

# Genetic diversity of *Prunus domestica* selected from ten countries across Europe

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## Abstract

A DNA marker-based study in European plum was performed within the project “Identification of a representative set of *Prunus domestica* accessions of European origin, well documented and characterized, to be included into the AEGIS system (PRUNDOC)”. A total of 46 local plum accessions from 10 European countries (Belgium, France, Germany, Greece, Italy, Latvia, Norway, Serbia, Slovakia and Sweden) were analysed using SSR loci. In addition, seven reference cultivars (Bistrica, Hanita, Mirabelle de Nancy, Reine Claude Violette, Stanley, Valor and Victoria) were analysed for standardization of allele sizes. The following nine primers were used; PacA33 is an EST-SSR developed in apricot, BPPCT039, BPPCT007, BPPCT014, BPPCT034, BPPCT040, UDP96 and UDP98 were developed from genomic peach DNA while CPSC026 was developed from genomic DNA of Japanese plum *Prunus salicina*. None of the 46 investigated local plum accessions were identical, nor were any of them identical to any of the 7 reference cultivars. Genetic similarity among accessions was examined using Jaccard's similarity coefficients. The obtained dendrogram showed that the plum accessions did not group in a pattern corresponding to their country of origin.

**Keywords:** DNA, genebank, molecular marker, plant conservation, plum, SSR

## INTRODUCTION

The European Cooperative Programme for Plant Genetic Resources (ECPGR) is aimed at ensuring the long-term conservation of important germplasm in Europe as well as facilitating an increased utilization of this germplasm in e.g. plant breeding. Within ECPGR a number of crop-specific working groups have been established. European plums (*Prunus domestica* and subspecies), are part of the responsibility of the WG *Prunus*. To date, curators

for 33 different plant collections have sent data to the European *Prunus* Database (EPDB), which now encompasses a total of 3,500 accessions.

A strategic framework for the implementation of a European Genebank Integrated System (AEGIS) has been developed with the aim to improve management of European crop collections, including also fruit crops. Special attention is given to the selection of Most Appropriate Accessions (MAA) for future conservation and characterization. While EPDB is a valuable tool for the application of selection criteria for identification of MAA in plums, there are also limitations due to problems of synonymy and trueness-to-name. A new project, PRUNDOC, was therefore initiated to develop and test MAA selection methodology using both DNA marker analysis and the EPDB on European plums. The final outcome will be a list of MAA accessions to be flagged as belonging to AEGIS by the respective National Coordinators.

Within this project, participants from 10 countries have supplied phenotypic data according to previously agreed standards (e.g. “General protocols for using the ECPGR descriptors for *Prunus* spp” and “AEGIS selection of MAA List of minimum passport descriptors for all *Prunus* species”) for a number of candidate genotypes. In addition, a subset of those candidates presumed to be the most genetically unique, were chosen for analysis with SSR markers. Since European plum is hexaploid, with up to six different alleles in a single locus in a single individual, allele scoring is rather complicated. A set of carefully selected and previously applied primer pairs (Halapija Kazija et al. 2014, Sehic et al. 2015) were, however, chosen for the genotyping.

## **MATERIAL AND METHODS**

### **Plant material**

A total of 46 local plum accessions, presently conserved by partners in 10 different countries, were analysed in this study (Figure 1). Seven international reference cultivars were also included in the analyses. Young leaves were collected in the spring or summer from a single tree for each of the investigated accessions, and then sent to Balsgård, SLU in Sweden, where all DNA analyses were performed.

### **SSR analyses**

DNA was extracted from frozen leaf material using the Qiagen Dneasy™ Plant Mini Kit (Qiagen AB) according to the manufacturer’s instructions. A set of nine previously published SSR primer pairs (Table 1) were employed for the analyses. For DNA sequences, references, amplification procedures and annealing temperatures, see Sehic et al. (2015) with the minor change that Taq DNA polymerase (recombinant) (Thermo Fischer Scientific) was used. Diluted PCR products were mixed with Hi-Di formamide (Applied Biosystems) and an in-house prepared size standard, after which the amplified fragments were separated on an ABI 3130xl Genetic Analyser (Applied Biosystems). Software package Gene-Marker v. 1.85 (SoftGenetics LLC) was used for the data analysis. In case of any uncertainty regarding the scoring process, PCR amplification was repeated.

## Population genetics analyses

Population genetics software SPAGeDI 1.3 (Hardy and Vekemans, 2002) was used to calculate allele frequencies and gene diversity (Nei, 1978). A UPGMA dendrogram, based on a matrix with pairwise comparisons using Jaccard's similarity coefficients, was calculated in NTSYS (Rohlf, 1993) and constructed in MEGA 5 software (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2011).

## RESULTS AND DISCUSSION

### SSR polymorphism

All accessions except a sample of the diploid *P. cerasifera* (MY BO 1 provided from Slovakia) produced up to six alleles per locus in keeping with their presumed hexaploidy. Nine primer pairs amplified 218 distinct alleles in this study, or on average 24.2 alleles per locus (Table 1). This is higher than the 18.7 alleles per locus reported by Halapija Kazija et al. (2014) for 62 plum accessions (traditional Croatian, regional and international) and the 22.7 alleles reported by Sehic et al. (2015) (traditional Norwegian, Swedish and international) analysed with the same nine SSR primer pairs. The average number of alleles per locus was much higher among local European accessions (24.0) compared to reference cultivars (12.7).

The highest number of different alleles was detected for BPPCT014, while BPPCT040 was the least polymorphic of all loci analyzed in our study. Similar results for the same SSR loci were reported by Halapija Kazija *et al.* (2014). The gene diversity was very similar for local European accessions (0.91) and reference cultivars (0.92). Gene diversity calculated for all analyzed samples (0.91) was very similar or identical to the values reported by Halapija Kazija et al. (2014) and Sehic et al. (2015).

Table 1. Allele size range (bp) for all the analysed plum accessions, number of alleles per locus and gene diversity (Nei, 1978), based on 9 SSR loci, for all analysed accessions, for 46 local European plum accessions and for 7 reference cultivars.

Locus	All analysed accessions (N = 53)			Local European accessions (N = 46)		Reference cultivars (N = 7)	
	Size range (bp)	No. of alleles	Gene diversity	No. of alleles	Gene diversity	No. of alleles	Gene diversity
UDP98	164/231	20	0.89	19	0.89	11	0.94
PacA33	169/254	34	0.93	34	0.93	15	0.92
CPSCT026	165/216	21	0.92	21	0.92	13	0.92
BPPCT040	120/154	15	0.88	15	0.88	8	0.89
BPPCT007	123/152	16	0.91	16	0.91	11	0.91
BPPCT039	122/179	28	0.93	28	0.92	16	0.94
BPPCT014	186/283	37	0.93	37	0.93	16	0.94
BPPCT034	215/260	20	0.91	20	0.90	12	0.92
UDP96	92/165	27	0.93	26	0.93	12	0.90
Mean		24.2	0.91	24	0.91	12.7	0.92

## Genetic relationships and differentiation

UPGMA cluster analysis grouped all 46 plum accessions and 7 reference cultivars into a dendrogram (Fig. 1). However, there was no notable separation in clusters of cultivars from different countries. No synonyms or homonyms were identified using the cluster analysis. Accessions 'Pozegaca' from Serbia and 'Bistrica' control from Croatia, which are supposed to be synonymous, clustered tightly together but were not identical. Small differences in SSR profiles among 'Pozegaca' accessions (syn. 'Bistrica' and 'Hauszwetsche') have previously been reported by Halapija Kazija et al. (2011) and attributed mostly to clonal polymorphism.

The accessions analysed in this study have also been evaluated for a number of morphological traits. This data will be used to provide a basis for investigating possible clustering according to commonly acknowledged morphological groups, sometimes treated as different subspecies or varieties. Previous SSR-based studies of plum germplasm (Horvath et al., 2011; Halapija Kazija et al., 2014; Sehic et al., 2015) indicate that clustering among accessions is largely influenced by adherence of individual genotypes to a certain morphological group (European plum, damson, mirabelle etc.) and not to specific geographical regions.

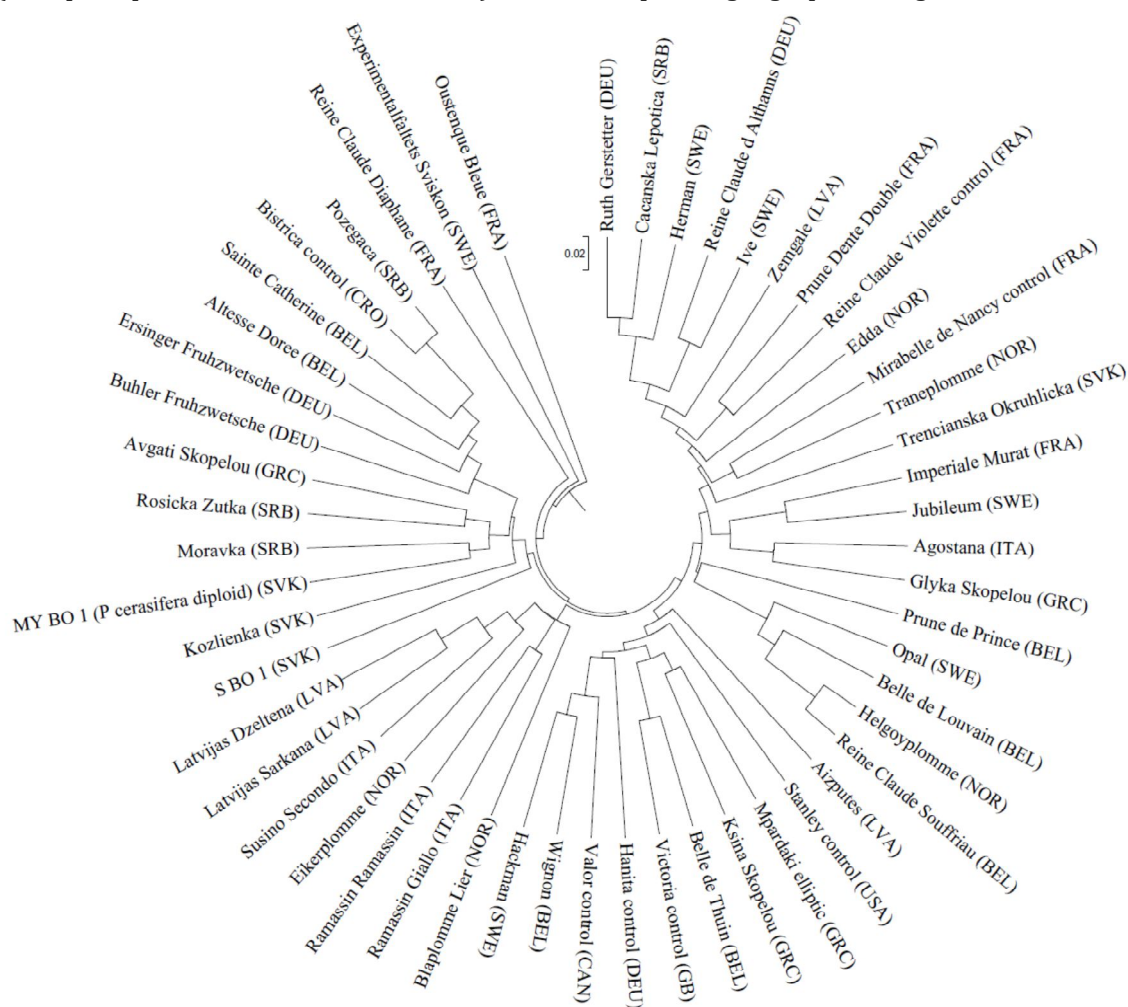


Figure 1. UPGMA cluster analysis based on polymorphisms of SSR data for 53 plum accessions using Jaccard's similarity coefficient.

## CONCLUSIONS

European plum is a diverse crop, but the partitioning of variability does not appear to be related to the geographic origin.

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