Prundoc – A Project to Define Accessions for the European Collection

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Abstract

PRUNDOC was a project financed by ECPGR (European Cooperative Programme for Plant Genetic Resources) in order to include *Prunus* germplasm into the European Collection. The project partners have offered approximately 100 unique genotypes for the European Collection, phenotypically described, and 36 of them were analysed by SSR. Data will be uploaded into the European *Prunus* Database (EPDB) and into the EURISCO catalogue, through the National Inventory Focal Point.

Keywords: *Prunus domestica*, cultivars, European collection, AEGIS, ECPGR

INTRODUCTION

According to the Convention on Biodiversity (CBD) (United Nations, 1992) and the International Treaty for Plant Genetic Resources for Food and Agriculture (the Treaty) (FAO, 2002), states have the sovereign right to exploit their own genetic resources, as well as the responsibility to conserve, sustainably use and provide appropriate access to them. For fruit and berry species, numerous field collections throughout Europe are held by research institutes, breeding companies and other organizations with the aim of conserving germplasm as part of their routine activities. These institutes have traditionally exchanged germplasm freely to national and international colleagues, with the exception of breeding material that has restrictions on propagation and exploitation. However, following the current regime regulating access to germplasm, resulting from the CBD and the Treaty, there has been a lot of uncertainty among collection holders on how to handle the germplasm material.

Contracting Parties to the International Treaty, which is in harmony with the CBD, have agreed to establish a Multilateral System (MLS) for facilitated access, covering plant genetic resources for food and agriculture (PGRFA) that are listed in Annex I of the Treaty. Facilitated access to germplasm included in the MLS is regulated by the SMTA (Standard

Material Transfer Agreement). By signature of this standard agreement, providers of germplasm agree with the recipients that no property rights would be claimed that would limit further access to the same material and that any benefit arising from its use will be equitably and fairly shared. Recipients of the material obtain facilitated access, but only for the purpose of utilization and conservation for research, breeding and training for food and agriculture. Whenever the material included in the MLS is made available for direct cultivation, no SMTA is necessary and it remains at the discretion of each country under which conditions the material can be delivered to the users. For example, Nordgen has worked out simplified MTAs for hobby use and for distribution to nurseries that will propagate the material for commercial sale (Rasmussen, 2015).

One of the limitations of the MLS is that it is only applicable to a short list of 35 crops and most of their wild relatives as well as 29 forage genera, all selected on the basis of their contributions to food security. This list is the result of a complex compromise among the negotiating Contracting Parties to the Treaty. For example, *Prunus* crops are not included in Annex I and therefore, are not part of the MLS. This means that users should not expect to generally have access to *Prunus* accessions under the terms of the SMTA, but that every country might decide to establish different and specific conditions to provide germplasm under its control, with bilateral rather than multilateral agreements, according to the principles of the CBD.

Conscious of the limitations of the scope of Annex I, as well as aiming at the establishment of an efficient and effective conservation system in Europe, the Steering Committee of the European Cooperative Programme for Plant Genetic Resources (ECPGR) promoted the creation of A European Genebank Integrated System (AEGIS) (ECPGR, 2009). European countries participating in AEGIS (see AEGIS Membership at: http://www.ecpgr.cgiar.org/aegis/aegis-membership/) agreed to select individual accessions for which they are prepared to assume long-term conservation responsibilities according to agreed technical standards, ensure their safety duplication and make these "European Accessions" available in accordance with the principles defined in the SMTA. The European Accessions that are part of AEGIS constitute the 'European Collection'. A conscious decision was made to open the European Collection to the inclusion of all crops and their wild relatives that are being conserved in European countries, thereby extending *de facto* the scope of the MLS in Europe. The decisions on which specific accessions to include ultimately remain at the discretion of the respective countries. Thus, also accessions that are not included in Annex I, such as *Prunus*, will be exchanged with the SMTA. The latter is provided on the AEGIS web site (http://www.ecpgr.cgiar.org/aegis/european-collection/), and includes an explanatory note to account for the fact that accessions other than those listed in Annex I are made available under the same conditions.

The PRUNDOC project, funded by the ECPGR Activity Grant Scheme, has the objective to help the countries to select from existing sources of information, such as the European *Prunus* Database (EPDB http://www.bordeaux.inra.fr/euprunusdb/), those *Prunus* accessions that are suitable for inclusion into the European Collection.

Accessions entering the European Collection must meet the general selection requirements agreed by the ECPGR Steering Committee. Material needs to be a PGRFA that is under the management and control of the member countries' governments and in the public domain. It also needs to be genetically unique within AEGIS, to the best available knowledge, and should be of European origin or introduced germplasm that is of actual or potential importance to Europe (for breeding, research, education or for historical and cultural reasons). The holding institute needs to be an "Associate Member" of AEGIS, which requires the signature of the AEGIS Associate Membership Agreement between the institute and the respective country National Coordinator. By this agreement, the holding institute accepts the responsibilities, among others, to ensure the long-term conservation of the

European Accessions according to approved standards. The Genebank Standards for Plant Genetic Resources for Food and Agriculture (FAO, 2014) are the starting reference for AEGIS, while crop specific standards are being developed by each of the ECPGR Working Groups, including the *Prunus* Working Group.

One of the main challenges for the institutes selecting genetic resources for the European Collection is the classification of the material. The collections normally consist of accessions that are old cultivars, chance-seedlings, old breeding material, commercial cultivars without plant breeders' right or patent protection and unclassified introduced material. All this material can be included into the existing databases, however material offered to the European Collection needs to be confirmed by the respective National Coordinator and registered as "part of AEGIS" into EURISCO (http:/eurisco.ecpgr.org) by the National Inventory Focal Point (NFP).

The PRUNDOC project intends to identify at least 100 suitable accessions to be proposed for inclusion into the European collection, on the basis of agreed selection criteria and the above mentioned requirements. The selected accessions will be fully characterized morphologically and a subset also molecularly with SSR. All the data will be made available through the EPDB.

MATERIAL AND METHODS

PRUNDOC partners met at the project's kick-off meeting in April 2015, in order to agree on protocols and workplan during the project period in 2015. The 9 partners, members of the Prunus WG (http://www.ecpgr.cgiar.org/working-groups/prunus/), represent as many European countries.

About one hundred *P. domestica* and *P. insititia* accessions of European origin were presented by the partners as candidates to be part of the European Collection.

Partners agreed on using common descriptors, categories and rankings per descriptor, with the aim of describing harmoniously the whole lot of PRUNDOC accessions but also providing a tool to harmonize future characterization of European Prunus collections. They also agreed to prioritize a subset of descriptors (named First Priority Descriptors, FPD) deemed as particularly useful to describe the salient features of plum varieties and also to provide a basic screening tool to highlight possible duplicates. Protocols for the descriptors were based upon Szalatnay & Bauermeister (2006), UPOV (1977) and IPGRI (Bioversity International, 2015). They finally agreed to choose a representative subset of 4 of their selections, and to send leaf samples in spring 2015 to the Swedish University of Agricultural Sciences (SLU) Balsgård for genotyping with SSR markers. Genetic analyses and interpretation of data were carried out at SLU Balsgård in Sweden, according to the method described by Sehic et al (2015).

Field observations were recorded throughout the season from flowering till harvest. Pictures of fruits in the field were also taken according to the method developed in Gembloux, Belgium (Lateur, 2015), whereas pictures of fruits in the laboratory were taken according to Szalatnay & Bauermeister (2006). The Manager of the European Prunus Database (EPDB), hosted by INRA, Bordeaux, France, prepared an Excel template and sent it to partners for filling it with their passport and characterization data.

Partners sent data to the National Inventory Focal Points for uploading to the EURISCO catalogue, and will check that the accessions they proposed for the European Collection are present in this catalogue. This is a prerequisite to initiate the procedure of flagging the proposed accession 'as part of AEGIS'.

RESULTS AND DISCUSSION

The project revealed a high degree of uncertainty among the partners about how to handle the material and what is required once the accessions would become part of AEGIS. Hence, the connections to the ECPGR Secretariat and the National Coordinators were important. Regarding the selection of unique accessions, a number of challenges had to be faced. Firstly, plums are named with a high number of synonyms. In fact, plum cultivars have been historically exchanged repeatedly between international partners and very often the original name of the cultivar has been translated by the recipient countries to make it more familiar. Additionally, mutations occurring to old cultivars have led to a large number of similar, though distinct types. These small changes might not be identified by the SSR analyses, therefore phenotyping is a key factor for distinguishing such varieties. As old varieties are found in many countries, it is often difficult to establish in which country the original type was bred and, therefore, to assign the responsibility of conservation to the actual country of origin.

As PRUNDOC was a project with limited resources, it was decided that each partner would only propose candidate varieties that, to their best knowledge and/or based on literature, had originated in their country, so as to minimize risks of proposing duplicates for the European Collection. Other criteria for selecting accessions to be offered to AEGIS were a historical link to local traditions or the possession of at least one phenological or pomological trait (e.g. rusticity, high sugar content, very good flavor, etc.) of potential interest for future use in breeding actions.

The Partners agree on a list of 12 descriptors to prioritize when performing the characterization of the European Collection. The list of First Priority Descriptors (FPD), detailed in table 1, together with pictures to help curators in assigning the correct category and rankings, will soon be made publically available on the ECPGR Prunus WG web site (http://www.ecpgr.cgiar.org/working-groups/prunus/).

The Excel template prepared by the EPDB manager was compiled by each partner with passport, evaluation and characterization data and sent to the DB manager together with the pictures of fruits. SSR molecular scores of 40 out of 100 PRUNDOC accessions were sent as well. All the data will be checked by the DB manager for coherence and will soon be available in the EPDB. The number of accessions phenotyped and genotyped by each partner is presented in Table 2. PRUNDOC phenotypical and genotypical data will be analysed in order to assess the diversity of a representative set of European plum genetic resources. In addition to these 9 partners, SLU Balsgård contributed with 5 genotypes, bringing the grand total to 100 accessions phenotyped and 40 accessions analysed by SSR.

CONCLUSIONS

The PRUNDOC project is giving a significant contribution to the process of establishing the European Collection in this crop and to develop a protocol for further selection of material to be elevated to the status of European Accession. PRUNDOC also highlighted bottlenecks of how fruit and berry germplasm could be included in the European Collection.

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Table 1. List of First Priority Descriptors (FPD) in plum

Descriptor name	Details
Phenology	
Time of beginning of flowering[1]	Time of beginning of flowering (1-9 numbered scale) using stage BBCH61
Time of beginning fruit ripening[1]	Season of maturity for picking (1-9 numbered scale) using stage BBCH89
Fruit	
Size[2]	Average weight of fruit (1-9 numbered scale)
Shape (in lateral view) [1]	Fruit shape (1-9 numbered scale)
Skin ground color (after removing bloom) [1]	Colour of the skin of fully mature fruits (1-9 numbered scale)
Skin overcolour (after removing bloom) [2]	Over colour of the skin (1-9 numbered scale)
Color of flesh[3]	Colour of flesh (1-9 numbered scale)
Degree of adherence to flesh[1]	Stone adherence to flesh (1-3 numbered scale)
Eating quality (global taste) [4]	At optimum eating time (1-9 numbered scale)
Sensorial analysis of sugar/acid ratio[1]	Subjective assesment (1-9 numbered scale)
Flesh firmness[2]	Subjective assesment (1-9 numbered scale)
Stone	
Shape (in lateral view) [1]	Stone shape in lateral view (1-9 numbered scale)

[1], Reference, Bioversity International (2006); [2], Reference, Szalatnay & Bauermeister (2006);
[3] Reference, Szalatnay & Bauermeister (2006) and UPOV; [4], Reference, Lateur (2015)

Table 2. Number of accessions characterized by the PRUNDOC partners in 2015.

Partner name	**Country**	**Number of phenotyped accessions**	**Number of SSR-analysed accessions**
Njøs næringsutvikling (NOR053)	Norway	5	3
Agr. Res. Council – Fruit Tree Research Unit of Forlí (ITA380)	Italy	13	4
French National Institute for Agricultural Research (INRA – FRA057)	France	16	4
National Agriculture and Food Center Luzianky, Research Institute of Plant Production Piestany, Gene bank of Slovak Republic (SVK001)	Slovakia	14	4
Julius Kühn-Institute, Institute for Breeding Research on Fruit Crops (DEU451)	Germany	14	4
Dep. of Deciduous Fruit Trees in Naoussa, HAO 'Demeter' (GRC012)	Greece	7	3
University of Novi Sad, Faculty of Agriculture, Department for fruit growing, viticulture and landscape architecture (SRB028)	Serbia	11	4
Latvia State Institute of Fruit- Growing (LVA015)	Latvia	9	4
Agricultural Research Centre (CRA-W) (Self-funded partner)	Belgium	6	6
	Total	95	36