessions with the assumption that sampled cultivars were from an unknown origin. Five independent runs were conducted for each K. A burn-in period of 200,000 and 500,000 iterations was used. The most probable K value was estimated using Structure Harvester python script (https://github.com/dentearl/structureHarvester (accessed on 8 February 2024)) [38] implementing the Evanno model [39]. After estimating the most probable K values, runs with the maximum likelihood were used to assign individuals to a specific Genetic Cluster (GC) [40]. The assignment of a cultivar to a GC was provided by the probability of membership qI chosen at 80%, according to corresponding studies on plums [5,20].

The Jaccard distance matrix between the analyzed plum cultivars was computed in R studio (version 4.3.2) using philentrophy [41] and usedist packages [42]. UPGMA (unweighted pair group method with arithmetic mean) dendrogram, based on the Jaccard distance matrix, was constructed using MEGA 6 software (Molecular Evolutionary Genetics Analysis) [43].

FCA (Factorial correspondence analysis) was plotted in R Studio using the following packages: gee [44], arules [45], combinat [46], rgl [47], ade4 [48], and scatterplot [49].

3. Results and Discussion

3.1. SSR Polymorphism

Nine ECPGR-selected primer pairs amplified 196 distinct alleles among the 40 genotyped plum accessions. This translates to 21.78 alleles per SSR locus (Table 2), ranging from 17 per locus for BPPCT040 to 28 for PacA33. It is important to note that the average effective allele number was much lower (10.54). Manco et al. [18] reported a similar ratio between the total and the effective number of alleles on 29 Italian plum accessions, while Pérez et al. [50] reported a somewhat higher percentage of effective alleles (65%) on 35 Spanish hexaploid plum accessions. The difference between the average numbers of detected and effective alleles obtained in our study is most likely due to the relatively high presence of rare alleles (135 alleles or 61.8%). A high presence of rare alleles can indicate that a large portion of the investigated germplasm has not been extensively used in breeding programs [12].

Table 2. Number of detected, rare (frequency < 0.05), and effective alleles, detected allele range, and gene diversity [36] for the plum accessions, based on nine ECPGR-recommended SSR loci.

Locus Code	No. of Alleles	No. of Rare Alleles	No. of Effective Alleles	Detected Alle Range	Gene Diversity (He)
UDP96	24	17	12.53	100/150	0.92
UDP98	18	12	8.85	156/203	0.89
BPPCT039	22	15	9.33	122/183	0.89
BPPCT034	25	20	10.15	215/271	0.90
CPSCT026	19	14	9.78	165/210	0.90
BPPCT007	18	9	12.76	121/157	0.92
BPPCT014	25	16	11.25	186/258	0.91
BPPCT040	17	9	10.82	113/174	0.91
PacA33	28	23	9.42	169/260	0.89
Average	21.78	15	10.54		0.90

The average number of alleles per locus obtained in our study is very similar to the values reported by Sehic et al. [23] (22.7) in a study on 76 Nordic plum accessions, maintained ex situ (germplasm collection, Balsgård in Sweden) and on farms (Southern Norway). Lower values for this parameter have been previously reported by Halapija Kazija et al. [16] (18.7) on 62 traditional Croatian, regional, and international plum accessions; Makovics-Zsohár et al. [19] (19.3) on 55 mainly Hungarian accessions, and Xuan et al. [51] (20.0) on 45 European plum accessions conserved in Germany. Slightly higher values (23.4 alleles per SSR locus) have been reported on 166 Spanish and international accessions [20]. In comparison, much higher values were reported on 80 accessions from the French National Collection (29.0) [15] and by the cross-European study, conducted on 104 plum accessions (29.25) [5]. The significance of the results obtained in this study is further highlighted by the fact that the Gasi et al. [5] study included accessions from several different collections, and the French study included three different *Prunus* species. On the other hand, the average values for gene diversity (0.90) obtained for the 40 plum accessions maintained at the Ullensvang heritage cultivar collection are almost identical to both the values reported on the Nordic plum accessions (0.90–0.91) [23] and the cross-European plum accessions (0.91) [5]. Based on the presented allele polymorphism, there is no doubt that the plum germplasm, maintained at the Ullensvang heritage cultivar collection, possesses a great deal of genetic diversity.

3.2. Genetic Identity and Relationships

Among the 40 analyzed plum accessions, no redundancies were detected (Figure 1), which starkly contrasts the results of a previous molecular study on plum samples collected through an on-farm inventory of Southern Norway [23], which reported one-third of the samples as duplicates. Sehic et al. [23] argued that the high frequency of duplicates in the germplasm was probably due to the "informal" names of sampled accessions, such as

'Blåplomme' and 'Gulplomme' ('Blue plum' and 'Yellow plum' in Norwegian, respectively) or the name of the village or town where the sampled tree was registered. Although some descriptive names are present among the accessions from the Ullensvang heritage cultivar collection, such as 'Sukkerplomme' ('Sugar plum' in Norwegian), a large part of the analyzed accessions represent old cultivars with a lengthy history of cultivation in Norway and a "proper" name. The most interesting case presents the accessions designated as the old English cultivar 'Victoria' (VEM, VIC, and RVR). Namely, all three accessions possessed distinct SSR profiles, but were, nonetheless, grouped tightly within the cluster analyses (Figure 1). A possible explanation could be tied to this cultivar's self-fertile nature, which can produce genetically similar offspring through selfing. The accessions designated as 'Victoria', 'Jefferson' (JEF) and 'Reine Claude Grøn' (RCG) grouped together in a large cluster, which is very similar to the results reported by Sehic et al. [23]. Furthermore, two well-known greengages, 'Reine Claude d'Oullins' (RDO) and 'Reine Claude Souffriau' (RCS), which were previously reported to be closely related [5], clustered closely in our study as well (Figure 1). 'Early Laxton' (ELA) is clustered relatively closely to 'Rivers Early Prolific' (REP) and is its' believed parent.

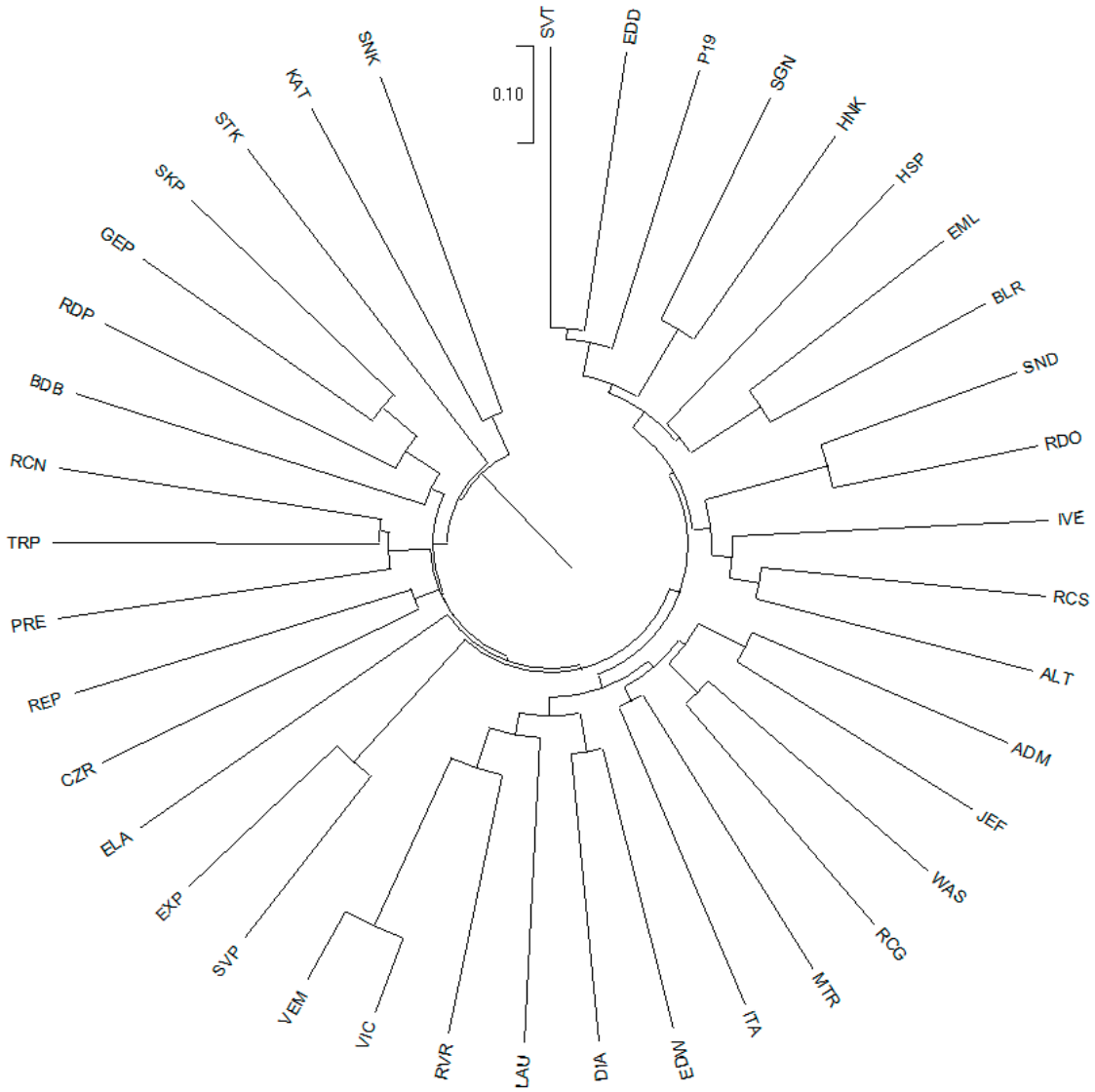

Figure 1. UPGMA cluster analysis based on polymorphisms of SSR data for 40 plum accessions using Jaccard's similarity coefficient.

Overall, the lack of redundancies indicates that the collection process that preceded the establishment of the Ullensvang heritage plum cultivar collection managed to avoid duplicates, thus contributing to the overall economic sustainability of the conservation effort. Genetic relationships of the analyzed plum accessions were investigated further through a multivariate analysis.

The factorial correspondence analysis (FCA) reveals that although the genotyped samples are far from a homogeneous group, it is difficult to identify distinct groups of accessions (Figure 2). The closest to this were the following samples: 'Victoria_EMLA' (VEM) and 'Victoria–UMB' (VIC), 'Blue rock' (BLR), 'Edda' (EDD), 'Ive' (IVE), 'Czar' (CZR), as well as two closely related 'Reine Claude' accessions ('Reine Claude Souffriau'—RCS and 'Reine Claude d'Oullins'—RDO), all of which formed a string together on the far right of the FCA graph. It is also worth mentioning that 'Edda' is an offspring of the cultivar 'Czar', so their close proximity was expected. 'Rivers Early Prolific' (REP), a progenitor of 'Czar' could also be found very close to this string. The most interesting were again the samples with 'Victoria' in their name. Namely, although these two 'Victoria' accessions grouped tightly, the third sample ('Rød Victoria Ruud'—RVR) was more distant, possibly indicating a hybridization between its 'Victoria' progenitor and an unknown cultivar.

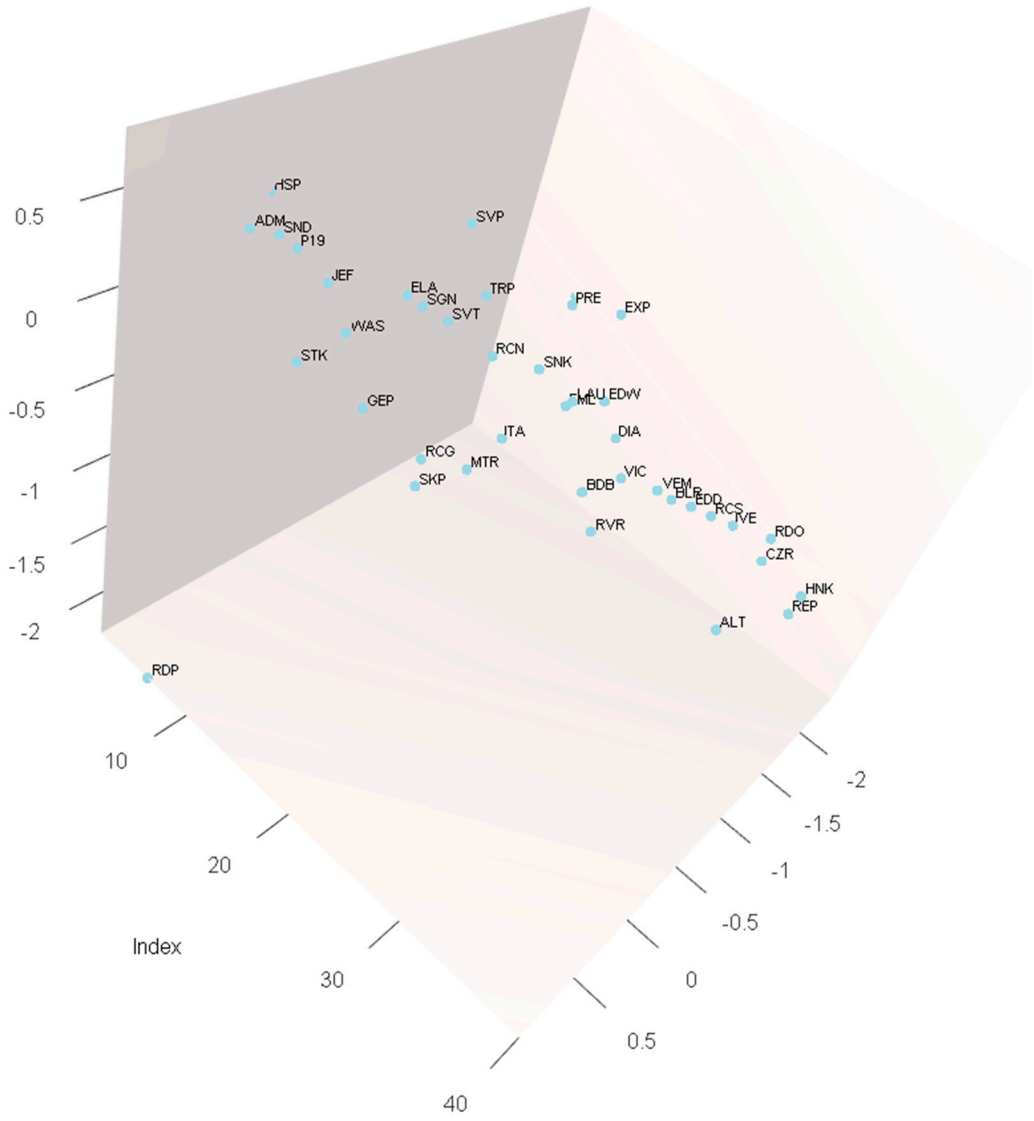

Figure 2. Factorial correspondence analysis (FCA) of SSR data on 40 plum accessions from the Ullensvang heritage cultivar collection. Accessions' abbreviations correspond to Table 1.

The positioning of individual accessions could not be entirely explained through their adherence to the pomological groups defined by Gasi et al. [5]. For instance, the accessions belonging to the 'Reine Claude' gage group (RCN, RCG, RCS, RDO, ALT, and PRE), were quite dispersed throughout the FCA graph. On the other hand, the cultivar 'Ive' (IVE), previously classified as a hybrid of greengage and egg plum parents [5], positioned itself close to two 'Reine Claude' gages (RDO and RCS). Furthermore, the well-known gages, 'Early Laxton' (ELA) and 'Jefferson' (JEF), were grouped very loosely together on the left of the graph, with the yellow gage cultivar 'Washington' (WAS).

The overall positioning of the investigated samples indicates a history of hybridization within and between pomological groups. An analysis of the genetic structure was performed to investigate this conclusion further.

3.3. Genetic Structure

A Bayesian analysis of the 40 plum accessions investigated and a subsequent ΔK analysis revealed a maximum value for K = 3. After assigning individual accessions to three genetic clusters, 12 analyzed accessions did not display a probability of membership above 80% to any of the genetic groups and were thus classified as admixed (Table 1, Figure 3). Overall, 8 plum accessions displayed a probability of membership above 80% to GC1, 9 to GC2, and 11 to GC3.

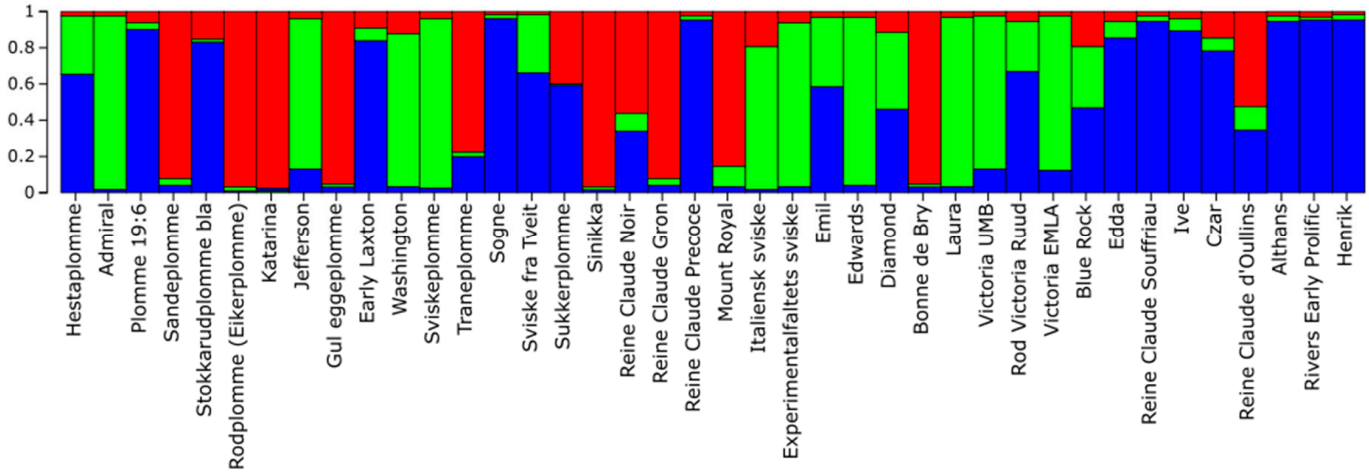

Figure 3. The bar plot of the results from the Bayesian genetic structure analysis conducted on 40 plum accessions from the Ullensvang heritage cultivar collection (K = 3). Red—GC1, Green—GC2, Blue—GC3. Plum accessions' numbers (1–40) correspond to Table 1.

The first genetic cluster (GC1) most notably included the greengages 'Reine Claude Gron' (synonyms 'Reine Claude', 'Reine Claude verte', 'Green Gage') and 'Bonne de Bry', which have been previously reported by Sehic et al. [23] to cluster among each other. This group also contained the old French dark purple cultivar 'Katarina'. The GC2 contained two of the Victoria accessions ('Victoria_EMLA' and 'Victoria_UMB'), which grouped tightly within the UPGMA and FCA cluster analyses, but not the third one ('Rod Victoria Ruud'—RVR), which differentiated from the others in the mentioned cluster analyses. 'Victoria' was previously classified as belonging to the egg plums pomological group [5]. Interestingly, the only cultivar with a name indicating an egglike shape, 'Gul eggeplomme' ('Yellow eggplum'), was grouped in GC1. However, it is important to note that the classification to pomological group was conducted purely based on the name of the accession. The yellow gage cultivar 'Washington' (WAS) also grouped within this GC2 (Figure 3, Table 1).

The GC3 was also the most populous cluster, containing 11 accessions that displayed a probability of membership above 80%. The blue-colored cultivar 'Rivers Early Prolific' grouped with its presumed gage offspring 'Early Laxton'. Its other offspring, 'Czar', displayed an adherence to GC3 only slightly lower than 80% (Figure 3). One of the 'Czar'

offsprings, 'Edda', also clustered in this GC. This cluster also included the cultivar 'Ive', classified as a hybrid of greengage and egg plum, 'Jefferson', a well-known gage, and most of the Reine Claude accessions ('Reine Claude Precoce', 'Reine Claude Souffriau' and 'Reine Claude d'Althanns').

The previous study on Nordic plum accessions [23], which included accessions collected on a farm from Southern Norway, detected a genetic structure of this germplasm loosely tied to pomological groups (greengages, damsons, prunes, etc.). The cross-European study on this crop noted a similar connection between pomological groups and genetic structure in [5]. Furthermore, this study concluded that the different admixture levels within the Bayesian Structure analysis indicate hybridization between genotypes belonging to different pomological groups of plum. On the other hand, Pérez et al. [50] reported that the genetic structure analyses differentiated plum samples based on their membership to each of the two investigated plum collections (CITA and La Palma). In a study on Slovenian plum accessions, the detected genetic structure among the samples was strongly correlated to two distinct ancestral populations [22].

The lack of a clear link between the genetic structure and adherence of the Ullensvang accessions to a pomological group is presumably the consequence of hybridization between plums of various pomological classifications.

4. Conclusions

The presented results indicate that the Ullensvang plum heritage cultivar collection contains a significant genetic diversity of Norwegian plum germplasm, previously held in decentralized sites. Considering this, as well as the lack of redundancies, the Ullensvang collection can certainly be the National Clonal Germplasm Repository for plums in Norway. The nine SSR markers selected by ECPGR and used in this study revealed a genetic structure not entirely tied to previously proposed pomological groups, possibly indicating a history of hybridization among members of the various groups. In future, the genetic information obtained from this study can guide conservation strategies, breeding programs, and the sustainable management of plum genetic resources in Norway.

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