Characterisation of the Latvian and Swedish Sweet and Sour Cherry Genetic Resources

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Abstract

A wide diversity of cherry varieties are collected in the Latvian and Swedish genetic resources collections, which consists of landraces and selections of local breeders, adapted to the local climate and growing conditions (winter hardy and disease resistant) as well as germplasm that results from years of scientific exchange and co-operation with the world's leading plant research institutes. The introduction of this material into the breeding programs is largely dependent on the level of characterization. The genetic diversity and internal structure of Latvian and Swedish sweet and sour cherry genetic resources collections has been investigated using phenotypical characterization and evaluation in combination with SSR and self-incompatibility gene specific molecular markers. Phenotypical and molecular characterization revealed high phenotypic and genetic diversity of analysed germplasm as well as the relatedness of Baltic and Scandinavian sweet and especially sour cherry landraces which indicate a possible common historical origin. Local Baltic-Scandinavian cherry varieties were also differentiated from other cherry germplasm by the frequency of self-incompatibility alleles detected using gene specific molecular markers. Self-incompatibility allele information gained from this study will be also useful in breeding programmes for the planning of crosses and conservation of alleles. The use of different characterization methods of cherry genetic resources also facilitated methodological observations, applicable to cherry germplasm characterization. It was concluded that thorough evaluation of genetic diversity and internal structure of cherry genetic resources collections should include both phenotypic and molecular characterization. The information of genetic relatedness revealed by SSR markers did not show direct correspondence with the relatedness information detected by phenotypic characterization, regardless of the number of analysed markers. Therefore a sufficient preliminary description of cherry genetic resources and discovery of internal genetic relatedness of germplasm could be obtained by using phenotypic description in combination with a small set of highly polymorphic SSR markers in combination with available gene specific markers.

Keywords: genetic diversity, germplasm, *Prunus avium*, *Prunus cerasus*, molecular markers, phenotypic characterization

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Lacis, G**., Kaufmane, E., Rashal, I., Trajkovski, V., Iezzoni, A.F. (2008). Identification of self-incompatibility (*S*) alleles in Latvian and Swedish sweet cherry genetic resources collections by PCR based typing. *Euphytica* 160, 155–163.

II **Lacis, G**., Kaufmane, E., Trajkovski, V., Rashal, I. (2009). Morphological variability and genetic diversity within Latvian and Swedish sweet cherry collections. *Acta Universitatis Latviensis* 753, 19–32.

III **Lacis, G.**, Rashal, I., Ruisa, S., Trajkovski, V., Iezzoni, A.F. (2009). Assessment of genetic diversity of Latvian and Swedish sweet cherry (*Prunus avium* L.) genetic resources collections by using SSR (microsatellite) markers. *Scientia Horticulturae* 121(4), 451-457.

IV **Lacis, G**., Trajkovski, V., Rashal, I. (2010). Phenotypical variability and genetic diversity within accessions of the Swedish sour cherry (*Prunus cerasus* L.) genetic resources collection. *Biologija* 56(1/4) (in press).

V **Lacis, G**., Rashal, I., Trajkovski, V. (2010). Comparative analysis of sweet cherry (*P. avium*) genetic diversity revealed by two methods of SSR marker detection. *Proceedings of the Latvian Academy of Sciences. Section B: Natural, Exact and Applied Sciences* 64(3/4) (in press).

VI **Lacis, G**., Rashal, I., Trajkovski, V. Assessment of genetic variability in Latvian and Swedish sour cherry (*Prunus cerasus* L.) genetic resources collections using microsatellite (SSR) markers (submitted).

VII **Lacis, G**., Rashal, I., Trajkovski, V. Comparative analysis of two SSR marker sets and morphological characterization sweet cherry (*P. avium*) for genetic diversity evaluation (manuscript).

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1 Introduction

1.1 Taxonomy, domestication, distribution

Cherries are members of the Rosaceae family, subfamily Prunoideae. Cherries belong to the *Cerasus* subgenus of the *Prunus* genus and are fairly distinct from the other stone fruit species, like almonds, apricots, peaches and plums (Badenes and Parfitt, 1995; Tavaud *et al*., 2004). The *Cerasus* subgenus is divided into seven sections and both widely cultivated species – sweet (*Prunus avium* (L.) L.) and sour (*Prunus cerasus* L.) cherries belong to the same section - *Eucerasus* (Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008; USDA, NRCS, 2010).

Sweet cherries (*Prunus avium* (L.) L. (syn. *Cerasus avium* (L.) Moench; *Prunus cerasus* var. *avium* L.; *Prunus macrophylla* Poir.)) are usually diploid (2n) with 16 chromosomes (Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008). The name *Prunus avium* for sweet cherries was given by Linneus in 1755. Sweet cherries are often also called Gean or Mazzard cherries (Faust, Syrányi, 1997). They are characterized by large trees with a pyramidal canopy, which can reach even 20 m in height. Their leaves are large – approximately 7.5 – 12.5 cm long, their width is about half of their length. Petioles are long with reddish glands. The flowers are white; with diameter of about 2.5 cm. Flowers are formed on the previous year's growth singly or in groups of five, covering the vegetative buds on older growth. Fruits of wild species are roundish, red or black and small (their diameter is about 2 cm) (Webster, 1996). Sweet cherries originate from the central Middle East, which includes Asia Minor, Iran, Iraq and Syria (Vavilov, 1951). As local plants they are found in Asia, particularly in the north of

present-day Iran, as well as in Ukraine and other countries to the south of the Caucasus Mountains. They grow well in Europe, mainly in Greece, Italy and Spain. It is considered that the species originated near the Caspian and Black Sea, from whence it slowly spread further. Originally they were distributed by migratory birds, but then by humans who travelled over long distances during the Stone Age (Brown *et al*., 1996; Webster, 1996; Faust, Syrányi, 1997). Hedrick (cited by Webster, 1996) describes the wild sweet cherry geographical boundaries as the whole mainland Europe, and in the east deep into Russia and South Asia, as well as North India, with the largest distribution area between the Caspian and Black Sea.

Sour cherry (*Prunus cerasus* L. (syn. *Cerasus vulgaris* Mill., *Prunus vulgaris* Schur)) is an allotetraploid species, originating from a natural hybridization between sweet cherry (*P. avium* L.; 2n = 2x = 16) and ground cherry (*P. fruticosa* Pail.; 2n= 4x = 32) (Olden, Nybom, 1968; Webster, 1996; Iezzoni 2008). The distribution of European sour cherry ranges from the Mediterranean islands to northern Russia, and within the range there is a wide diversity of different plant habitats and fruit characters (Krahl *et al*., 1991; Beaver *et al*., 1995; Trajkovski, 1996). Sour cherries grow as small trees or shrubs. They have smaller leaves than sweet cherries, oval or ovoid, 4-7 cm long, and leaf width is about half of the length. Flowers, which form abundantly on one-year growth is white, the diameter of 1.75 to 2.5 cm. Wild type fruits are red or nearly black, circular with sour pulp. De Candolle (1884, according to Webster, 1996) concluded that sour cherries originated approximately in the same area as sweet cherries. According to other authors, the possible area of sour cherry origin is much wider: from Switzerland to the Adriatic Sea in one direction and from the Caspian Sea to the north of Europe in the second direction (Webster, 1996). Kolesnikova (1975) divided native sour cherries into two ecological groups: Western European and Middle Russian groups. The Western European sour cherry group is characterized by lower winterhardiness and higher fruit quality. Typical representatives of this group are cultivars ‘Kentish’ and ‘Griot Ostheim’. The sour cherries of Middle Russian group have high cold-resistance, but lower fruit quality. Typical representatives of this sour cherry ecological group are cultivars ‘Vladimirskaja’ and ‘Ljubskaya’ (Webster, 1996).

Nowadays sweet and sour cherries still grow wild in various parts of Europe from Scandinavia in the north to Turkey in the south and show wide genetic diversity. Most of the European cherry germplasm consists of

local forms unique to a particular place. These local forms, which show high variation in tree and fruit size, productivity, ripening time, fruit quality and disease resistance also form a majority of clonal collections (Webster, 1996; Iezzoni, 2008).

Cultivation of sweet cherries began in the southern part of the northern hemisphere, in temperate and partly subtropical zones (Zhukovsky, 1971). Cherry pits have been found in Neolithic period archaeological excavations in Switzerland, Germany, northern Italy and southern Sweden (Bahteev, 1970; Kolesnikova, 1975). Cherries were first described by Teofrast in 300 B. C. Since Roman times cherries have been popular as garden and roadside trees. In antiquity sweet cherry gum was also used to ease coughing (Brown *et al*., 1996; Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008).

The history of cherry development is very uneven. Nearly 2000 years (from 300 B. C. to 1700) cherry fruits were mediocre, harvested from locally grown trees. Farmers and gardeners selected and reproduced the cherry trees that were the most productive and had the highest fruit quality. The best clones, propagated by rooting shoots or grafting, were planted in gardens or along roads (Brown *et al*., 1996; Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008). Several locally selected sour cherry landraces still are grown in the northern and eastern regions of cherry cultivation: 'Cigany', 'Oblacinska', 'Stevnsbær', 'Vladimirskaya' (Brown *et al*., 1996; Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008). Targeted cherry breeding using hybridization started only in 18th century. During the next 200 years numerous outstanding varieties were bred. Most of them have already disappeared because they had no commercial value, but many are still available and are used in breeding (e.g. 'Drogan's Gelbe Knorpelkirsche', **'**Donissens Gelbe Knorpelkirsche'). Cherry growing during the 20th century in Western Europe increased rapidly, by planting of large orchards, use of modern farming technologies and modern varieties suitable to local conditions (Brown *et al*., 1996; Webster, 1996; Faust, Syrányi, 1997; Iezzoni, 2008).

1.2 Sweet and sour cherries in Latvia and Sweden

Cherries are an important horticultural crop - approximately 2.2 million tons of cherries were produced worldwide in 2009 (Anonymous, 2010).

Around 40% of cherry world production originates in Europe. The biggest producers of cherries are Turkey, USA, Iran, Italy and Spain (Anonymous, 2010). Russian Federation and Poland are the largest sour cherry producers in the world (Anonymous, 2010). Neither Latvia nor Sweden are among the world's biggest producers of cherries (Table 1), but it is still an important fruit crop in both countries (Trajkovski 1996; Ruisa 1998).

Table 1. *Cherry production in Latvia and Sweden (Anonymous, 2010)*

Country	Rank in the world according growing area	Growing area, ha	Yield, kg/ha	Production, t
Latvia	54	224	11 517	258
Sweden	65	160	9 375	150

1.2.1 Introduction and growing of cherries in Latvia

Cherries have been cultivated in Latvia for more than 150 years. Predominantly forms of local origin have been grown, which have emerged over the past 100 years. Sour cherries have been grown more widely due to the better adaptation to the local climate than sweet cherries. Since the main limitation of sweet cherry growing is winterhardiness, they are distributed mostly in the western and southern part of country (Kurzeme, Zemgale) where some stands of wild or semi-wild sweet cherries have been found (Ābelnieks, 1956; Ruisa, Kaufmane, 2008).

It is assumed that sweet and sour cherries were introduced in Latvia by German nobility, they were planted in manor gardens and afterwards disseminated and formed the local cherry populations. Local sweet cherries usually have large, high trees with soft, juicy fruits and fruits of various colours, size and maturation time. The popular sweet cherry landrace 'Vidzemes Sārtvaidzis' has been developed from these local populations. The most widely grown and nowadays the most important sour cherry variety 'Latvijas Zemais' originated from the local cherries in the southern part of Latvia close to the Lithuanian border, therefore it has several synonyms – 'Lietuvas Zemais' ('Lithuania's Low'), 'Leišu ķirsis' (Lithuanian cherry) etc. Other local sour cherry varieties like 'Latvijas Augstais', 'Kazdangas' and 'Daugmales Stikla' have less importance in cherry growing (Ābelnieks, 1956; Ruisa, Kaufmane, 2008).

Later, during the development of cherry cultivation, varieties from Germany, France and Russia were imported and planted in Latvia. This is shown by nursery catalogues, which have offered sour cherry varieties such as ‘Kentish’, ‘Ostheimer Weichsel’, ‘von Hindenburg Exzellenz’, ‘Diemitzer Amarelle’, ‘Montmorency’, ‘Doppelte Natta’, ‘Plodorodnaya Mitchurina’, ‘Yubileinaya Mitchurina’ and sweet cherry varieties ‘Dönissen's Gelb Knorpelkirsche’, ‘Drogans Gelbe Knorpelkirsche’, ‘Elton’, ‘Fromm's Schwarze Herzkirsche’, ‘Hedelfinger Riesenkirsche’, ‘Imperatrice Eugene’, ‘Kassins Frühe’, ‘Napoleon’ and others. Most of the imported cultivars had insufficient winterhardiness and adaptation to the local climate. Harsh winters of 1939/40, 1955/56 and 1965/66, when the temperature fell to -40°C, and sometimes even to -44°C destroyed most of the sweet cherry orchards (Ruisa, Kaufmane, 2008). Therefore breeding of locally adapted cherry cultivars is a priority.

Sweet cherry breeding in the 1950’s was launched by Pēteris Upītis in Dobele (in the south of Latvia). The aim of his breeding activities was to obtain winter-hardy and productive cherry varieties. Breeding was based mostly on the local sweet cherries, especially those that grew around Dobele. P. Upītis made many expeditions around Latvia to collect the best local fruit trees and used them in cherry breeding. Since P. Upītis had also good cooperation with many breeders outside Latvia, some imported plant material and pollen were used in the crosses. At the same time in 1950’s sweet cherry breeding was started also by R. Āboliņš and A. Maizītis at the "Iedzēni" experimental station (in the central part of Latvia). Their breeding program was focused on increased winter hardiness and fruit season extension. To reach this goal breeders used crosses among locally well adapted varieties and introduced cultivars with high fruit quality (Blukmanis *et al*., 1997; Ruisa, 1998; Ruisa, Kaufmane, 2008). During the Soviet period, a wide spectrum of cultivars from other countries (Estonia, Lithuania, Russia, Byelorussia, Ukraine etc.) were tested and used in breeding.

The current sweet cherry breeding program at the Latvia State Institute of Fruit-Growing (LIFG, former Dobele Horticultural Plant Breeding Experimental Station) was started in early 1990’s by breeder Silvija Ruisa. The breeding program was based on the plant material of P. Upītis. In 1998 the cultivar ‘Aija’ was registered, but in 2002 the cultivars ‘Indra’ and ‘Jānis’ were recommended for cultivation in Latvia (Ruisa, Kaufmane, 2008).

1.2.2 Cherry breeding and growing in Sweden

The remains of old groves of sour cherries found at former convent areas confirm the presence of cherries in monastery orchards. There is also written information about a drink made from cherries in Sweden in the late 13th century. The drink, the name of which appears in different variations such as kirse-, kerss-, karsse-, kyrss-drank, seems to have been popular both in simple and noble households (Dahl, 1988).

King Gustav Vasa had an important influence on the development of fruit growing in Sweden and was interested in horticultural crops and facilitated the building of large orchards in the various royal palaces. There is information, that already in 1556 there were cherry groves and a cherry garden at Gripsholm. At about the same time or perhaps even earlier, there was an orchard of cherries and plums at Svartsjö, which was developed by planting young saplings taken from other locations. Swedish nobility followed the example of the king and started to cultivate gardens and cherry trees (Dahl, 1988).

One of the first reccomendations on cherry cultivation was produced by French garden architect André Mollet, who in 1651 gave a tutorial in the Swedish language about the orchard management and mentioned three groups of cherries: Griotiers, Bigareautiers and Guigniers (Dahl, 1988). More detailed information about the cherry varieties introduced in Sweden was presented in the publication "Handbook i svensk pomologi" (Eneroth, 1866 cited by Dahl, 1988), which mentioned the following cherry varieties: ‘Guines’, ‘Bigarreaux’, ‘v.d. Natt’, ‘Courtequeux’, ‘Moreller’, ‘Hvita spanska’, ‘Svarta d:o’, ‘Röda d:o’, ‘Prager-kirschen’, ‘Spanska Weichsel’, ‘Dubbla Maikirschen’, ‘Prinzenkirschen’, ‘Lödkörsbär’, ‘Klara’, ‘Oranienkirschen’, ‘Brussische Bruyne’, ‘Prinzessinkirschen’, ‘May Dukes’ and ‘Kentiska röda’. This shows an impressive number of varieties, which clearly demonstrate the efforts of owners to obtain almost everything available at that time. All mentioned varieties originated in other European countries: France, Germany, Great Britain, and Spain (Dahl, 1988).

The notable event for the development of agriculture and horticulture inter alia was the founding of the Royal Swedish Academy of Agriculture in 1811, which soon after foundation organized training in gardening as well as the use of nurseries. Significant areas of land in Djurgården in Stockholm were allotted to the Academy of Agriculture for the construction

of experimental fields in agriculture and horticulture. In 1862, the Institute of Horticulture at Alnarp was established, which acquired a very significant collection of fruit and ornamental plants. The first plant catalogue, issued for year 1862-1863 mentioned 19 cherry varieties. A very critical period for cherries was the turn of the century - around 1900 there occurred three years of severe devastation by all kinds of pests, diseases and inclement climatic conditions (Dahl, 1988).

In 1938 a governmental research organization, Statens Träsgårdsförsök (ST) was established and a number of foreign cherry varieties and rootstocks were tested. According to the census completed in 1943, the number of at least five year old cherry trees was approximately 0.930 millions, around 14 percent of the total number of fruit trees grown in Sweden. In 1963 Statens Träsgårdsförsök (ST) was incorporated in the Department of Pomology at the Royal Swedish Agriculture College, which in turn became a faculty of the Swedish University of Agricultural Sciences (SLU) in 1977.

A significant push in cherry crop development in Sweden was the initiative of the Association for Plant Breeding of Fruits and Berries in 1941 to start activities at the Balsgård Fruit Breeding Institute. The aim was the development of locally adapted cherry varieties, since foreign cultivars were not suitable for local growing conditions. In 1970 Balsgård was incorporated into the governmental research organization and in 1977 it became a part of SLU. As a result of the cherry breeding program, well known sweet cherry varieties ‘Almore’ and ‘Heidi’ (Trajkovski, 1982) and sour cherry cultivars ‘Kirsa’ and ‘Pernilla’ (Fernqvist, 1988; Trajkovski, Andersson, 1988), and ‘Nordia’ (Trajkovski, Andersson, 1992) were released and marketed at the SLU - Balsgård. Unfortunately cherry breeding programme is not active at the SLU – Balsgård at present. However, still today SLU - Balsgård holds the national genetic resources collection for fruit and berries and ensures their use in breeding and research as well as continues the germplasm investigations.

2 Importance of plant genetic resource characterization for maintenance and use

Plant genetic resource (PGR) activities comprise collection, maintenance, characterization and evaluation of the genetic diversity within a crop, and use in breeding and research. All mentioned activities are interdependent and should be performed for sustainable PGR conservation. Characterization and evaluation is an important and critical connection point for all other PGR activities, because it will also determine the success of collection, maintenance and utilization efforts. A high level of characterization and evaluation will improve approaches for more secure PGR conservation; increase the level of potential user informativeness and facilitate utilization in breeding and research.

Knowledge about the amount, extent and distribution of genetic variation is the key for developing effective maintenance and management strategies of PGR. It is particularly important for the fruit crop genetic resources, which are usually preserved in field collections with safety duplications for each accession. Maintaining of more than one plant for each accession as well as duplication of germplasm in another location increases the security of the collection, but also the costs related to the preservation. Therefore it is necessary to develop PGR collections including the maximal possible genetic diversity of certain species as well as excluding possible duplications (Frankel and Soulé, 1981; Trajkovski, Hjalmarsson, 2007).

PGR characterization and evaluation data could be used in three main maintenance activities: evaluation of genetic diversity, proper identification of germplasm and control of maintenance.

Application of various characterization methods provides information about the genetic diversity of PGR collections, the degree of similarity among individual genotypes within an accession or among accessions in the collection, and the amount of genetic variation in the collection and it's partitioning among accessions or among genotypes. Available genetic diversity information can reveal gaps of genetic diversity in the collections, which could be filled by targeted collection missions, and can be used in the optimization of existing germplasm collections. One of the approaches for PGR collection optimization is the establishment of core collections – subsets of particular crop genetic resources representing maximal genetic diversity of the entire collection (Brown, 1995; van Hintum *et al*., 2000). Such representative set of accessions could only be selected using various characterization approaches (morphological, biochemical, molecular, statistical etc.).

Accurate identification of plant material in the PGR collection also has high importance in the PGR maintenance activities. During the process of collection development there is considerable accumulation of documentation errors, which also leads to the duplication of accessions during collection renewal and loss of particular germplasm. Such errors are impossible to detect without detailed characterization data about the collection (Gilbert *et al*., 1999; Engels, Visser, 2003). Implementation of DNA based genotyping in PGR characterization and development of DNA fingerprints for all accessions has especial importance in the confirmation of the identity of each accession in the collection. Obtained accession-specific band patterns or fingerprints can serve as standard profiles for identifying a particular accession (Karp *et al*., 1997; Hodgkin *et al*., 2001; Weising *et al*., 2005). Application of DNA based fingerprinting methods is very important for fruit crops, because phenotypic characters are generally influenced by environment and the growth stage of the plant. Thus they can overcome the necessity for long and expensive field evaluations to obtain satisfactory phenotypical data for accession identification. This would reduce the investment necessary for the assessment of a PGR accession candidate (Laurentin, 2009).

The objective of *ex situ* conservation is to maintain the accessions of particular crop without change in its genetic constitution (Frankel and Soulé, 1981). Therefore a very important issue and challenge of PGR conservation is to minimize the possibility of changes occurring through mutation, selection, random drift or contamination. The characterization

and evaluation data define the point of reference for an accession and could be used for testing of stability and confirmation of identity. Implementation of new methods for maintenance of vegetatively propagated PGR as *in vitro* cultures and by cryopreservation increases the possibilities for control of genetic stability, which could be ensured by characterization information, especially molecular characterization data (Reed *et al*., 2004; Panis, Lambardi, 2006).

Conservation of PGR itself has little value without utilization of the stored plant material. The main areas of PGR utilization are breeding and research. Effective use of PGR in breeding is mainly dependent on the level of germplasm characterization. Breeders are usually looking for well evaluated plant material containing desirable agricultural traits (e.g. disease resistance, winterhardiness, specific tree or fruit traits etc.). Application of molecular markers linked to loci controlling particular traits could also facilitate the use of particular germplasm in breeding, especially MAS (Marker Assisted Selection) (Spooner *et al*., 2005; Ferreira, 2006). PGR collections usually contain a large number of locally adapted landraces and wild relatives, which contain many useful characters that could be introduced into crop cultivars. The effectiveness of introduction is dependent on the degree of relatedness of the landrace or wild species with the target crop. Detailed characterization and evaluation of such germplasm could facilitate its application in breeding (Hodkin, 1997).

PGR characterization and evaluation data could be used in different research activities and provide information about the effect of human selection in particular environments as well as the history of crop development in different locations, using comparison of characterization and evaluation data from different germplasm collections. Information acquired using molecular markers allows worldwide population studies using PGR collections and comparison of plant material in different regional collections. Available PGR characterization data could also be used in research on development of new characterization and evaluation approaches.

3 The aim, main objectives and hypothesis

The aim of the investigations of this thesis was to characterize the sweet and sour cherry germplasm in the Latvian and Swedish collections using complex description and evaluation approaches.

The main objectives of the studies were to show how the use of different and complex germplasm characterization approaches can be applied

- in the characterization of particular fruit crop genetic resources to collect genetic diversity,
- for identification of accessions in the genetic resources collection and detection of internal relationships,
- for facilitation of characterized genetic resources utilization in the breeding.

Hypothesis of thesis - Latvian and Swedish sweet and sour cherry genetic resources collections reflect the introduction, breeding and growing history of cherries in both countries; complex application of phenotypical description, genotyping using small number of unspecific molecular markers and gene specific markers ensure that cherry genetic resources are not duplicated in identification and for collection optimization.

4 Phenotypical diversity of Latvian and Swedish cherry genetic resources

Latvian and Swedish cherry genetic resources representing landraces, old and modern cultivars and breeding material were investigated. Collections have been evaluated using set of morphological, phenological, fruit quality traits as well as disease resistance and hardiness evaluation. The characterisation was based on UPOV (UPOV, 1995) and IPGRI (IPGRI, 1985) descriptor lists supplemented by local reference cultivars as well as by morphometric measurements.

4.1 Phenotypical characterization of Latvian and Swedish sweet cherry collections

Forty nine and ninety one sweet cherry accessions from the Latvian and Swedish collections respectively were characterized using morphological, phenological and agronomical trait descriptors (Paper II). Principal coordinate analysis (PCA) of phenotypical data gave a general impression about the overall variability in collections and revealed traits with high impact on the determination of similarity and relatedness.

The most useful traits for both sweet cherry collections were fruit and tree architecture traits, which were represented in the first PC with a high percent of variability. Because those traits are also important in breeding, more attention should be paid to them during further evaluation activities in sweet cherry collections. Fruit traits are especially important, having high heritability (Hjalmarsson, Ortiz, 2000).

The level of similarity between accessions revealed by cluster analysis was relatively high, especially for the Swedish collection. Nevertheless, cluster analysis discovered grouping of accessions within the collections. Although analysis was based only on phenotypical data, the results were supported by pedigree data, which was available for the sweet cherry collection in Sweden. Particular accessions showed a distinct separation from the other groups (Fig. 1 and 2). In the Swedish collection it was cultivar 'Regina', characterized by a spur tree type. Cultivars 'Leningradskaya Czornaya' from the Latvian collection showed separation based on small leaf size. As it is already mentioned in another publication (Peeters, Martinelli, 1989), cluster analysis is useful for grouping of accessions to facilitate an understanding of the internal structure of germplasm collections. It was especially important for the Latvian sweet cherry collection, because of the lack of information on origin of most of the accessions.

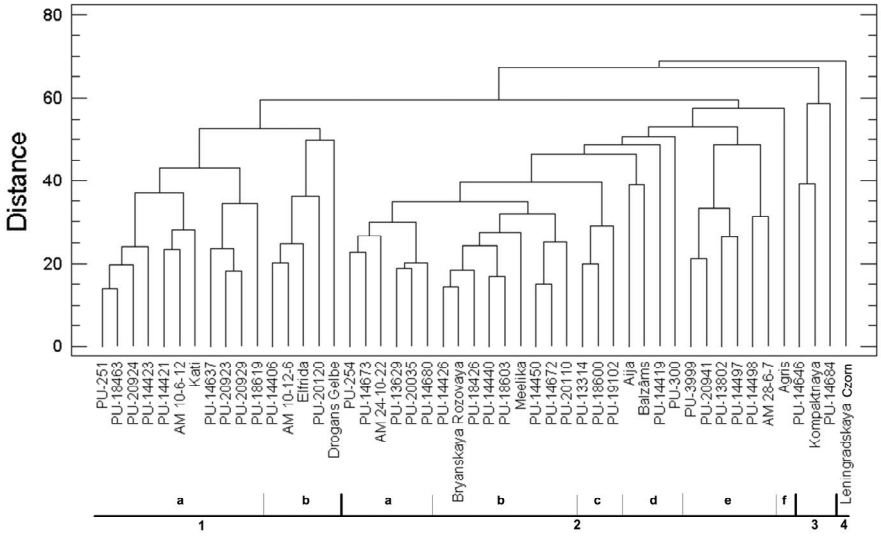

Figure 1. Hierarchical analysis dendrogram obtained by the Group Average Method (Squared Euclidean) using morphological traits of sweet cherry accessions of the Latvia State Institute of Fruit-Growing (Fig. 1, Paper II)

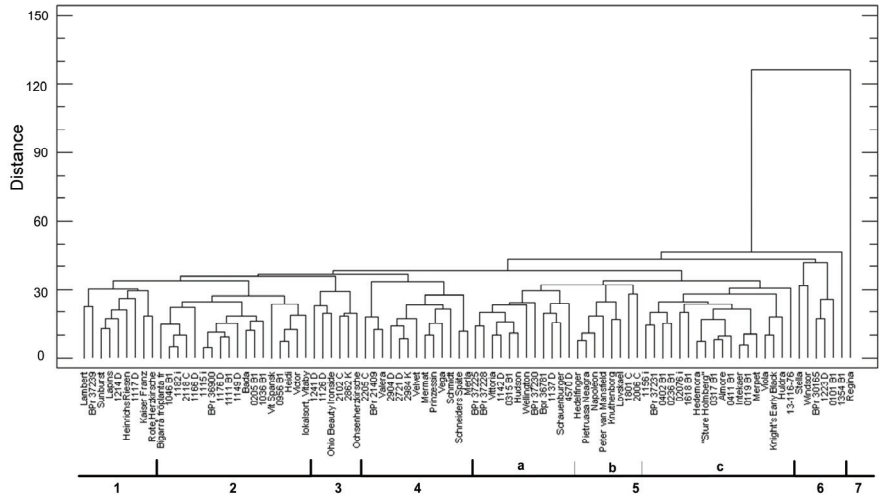

Figure 2. Hierarchical analysis dendrogram obtained by the Group Average Method (Squared Euclidean) by using morphological traits of the of sweet cherry accessions of the Division of Horticultural Genetics and Plant Breeding at Balsgård, Department of Crop Sciences, Swedish University of Agricultural Sciences sweet cherry accessions (Fig. 2, Paper II)

4.2 Phenotypical characterization of Swedish sour cherry collection

Detailed description of accessions and application of multivariate statistics to characterization data revealed the most useful traits for the characterization of sour cherry germplasm: fruit features, tree architecture traits and susceptibility to diseases (Paper V). High heritability and usefulness of fruit traits has also been found for sweet cherries (Hjalmarsson and Ortiz 2000). The PCA ordination of phenotypical traits did not successfully find complex factor gradients of accessions in the sour cherry collection, as the first four PC explained only 50% of the total variation. This might be due to a common gene pool of accessions. A high degree of relatedness of accessions in this collection is confirmed by pedigree data. Phenotypical similarity of breeding material is mostly a result of breeding targeted towards valuable traits.

Clustering of sour cherry accessions according to the evaluated phenotypic traits was quite clear and four distant clusters were detected. The clustering

results did not reveal good separation of sour cherry accessions on the basis of West-European and Middle-Russian sour cherry types, as designated by Kolesnikova (1975). However, varieties from Russia and Eastern Europe, as well as hybrids developed using these varieties, were located mostly in Clusters 1 and 2, while the most typical Western-European cultivars (e.g. 'Heiman's Rubin', 'Montmorency', 'Ostheimer') were located in Cluster 4 (Fig. 3). Clustering according to the ecogeographical grouping of sour cherries likely did not occur because several varieties and hybrids in the SLU-Balsgård collection were acquired from crosses between representatives of these groups. These results confirm the arguments against the existence of ecogeographical sour cherry groups proposed by Hillig and Iezzoni (1988) and suggest that there is only a range of morphological variation. Correspondence between grouping results and pedigree data was found, these data analysis approaches can also probably be used to estimate the relatedness of accessions to plant material of unknown origin. This is particularly important for Baltic and Nordic sour cherry landraces, as there is little information about their origin.

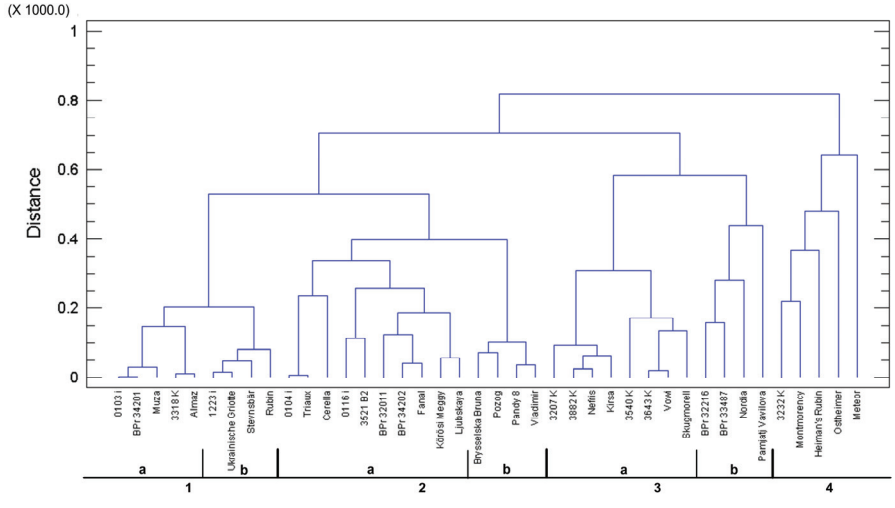

Figure 3. Hierarchical analysis dendrogram obtained by the Ward's Method (Squared Euclidean) using morphological traits of sour cherry accessions of the Division of Horticultural Genetics and Plant Breeding at Balsgård, Department of Crop Sciences, Swedish University of Agricultural Sciences (Fig. 1, Paper IV)

5 Molecular diversity of Latvian and Swedish cherry genetic resources

The characterization of sweet cherry PGR collections requires fast, reliable, cost effective and objective methods to identify and describe plant material. Unfortunately, even using a high number of detailed phenotypical descriptors, in some cases it is difficult to describe accessions due to the influence of environmental factors and growing conditions. Therefore DNA based technologies have been implemented in the characterization of germplasm. In general, two main groups of molecular markers could be utilised for PGR characterization:

- gene specific markers, subsequently applicable for Marker Assisted Selection (MAS),
- markers for detection of the structure of genetic resources collections and relatedness of available plant material.

5.1 Self-incompatibility in Latvian and Swedish sweet cherries

Sweet cherry (*P. avium* L.) is a typical out-crossing species with a mono-factorial and multi-allelic gametophytic incompatibility system (Crane and Lawrence, 1929; Lewis, Crowe, 1954; Tehrani, Brown, 1992). In commercial sweet cherry orchards, suitable pollinator cultivars must be planted to ensure fertilization and subsequent fruit development.

In many plant species, self-incompatibility is a mechanism that overcomes inbreeding depression and facilitates out-crossing. In most cases gametophytic self-incompatibility is governed by a single *S*-locus with multiple alleles (de Nettancourt, 1977). If a pollen *S*-allele matches the *S*-

alleles of the pistil, the growth of the pollen tube is arrested in the style, inhibiting fertilization. In Rosaceae, the recognition reaction involves an allele specific ribonuclease (RNase) (Sassa *et al.,* 1996) expressed in stylar tissue and an F- box pollen component (Entani *et al*., 2003; Ushijima *et al.*, 2003; Ikeda *et al*., 2004; Yamane, Tao, 2009).

Pollen-incompatibility groups and *S*-alleles have been determined for many sweet cherry cultivars using controlled crosses and evaluation of fruit set numbers as well as cytoembryological assessments of the pollen tube growth within the style. Based on crossing experiments, six widely distributed sweet cherry *S*-alleles were determined (*S1* to *S6*) (Crane, Brown, 1937; Sonneveld *et al.,* 2003).

In recent years, molecular methods based on PCR have been developed to facilitate analysis of allele type (Tao *et al.,* 1999; Hauck *et al.,* 2001; Sonneveld *et al*., 2001; Wiersma *et al*., 2001; Sonneveld *et al.,* 2003). Several new *S*-alleles have been discovered by molecular analysis. Currently, 16 sweet cherry *S*-alleles have been identified, and 28 incompatibility groups have been confirmed (Tobutt *et al.* 2004).

The cross-incompatibility of Latvian and Swedish sweet cherries was not previously tested using molecular markers. In our early investigations some controlled crosses and subsequent cytoembriological investigations have been done on six selected sweet cherry cultivars and their cross-compatibility was detected (Lacis *et al*., 2000). Unfortunately these applied methods are time and labour consuming and also are highly influenced by environmental factors, and are not suitable for large scale self-incompatibility investigations. Therefore introduction of molecular genotyping is essential. This study represents the first comprehensive *S*-allele determination for the Latvian and Swedish sweet cherry collections using *S* gene specific molecular markers, which will be useful in breeding programmes in the planning of crosses, for conservation of alleles and population genetics studies (Paper I).

Genotyping was performed using allele specific markers for the first six *S*-alleles, identified by crossing experiments. All tested *S*-alleles were found in both sweet cherry collections (Fig. 4). Significant allele frequency differences between collections were found only for alleles *S4* and *S5*. The Swedish collection contains mainly accessions from the Swedish sweet cherry breeding programme, in which three sweet cherry cultivars

'Heinrichs Riesen' (*S3S4*), 'Kaiser Franz' (*S3S4*), and 'Schmidt' (*S2S4*) were the most common parents. The presence of *S4* in all the parents and absence of *S5* is consistent with the dominance of *S4* and the lower frequency of *S5* in the Swedish germplasm. The acquired *S*-allele frequency data were compared with previous work on *S*-allele genotyping, conducted by various authors (Bošković and Tobutt, 2001; Hauck *et al*., 2001; Sonneveld *et al*., 2001; Wiersma *et al*., 2001; Sonneveld *et al*., 2003 and others) summarized by Tobutt *et al*. (2004).

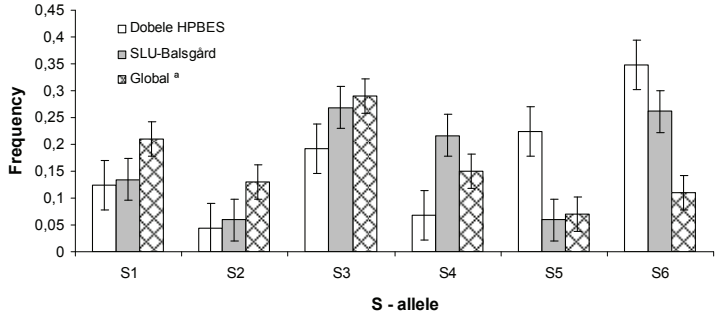

Figure 4. S-allele frequencies in accessions of the Dobele HPBES and the SLU-Balsgård sweet cherry collections
a - allele frequencies calculated from S-allele identification data published by Tobutt et al. (2004). Standard error bars shows the 0.95 confidence level ($P < 0.05$) (Fig. 1, Paper I)

One interesting question raised during this investigation was the reason for the high frequency of the *S6* allele in Latvian and Swedish collections (Fig. 4). Could it be explained by its presence in the founder clones that were widespread in Latvia and Sweden or as a result of linkage of alleles with adaptation to the northern European conditions? Generating insights to this question may be possible as *S*-allele genotypes for accessions in more sweet cherry collections become available. Additional genotyping of accessions with exactly known pedigrees or the use of mapping populations with known *S6* allele carriers may elucidate inheritance patterns of this allele.

These results discovered differences in *S*-allele and *S*-genotype frequency compared to other *S*-allele studies, suggesting a particular distinctness of the two tested germplasm collections. Latvian and Swedish sweet cherry collections have a common feature, in that they contain a high proportion of accessions adapted to north central European growing conditions, which is not typical for most of the sweet cherry material analyzed to date.

5.2 Molecular characterization of Latvian and Swedish cherry genetic resources collections

Several types of molecular markers have been utilized in the characterization of cherry genetic resources. One of the most widely used markers are SSRs or microsatellites (Wunsch and Hormaza, 2002a; 2002b; Schueler *et al*., 2003; Struss *et al*., 2003; Canli *et al*., 2004; Kacar *et al*., 2005; Höltken and Gregorius, 2006; Pedersen, 2006). The characterization of Latvian and Swedish cherry genetic resources using SSR molecular markers was undertaken to support the previous phenotypic evaluation. A very high level of polymorphism and successful application in sweet cherries (Kacar *et al*., 2005; Pedersen, 2006) and sour cherries (Cantini *et al*., 2001; Pedersen, 2006) was found for SSR markers PceGA25 (developed for *P. cerasus* L.), PMS3 and PMS49 (developed for *P. avium* L.). These three highly informative markers were used for genetic diversity evaluation of Latvian and Swedish sweet cherry genetic resources collections.

5.2.1 Molecular diversity of Latvian and Swedish sweet cherry accessions

The SSR markers used in this work (Paper III) identified a higher number of alleles in sweet cherry than any other set of SSR markers in previous studies (Wünsch and Hormaza, 2002b; 2004; Schueler *et al*., 2003). This difference could be due to the larger number of accessions analysed in our experiment and their potentially greater genetic diversity. The utilised polymorphic primer pairs allowed the differentiation of 90 of the 126 sweet cherry accessions (71.4%). Accession unable to be differentiated using these markers probably shared a common origin, and in fact some of these undifferentiated genotypes represent results of particular breeding programs and according to pedigree data are either full- or half-sibs. The Latvian sweet cherry collection had a slightly higher proportion of unique genotypes than the Swedish collection (74.1% and 70.6%, respectively), indicating higher genetic diversity. This was also confirmed by the PCA of genotypic data, which showed a wider genetic distribution of Latvian sweet cherry accessions (Fig. 5).

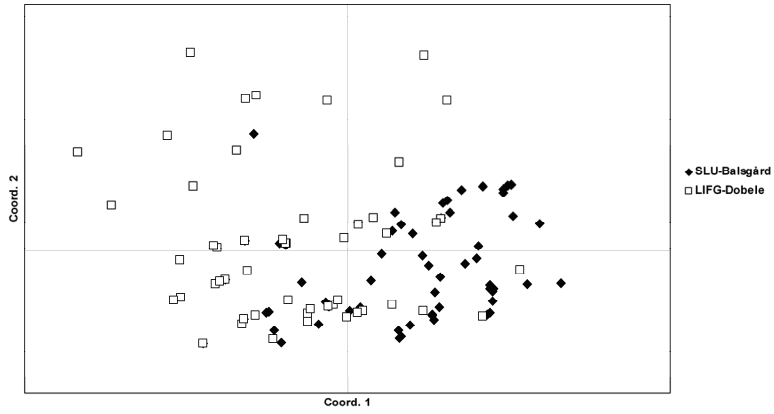

Figure 5. Distribution of accessions of the Latvian and Swedish sweet cherry collections according to values of the two main principal coordinates calculated from allele composition at three SSR loci. (Fig. 4, Paper III)

5.2.2 Molecular diversity of Latvian and Swedish sour cherry genetic resources

The analysed combination of three polymorphic primer pairs allowed the differentiation of all of the 41 sour cherry accessions (Paper VI). Four sour cherry cultivars (‘Kirsa’, ‘Ljubskaya’, ‘Nordia’ and ‘Oblachinska’) were present in both collections and had completely identical allele profiles. This proves the repeatability and transferability of the SSR analysis method, because plant material of the common sour cherry cultivars has the same origin – the accessions in the LIFG sour cherry collection were acquired from the SLU-Balsgård collection.

The number of different SSR alleles identified in the Latvian and Swedish sour cherry collections as well as characterization of population parameters, indicates that these collections contain a high level of genetic diversity and represent valuable material for breeding and research. Genetic similarity analysis showed higher genetic similarity between Baltic and Scandinavian sour cherry varieties, than between Baltic and Central and Eastern European (Hungarian, Polish and Russian) varieties. Baltic (Latvian and Lithuanian) landraces, included in the investigation showed close genetic relatedness with Scandinavian landraces, e.g. Latvian landrace ‘Latvijas Augstais’ showed high relatedness with Swedish landrace ‘Surkörsbar från H.Björkman’ as well as with Danish landrace

'Stevnsbær' (Fig. 6). The Lithuanian landrace 'Jonisku' showed high genetic relatedness with Swedish cv. 'Skuggmorell'. These tendencies in the sour cherry genetic relatedness indicate the possible common historical origin of the Baltic and Scandinavian landraces.

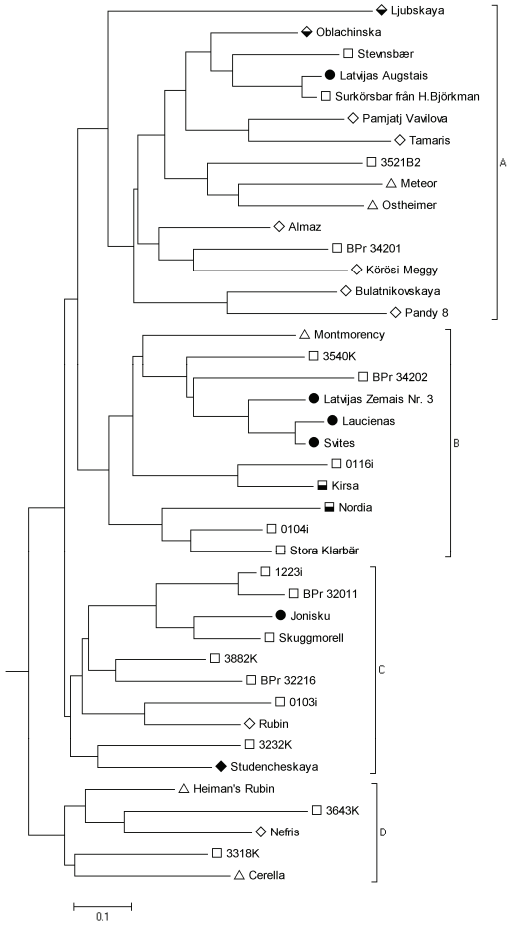

Figure 6. Distribution of accessions in the LIFG and SLU-Balsgård sour cherry collections using three SSR primer pairs based on Nei and Li/Dice similarity index and Neighbor-Joining clustering method.
Origin: ○ - Baltic, □ - Nordic, ◇ - Central European, △ - Other (incl. Western European, USA and unknown origin). Empty symbols represent accessions of the SLU-Balsgård collection, filled - accessions of the LIFG collection, half-filled – accessions presented in both collections. (Fig. 2, Paper VI)

6 Methodological aspects of cherry genetic resources characterization

The application of different methods for characterization of genetic resources increases the value of the obtained information, but at the same time creates problems with interpretation of results as well as comparison of results among investigations. Phenotypical and molecular characterization has been applied for the Latvian and Swedish cherry genetic resources, which raised some methodological questions.

6.1 Comparative analysis of different marker detection methods

Analysis of published and experimental data (Cantini *et al*., 2001; Kacar *et al*., 2005; Pedersen, 2006) discovered high variation in polymorphism level of sweet cherries using the same SSR markers, utilising different fragment separation and detection methods. Therefore it was troublesome to compare different results and make valid decisions about the most appropriate SSR markers. Due to different SSR fragment detection methods and genotyping differences it is complicated to combine data from different laboratories and projects for genetic diversity evaluation. A similar problem was encountered in the genotyping of Latvian and Swedish sweet cherry genetic resources, which was started using SSR fragment detection by PAGE (polyacrylamide gel electrophoresis) and continued by ASCE (automated sequencer capillary electrophoresis).

Utilisation of different SSR detection methods caused differences in the number of putative alleles and SSR marker characteristics (Paper IV). SSR marker analysis utilising PAGE revealed a higher number of putative

alleles in comparison with ASCE. Using PAGE for SSR marker analysis, some discrepancies in sweet cherry accession pedigree were also observed, whereas the ASCE data set was more in accordance with reported pedigree data.

DNA fingerprinting data obtained by different detection methods showed slightly different phylogenetic UPGMA dendrograms. There were no identical groups among dendrograms calculated from both detection methods data. Since clustering analysis is greatly affected even by small differences in sample allele composition, slight allele sizing shifts in the accession genotypes can cause significant changes in the clustering. Overestimated genetic differences could be also a reason for troublesome accession group determination, due to incorrect assessment of genetic relatedness. Therefore the applied SSR detection method should be taken into account in the comparison and analysis of genetic relatedness data. In principle, the data acquired by different detection methods should not be used in direct comparisons, as this could lead to inaccurate or even incorrect conclusions.

The comparison of two SSR allele detection methods (ASCE and PAGE) revealed that more precise genotyping could be acquired using ASCE. These results did not show a similar level of ladder-like stutter allele patterns, frequent for PAGE and also had a higher correspondence with known pedigree. Therefore this SSR allele detection method is suitable for cultivar genotyping and identification purposes. Utilising PAGE detection, a higher number of putative alleles was detected, possibly due to detection biases and manual scoring errors, which leads to overestimation of polymorphism level and subsequent genetic diversity and relatedness characterisation. This should be taken into account in the comparison and discussion of research results obtained using different SSR analysis methods.

6.2 Comparison of phenotypical and molecular characterization approaches

The Latvian and Swedish sweet and sour cherry genetic resources collections were characterized using three SSR markers which revealed a high level of polymorphism and successfully characterised both cherry species. Both cherry genetic resources collections were also evaluated and

characterized using phenotypic description, which shows slightly different relatedness of accessions than molecular data analysis, which could be influenced by the level of correspondence between data types, insufficient number of SSR markers or method of marker selection (Paper VII).

Standardized data analysis methods (pairwise Euclidean distance and Neighbour-Joining clustering) were applied to a representative set of sweet cherry accessions to evaluate correspondence between phenotypic and molecular data as well as the impact of SSR marker number and selection method on this correspondence. Three sets of SSR markers were tested: the initial three marker set selected from the published data, a set of eleven SSR markers and a set of three highly polymorphic SSR markers selected from the experimental data acquired using the eleven SSR markers. Comparison of distance matrices by the Mantel test showed low correlation between phenotypical and all molecular data sets ($r = –0.0956$ for the initial set of three SSR markers; $r = –0.0959$, for the experimentally selected three SSR markers and $r = –0.0460$ for the total set of eleven SSR markers, $p < 0.001$). Similar results have also been reported in other investigations on different crops (Beyene *et al*., 2005; Fufa *et al*., 2005). Whereas correlation between all tested SSR marker methods were high: ($r = 0.6415$ for the initial set of three SSR markers and eleven SSR markers, $r = 0.5852$ for the three experimentally selected SSR markers and eleven SSR markers, $p < 0.001$). This indicates that the information about genetic relatedness revealed by SSR markers has no direct correspondence with relatedness information detected by phenotypic characterization, without reference to the number of analysed markers and/or method of marker choice. Therefore, using phenotypic description in combination with a small set of highly polymorphic SSR markers could be sufficient for the preliminary description and determination of internal genetic relatedness of sweet cherry genetic resources collections. The possibility of replacing a large number of SSR markers with a small, well described and highly polymorphic marker set has already been discussed for several crops (Bianchi *et al*., 2004; Karaagac *et al*., 2010).

7 Concluding remarks and future perspectives

The availability of information about plant germplasm becomes more and more important for the future preservation and sustainable use of genetic resources. Investigations of Latvian and Swedish cherry genetic resources collections resulted in the identification of accessions, systematic evaluation of available germplasm and valuable information for sweet and sour cherry breeding programs, thus promoting their utilization. Phenotypical and molecular characterization revealed high phenotypic and genetic diversity of analysed germplasm as well as the relatedness of Baltic and Scandinavian sweet and especially sour cherry landraces that indicate a possible common historical origin. Local Baltic-Scandinavian cherry varieties were also differentiated from other cherry germplasm by the frequency of self-incompatibility alleles detected using gene specific molecular markers. The self-incompatibility allele information gained from this study will be as well useful in breeding programmes for the planning of crosses and conservation of alleles. The use of different characterization methods of cherry genetic resources also facilitated methodological observations, applicable to cherry germplasm characterization. It was concluded that thorough evaluation of genetic diversity and internal structure of cherry genetic resources collection should include both phenotypic and molecular characterization.

Introduced and adapted characterization approaches and methodological considerations will facilitate further characterization of cherry genetic resources and could be applied to broader plant material, especially in case of accessions originated in Baltic and Scandinavian regions. There could be considered also the possibility of knowledge transfer to the investigations

on other *Prunus* species. Available information on genetic structure of sweet and sour cherry genetic resources collections as well as accession identity profiles will be useful for the improvement of maintenance strategies to ensure secure and cost effective conservation as well as increase the germplasm potential utilization in breeding. Acquired genetic information of local cherry will broaden the utilization of cherry genetic resources for breeding in Baltic States. Since breeding of cherries in Nordic countries is limited or completely stopped, common Nordic – Baltic and breeding programmes could be initiated and facilitated based on the acquired information about genetic diversity, structure as well as relationships of available accessions.

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