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Genetic diversity in Swedish and Finnish heirloom apple cultivars revealed with SSR markers

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ABSTRACT

A set of 85 heirloom apple cultivars aimed for long-term preservation in two germplasm collections in Sweden and Finland was evaluated with 8 SSR primer pairs to evaluate genetic diversity and genetic relatedness. An additional set of 16 European cultivars was included for comparison. The eight SSR primer pairs amplified 9 loci and 105 alleles. Genetic analyses performed by MDS indicated some differentiation between Swedish and Finnish cultivars, with European cultivars intermixed with the Swedish. The existence of three groups was, however, indicated by a Bayesian model-based clustering. One of the groups was clearly dominated by Swedish cultivars and another by Finnish. The third group included almost equal proportions of representatives from all three areas. The obtained results confirmed the genetic distinctness of Finnish apple cultivars, which can be explained by climate adaptation and admixture with a Russian gene pool.

1. Introduction

Apple (*Malus* × *domestica* Borkh.) is one of the economically most important fruit crops in the temperate zone, and is the fourth most important fruit crop world-wide (Gharghani et al., 2009). Sweden and more so Finland are on the northern edge of commercial apple growing. In both countries, a harsh climate with fluctuating spring and winter temperatures put special demands on climate adaptation of apple cultivars. Cultivars suitable for cultivation in the Nordic climate should have winter-hardiness, frost tolerance and short maturing period. Furthermore, high relative humidity and high amount of precipitation create favourable environments for fungal diseases.

In Sweden, apples were introduced around the 12th century from Central and Southern Europe and grown mainly in monastery orchards. Apple production for commercial sale started in 16th century. It relied mainly on foreign cultivars from e.g. Germany and England, as well as local cultivars selected among chance seedlings due to their large and tasty fruits. Modern plant breeding, based on controlled crosses, has been undertaken in Sweden since 1920, and both foreign and indigenous cultivars have been used as parents for producing new cultivars adapted to the Nordic climate.

The first apple trees were imported to Finland around 16th century, but it took a couple of hundred years before apple became a commercial crop. Fruit cultivation was attempted several times, but occasional severe winters caused major setbacks. Cultivars were brought to Finland from all neighbouring countries: Sweden, Estonia and Russia as well as from Denmark and Germany. Seedling plants originating from imported cultivars were selected as local landraces. In the beginning of 20th century apple material was imported from more distant locations, especially many Canadian cultivars were tested at that time (Kinnanen and Antonius, 2006; Krannila and Paalo, 1997). As in Sweden, the modern apple breeding was based on local hardy material, that was combined with improved foreign cultivars.

Plant breeders are constantly looking for new sources of genes, which confer climate adaptation, resistance to pests and diseases and tolerance to abiotic stresses. Modern resistant cultivars, but

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also some old, locally adapted cultivars serve as sources of desirable genes.

In Sweden and Finland, as in some other European countries, conservation of apple genetic resources is directed mainly to so-called heirloom cultivars, i.e. old and presumably indigenous cultivars (Kinnanen and Antonius, 2006; Nybom and Garkava-Gustavsson, 2009). Many of these cultivars have arisen as open-pollinated seedlings and their origin is often unknown. Furthermore, the choice of cultivars for preservation is seldom based on proper genetic analysis, and no attempts are made to maximise genetic diversity.

In Sweden, the preservation of apple genetic resources is presently managed by the 'National Program for Diversity of Cultivated Plants', which has appointed a set of 220 mandate cultivars including both indigenous cultivars which have been bred, named, propagated and marketed in Sweden as well as some foreign cultivars with a long history of cultivation in Sweden (Hjalmarsson and Wallace, 2004). These mandate cultivars are now conserved mainly in 13 clone archives located at outdoor museums and other public places. The largest apple collection in Sweden is, however, the research- and breeding oriented material grown at Balsgård, Swedish University of Agricultural Sciences, situated in the southernmost province Skåne. This collection holds about 600 cultivars including about 100 mandate cultivars.

The Finnish apple germplasm collection is managed by MTT Agrifood Research Finland. The field collection is located in Southern Finland, in the municipality of Kaarina. 197 cultivars have been preliminary listed as the Finnish mandate collection, based on their documented importance for Finnish apple cultivation in literature references. However, some of these cultivars have not been recovered in present-day inventories, and many foreign cultivars are present in the collections of the original countries. Therefore the final list of cultivars for long-term conservation is still under development (Kinnanen and Antonius, 2006).

Proper characterisation of germplasm collections is of outmost importance for more effective management and utilisation of genetic resources in research and breeding.

Molecular markers, especially Simple Sequence Repeats (SSR), are commonly used for quantification of the amount of diversity in national collections of various plant crops (Nybom and Weising, 2010). SSR markers have thus been widely used for evaluation of genetic diversity and genetic relatedness in apple germplasm collections (Guarino et al., 2006; Pereira-Lorenzo et al., 2007; Garkava-Gustavsson et al., 2008; Farrokhi et al., 2011; Lacis et al., 2011; Naseri et al., 2011; Patzak et al., 2012).

Provided that the same SSR primers are used and that allele sizing is standardised, data obtained in different studies can be pooled, thus allowing the scientists to estimate not only diversity but also the degree of differentiation among germplasm collections preserved in different national gene banks (Garkava-Gustavsson et al., 2012). Furthermore, the characterisation of collections in different countries using a common set of SSR markers allows to clarify doubtful cultivar identification (Sehic et al., 2012). Bayesian model-based structure analysis has been used as a tool to extrapolate the level and/or the presence of admixtures between collections. Furthermore, information about the structure and pattern of genetic diversity within and among different collections of apples will also be useful in developing further conservation programmes.

The objectives of this study were: (1) to evaluate genetic diversity in the subset of Swedish and Finnish heirloom cultivars in comparison to European cultivars; (2) to evaluate genetic relatedness among Swedish, Finnish and European cultivars; (3) to estimate structure of genetic diversity in the studied germplasm.

2. Materials and methods

2.1. Plant material

Swedish (SWE) and Finnish (FIN) national apple collections were characterised with SSR markers. In the present study, we used a subset of old diploid heirloom cultivars: 43 SWE and 42 FIN. In addition, a group of cultivars originating from other European countries (EUR), in total 16, grown in the germplasm collection in Sweden, was included for comparison (Table 1).

In both countries, newly expanded leaves of analysed cultivars were collected in April–May and stored at −80 °C until use.

2.2. DNA isolation and PCR amplification

Genomic DNA was isolated from approx. 100 mg of leaf tissue using the Qiagen Dneasy™ Plant Mini Kit and following the manufacturer's protocol. In total, 11 SSR primer pairs were used in Sweden and 10 primer pairs were used in Finland to characterise each of the national collections, while 8 primer pairs from a 'standard set' defined by the European working group on apple genetic resources (Guarino et al., 2006; Garkava-Gustavsson et al., 2008), were in common: CH01h02, CH02C06, CH02c09, CH02c11, CH02d08, CH04c06, CH04e05 and COL.

PCR amplifications and allele sizing of FAM and HEX labelled amplification products for SWE and EUR cultivars were performed at SLU-Balsgård in Sweden as in Garkava-Gustavsson et al. (2008). Three reference cultivars, 'Discovery', 'Golden Delicious' and 'Guldborg', were included in each PCR run.

PCR amplifications and allele sizing for FIN cultivars were performed at MTT, Jokioinen in Finland. Reactions and amplifications were conducted as in Antonius-Klemola et al. (2006). Labelled (HEX, TET or FAM) amplification products were detected by capillary electrophoresis using MegaBACE™-1000 DNA sequencer (Amersham Biosciences Ltd., UK), with ET400-R as an internal standard. 'Discovery' and 'Summerred' were included as reference cultivars in each run.

To enable the combining of the two separately produced data sets (in Sweden and Finland, respectively), 12 common cultivars ('Discovery', 'Golden Delicious', 'Gyllenkrok's Astrakan', 'Katja', 'Rödluvan', 'Signe Tillisch', 'Silva', 'Snövit', 'Suislepper', 'Summerred', 'Sävstaholm' and 'Åkerö') were included in the analysis of cultivars from both countries and used for correction of allele sizes. However, these cultivars, except 'Sävstaholm' and 'Åkerö', were not included in the statistical analyses.

2.3. Data analyses

All genetic analyses were performed in an Excel (Microsoft Excel v. 2007, Redmond, USA) VBA application package developed at the Institute of Agriculture and Ecology, University of Copenhagen. Nei's gene diversity (Nei, 1973) for each marker was calculated according to the formula: $1 - \sum_i^n p_i^2$ where p_i is the frequency of the single allele of ith individual and n is the number of individuals. The PIC was calculated using the formula: $\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$ where p_i is the frequency of the allele in the ith individual and n is the number of alleles per marker (Botstein et al., 1980). The genetic distance was calculated using modified Roger's distance (Wright, 1984) according to the following formula: $(1/\sqrt{2m})\sqrt{\sum_{i=1}^{n}\sum_{j=1}^{nj}(p_{ij} - q_{ij})^2}$ where p and q are allele frequencies of the two accessions, n is the number of alleles and m is the number of loci. The resulting matrix of genetic distances was used in a non-parametric multi-dimensional scaling (MDS) by applying