Genetic diversity within Swedish national heritage cultivars of gooseberry, *Ribes uva-crispa*

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Josefine Nordlander

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Josefine Nordlander

Supervisor:	**Larisa Gustavsson, SLU, Department of Plant Breeding**
Assistant supervisor:	Jonas Skytte af Sätra, SLU, Department of Plant Breeding
Assistant supervisor:	Helena Mattisson, SLU, Department of Plant Breeding
Examiner:	Helena Persson Hovmalm, SLU, Department of Plant Breeding

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Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Department of Plant Breeding

Abstract

In the year 2000, the *National program for diversity of cultivated plants (Pom)* was established, aiming to recover old, Swedish fruit and berry cultivars that historically have been important for breeding and commercial production. A collection of mandate cultivars of *Ribes uva-crispa* (gooseberry) containing valuable accessions was created. Currently, 16 accessions from the mandate list are missing in the collection and missing mandate cultivars could be found through a national inventory of plant resources. In this study, mandate cultivars and inventory findings collected by Pom, were analyzed though molecular characterization (SSR-markers), to find duplicates, mislabelings and to analyze the genetic variability of the collection.

The present work uncovered several cases of duplication among the inventory findings and one, possible case of mislabeling. All the mandate cultivars had unique SSR-profiles, which was expected. Furthermore, of the 15 SSR markers used, 6 markers are particularly effective at describing the variability of the gooseberry germplasm. The PCoA analysis revealed the presence of a relatively more homogenous group among the analyzed plant material. These accessions display more genetic similarities, compared to the rest of the analyzed plants.

The data constitutes an important basis for evaluation and addition of accessions to the present collection of mandate cultivars and for removal of duplicates among the inventory findings.

Keywords: genetic diversity, heterozygosity, molecular characterization, PCoA, pomological heritage, *Ribes grossularia*, *Ribes uva-crispa*, SSR markers

Sammanfattning

Under år 2000 grundades Programmet för odlad mångfald, (Pom), med syftet att bevara gamla kultursorter av bland annat frukt och bär. För att en sort ska bevaras behöver den dels inneha ett kulturhistoriskt värde, dels måste sorten ha varit viktig för Sverige antingen i förädlingssammanhang eller i kommersiell produktion. På denna grund valde man ut ett antal krusbärssorter som fick benämningen mandatsorter och de sorter som kunde hittas och valideras fick ingå i en samling i Alnarp (SLU). För tillfället saknas dock 16 accessioner från mandatlistan i samlingen. Äldre, saknade sorter kan hittas genom nationella inventeringar, där man samlar in intressant växtmaterial för analys. I denna studie genomfördes en molekylär karaktärisering med hjälp av SSR-markörer av mandatsorterna i samlingen och de inventeringsfynd som Pom samlat in. Målet med analysen var att hitta duplikat eller felmärkningar samt analysera samlingens genetiska variabilitet.

Studien visade att det fanns ett flertal duplikat bland inventeringsfynden och ett fall av felmärkning. Alla mandatsorter hade unika SSR-profiler, vilket var förväntat. Det visade sig också att av de 15 SSR markörer som användes, så var 6 markörer särskilt effektiva för att beskriva variabiliteten i arvsmassan hos krusbär. PCoA analysen visade att det fanns en relativt homogen grupp bland det analyserade växtmaterialet. Dessa accessioner visade på större genetiska likheter i jämförelse med resten av de analyserade krusbärsbuskarna.

Resultaten från denna studie kan ligga till grund för utvärdering av samlingen av inventeringsfynd, borttagning av duplikat och eventuell addering av accessioner till den nuvarande samlingen av mandatsorter.

Nyckelord: genetisk diversitet, heterozygositet, molekulär karakterisering, PCoA, kultursorter, *Ribes grossularia*, *Ribes uva-crispa*, SSR markörer

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Abbreviations

EPS	Swedish Elite Plant Station
PCR	Polymerase Chain Reaction
Pom	National program for diversity of cultivated plants
SSR	Simple Sequence Repeat

1. Introduction

1.1. History of *Ribes* L.

Ribes L. is a genus within the plant family *Grossulariaceae* where black currant (*R. nigrum*), red currant (*R. rubrum*) and gooseberry (*R. uva-crispa* or *R. grossularia*) belong. There are about 150 known species of currants and gooseberries, mainly found in temperate regions of Europe and North America. European gooseberry species *R. uva-crispa* (synonym *R. grossularia*) is more distributed across the world than the North American species *R. hirtellum* (Anastasiadi et al. 2016; Pluta 2018). *Ribes uva-crispa* is a small bush approximately 1.0-1.8 meters tall and 1.0-1.2 meters wide. Most of the gooseberry cultivars have spines on their branches and their leaves are small and hairy with three to five lobes. The flowers are green, sometimes with pink tendencies, and form clusters of two to three flowers. The berries are oval, the color varies from white to yellow, green, or red, and they have a hairy or smooth surface (Heiberg 2003; Pluta 2018).

According to Nilsson (1958), the domestication of berries in Europe probably began in the Middle Ages, but the cultivation of berries became more common in the 18th century. The first Swedish record of gooseberry cultivation is from 1638 (Norstedt 1920). Even though gooseberry can be found naturally in Sweden , it is not considered an indigenous species in contrast to red and black currant which are indigenous to Scandinavia.

Foreign gooseberry cultivars became important in the 19th century, and Sweden began to import cultivars from Great Britain, where horticultural practices and knowledge were more advanced, and North America, where cultivars resistant to powdery mildew could be found. Many imported cultivars also had higher yields and tastier berries. A serious outbreak of powdery mildew (*Sphaerotheca mors-uvae*) in Sweden 1901 (Hjalmarsson & Wallace 2007), resulted in a drastic decrease in the cultivation of gooseberries.

At the beginning of the 20th century, C. G. Dahl initiated a breeding program at Alnarp, focusing on developing disease-resistant cultivars suitable for the changing growth conditions. North American gooseberry cultivars, resistant to powdery mildew, were imported and crossed with the European species *R. uva-crispa*. The imported, North American cultivars 'Houghton' and 'Mountain' originate from crosses between *R. hirtellum*, a wild, disease-resistant gooseberry species native to Canada, and the European species *R. uva-crispa* (Hjalmarsson & Wallace 2007). Information about C. G. Dahls work was unfortunately not well preserved and parental lines got lost along the way. It is however known that the very popular cultivars 'Scania' and 'Dr. Törnmarck' were a result of C. G. Dahls extensive breeding work. During the 1940s, cultivar trials were conducted at 10 different locations in Sweden, focusing on hardiness and yield (Hjalmarsson & Wallace 2007). The very popular cultivar 'Jacob' was marketed in 1979 and is a cross between the hybrid 'Whitesmith' x *R. oxyacanthoides* and the cultivar *R. uva-crispa* 'Achilles'. 'Jacob' is known to be both hardy and resistant to powdery mildew.

1.2. Conservation of pomological heritage

In the year 2000, the *National program for diversity of cultivated plants (Pom)* was established, aiming to recover old, Swedish fruit and berry cultivars that have been important in breeding and commercial production (Hjalmarsson, 2020). Important cultivars that are a part of Sweden's pomological heritage are called *mandate cultivars*. As a result of Pom's efforts, the Swedish National Gene Bank was established in 2016. A collection of mandate cultivars of *Ribes* was created with the aim to prevent loss of genetic variability and to conserve the valuable germplasm. Moreover, these old Swedish cultivars are also valuable to maintain due to their high cultural values.

The Swedish National Gene Bank keeps the mandate cultivars for long-term conservation in their central collection at Alnarp, along with inventory findings waiting to be analyzed. A virus-free backup of each mandate cultivar is kept at the Swedish Elite Plant Station (EPS) in Kristianstad. Furthermore, 32 gooseberry cultivars are kept in the field collection at Balsgård outside Kristianstad, which is a part of the Swedish University of Agricultural Sciences (SLU).

A cultivar can only be assigned as a mandate cultivar if it originates from Sweden, either as a chance seedling or as a result of conscious breeding, or if it has been cultivated in the

country for a long time (Hjalmarsson and Wallace, 2004). Historical or cultural values are important parts when deciding which cultivars should be denoted as Swedish mandate cultivars.

The selection of accessions to be included in a collection can also be based on the output of phenotypic and molecular characterization. During the phenotypic characterization, different plant traits are evaluated and documented. It includes both morphological characterstics and agronomic traits such as hardiness, fruit production, fruit quality, and disease resistance (Karhu et al. 2007; Karhu et al. 2012). Environmentally induced variation can, however, alter the phenotypic characters, which sometimes makes identification difficult (Garkava-Gustavsson et al. 2008). Molecular characterization can uncover duplicates among the accessions, secure variety purity, and trueness-to-type; it can give an indication of which accessions would contribute to a maximum amount of genetic variation in a core collection and estimate the genetic relationships among accessions. Molecular marker technology, such as SSR markers, can be used for molecular characterizations of germplasm collections (Karhu et al. 2007; Garkava-Gustavsson et al. 2008; Testolin & Cipriani 2010; Sehic et al. 2012; Karhu et al. 2012; Yu et al. 2012; Mengistu et al. 2016).

The current list of mandate gooseberry cultivars comprises 35 cultivars and has been determined in advance, based on literary studies (Hjalmarsson 2020). The mandate cultivars that were found earlier have been phenotypically identified and added to the collection in Alnarp. However, several mandate cultivars are yet to be found. Missing cultivars could be found through national inventory of plant resources, but some are harder to trace than others. Names can easily be forgotten, especially if a cultivar only has been cultivated in a restricted, geographical area. It is more likely that cultivars with good resistance to diseases remain in cultivation, and names of such cultivars are therefore more frequently mentioned in literature.

Currently, 16 accessions from the mandate list are missing in the collection and missing mandate cultivars could be found through national inventory of plant resources.

The National gene bank in Sweden has mainly collected gooseberry accessions from the older collections in Alnarp and Balsgård. Many accessions have also been collected through Pom's inventory project. These "inventory findings" come from private gardens and are unidentified cultivars. The collection of inventory findings hopefully contains missing mandate cultivars or new interesting accessions that can be added to both the

collection and the mandate list. The inventory findings, therefore, need to be identified and validated to secure trueness-to type.

Apart from detection of possible synonyms, molecular characterization can assign an un-labeled accession a cultivar epithet or give information about which accessions that might be mislabeled. It is also possible that cultivars mutate, while keeping the same name, or that they are seedlings displaying a different genotype compared to the mother plant. Many cultivars that are imported from other countries are also given a new name in the native language, which might complicate the identification (Hjalmarsson 2020; Karhu et al. 2007).

By conservation of mandate cultivars of berries and fruit trees, the loss of genetic resources can be prevented, and will lead to an increase in biodiversity. Rich biodiversity strengthens the ability of species to resist diseases and pests, changes in climate, and other factors affecting the survival of a population (Sunderland 2011). A high genetic variability will increase the fitness of a species. The collection of mandate cultivars can become a source of favorable alleles that can be utilized in breeding programs and traits relevant for local adaptation can be maintained. The mandate cultivars are also an important part of our history and cultivars that are not preserved will vanish.

1.3. Molecular markers

Molecular markers have revolutionized the ability to characterize germplasm and identify traits and their inheritance. Genetic markers can be divided into three classes; markers based on morphological and agronomic traits (visual), markers based on gene products (biochemical), or markers requiring a DNA assay (molecular). Molecular markers may, or may not, have a biological effect (Khan 2015).

There are several molecular marker methods such as amplified fragment length polymorphism (AFPL), inter-simple sequence repeats (ISSRs), single nucleotide polymorphisms (SNPs), or simple sequence repeats (SSRs) (Grover & Sharma 2016). SSRs, also known as microsatellites, are short, nucleotide motifs of 1 - 6 bases, widely distributed in all genomes (Grover & Sharma 2016; Lanham & Brennan 1998). They can be amplified by PCR, using locus-specific oligonucleotide primers. The PCR product can then be visualized by electrophoresis on a high-resolution agarose gel (Grover & Sharma 2016; Litt & Luty 1989) or by capillary electrophoresis (Rubtsova et al. 2016). Different

marker methods have different features, and thus the application determines which marker system should be used. In 1999, Lanham & Brennan analyzed the genetic diversity among gooseberries using molecular markers such as ISSRs and AFLPs. However, these marker systems produce dominant markers and thus cannot differentiate between homo- and heterozygous loci, an important aspect when analyzing genetic variability. SSR markers, on the other hand, are reliable and easy to use, codominant and effective in detecting polymorphism (Antonius et al. 2011; Grover & Sharma 2016). The high information content of SSRs have made them popular when analyzing genetic diversity, identifying synonyms, and possible genetic relatedness (Cavanna et al. 2009). Especially in diploid species, such as *Ribes*. In 2002, Brennan et al. were able to identify 11 polymorphic SSR markers in the *Ribes* species, which have proved very useful. In 2017 Mezhnina & Urbanovich proposed another set of SSR-markers that could be used to identify cultivars of gooseberry and black currant. SSR-markers have been used previously to study the national collection of fruits and berries, especially in apple, pear and black currant (Antonius et al. 2011; Garkava-Gustavsson et al. 2008; Garkava-Gustavsson et al. 2013; Sehic et al. 2012).

Black currant (*Ribes nigrum* L.) is the most commercially important species in the *Ribes* genus and thus is more studied than related species such as gooseberries (Brennan et al. 2002). One of the most damaging and serious pests of black currant is the gall mite (*Cecidophyopsis ribis).* This pest can be controlled by the introgression of resistance genes from other *Ribes* species. In *R. uva-crispa,* the resistance towards *C. ribis* is controlled by a single gene, *Ce*. Breeding programs for black currant have therefore attempted to develop molecular markers linked to the *Ce* gene in gooseberry (Brennan et al. 2009; Mezhnina & Urbanovich 2017).

2. Aim

This project aims to evaluate the status of the Swedish national collection of gooseberry heritage cultivars and analyze inventory findings from Pom by molecular characterization, using SSR markers. Duplicates and possible mislabelings in the collection and among the inventory findings kept at Alnarp, Balsgård and Pom will be therefore revealed. The results from this study will be useful when searching for old, missing mandate cultivars and will contribute to a well-cured Swedish collection of national heritage cultivars of gooseberry, *Ribes uva-crispa.*

3. Materials and methods

3.1. Plant material

Branches from both verified, mandate gooseberry cultivars, un-named and un-identified inventory findings were collected from collections in Alnarp, Balsgård, the Elite Plant Station (EPS), local clonal archives (Sofiero slottsträdgård, Bergianska trädgården) and private gardens. The studied accessions in this project are listed in Appendix 1. The total number of analyzed plants was 105.

3.2. DNA extraction

Branches were collected in February and March and put in glass bottles filled with water and a liquid fertilizer Chrysal White at a ratio of 200:1 with a plastic cover to initiate bud bursting. Leaf material was collected from 105 gooseberry accessions. Most of the leaf material was collected in tubes and lyophilized, but the first DNA extractions were carried out using juvenile, fresh leaf material since it took several days for the lyophilizer to freeze-dry the samples. The lyophilized material was stored at -80°C.

Genomic DNA from 80 – 100 mg fresh leaf material or 20 mg lyophilized tissue was isolated with DNeasy Plant Mini Kit (Quiagen) according to the manufacturer's instructions.

DNA purity and concentration were measured on a NanoDrop (ThermoFisher Scientific Inc.). The samples were then separated through gel electrophoresis using 1.5 % agarose gel and TAE buffer (Tris-Acetate-EDTA), stained with ethidium bromide, to confirm that no degradation of the DNA occurred during extraction. The gel was run for 40 minutes at 100 V. The DNA was then visualized under UV light.

DNA was diluted with Milli-Q water to the concentration of 20 - 40 ng/µl, with a total amount of 60µl, for immediate analysis. Residual DNA was stored at -80°C for later use.

3.3. SSR analysis

The markers were chosen based on previous publications where they were reported to detect polymorphism in currants and gooseberries (Droz et al., 2019; Gunnarsson 2009; Mezhnina & Urbanovich 2017; Palmieri et al. 2013; Pikunova et al. 2015) and through personal communication with Dr. E. Droz (Agroscope, Switzerland). In this study, 15 primer pairs were chosen and analyzed for their ability to amplify different SSR loci (Table 1). All the forward primers had a specific fluorescent tag (6FAM, PET, HEX, NED).

Table 1. *Primer pairs used to amplify the SSR loci.*

SSR locus	Size range	Forward sequence	Reverse sequence	Fluorescent dye	Annealing temperature
e1-O01	131-160	CCTTTCCAGAGAAAACTCAAACA	AAGTATGGGAACAACGGCAG	6FAM	60
e1-O21	290-318	TCTCTCCAACTGAGAAGGAAAA	GATTTGTTCTTGTGCAGCGA	6FAM	54
e4-D03	160-238	CCCAAAAGCAAATTTAGGGT	GTGAGGCATGGAACCACTTT	PET	58
g1-A01	205-254	CGAAGGTTGAATCGGTGAGT	CGTAGCCACGTAGTTCCACA	HEX	60
g1-K04	277-298	TGTTCCCTGTTTCCTTCAAAA	GGACGTGGACGATGAGAGTT	6FAM	58
g1-M07	199-232	TCCGTTACTGGAGTGGTGT	CCATGGTTTTCCGATTTGTT	PET	48
g2-B20	139-191	CTCCATCAAATCCCTCGTTT	TCTTGCTTCCCAAACAGTATCA	PET	54
g2-G12	164-194	GTGACCCACCTAAACCGTCC	GGAGTGGAGGGTTGGAAAAT	6FAM	62
g2-H21	238-272	TGCCCTTTTTGGTCATTTTC	CAATCGTCGATGAAGGTCTG	HEX	54
g2-J08	140-181	CGCCGAGCTCTAATCACTGT	ATAGCCCATGCCCATATTCA	NED	60
g2-L17	114-166	TTTGGAAAACCTCCCCTTT	GAGCTGTTGCTGTTGCCATA	HEX	56
gr2-J05	160-186	CAAAACTGATTAGGGATCA	TTTGAAGAAGAGATGGCGAAA	HEX	54
MTT-5	144-244	GCGATTCCATTACGACACTTTGCA	ATAGGCAAGCATCACCTCACC	NED	52
RJL-7	200-238	TCCCGTTACTGGAGTGGTGT	CCATGGTTTTCCGATTTGTT	NED	50
RJL-11	215+	CGAAGGTTGAATCGGTGAGT	TTGTGAGCCGTAACCACGTA	PET	56

A DreamTaq PCR Mastermix (ThermoFisher Scientific Inc.) was prepared according to the manufacturer's instructions. The mastermix and the DNA were loaded onto a PCR plate and DNA amplification was performed in a PCR machine (VWR Unocycler, VWR International or Eppendorf Mastercycler ep gradient S., Eppendorf) with a specific PCR program for each primer pair (Table 1). The first 5 samples of each plate were amplified twice. Those samples where the amplification failed were run again in the second plate, together with the remaining DNA samples and 5 reference samples from the first run. Two PCR runs for each primer pair were needed to include all the DNA samples. The results from the repeated samples were compared to assure consistency of the results.

The amplification of the PCR products was verified on a 1.5% agarose gel together with O'RangeRuler 50 bp DNA ladder (ThermoFisher Scientific Inc.).

After amplification, the PCR products were diluted with Milli-Q water and separated by capillary electrophoresis (3500 Series Genetic Analyzer, ThermoFisher Scientific Inc.). A test run with 8 samples was performed prior to the final analyses to adjust the concentration of the PCR products to appropriate levels.

3.4. Statistical analysis/Data analysis

The data from the capillary electrophoresis were processed with GeneMarker 3.0.1 software (SoftGenetics LLC). The markers were first called automatically and thereafter curated manually, assuming that all analyzed accessions were diploid. Lynch & Ritland's estimator of pair-wise relatedness was calculated in Excel using the genetic analysis software GenAlEx 6 (Peakall & Smouse 2006). The marker calls of these samples were double-checked in GeneMarker and errors were corrected. Duplicates were identified by calculating the genetic distance and were then excluded from further analysis. Based on the output data, the samples were classified into four different categories based on the classification system proposed by de Andrés et al (2007);

Class 1 - Single samples with a unique SSR-profile.
Class 2 - Samples with similar names sharing the same SSR-profile.
Class 3 - Samples with different names sharing the same SSR-profile (known synonyms or sports).
Class 4 - Samples with the same/similar names having different profiles (4a) or different samples sharing the same SSR-profile (4b) (putative cases of mislabeling).

The marker status was evaluated and allele frequencies for each locus were calculated and presented in a bar chart.

The genetic diversity was estimated by calculating allele frequencies for each locus (A), expected heterozygosity (H_e), and observed heterozygosity (H_o). Expected heterozygosity is the probability that a randomly selected individual is heterozygous at a given locus under Hardy-Weinberg equilibrium (Nei 1973; Alexandre & DeGiorgio 2017). This was calculated in GenAlex, using the following equation:

$$H_e = 1 - \sum (p_i)^2$$

Observed heterozygosity (H_o) is calculated by dividing the number of heterozygous individuals by the total number of individuals.

Fixation index (*F*) (providing a value between -1 and +1) was also calculated for each locus and averaged over all loci. Positive values indicate inbreeding or undetected null alleles and negative values indicate an excess of heterozygosity. The fixation index is expected to lie close to zero if random mating occurs in the population (Wright, 1965).

After duplicates were excluded, a genetic distance matrix was used as input for a principal coordinate analysis (PCoA). A PCoA plot was created and used to evaluate a potential genetic structure and discover similarities or dissimilarities among the analyzed genotypes.

4. Results

4.1. SSR-fingerprints

In some cases, it was not possible to identify any peaks in the electropherogram. The following 10 accessions and inventory findings had missing values at 1 - 6 loci; 'Flottskär gröna', Flottskär röda', 'Houghton', 'Lilla busken – Släta bär', 'POM okänd 2', 'Sandgren', 'Scania', 'Sofiero 4', 'Worchesterberry' and 'Ångermanlands röda'. They were therefore excluded from PCoA, genetic diversity analyses, and classification with the de Andrés classification system.

The SSR-profiles of all 105 studied accessions are presented in Appendix 1.

4.2. Identification of cultivars and duplicates

Following the de Andrés classification system, 60 accessions were assigned class 1 (single samples with a unique SSR-profile). Three pairs (6 accessions) had similar names and similar profiles and were therefore assigned class 2. No accession was assigned class 3 (known synonyms/sports). Three pairs (6 accessions) had similar names, but different profiles and were therefore assigned class 4a. Finally, 24 cultivars were attributed as mislabeled or identical to other accessions (class 4b). The classification of all studied accessions and inventory findings is shown in Table 2. All of the mandate cultivars included in the collection at Alnarp had individual SSR-profiles, or shared SSR-profile with an un-labelled inventory find.

The accessions where 1 - 6 loci failed to amplify were excluded from the statistical analyses and classification, except for 'Worchesterberry', which was not excluded from the classification. In this case, the amount of the data was sufficient to be able to make a fair comparison with the accession "Worchester."

4.2.1. Class 1 - Single samples with unique SSR-profiles

Of 96 compared accessions and inventory findings, 60 had unique SSR-profiles.

The following inventory findings had unique SSR-profiles: ‘POM 1’, ‘POM 2’, ‘POM 5’, ‘POM 7’, ‘POM 9’ ‘POM 10’, ‘POM 11’, ‘POM 13’, ‘POM 14’, ‘POM 15’, ‘POM 17’, ‘POM 19’, ‘POM 22’, ‘POM 23’, ‘POM 24’, ‘POM 25’, ‘POM 27’, ‘POM 28’, ‘POM 30’, ‘POM 31’, ‘POM 33’, ‘POM 37’, ‘POM 39’, ‘Björn K.’, ‘Frick’, ‘Hönings Früheste’, ‘Invicta’, ‘Kalvstorp’, ‘Catharina Ohlenberg’, ‘Kerstin M.’, ‘Kopparbro’, ‘Kozachok’, ‘Landströms gröna - Avvikande’, ‘Linda’, ‘Linköping’, ‘Lottas’, ‘Mette P.’, ‘Perle von Münchenberg’, ‘POM gården 1’, ‘POM gården 2’, ‘POM okänd 1’, ‘Resistenta’, ‘Robustenta’, ‘Ronja’, ‘Roskil’, ‘Smiling beauty’, ‘Snickarmoa’, ‘Sofiero 3’, ‘Sofiero 5’, ‘Spine free’, ‘Stora busken - Päronträd’, ‘Söderbrunn’, ‘Trasher white’, ‘Villa Virginia’, ‘Åsgatan’, ‘Österhammarsbruk’ and ‘Övertjärn’.

The four mandate cultivars, ‘Aaron’, ‘Dr. Törnmark’, ‘Jacob’ and ‘London’ also displayed unique SSR-profiles.

4.2.2. Class 2 - Samples with similar names sharing the same SSR-profile

Class 2 cultivars are described as several cultivars with the same name, from different locations that share the same SSR-profile. The mandate cultivars ‘Bloodhound’ and ‘Landströms gröna’ were collected from both Balsgård and Alnarp and the mandate cultivar ‘Mountain’ was collected from EPS and Alnarp. Both accessions of each cultivar had identical SSR-profiles.

4.2.3. Class 3 – Samples with different names sharing the same SSR-profile

Known synonyms or sports are divided into class 3. Since no known synonym/sport was found in this project, no cultivar was assigned class 3.

4.2.4. Class 4 – Mislabelings, duplicates, and deviating samples

Class 4 is divided into two subgroups. Class 4a refers to accessions with the same or similar name, having different profiles. ‘Worchester’ from POM and ‘Worchesterberry’ from Balsgård have similar names but did not share the same SSR-profile. So was also the case

with the mandate cultivar ‘Achilles’ (Balsgård) and ‘Akilles’ (Alnarp). The cultivar ‘Alicant’ was collected from both Alnarp and Balsgård, but these accessions had different SSR-profiles.

Class 4b refers to different accessions sharing the same SSR-profile. ‘POM 3’, ‘POM 20’, ‘POM 34’, ‘Hinnonmäen keltainen’ (mandate cultivar), ‘Olavi’, ‘Smedjebacken’ had similar SSR-profile, but ‘POM 3’ differed by being homozygous at one locus.

‘POM 4’, ‘POM 38’ had almost identical SSR-profiles, but ‘POM 38’ was heterozygous at one locus where ‘POM 4’ was homozygous.

‘POM 8’, ‘POM 16’, ‘POM 35’, ‘Industry’ and ‘Sofiero 7’ had identical SSR-profiles, but ‘POM 16’ differed from the rest by one base pair in one allele at one locus

The following pairs are possible synonyms, displaying identical SSR-profiles; ‘POM 6’ and ‘POM 9’, ‘POM 12’ and ‘POM 29’, ‘POM 32’ and ‘POM 36’, ‘Sofiero 6’ and ‘Stora busken – Landet’. The three accessions ‘POM 26’, ‘POM 40’ and ‘POM 41’ do also have identical SSR-profiles.

Table 2 – *Studied accessions and sampling locality, classified into four different categories based on the classification system used by de Andrés et al (2007). Class 1 - Single accessions with a unique SSR-profile. Class 2 - Accessions with similar names sharing the same SSR-profile. Class 3 - Accessions with different names sharing the same SSR-profile (known synonyms or sports). Class 4 - Accessions with the same or similar name having different profiles (4a), or accessions with different names with SSR-profile identical to at least one other accession (4b). The asterisk (*) denotes accessions that differ in only one allele when compared to SSR-profiles of at least one other accession. The cultivars denoted with "-", were excluded from the comparison.*

Analysed accession		Location	Class	Analysed accession		Location	Class
1	POM 1	POM	1	54	Hönings Früheste	EPS	1
2	POM 2	POM	1	55	Industry	EPS	4b
3	POM 3	POM	4b*	56	Invicta	EPS	1
4	POM 4	POM	4b	57	Jacob	Alnarp	1
5	POM 5	POM	1	58	Kalvstorp	External	1
6	POM 6	POM	4b	59	Catharina Ohlenburg	External	1
7	POM 7	POM	1	60	Kerstin M.	Alnarp	1
8	POM 8	POM	4b	61	Kopparbro	External	1
9	POM 9	POM	4b	62	Kozachok	Balsgård	1
10	POM 10	POM	1	63	Landströms gröna	Balsgård	2
11	POM 11	POM	1	64	Landströms gröna	Alnarp	2
12	POM 12	POM	4b	65	Landströms gröna (Avvikande)	Alnarp	1
13	POM 13	POM	1	66	Lilla busken - Släta bär	External	-
14	POM 14	POM	1	67	Linda	EPS	1
15	POM 15	POM	1	68	Linköping	Alnarp	1
16	POM 16	POM	4b*	69	London	Balsg.	1
17	POM 17	POM	1	70	Lottas	POM	1
18	POM 19	POM	1	71	Mette P.	POM	1
19	POM 20	POM	4b	72	Mountain (Alnarp)	Alnarp	2
20	POM 22	POM	1	73	Mountain (EPS)	Balsgård	2
21	POM 23	POM	1	74	Olavi	External	4b
22	POM 24	POM	1	75	Perle von Münchenberg	Balsgård	1
23	POM 25	POM	1	76	POM gården 1	POM	1
24	POM 26	POM	4b	77	POM gården 2	POM	1
25	POM 27	POM	1	78	POM okänd 1	POM	1
26	POM 28	POM	1	79	POM okänd 2	POM	-
27	POM 29	POM	4b	80	Resistenta	Balsgård	1
28	POM 30	POM	1	81	Robustenta	Balsgård	1
29	POM 31	POM	1	82	Ronja	Balsgård	1
30	POM 32	POM	4b	83	Roskil	Balsgård	1
31	POM 33	POM	1	84	Sandgren	POM	-
32	POM 34	POM	4b	85	Scania	EPS	-
33	POM 35	POM	4b	86	Smedjebacken	POM	4b
34	POM 36	POM	4b	87	Smiling beauty	Balsgård	1
35	POM 37	POM	1	88	Snickarmoa	Alnarp	1
36	POM 38	POM	4b*	89	Sofiero 3	POM	1
37	POM 39	POM	1	90	Sofiero 4	POM	-
38	POM 40	POM	4b	91	Sofiero 5	POM	1
39	POM 41	POM	4b	92	Sofiero 6	POM	4b
40	Achilles	Balsgård	4a	93	Sofiero 7	POM	4b
41	Akilles	Alnarp	4a	94	Spine free	Balsgård	1
42	Alicant	Balsgård	4a	95	Stor buske - Päronträd	External	1
43	Alicant	Alnarp	4a	96	Stora busken - Landet	External	4b
44	Aaron	Alnarp	1	97	Söderbrunn	POM	1
45	Björn K.	Alnarp	1	98	Trasher white	Balsgård	1
46	Bloodhound	Balsgård	2	99	Villa Virginia	Alnarp	1
47	Bloodhound	Alnarp	2	100	Worchester	POM	4a
48	Dr. Törnmarck	Balsgård	1	101	Worchesterberry	Balsgård	4a
49	Flottskär gröna	External	-	102	Ångermanlands röda	EPS	-
50	Flottskär röda	External	-	103	Åsgatan	External	1
51	Frick	Alnarp	1	104	Österhammarsbruk	External	1
52	Hinnonmäen keltainen	Balsgård	4b	105	Övertjärn	External	1
53	Houghton	Alnarp	-				

4.3. Marker status and allele frequencies

The number of alleles (Na) and allele frequencies varied among different loci (Table 4 and Appendix 2).

The locus MTT-5 had a higher number of alleles (24) with allele frequencies ranging from 0.006 to 0.268, compared to other loci. Markers such as g1-K04, RLJ-11, RLJ-7, g1-A01, and g1-M07 did also display high variability. Markers g2-B20, g2-L17, e1-021, g2-J08, gr2-J05, g2-H21, and e1-O01 had 2 - 3 alleles with high frequency.

For the three markers g2-B20, e1-021, and g2-G12, the population was almost fixated for 1 - 2 alleles.

The SSR markers e4-D03, g1-A01, g1-K04, g1-M07, g2-B20, g2-G12, g2-L17, gr2-J05 and MTT-5 failed to amplify some alleles and thus accessions with missing alleles had to be excluded from statistical analysis.

All repeated samples, which purpose was to assure consistency of the results, had the same allele size and composition as the original sample. Several cases of discrepancy were, however found in the allele size and allele composition when some of the data from this study was compared to the data from a previous study by Gunnarsson (2009) (Table 3). The differences in size of the amplified fragments were 1 - 25 base pairs. The cultivars ‘Achilles’ (MTT-5), and ‘Hönings früheste’ (RLJ-7) were attributed as triploid in Gunnarsson’s study and were excluded from comparison. The cultivars ‘Invicta’ (MTT-5) and ‘Hönings Früheste’ (RLJ-11) were homozygous in this study, but heterozygous in Gunnarsson's study, while ‘Worchesterberry’ (MTT-5 and RLJ-7) was homozygous in Gunnarsson's study and heterozygous in this study.

Table 3. *Data comparison between this study and a previous study by Gunnarsson (2009), displaying the allele composition and allele size in loci MTT-5, RLJ-7, and RLJ-11. Missing values are denoted mv. "b.p. dif." denote the differences in allele size between this and the previous study.*

Accession	MTT-5 This study	Gunnarsson 2009	*b.p. dif.*	RLJ-7 This study	Gunnarsson 2009	*b.p. dif.*	RLJ-11 This study	Gunnarsson 2009	*b.p. dif.*
Achilles	201/238	202/204/243		205/205	210/210	-5	250/261	252/262	-2/-1
Alicant	212/238	216/243	-4/-5	205/205	*mv*		244/261	245/262	-1
Bloodhound	201/212	204/216	-3/-4	205/205	210/210	-5	239/261	241/262	-3/-1
Dr. Törnmark	180/217	183/221	-3/-4	226/230	232/236	-6	235/261	235/262	0/-1
Hinnonmäen keltainen	180/236	183/241	-3/-5	204/206	208/210	-4	261/261	262/262	-1
Hönings früheste	212/219	*mv*		205/230	198/210/236		250/250	241/252	+9/-2
Invicta	212/212	200/216	+12/-4	205/205	210/210	-5	239/250	241/252	-2
Jacob	201/240	*mv*		206/206	210/210	-4	250/261	252/262	-2/-1
Kozachok	221/225	225/229	-4/-4	206/206	210/210	-4	235/250	235/252	0/-2
Mountain	211/211	*mv*		206/212	210/216	-4	231/261	232/262	-1
Perle von Münchenberg	191/238	194/243	-3/-5	206/228	*mv*		247/250	249/252	-2
Resistenta	191/238	194/243	-3/-5	206/206	210/210	-4	243/250	241/249	+2/+1
Robustenta	238/240	243/245	-5/-5	206/206	210/210	-4	243/261	245/262	-2/-1
Smiling beauty	221/236	225/241	-4/-5	205/205	210/210	-5	250/261	252/262	-2/-1
Spine free	200/200	202/202	-2	212/214	216/218	-4	237/261	239/262	-2/-1
Trasher white	180/212	*mv*		205/205	208/208	-3	239/261	241/262	-2/-1
Worchesterberry	198/225	200/200	-2/+25	202/205	208/208	-6/-3	237/250	239/252	-2/-2

4.4. Genetic diversity

Observed heterozygosity (Table 4) is slightly higher than expected heterozygosity for the 9 loci e1-O01, g1-K04, g1-M07, g2-B20, g2-G12, g2-H21, g2-L17, MTT-5, and RLJ-11. This is also reflected in the mean value of H_o and H_e.

The fixation index is close to zero among 10 loci, while 5 loci deviate somewhat. The marker RLJ-7 has a fixation index of 0.37, and e4-D03 0.26. The marker e1-O01 has a value of -0.24, g1-K04 -0.33, and g2-L17 -0.19. Averaged over all loci, the H_o and H_e are very similar, and the fixation index is very close to zero.

Table 4. *Observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index for the 15 different markers. Na stands for "Number of alleles", which displays the number of unique alleles detected in each SSR locus.*

Locus	Na	Ho	He	F
e1-O01	8	0.73	0.59	-0.24
e1-021	8	0.13	0.14	0.11
e4-D03	10	0.38	0.51	0.26
g1-A01	13	0.73	0.77	0.05
g1-K04	9	0.93	0.70	-0.33
g1-M07	13	0.79	0.73	-0.07
g2-B20	5	0.42	0.39	-0.10
g2-G12	2	0.17	0.16	-0.09
g2-H21	10	0.56	0.54	-0.04
g2-J08	9	0.49	0.51	0.04
g2-L17	15	0.76	0.64	-0.19
gr2-J05	7	0.55	0.60	0.08
MTT-5	24	0.88	0.87	-0.01
RLJ-7	14	0.45	0.72	0.37
RLJ-11	14	0.83	0.79	-0.05
Mean		0.59	0.58	-0.01

4.5. Proximities between accessions (PCoA)

The proximities between the accessions are visualized in a two-dimensional principal coordinate analysis ordination plot (Figure 1), where the percentage of variation is explained by the principal coordinates. PCoA axis 1 accounts for 20 % of the variation of the data, axis 2 accounts for 13 % of the variation, and axis 3 (not presented in the plot) accounts for 10 % of the variation. Individuals positioned closer to each other have lower dissimilarity values than objects placed further apart. In this case, accessions far apart display more genetic divergence than accessions closer to each other. Duplicates and accessions with missing values were excluded from the PCoA.

The results indicate some structure within the analyzed plant material. The PCoA analysis revealed the presence of a relatively more homogenous group (accentuated with a red circle in Figure 1) compared to the rest of the analyzed samples. This group is formed by the following accessions: ; 'POM 2', 'POM 9', 'POM 10', 'POM 14', 'POM 15', 'POM 16', 'POM 17', 'POM 19', 'POM 23', 'POM 24', 'POM 28', 'POM 29', 'POM 31', 'POM 38', 'POM 40', 'Alicant', 'Björn K.', 'Flottskär gröna', 'Hönings früheste', 'Invicta', 'Landströms gröna - Avvikande', 'Linda', 'Linköping', 'Lottas', 'Mette P.', 'POM gården 1', 'POM gården 2', 'POM okänd 2', 'Ronja', 'Sandgren', 'Smiling beauty', 'Snickarmoa',

‘Sofiero 3’, ‘Sofiero 4’, ‘Sofiero 5’, ‘Stor buske - Päronträd’, ‘Stora busken - Landet’, ‘Söderbrunn’, ‘Trasher White’ and ‘Villa Virginia’. There are also 7 mandate cultivars within this homogenous group: ‘Akilles’, ‘Aaron’, ‘Bloodhound’, ‘Jacob’, ‘Catharina Ohlenburg’, ‘Landströms gröna’ and ‘London’.

There are also some accessions positioned to the far right of the plot (accentuated with a green circle in Figure 1) displaying high dissimilarities (high genetic divergence) compared to the accessions within the cluster. These accessions and inventory findings are ‘POM 5’, ‘POM 20’, ‘POM 25’, ‘POM 39’, ‘Dr. Törnmark’, ‘Kerstin M.’, ‘Kozachok’, ‘Mountain’, ‘Robustenta’, ‘Scania’, ‘Spine free’ and ‘Åsgatan’. Among these, ‘Dr. Törnmark’, ‘Mountain’ and ‘Scania’ are mandate cultivars.

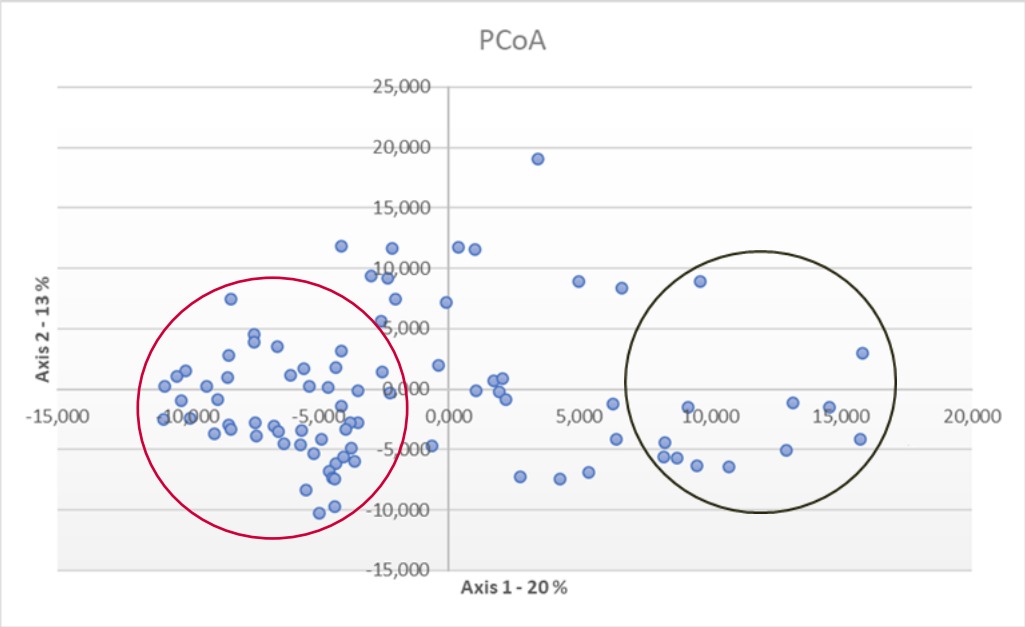

Figure 1. *Principal coordinate analysis (PCoA) ordination computed from a distance matrix. Each blue dot represents one of the 86 accessions with a unique SSR profile. In total, 9 duplicates and 10 accessions with missing values were excluded from the principal coordinate analysis and are not displayed in this plot.*

5. Discussion

5.1. Methodology

The SSR primer pairs used in this study generally amplified clearly and easily scored alleles. However, ten cultivars in this project had missing data for alleles in one or more loci, and therefore they had to be excluded from the statistical analyses. PCR could fail due to technical reasons such as poor DNA quality or low concentration, however, in these cases, there was no remarkable difference in DNA concentrations or quality compared to the rest of the analyzed cultivars. The excluded cultivars will be analyzed again within a successive project.

Missing data or inaccurate data where the observed genotype does not correspond to the true genotype of an individual can be a result of genotyping errors (Bonin et al. 2004). Genotyping errors are important to take into account to avoid erroneous conclusions. Understanding the consequences of possible genotyping errors helps to prevent and limit their occurrence. The four different factors causing genotyping errors are; errors linked to the DNA sequence (such as *null alleles*), errors caused by low DNA quality or low DNA concentration, biochemical artifacts, and human errors (Pompanon et al. 2005).

According to Dakin & Avise (2004), null alleles are alleles at an SSR locus that do not amplify to detected levels via PCR (despite repeated attempts). They are problematic since they can create false homozygotes. There are two potential biological reasons for null alleles. One reason could be poor primer annealing because of nucleotide sequence divergences such as indels or point mutations. Null alleles (or in this case, ‘partial nulls’) can also be generated due to differential amplification if the alleles have different sizes. Shorter fragments tend to amplify more efficiently than larger fragments during PCR. This may result in detection of only the smallest allele (Wattier et al. 1998). This problem can be avoided by loading more of the sample.

The possible presence of null alleles should be considered when interpreting the data. Several cultivars that are called as homozygotes might be heterozygotes for null-allele and can therefore be similar to other cultivars with similar, but not identical, SSR-profiles.

The phenomenon of an allele having an insufficient amplification is called allelic dropout. Allelic dropout affects allele frequency and decreases the observed heterozygosity, thus increasing the level of inbreeding at the specific loci. The observed molecular genotype will therefore not correspond with the actual genotype. Preferential amplification can occur if the *Taq*-polymerase is limiting, the DNA is degraded or if there is a mismatch between the primer and the allele template (Walsh et al. 1992). If the amplification of the smaller allele is preferential in a heterozygous genotype, the peak of the larger allele might be smaller in an electropherogram, or both may fail to amplify. Large-allelic dropout is solely linked to allele size, but allelic dropout can also occur in low-quality samples when the DNA concentration is very low (de Woody et al. 2006; Hunt 2009).

Biochemical artifacts are another cause of genotyping error. A biochemical artifact is a result that does not correspond to the true genotype and arises from the technical process. Taq-polymerase used when preparing the DNA for PCR, can sometimes carry out a non-templated nucleotide addition to the 3´-end of a synthesized strand. This non-templated nucleotide (usually an adenine) can result in an artefactual band on a gel, or a peak in a genotyping assay (Pompanon et al. 2005). *Taq*-polymerase can also cause replication slippage and lead to SSR instability, which generates "stutter bands" (de Woody et al. 2006; Castillo-Lizardo et al. 2014). This aggravates the interpretation of the electrophoretic output.

Human errors are another cause of genotyping errors. Extensive handling of samples and data can lead to a mix-up of samples/data, contamination, or pipetting errors. Manual scoring of data is also a possible source of errors. In this study, the alleles were manually called based on evaluation of the peaks in an electropherogram and the expertise of the researcher influences the number of scoring errors. The difficulty of allele-calling can, however, be reduced by having high-quality data (Pompanon et al. 2005).

One way to improve data quality is through the process of binning. Binning is referred to as a pre-processing method for data averaging and is used to reduce the effects of minor observation inaccuracies (Amos et al. 2006). When it comes to allele binning in SSR markers, alleles mostly need to be assigned different sizes and put into the corresponding allele "bin" (Idury & Cardon 1997). The process of binning can be a source of errors and poor binning can have an impact on the identification of individuals and their parental lines.

Different fragments of the same allele can differ in size with 1 base pair, thus reduce the accuracy of binning. The allele length can also be altered if a mutation occurs in the flanking sequence (Amos et al. 2006). This affects the allele frequencies estimates as well as peak alignment during marker calling. The mobility of a DNA fragment depends on both sequence and length. GC-content (genomic DNA base composition), mostly associated with the stability of the DNA (Benjamini & Speed 2012), can affect the mobility and a fragment with high GC content migrates differently from a fragment with low GC content.

Several of the accessions in this project had SSR-profiles with very high similarities, but differed from one another by 1 - 2 base pairs, or by being homo- or heterozygous at one locus. This difference can be caused by any of the previously mentioned genotyping errors. A minor mismatch in one or two SSR-locus can be accepted to count two genotypes as identical (Urrestarazu et al. 2016). 'POM 3', for example, differs from the other accessions with identical SSR-profiles by being homozygous at one locus. 'POM 3' may have failed to be identified as a heterozygote at this specific locus due to the presence of null alleles or if allelic dropout may have occurred.

Many of the inventory findings have been collected from private gardens, where the owner does not have the name of the cultivar. Private gardens are a valuable source of genetic resources, but one must keep in mind that since the entry of powdery mildew at the beginning of the 20th century, many old cultivars have disappeared.

A limitation of this project is that the inventory findings were only analyzed with molecular markers. Phenotypical analyses, together with the genotypical analyses, would have contributed with much more information on whether some accessions are indeed duplicates or sports. Therefore, molecular, and phenotypical characterization should be combined.

5.2. Identification of cultivars and duplicates

The cultivars 'Worchester' (POM) and 'Worchesterberry' (Balsgård), 'Achilles' (Balsgård) and 'Akilles' (Alnarp) and 'Alicant' (Alnarp and Balsgård) had similar or identical names but different SSR-profiles (class 4a). These pairs were expected to share identical SSR-profiles. When the SSR-profiles of each pair were compared individually, it was discovered that 'Achilles', 'Akilles', and 'Alicant' from Balsgård and Alnarp had very similar profiles but differed at several loci with only 1 - 2 base pairs. There could be various reasons for this, it could for example be a large-allelic dropout, a mutation in flanking

regions, or a non-templated nucleotide addition caused by the *Taq*-polymerase. These accessions should therefore be considered as possible identical cultivars.

‘Worchester’ from POM and ‘Worchesterberry’ from Balsgård have similar names but did not share the same SSR-profile. When the profiles were compared individually, the differences were significant, and these accessions are most likely different cultivars.

‘POM 20’, ‘POM 34’, ‘Hinnonmäen keltainen’, ‘Olavi’ and ‘Smedjebacken’ shared the same SSR profile and most probably all represent the same cultivar, (‘POM 3’ is also considered identical to these accessions.) ’Hinnonmäen keltainen’, commonly named ‘Hinnonmäki gul’ is a common cultivar in Sweden, denoted as a mandate cultivar. ‘Olavi’ is described in the literature as a completely different cultivar (Larsson, 1961) and was not expected to share SSR-profile with ‘Hinnonmäen keltainen’. The inventory finding ‘Olavi’, was collected from a private garden, while ‘Hinnonmäen keltainen’ at Balsgård was received from The Natural Resources Institute of Finland (Luke). Since ‘Hinnonmäen keltainen’ was obtained from a more reliable source, ‘Olavi’ is most likely mislabeled. The accession ‘Smedjebacken’ was collected from a private garden and is most probably also mislabeled. This result suggests that all accessions above are, in fact, ‘Hinnonmäen keltainen’.

‘POM 4’and ‘POM 38’ had almost identical SSR-profiles, but ‘POM 38’ was heterozygous at one locus where ‘POM 4’ was homozygous. These accessions are considered as identical.

‘POM 8’, ‘POM 35’, ‘Industry’ and ‘Sofiero 7’ shared a similar SSR-profile. ‘POM 16’ had a very similar SSR-profile and was considered identical with these four accessions. ‘Industry’, also called ‘Whinham’s industry’ was introduced by Robert Whinham in 1858 (Tunblad 1985). There is reason to believe that all the analyzed accessions with identical SSR-profiles are ‘Whinhams industry’.

The SSR-profile of ’Landströms gröna - Avvikande’ was different from the accession ‘Landströms gröna’ from Alnarp and Balsgård, which was expected. This confirms that it might be a seedling from ‘Landströms gröna’ or another cultivar.

5.3. Allelic diversity

Loci with high variability, i.e., high allele number and with more even distribution of allele frequencies, are more useful since they contribute with more information than loci with uneven distribution of allele frequencies, e.g., those where one or two alleles are very common, and the rest are found at low frequency. The locus MTT-5 had 24 different alleles with frequencies ranging from 0.006 to 0.268. This marker proved to be very useful for differentiating between accessions. Markers such as g1-K04, RLJ-11, RLJ-7, g1-A01, and g1-M07 do also display high variability and have proved very useful. Contrary, markers g2-L17, g2-J08, gr2-J05, g2-H21, and e1-O01 have high allele frequencies for few alleles which makes them less efficient as markers.

Based on both allele frequency and the number of alleles, the markers g2-B20, e1-021, and g2-G12 are the least efficient for genetic fingerprinting among gooseberry accessions. For all these markers, 1 - 2 alleles are fixed in the studied plant collection.

There were several differences in allele sizes when the data from this study were compared to the data from a previous study by Gunnarsson (2009) (Table 4). The differences in size of the amplified fragments were 1-12 base pairs. For example, the cultivar 'Invicta' had allele sizes 205/205 using the marker RLJ-7, while the same cultivar had allele sizes 210/210 in Gunnarsson's study. The size of the alleles differs with 5 base pairs, but both are homozygous. Only three major differences could be found. The cultivars 'Hönings Früheste' (RLJ-11) and 'Invicta' (MTT-5) were homozygous in this study, but heterozygous in Gunnarsson's study, while 'Worchester berry' (MTT-5) were homozygous in Gunnarsson's study and heterozygous in this study. The cultivars 'Achilles' (MTT-5), and 'Hönings früheste' (RLJ-7) were attributed as triploid in Gunnarsson's study. A third peak was also present in this study for each cultivar, but it was considered a stutter peak.

True alleles could be expected to differ with one base pair due to the occurrence of mutations in DNA flanking regions (Guarino et al. 2006), but in this case, the allele size discrepancy ranged from 1-12 base pairs. A likely explanation for these discrepancies is experimental artifacts and differences in binning procedures. The compared accessions that differed by being homo- or heterozygous at several loci are most likely different due to allelic dropout or null alleles. Taking all these possible causes for allele size differences into consideration is important when results from different studies are pooled together, to avoid inaccurate output data.

5.4. Genetic diversity within the plant collection

High expected heterozygosity (H_e) implies a high level of genetic variability, while low heterozygosity indicates low genetic variability. If the observed heterozygosity is lower than expected in an outcrossing species, this might indicate inbreeding. In this study, the mean values of H_o and H_e are close to each other. However, 9 loci displayed somewhat higher observed heterozygosity than expected heterozygosity. This indicates that these loci do not comply with the Hardy-Weinberg principles. Forces such as non-random mating can disturb the Hardy-Weinberg equilibrium. Gooseberry is an outcrossing plant (Lim 2012). This might be the reason why H_o is higher than H_e in the majority of the SSR markers. High genetic variability is desirable when establishing a germplasm collection, especially a core collection. However, it is important to consider that the presence of null alleles can influence the diversity indexes, especially the estimates of observed heterozygosity.

The fixation index is close to zero among 10 loci, while loci RLJ-7 and e4-D03 deviate by having a higher positive value. This indicates that un-detected null-alleles could exist in these loci. The markers e1-O01, g1-K04 and g2-L17 differentiate by having a higher, negative fixation index compared to other markers, which may indicate an excess of heterozygosity due to negative assortative mating. Negative assortative mating can lead to an increase of heterozygosity in a population since individuals with different phenotypes are more likely to mate than individuals with similar phenotypes (Hedrick, 2016).

5.5. PCoA

An area could be identified within the PCoA-plot, where the accessions were more homogenous, compared to the rest of the studied cultivars. An explanation as to why these accessions were more homogenous could not be found in the literature. It is possible that the accessions within the group are related somehow, and therefore display more genetic similarities. It is, however, difficult to draw such a conclusion based on data from a low-density marker system. Application of high-density single nucleotide polymorphism markers (SNPs) allowed more detailed clarification of genetic relationships among Swedish mandate cultivars in apple (Skytte af Sätra et al., 2020). Thus, further analyses of a larger number of individuals with SNP markers would help to clarify parent-offspring and other types of relationships in the gooseberry germplasm.

Accessions far apart display more genetic divergence than accessions closer to each other In this case, ‘POM 5’, ‘POM 20’, ‘POM 25’, ‘POM 39’, ‘Dr. Törnmarck’, ‘Kerstin M.’, ‘Kozachok’, ‘Mountain’, ‘Robustenta’, ‘Scania’, ‘Spine free’ and ‘Åsgatan’ (positioned to the far right of the plot) display the highest genetic divergence compared to the accessions with more genetic similarities. Interestingly, ‘Dr. Törnmarck’, ‘Mountain’ and ‘Scania’ are all cultivars with an American background (Hjalmarsson & Wallace 2007; Hjalmarsson 2020; Niggli & Frei 2019). As to date, only ‘Dr. Törnmarck’, ‘Kozachok’, ‘Mountain’ and ‘Scania’ in this deviating group are denoted as mandate cultivars. If the collection aims to cover up as much genetic diversity and resources as possible, it would be a good idea to include accessions that are more distantly related.

5.6. Conclusion

The present work uncovered several cases of duplication among the inventory findings and one, possible case of mislabeling. All mandate cultivars in Pom’s collection at Alnarp had individual SSR-profiles, which was expected since these accessions previously have been identified through phenotypical analyses. This study furthermore revealed that 6 SSR markers (MTT-5, g1-K04, RLJ-11, RLJ-7, g1-A01 and g1-M07) were more effective compared to the others and proved very useful for distinguishing between gooseberry accessions and describing the variability of the gooseberry germplasm. The data constitutes an important basis for evaluation and addition of accessions to the present collection of mandate cultivars and for removal of duplicates among the inventory findings.

5.7. Future outlook

In this study, 105 accessions and inventory findings were analyzed. The results are valuable for the preservation of gooseberry cultivars that are a part of Sweden’s pomological heritage. It would, however, be interesting to perform a molecular characterization on remaining inventory findings and accessions from other, interesting sources. Molecular characterization of the inventory findings could also be complemented with a phenotypical characterization.

Further research will be done in an upcoming project that aims to study genetic diversity, identify duplicates and mislabeling, and hopefully will also reveal parent-offspring

relatedness. The remaining Swedish cultivars will be analyzed along with cultivars from collections in the Ukraine, Latvia, and Denmark. The result from the upcoming study will be compared with the results of this study.

At the moment, there is no standardized way for analyzing the germplasm of *Ribes*. Primers with high reproducibility and consistency that can be used to differentiate between different genotypes could for example be used as an international, molecular standard. If analyzation of *Ribes* germplasm was conformed to a standard, it would be easier to compare and pool together results from different studies, which would benefit all parties.

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Appendix 1

SSR profiles of 105 gooseberry cultivars generated with 15 different primer pairs. Missing values are denoted as mv and the asterisk (*) denotes mandate cultivars. (See page 36.)

Analysed	accessions	e1-O01		e1-021		e4-D03		g1-A01		g1-K04		g1-M07		g2-B20		g2-G12	
1	POM 1	139	139	297	297	165	171	240	251	291	291	208	232	146	146	164	164
2	POM 2	139	141	293	293	165	165	229	240	291	297	207	232	144	146	164	164
3	POM 3	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
4	POM 4	133	141	293	311	165	165	229	240	291	297	207	208	146	146	164	164
5	POM 5	139	139	293	293	165	165	233	251	281	287	206	208	144	146	164	164
6	POM 6	139	141	293	293	171	171	240	240	291	297	208	232	146	146	164	164
7	POM 7	133	139	290	293	165	165	236	240	287	297	208	208	144	146	164	166
8	POM 8	139	141	293	293	165	165	229	240	291	297	207	208	144	146	164	164
9	POM 9	139	141	293	293	171	171	240	240	291	297	208	232	146	146	164	164
10	POM 10	133	141	293	293	165	186	240	242	287	297	207	221	144	146	164	166
11	POM 11	139	145	293	293	165	177	229	240	283	289	226	238	146	146	164	164
12	POM 12	141	141	293	293	171	186	229	240	283	291	207	232	144	146	164	164
13	POM 13	139	141	293	293	164	186	229	240	289	291	232	238	146	146	164	164
14	POM 14	139	141	293	293	165	171	233	240	291	297	207	208	144	146	164	164
15	POM 15	139	141	293	293	164	164	229	240	291	297	207	232	144	146	164	164
16	POM 16	139	141	293	293	165	165	229	240	291	297	207	208	145	146	164	164
17	POM 17	139	139	293	293	164	164	233	251	291	297	207	208	144	146	164	164
18	POM 19	139	141	293	293	165	171	229	240	289	291	208	232	146	146	164	164
19	POM 20	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
20	POM 22	139	139	293	293	165	171	240	240	289	291	208	238	144	146	164	164
21	POM 23	139	139	293	293	165	165	233	240	297	297	208	208	146	146	164	164
22	POM 24	139	149	293	293	165	171	240	240	291	297	208	232	146	146	164	164
23	POM 25	139	141	293	293	165	165	251	251	289	291	232	232	146	146	164	166
24	POM 26	139	141	293	293	165	171	240	240	291	297	207	232	146	146	164	164
25	POM 27	139	139	293	293	165	165	229	251	281	297	216	222	144	146	164	164
26	POM 28	139	141	293	293	165	165	240	240	291	297	207	208	146	146	164	164
27	POM 29	141	141	293	293	171	186	229	240	283	291	207	232	144	146	164	164
28	POM 30	139	145	293	293	165	165	229	231	291	291	232	232	146	146	164	164
29	POM 31	139	141	293	293	165	165	229	251	291	297	207	208	146	146	164	164
30	POM 32	139	141	293	293	165	165	219	240	281	287	206	208	144	146	164	164
31	POM 33	139	139	293	293	165	165	229	240	283	291	208	226	146	146	164	164
32	POM 34	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
33	POM 35	139	141	293	293	165	165	229	240	291	297	207	208	144	146	164	164
34	POM 36	139	141	293	293	165	165	219	240	281	287	206	208	144	146	164	164
35	POM 37	139	145	293	293	165	165	229	229	291	291	232	232	146	146	164	164
36	POM 38	133	141	293	311	165	165	229	240	291	297	207	208	146	146	164	164
37	POM 39	139	143	290	293	164	164	232	251	291	297	208	208	146	146	164	166
38	POM 40	139	141	293	293	165	171	240	240	291	297	207	232	146	146	164	164
39	POM 41	139	141	293	293	165	171	240	240	291	297	207	232	146	146	164	164
40	Achilles * (Balsgård)	139	141	293	293	165	165	240	251	291	297	207	208	142	146	164	164
41	Akilles * (Alnarp)	139	141	293	293	164	164	240	251	291	297	207	208	144	146	164	164
42	Alicant (Balsgård)	139	141	293	293	165	165	233	251	291	297	207	208	144	146	164	164
43	Alicant (Alnarp)	139	141	293	293	164	164	251	251	291	297	207	208	144	146	164	164
44	Aaron *	139	141	293	293	165	171	229	229	291	297	207	208	146	146	164	164
45	Björn K.	139	139	293	293	165	171	240	251	291	297	207	232	146	146	164	164
46	Bloodhound * (Balsgård)	141	143	293	293	165	165	229	251	291	297	207	208	144	146	164	164
47	Bloodhound * (Alnarp)	141	143	293	293	165	165	229	251	291	297	207	208	144	146	164	164
48	Dr. Törnmarck *	135	139	293	307	188	198	225	251	289	291	208	232	144	146	164	166
49	Flottskär gröna	139	141	293	293	165	165	*mv*	*mv*	291	297	207	208	144	146	164	164
50	Flottskär röda	139	141	293	293	165	165	*mv*	*mv*	*mv*	*mv*	208	208	*mv*	*mv*	164	164
51	Frick	141	145	293	293	165	186	231	240	283	291	226	232	146	146	164	164
52	Hinnonmäen keltainen *	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
53	Houghton	139	139	293	293	165	165	*mv*	*mv*	*mv*	*mv*	206	208	144	146	164	166

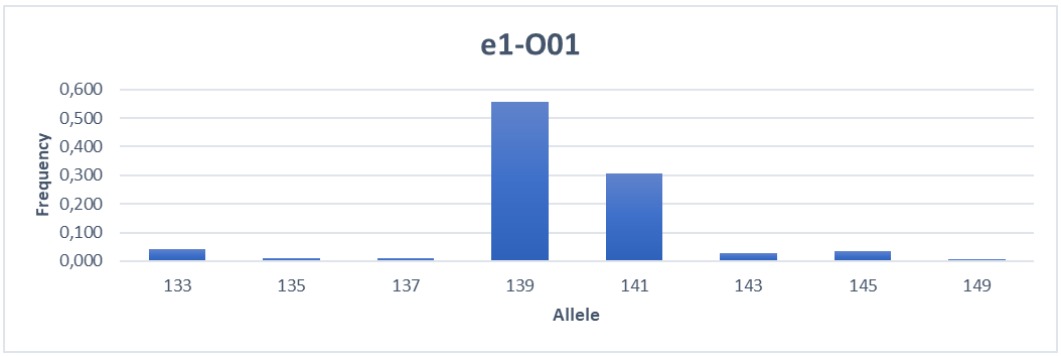
Analysed	accessions	g2-H21		g2-J08		g2-L17		gr2-J05		MTT-5		RLJ-7		RLJ-11	
1	POM 1	246	254	154	154	136	136	164	170	201	219	206	230	250	261
2	POM 2	246	254	154	154	120	136	164	170	212	219	205	230	239	250
3	POM 3	246	254	149	156	120	136	164	170	180	180	204	206	261	261
4	POM 4	246	254	154	154	120	153	164	164	212	212	205	205	239	250
5	POM 5	246	272	149	156	136	138	164	178	200	213	204	206	243	261
6	POM 6	254	254	154	154	120	136	170	170	201	212	206	230	243	250
7	POM 7	250	254	154	154	136	136	170	170	191	201	206	206	247	250
8	POM 8	254	254	154	154	120	120	164	170	212	212	205	205	239	250
9	POM 9	254	254	154	154	120	136	170	170	201	212	206	230	243	250
10	POM 10	246	254	154	154	120	128	170	178	207	212	205	218	250	252
11	POM 11	246	254	154	154	118	120	164	170	238	242	223	236	239	250
12	POM 12	246	254	154	154	136	136	164	170	212	219	205	230	239	250
13	POM 13	246	246	154	156	118	136	164	174	215	240	230	236	239	250
14	POM 14	254	254	154	154	120	136	164	170	221	236	205	205	244	250
15	POM 15	246	254	154	154	120	136	164	170	212	219	205	230	239	250
16	POM 16	254	254	154	154	120	120	164	170	212	212	205	205	239	250
17	POM 17	254	254	154	154	120	136	164	170	212	219	205	205	243	261
18	POM 19	246	254	154	156	120	136	164	170	201	213	206	230	239	250
19	POM 20	246	254	149	156	120	136	164	170	180	236	204	206	261	261
20	POM 22	246	254	154	156	120	136	164	164	213	225	206	236	250	250
21	POM 23	254	254	154	154	120	120	170	170	201	212	206	206	244	250
22	POM 24	254	254	154	154	120	136	170	170	201	212	206	230	243	250
23	POM 25	260	262	149	154	136	146	164	164	197	240	230	230	261	261
24	POM 26	246	254	154	154	120	120	164	164	219	225	205	230	250	250
25	POM 27	254	262	154	158	120	136	164	170	209	240	214	220	239	261
26	POM 28	254	254	154	154	120	136	164	170	201	212	205	205	244	250
27	POM 29	246	254	154	154	136	136	164	170	212	219	205	230	239	250
28	POM 30	246	254	154	154	120	136	164	170	238	240	230	230	239	242
29	POM 31	254	254	154	154	120	136	170	170	201	212	205	205	239	261
30	POM 32	254	254	154	154	120	120	164	164	212	212	204	206	229	250
31	POM 33	254	254	149	156	120	136	164	178	197	215	206	223	239	250
32	POM 34	246	254	149	156	120	136	164	170	180	236	204	206	261	261
33	POM 35	254	254	154	154	120	120	164	170	212	212	205	205	239	250
34	POM 36	254	254	154	154	120	120	164	164	212	212	204	206	229	250
35	POM 37	246	254	154	154	120	136	164	170	238	240	230	230	239	239
36	POM 38	246	254	154	156	120	153	164	164	212	212	205	205	239	250
37	POM 39	250	254	156	156	120	136	164	164	238	240	206	206	243	261
38	POM 40	246	254	154	154	120	120	164	164	219	225	205	230	250	250
39	POM 41	246	254	154	154	120	120	164	164	219	225	205	230	250	250
40	Achilles * (Balsgård)	254	254	154	156	120	137	164	170	201	238	205	205	250	261
41	Akilles * (Alnarp)	254	254	154	156	120	136	164	170	201	238	205	205	250	260
42	Alicant (Balsgård)	254	254	154	156	120	136	170	170	212	238	205	205	244	261
43	Alicant (Alnarp)	254	254	154	156	120	136	170	170	212	238	205	205	244	261
44	Aaron *	254	254	154	154	120	120	164	170	201	212	205	205	239	239
45	Björn K.	246	254	154	156	120	136	170	170	212	212	205	230	250	261
46	Bloodhound * (Balsgård)	246	254	154	154	136	136	164	164	201	212	205	205	239	261
47	Bloodhound * (Alnarp)	246	254	154	154	136	136	164	164	201	212	205	205	239	261
48	Dr. Törnmarck *	254	256	149	156	120	128	170	178	180	217	226	230	235	261
49	Flottskär gröna	254	254	154	156	120	136	164	170	201	238	205	205	250	261
50	Flottskär röda	246	254	154	156	120	136	164	170	212	236	206	206	250	261
51	Frick	246	254	154	154	118	120	164	174	225	240	223	230	242	250
52	Hinnonmäen keltainen *	246	254	149	156	120	136	164	170	180	236	204	206	261	261
53	Houghton	254	271	149	156	*mv*	*mv*	164	178	200	213	204	206	229	261

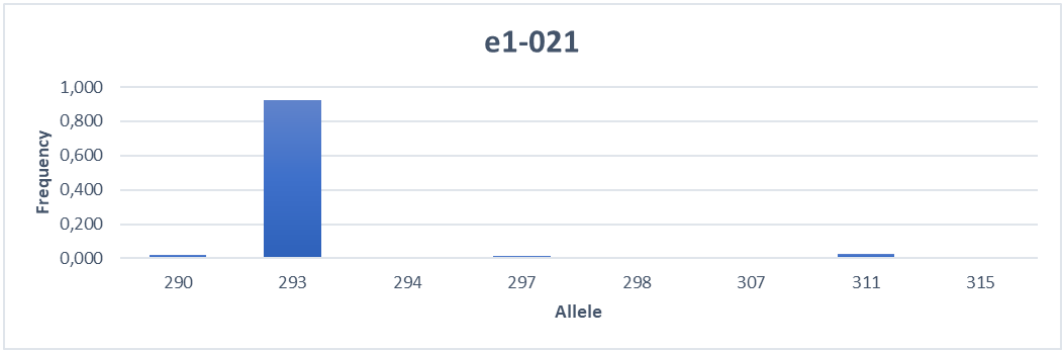
Analysed	accessions	e1-O01		e1-021		e4-D03		g1-A01		g1-K04		g1-M07		g2-B20		g2-G12	
54	Hönings Früheste	139	141	293	293	165	171	240	240	291	297	207	232	146	146	164	164
55	Industry *	139	141	293	293	165	165	229	240	291	297	207	208	144	146	164	164
56	Invicta	133	141	293	311	165	165	229	240	291	297	207	208	146	146	164	164
57	Jakob *	137	141	293	293	165	180	240	251	291	297	208	208	146	146	164	164
58	Kalvstorp	139	139	293	293	165	165	229	240	283	289	232	232	146	146	164	164
59	Catharina Ohlenburg *	139	141	293	293	165	171	229	229	291	297	207	208	146	146	164	164
60	Kerstin M.	139	139	293	293	165	165	251	251	281	287	206	208	144	146	164	164
61	Kopparbro	139	139	293	293	165	165	240	251	283	283	216	226	144	146	164	164
62	Kozachok	133	139	293	293	165	165	225	240	281	291	208	208	144	146	166	164
63	Landströms gröna * (Balsgård)	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
64	Landströms gröna * (Alnarp)	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
65	Landströms gröna (Avvikande)	139	141	293	293	165	165	233	251	291	299	207	208	146	146	164	164
66	Lilla busken - Släta bär	139	139	293	293	*mv*	*mv*	229	229	291	297	207	232	146	146	*mv*	*mv*
67	Linda	139	141	293	293	165	165	240	240	291	297	207	208	146	146	164	164
68	Linköping	139	139	293	293	165	165	240	251	291	297	207	208	146	146	164	164
69	London *	139	141	293	293	165	171	229	240	291	297	207	208	144	146	164	164
70	Lottas	139	141	293	293	165	171	240	251	291	297	207	208	146	146	164	164
71	Mette P.	139	139	293	293	165	171	229	240	291	297	207	208	146	146	164	164
72	Mountain * (Alnarp)	135	141	293	294	165	192	221	251	280	291	208	214	144	148	164	164
73	Mountain * (EPS)	135	141	293	294	165	192	221	251	280	291	208	214	144	148	164	164
74	Olavi	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
75	Perle von Münchenberg	133	139	293	311	165	165	236	240	289	297	208	231	146	146	164	166
76	POM gården 1	139	141	293	293	165	165	239	239	291	297	208	232	146	146	164	164
77	POM gården 2	139	141	293	293	165	165	240	240	291	297	208	232	146	146	164	164
78	POM okänd 1	139	139	293	293	165	171	231	240	291	297	207	207	144	146	164	164
79	POM okänd 2	139	141	293	293	164	164	*mv*	*mv*	291	297	*mv*	*mv*	144	146	*mv*	*mv*
80	Resistenta	139	143	293	311	165	165	232	240	291	297	208	208	146	146	164	166
81	Robustenta	139	143	290	293	165	165	232	251	291	297	208	208	146	146	164	166
82	Ronja	141	141	293	293	165	180	240	240	297	297	208	208	144	146	164	164
83	Roskil	139	139	293	293	165	165	240	251	281	291	208	208	144	146	164	164
84	Sandgren	139	141	293	293	165	171	*mv*	*mv*	291	297	*mv*	*mv*	146	146	*mv*	*mv*
85	Scania *	139	139	293	293	165	165	*mv*	*mv*	281	287	206	208	144	146	164	166
86	Smedjebacken	139	139	293	293	165	188	251	251	281	287	206	208	146	146	164	164
87	Smiling beauty	139	141	293	293	165	165	240	251	291	297	207	208	146	146	164	164
88	Snickarmoa	139	141	293	293	164	164	240	240	291	297	207	208	144	144	164	164
89	Sofiero 3	139	141	293	293	164	164	251	251	291	297	207	208	144	146	164	164
90	Sofiero 4	139	141	293	293	165	171	229	229	291	297	207	208	146	146	164	164
91	Sofiero 5	139	141	293	293	165	165	240	251	291	297	207	208	144	146	164	164
92	Sofiero 6	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
93	Sofiero 7	139	141	293	293	165	165	229	240	291	297	207	208	144	146	164	164
94	Spine free	141	143	293	293	164	164	227	251	291	295	214	216	144	146	166	164
95	Stor buske - Päronträd	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
96	Stora busken - Landet	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
97	Söderbrunn	139	141	293	293	165	171	229	233	291	297	207	208	146	146	164	164
98	Trasher white	139	141	293	293	165	165	229	251	291	297	207	207	146	146	164	164
99	Villa Virginia	139	139	293	293	165	171	240	251	291	297	207	232	146	146	164	164
100	Worchester	137	141	293	298	165	165	227	240	289	297	207	212	144	144	164	166
101	Worchesterberry	133	139	293	315	165	176	227	240	289	297	204	207	144	146	*mv*	*mv*
102	Ångermanlands röda *	139	145	293	293	165	165	229	231	283	297	207	226	144	146	164	164
103	Åsgatan	139	139	293	293	165	165	219	251	281	287	206	208	144	146	164	166
104	Österhammarsbruk	139	141	293	293	165	165	229	240	283	291	232	232	146	146	164	164
105	Övertjärn	139	145	293	293	165	165	231	231	283	291	226	226	144	144	164	164

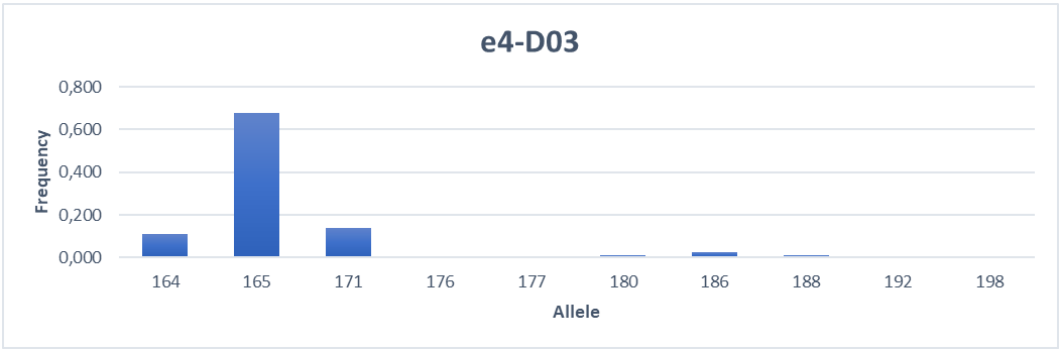
Analysed	accessions	g2-H21		g2-J08		g2-L17		gr2-J05		MTT-5		RLJ-7		RLJ-11	
54	Hönings Früheste	246	254	154	154	120	136	164	170	212	219	205	230	250	250
55	Industry *	254	254	154	154	120	120	164	170	212	212	205	205	239	250
56	Invicta	246	254	153	153	120	153	164	164	212	212	205	205	239	250
57	Jakob *	254	254	154	154	120	120	170	170	201	240	206	206	250	261
58	Kalvstorp	246	254	154	156	118	120	164	170	213	238	230	230	239	250
59	Catharina Ohlenburg *	254	256	154	154	120	120	164	170	201	212	205	205	239	239
60	Kerstin M.	246	271	149	156	136	138	164	178	200	213	204	206	244	261
61	Kopparbro	254	254	154	154	118	120	164	174	212	214	214	223	250	261
62	Kozachok	254	262	154	156	131	139	176	176	221	225	206	206	235	250
63	Landströms gröna * (Balsgård)	246	254	154	154	120	136	170	170	201	219	205	205	239	244
64	Landströms gröna * (Alnarp)	246	254	154	154	120	136	170	170	201	219	205	205	239	244
65	Landströms gröna (Avvikande)	246	254	154	160	120	136	170	186	201	217	205	205	243	261
66	Lilla busken - Släta bär	246	246	154	156	136	136	*mv*	*mv*	212	212	206	230	239	239
67	Linda	254	254	154	156	120	120	164	164	223	225	205	205	250	250
68	Linköping	254	254	154	156	120	136	164	170	216	238	205	205	250	261
69	London *	254	254	154	154	120	136	170	170	212	212	205	205	239	250
70	Lottas	254	254	154	156	120	136	164	170	212	236	205	205	250	261
71	Mette P.	254	254	154	156	120	120	164	170	212	238	205	205	239	250
72	Mountain * (Alnarp)	254	258	154	156	132	145	170	178	211	211	206	212	231	261
73	Mountain * (EPS)	254	258	154	156	132	145	170	178	211	211	206	212	231	261
74	Olavi	246	254	149	156	120	136	164	170	180	236	204	206	261	261
75	Perle von Münchenberg	246	254	156	172	120	153	164	164	191	238	206	228	247	250
76	POM gården 1	254	254	154	154	120	136	170	170	201	212	206	230	243	250
77	POM gården 2	254	254	154	154	120	136	170	170	201	212	206	230	243	250
78	POM okänd 1	246	246	154	154	136	136	164	164	219	240	205	230	241	250
79	POM okänd 2	254	256	154	154	120	136	164	170	201	238	205	205	250	261
80	Resistenta	246	254	156	172	120	153	164	164	191	238	206	206	243	250
81	Robustenta	250	254	156	156	120	136	164	164	238	240	206	206	243	261
82	Ronja	248	254	154	154	120	136	170	170	195	212	206	206	250	250
83	Roskil	254	254	154	156	120	136	164	178	215	225	206	206	250	261
84	Sandgren	254	254	154	154	120	136	170	170	201	212	206	230	243	250
85	Scania	254	271	149	156	138	141	164	178	200	213	204	206	229	261
86	Smedjebacken	246	254	149	156	120	136	164	170	180	236	204	206	261	261
87	Smiling beauty	246	254	154	156	120	136	164	170	221	236	205	205	250	261
88	Snickarmoa	254	254	154	158	136	136	164	164	201	209	205	205	244	250
89	Sofiero 3	254	254	154	156	120	136	170	170	212	238	205	205	243	261
90	Sofiero 4	254	254	154	154	120	120	164	170	*mv*	*mv*	205	205	239	239
91	Sofiero 5	254	254	154	156	120	136	164	170	201	238	205	205	250	261
92	Sofiero 6	254	254	154	154	120	120	164	170	201	212	205	205	239	239
93	Sofiero 7	254	254	154	154	120	120	164	170	212	212	205	205	239	250
94	Spine free	260	271	149	154	133	138	178	178	200	200	212	214	237	261
95	Stor buske - Päronträd	254	254	154	154	120	136	164	170	201	212	205	230	239	239
96	Stora busken - Landet	254	254	154	154	120	120	164	170	201	212	205	205	239	239
97	Söderbrunn	254	256	154	154	120	120	164	170	201	212	205	205	239	244
98	Trasher white	246	254	149	154	120	136	164	164	180	212	205	205	239	261
99	Villa Virginia	246	254	154	156	120	136	170	170	212	215	205	230	250	261
100	Worchester	254	256	154	170	136	136	177	177	212	219	205	210	237	250
101	Worchesterberry	246	246	154	162	120	149	164	164	198	225	202	205	237	250
102	Ångermanlands röda *	246	246	154	154	118	136	164	164	*mv*	*mv*	205	223	239	241
103	Åsgatan	254	271	149	156	120	138	164	178	200	213	204	206	229	261
104	Österhammarsbruk	246	262	154	154	118	136	164	170	240	240	230	230	239	250
105	Övertjärn	246	254	154	154	118	120	164	164	225	238	223	223	241	241

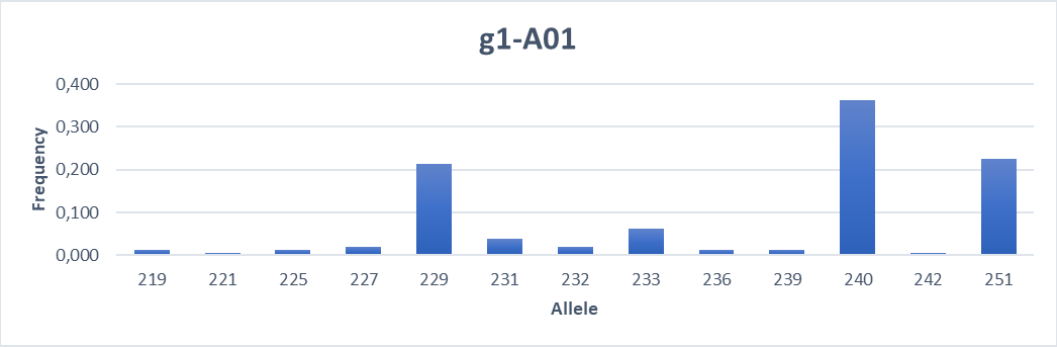
Appendix 2

Allele frequencies with graphs by locus for codominant data.





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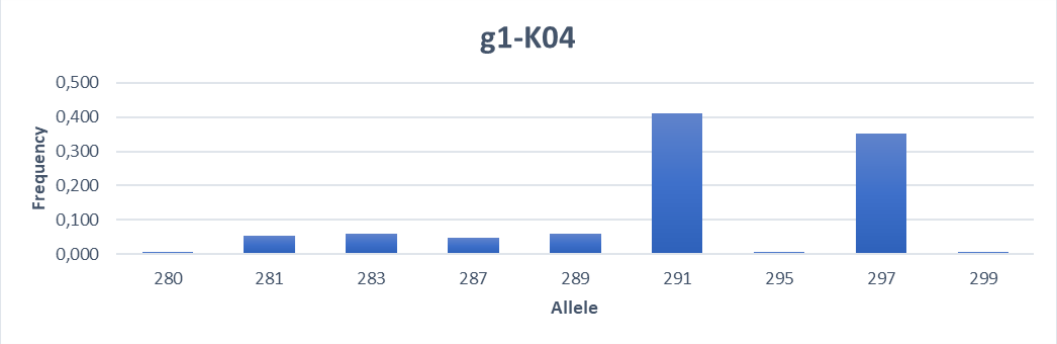
221
225
227
229
231
232
233
236
239
240
242
251
Allele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289
291
295
297
299
Allele


g1-M07
Frequency
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0,400
0,300
0,200
0,100
0,000
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212
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216
221
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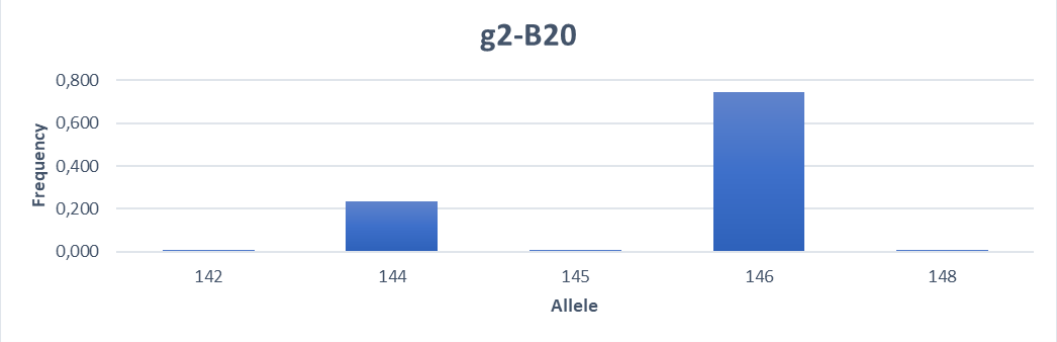
231
232
238
Allele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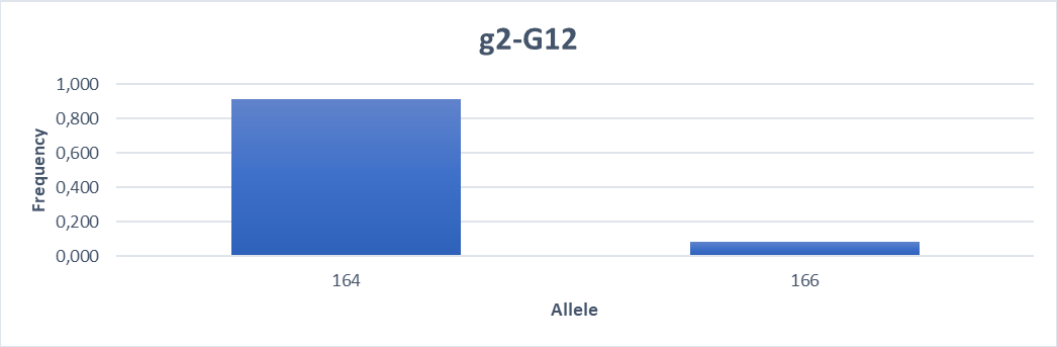
146
148
Allele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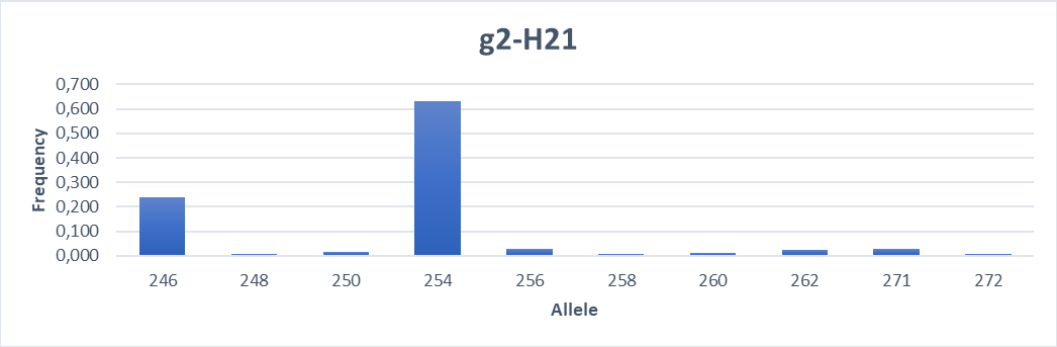
166
Allele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Frequency
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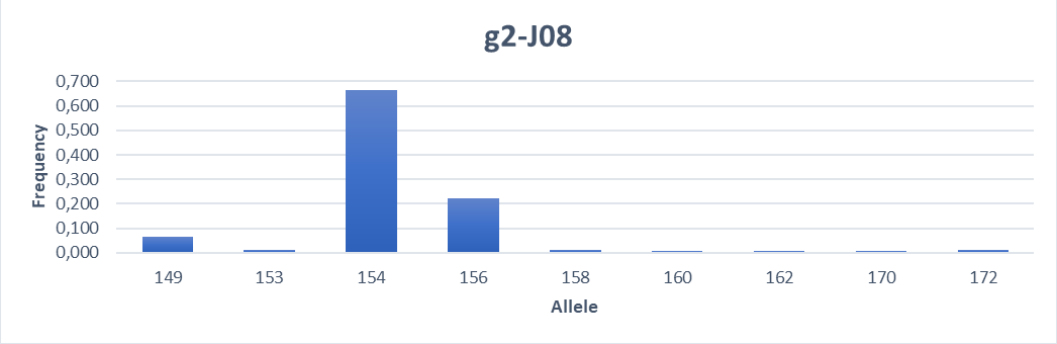
250
254
256
258
260
262
271
272
Allele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Frequency
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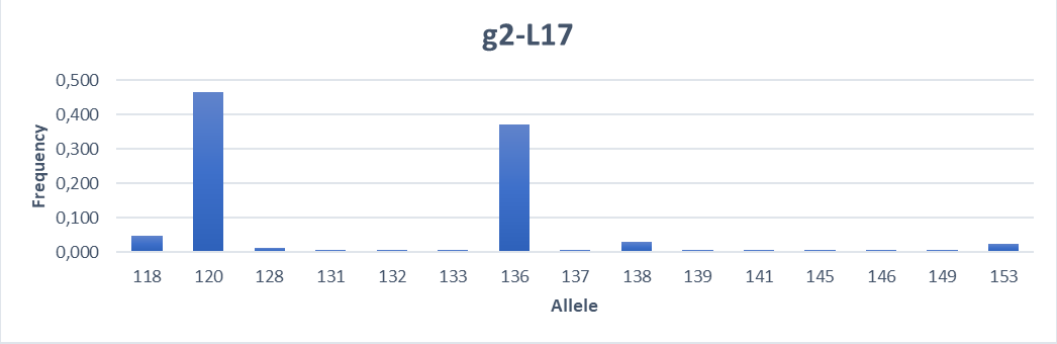
170
172
Allele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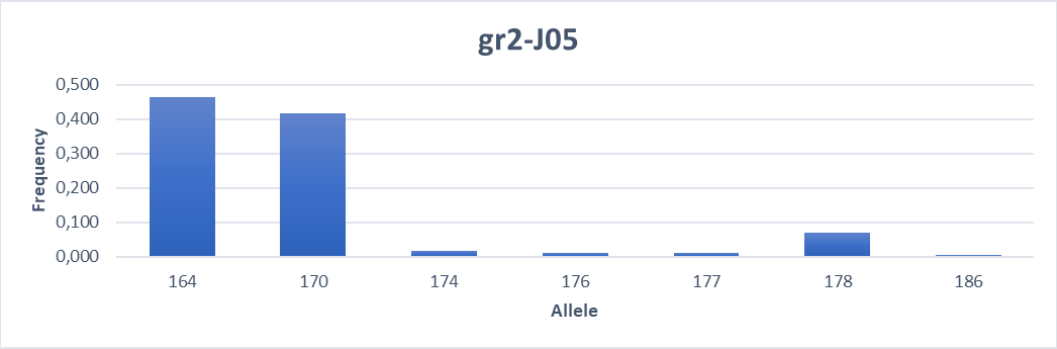
149
153
Allele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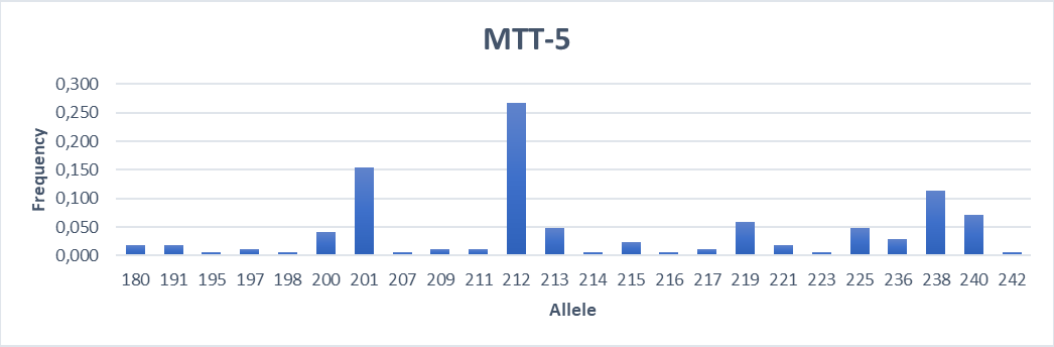
186
Allele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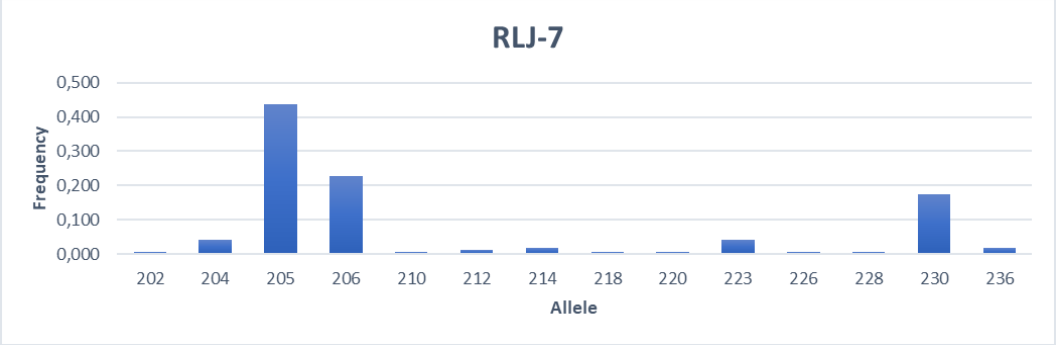
Allele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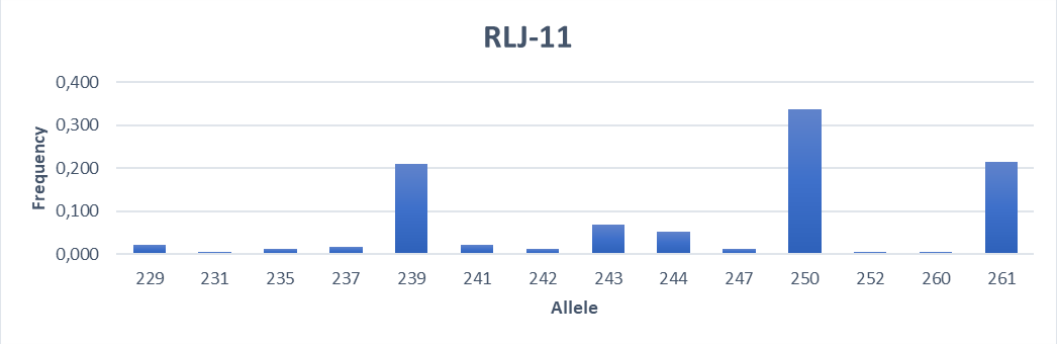
Allele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Allele

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