



Phenotypic Characterization and Evaluation of European Cherry Collections: A Survey to Determine the Most Commonly used Descriptors

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Abstract

Standardization of descriptors and protocols is a precondition when aiming to harmonize characterization data records from different plant collections. Improved information on the accessions maintained at the European collections has been a priority target of the Prunus Working Group (WG) of the European Cooperative Programme for Genetic Resources network (ECPGR) since its establishment in 1983. Recently, in order to prioritize in the characterization of the *Prunus spp.* accessions, the Prunus WG began selecting a subset of descriptors (First Priority Descriptors, FPD) considered most informative, from the crop-specific descriptors published by International organizations such as Bioversity International (formerly IPGRI) [1] and UPOV (International Union for the Protection of New Varieties of Plants) [16]. In 2013, a survey was carried out among the cherry collection holders, in the framework of the COST Action FA1104 'Sustainable production of high-quality cherries for the European market', to ascertain which descriptors, categories and scales were most in use for the evaluation and characterization of cherry cultivars in Europe [2]. Sixteen descriptors were routinely used by at least two-thirds of respondents (15 descriptors for sweet and 14 for sour/duke cherry), and deemed most effective in describing cherry accessions, based on the answers provided by 22 sweet cherry and 13 sour/duke cherry curators. This list of most used descriptors, relating to the phenology of blooming and harvesting, tree habit and vigor, fruit size, morphology and internal quality, are now under consideration by the Prunus WG for inclusion in the FPD list recommended for the characterization of the ECPGR cherry resources.

Keywords

Characterization, Evaluation, Descriptors, Plant genetic resources, *Prunus avium*, *Prunus cerasus*

Introduction

The utilization of plant genetic resources preserved in *ex situ* genebank collections is dependent upon the degree and the quality of information available [3]. Frankel and Soule [4] summarized the objectives for conservation and utilization of genebank centers as follows: "The goals are to assemble... to see that it is preserved against loss and deterioration, to make it generally available to those who can evaluate and use it, and to process and publish all available evaluation records for the benefit of all users". The characterization and evaluation (C&E), as well as the documentation of plant genetic resources are the key of their utilization [5]. With the ratification of the Convention on Biological Diversity in 1992, considerable awareness was raised about the importance of the conservation and sustainable use of biodiversity, and the need for benefit-sharing arrangements [6]. In 1996, a Global Plan of Action (GPA) for Conservation and sustainable Use of Plant Genetic Resources for Food and Agriculture (PGRFA) was launched, aiming to better rationalize the conservation system through better planning and increased collaboration and coordination among genebanks. The GPA listed 20 priority activities (reduced to 18 in the Second GPA) in the areas of *in situ* and *ex situ* PGRFA conservation, utilization and institutions capacity building [7]. Improved C&E was one of the activities to which priority was given. The identification of accessions endowed with traits with

potential for further improvement by breeders and farmers, as well as for direct use by farmers for production and marketing, was a way to encourage the more efficient use of PGRFA.

Prunus genetic resources in europe: the ECPGR

Prunus species are clonally propagated heterozygous fruit crops; therefore, maintaining the genetic diversity as specific genotypes such as old cultivars, is more demanding than for most inbred seed-producing plants. Primary collections of clonal crops are maintained in the field as active collections, where accessions are available for

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comprehensive characterization, evaluation and distribution. In Europe, *ex situ* and *in situ* conservation of PGRFA is facilitated by the European Cooperative Programme for Plant Genetic Resources (ECPGR), coordinated since its establishment, in 1980, by Bioversity International (hereafter Bioversity, formerly IBPGR 1974-1991; IPGRI 1991 -2006). ECPGR was launched with the main scope of fostering, on a cooperative basis, the effective and long-term preservation of PGRFA, their documentation, exchange and utilization [8]. In the current phase (Phase IX, 2014-2018) of its long-term workplan, ECPGR is putting major efforts towards the creation of 'a European genebank integrated system' (AEGIS) for PGRFA. The AEGIS approach, i.e. the sharing among Associate Members (genebanks and other institutions holding collections or providing PGRFA conservation-related services) of the responsibilities of the conservation, management, and access to PGRFA genetically unique and important to Europe, offers great potential to improve the quality of management and the use of germplasm collections [9].

The ECPGR program operates through 18 crop and 3 thematic Working Groups (WG). *Prunus* species are responsibility of the *Prunus* WG (<http://www.ecpgr.cgiar.org/working-groups/prunus/>), established in 1983 and currently involving a pool of 87 experts (mainly crop specialists, curators and breeders) from 39 countries. In line with ECPGR objectives for Phase IX, the *Prunus* WG is prioritizing activities aimed to lay the foundation of a decentralized European *Prunus* Collection (hereafter EPC) under the rules of AEGIS [10,11]. These activities include: promote the inclusion of valuable and unique accessions into the EPC; foster the harmonized documentation of the EPC accessions; prioritize sets of most informative descriptors; agree on technical standards across collections for the management of ECP accessions [12]; and, in general, set up any other aspect helpful to identify gaps or redundancies, improve conservation and documentation, enhance exchange and use of ECP accessions.

Currently, 13,635 *Prunus spp.* accessions (status: 30/6/2016) belonging to European collections are documented in the European *Prunus* Database (EPDB), developed by the *Prunus* WG and managed by INRA-Bordeaux (France) [13]. The EPDB portal (<http://www.bordeaux.inra.fr/euprunusdb/index.html>) provides access to 5 distinct databases: almond, apricot, cherry, peach and plum. The cherry database (eucherrydb) is the most developed, hosting 5,585 accessions, maintained by 42 institutes from 17 countries (status: 30/06/2016). Although the EPDB can host passport, C&E, molecular data and photographs of each accession, most of the information contained in the EPDB is still limited to passport data. In the database 85 specific traits are included, ranging from 7 to 22 for each *Prunus* crop.

Since genetic uniqueness and valuable phenotypic and agronomic traits are among the prerequisites for the eligibility of accessions to be part of the European AEGIS Collection, advancement in the C&E of accessions is a top priority of WGs operating in ECPGR.

Crop descriptors lists (DLs)

Since 1976, Bioversity has engaged groups of international experts in the development of a standard documentation system and an internationally agreed terminology to describe the accessions maintained at *ex situ* collections [14]. The goal was to improve the quality of accession-level information, rationalize conservation efforts reducing duplicates, and facilitate data comparison and exchange across collections. Overall, Bioversity has published over 100 crop descriptor lists (DLs). While the first DLs simply provided a minimum set of traits useful to describe the specific crop, the following revisions were more comprehensive, including passport, management, environment and site C&E descriptors. Multi-crop DLs were also published, the most known and used worldwide being the Multicrop Passport Descriptors (MCPDs) designed jointly by Bioversity and

FAO in 2001, updated in 2013 and 2015 [15] as a tool to provide basic information on accession origin and botanical positioning, as well as on the institution maintaining it. Another example of multi-crop list is the Descriptors for Genetic Marker Technologies, designed to allow the generation of standardized and replicable genetic marker data and foster the exchange of molecular data among labs [16]. In the survey carried out by Gotor, et al. Bioversity descriptors were the best known in the PGR stakeholder's community and the most used in documenting accessions in gene bank collections. However, based on the analysis of the answers collected through questionnaires, they found that the majority of users adapted the original descriptors to their needs, and only 20% used them unchanged [14].

Bioversity DLs are preferred by agricultural and biological scientists to facilitate the documentation and use of plant genetic resources, although quite often these are supplemented with additional descriptors such as the UPOV (International Union for the Protection of New Varieties of Plants, <http://www.upov.int/portal/index.html.en>) or USDA-GRIN (United States Department of Agriculture-Germplasm Resources Information Network) [17] descriptors, or other descriptors that curators design on their own for specific needs [18]. But, the UPOV Technical Guidelines mainly assist national authorities in dealing with registration of new plant varieties.

Sweet and sour cherry DLs

Bioversity published the first DLs for cherry (IBPGR/85/37) in 1985 [19], proposing three main categories of descriptors: passport, characterization (for highly heritable traits), and evaluation (for traits influenced by environment). Scales of 1-9 for scoring traits showing continuous variability were proposed and international cultivated varieties of both sweet and sour cherry included as references. Due to the genotype x environment effect on the expression of quantitative traits, site-specific descriptors were also included to better contextualize the data collected. In 1995, UPOV published Technical Guidelines for cherry (TG/35/6), listing 38 descriptors that applied generally to clonally propagated *Prunus avium* L. (sweet cherry), *Prunus cerasus* L. (sour cherry) and their hybrid *Prunus × gondouinii* Rehd. (duke cherry) accessions. However, cultivated varieties from all three groups were included as references and a separation of sweet, sour and duke cherry DLs were recommended. In the revised UPOV cherry Guidelines (2006), the split between sweet cherry (TG/35/7) and sour/duke cherry (TG/230/1) was implemented, and the number of descriptors increased to 41 for sweet cherry and 47 for sour/duke cherry. These were grouped into four categories: tree and shoots, leaf, fruit morphological and quality traits, and phenology descriptors.

In 2011, the *Prunus* WG of ECPGR published a revised list of passport and C&E descriptors chosen for documenting the accessions in the EPDB (European *Prunus* Database). These included 34 MCPD of the FAO/IPGRI list, 15 *Prunus*-specific descriptors related to rootstocks, the phytosanitary status, the prevalent use or the storage type of the accession, and crop-specific C&E descriptors (from 11 to 22, according to the species) [13]. The 15 descriptors proposed for cherry were mainly related to fruit features and degree of susceptibility to biotic and abiotic stress, based on a scale 1 to 9; reference cultivated varieties from Eastern and Western European countries were included as well.

In the early 2000s, in order to facilitate the detection of duplicates and support the trueness-to-type confirmation of ECPGR accessions, the *Prunus* WG, together with the *Malus/Pyrus* and *Vitis* WG embarked on the initiative of also standardizing the molecular markers and the fingerprinting protocols. Following a joint workshop held in 2006, a set of 16 unlinked, polymorphic microsatellite markers (SSRs), as well as 8 international reference accessions were recommended for sweet cherry genotyping [20]. This SSR set has

been widely used worldwide for fingerprinting both the sweet and sour cherry collections [21-23], and based on the experience gained on these fingerprinting studies, the *Prunus* WG is currently revising the original set to improve discrimination effectiveness.

During the 8th meeting of the *Prunus* WG, a panel of experts was engaged to develop crop-specific guidelines for harmonizing the documentation of the *Prunus* collections [24]. It was decided to use, whenever possible, already published descriptors and to refer to the most updated guidelines on descriptors as the basis for the development of species-specific documents. Moreover, the panel prioritized a subset of descriptors deemed most useful for the C&E of the accessions [25], referred to as First Priority Descriptors (hereafter FPD). The identification of a second set of descriptors, named Second Priority Descriptors (SPD), was also considered to supplement the FPDs aiming at increasing completeness of the C&E work. The list of FPDs and SPDs was recently endorsed for peach by the *Prunus* WG [26], and that for plum is in progress. The preparation of the FPD List for cherry is planned under the framework of the EU.CHERRY project ‘Collaborative action for updating, documenting and communicating the cherry patrimonial richness in EU’ (<http://www.ecpgr.cgiar.org/working-groups/prunus/eucherry/>), recently approved for funding by ECPGR.

Material and Methods

In the framework of the COST Action FA1104 ‘Sustainable production of high-quality cherries for the European market’ (<https://www.bordeaux.inra.fr/cherry/>) [2] for cherry, a survey was carried out aimed to i) ascertain which descriptors, categories and scales were most in use for the evaluation and characterization of cherry genetic resources in Europe, ii) compile evaluation protocols and iii) develop innovative protocols to phenotype important agronomic traits for breeders. Two excel tables, one for sweet cherry and the other for sour/duke cherry, were prepared and sent to the COST FA1104 member list, specifically addressing collection curators. Altogether, the survey was sent to 29 participating countries of the COST Action.

The tables included the 21 Bioversity/UPOV descriptors for sweet cherry and 28 for sour/duke cherry used at Julius Kuhn Institute, Dresden, the group leading this survey, for evaluating and characterizing cherry collections, and included descriptor states and rankings. The following categories of descriptors were considered: tree,

leaf, flower, yield, fruit external and internal quality, and phenology. The survey was delivered electronically in 2013. The recipients were asked to indicate i) which of the descriptors listed in the tables they used unchanged (i.e. with the same categories/scales proposed in the table), ii) which ones with modifications in the categories/scales, and to add iii) descriptors that they currently used for describing their cherry cultivars that were not included in the survey table. Based on the responses, the total number of descriptors used by respondents, the frequency of use of each, as well as the number of those most used (MU, i.e. used by at least two-thirds of the curators) was calculated considering the categories of descriptors: tree, leaf, flower, yield, fruit external and internal quality, and phenology. Finally, detailed scales were defined for the MU for the C&E of cherry genetic resources according to the survey.

Results and Discussion

Twenty-two COST partners participated to the survey for sweet cherry and 13 for sour/duke cherry; 10 provided answers and data for both species. Altogether, 25 genebank curators from 20 countries belonging to 25 institutions answered. Therefore the results of the survey got could be considered as representative of the COST FA1104 members.

The number of unique descriptors per category and the number of MU ones are reported in table 1. Distinct descriptors reported included 84 for the C&E of sweet cherry and 64 for the sour/duke cherry accessions. In either species, the tree, the leaf, the yield and the fruit quality were the categories for which the highest number of unique descriptors is used (Table 1). However, a large number of descriptors in use did not always reflect the importance of a certain category in the context of the C&E of cherry accessions. For example, while only 4 phenological descriptors are used by respondents curators, two of them, i.e. the time of beginning flowering and the time of beginning ripening, are MU descriptors; on the other hand, although up to 15 distinct descriptors are used to feature the leaf in each of the two species, their use is very variable across collections, and none of them is used by the majority of the curators surveyed. The category that was the most important to describe cherry characteristics for all curators was related to the assessment of the internal quality of fruit (Table 1): 6 of the 14 descriptors listed by the respondents were MU, i.e. flesh and juice color, firmness, juiciness, sweet/acid balance, eating quality and stone shape (Table 2). According to the survey results, 15

Table 1: Number of unique descriptors/category used for C&E of cherry resources, total number of descriptors/category used by the respondents and number of the same descriptor used by at least two-thirds of the respondent, as resulting from the survey carried out in the COST Action 1104.

Fruit species	Sweet cherry			Sour/duke cherry			
	Descriptor categories	No. of descriptors used per category	No. of the same descriptors used by at least 2/3 of respondents (absolute and %) - 22 respondents	No. of descriptors used	No. of the same descriptors used by at least 2/3 of respondents (absolute and %) - 13 respondents		
Tree		15	2	13	11	2	18
Leaf		15	0	0	15	0	0
Flower		6	0	0	2	0	0
Yield		17	2	12	11	2	18
Fruit (outer quality)		13	3	23	9	2	22
Fruit (inner quality)		14	6	43	14	6	43
Phenology		4	2	50	2	2	100
Σ		84	15		64	14	

Table 2: List of the descriptors used for C&E of the cherry genetic resources by at least two-third of the respondents, as resulting from the survey carried out in the COST action 1104.

Category of descriptors	Descriptors used both in sweet and sour cherry		Used exclusively in sweet cherry	Used exclusively in sour/duke cherry
Tree	vigor	habit		
Yield	fruit size	fruit weight		
Fruit (outer quality)	shape	color of skin	length of stalk	
Fruit (inner quality)	color of flesh	color of juice		
	firmness	sweetness/ acidity		taste/eating quality
	juiciness		stone: shape in ventral view	
Phenology	time of flowering	time of ripening		

Table 3: List of descriptors, categories and scales most used (MU) for the C&E of cherry genetic resources according to the survey carried out in the COST action 1104.

Descriptor	Sweet cherry UPOV (TG/35/7) 2006	Sour cherry UPOV (TG/230/1) 2006	Sweet and sour cherry IBPGR/85/37	Unit of measure/scores
Tree				
Vigor	UPOV 1	UPOV 1	IBPGR 6.1.2.	very weak (1), weak (3), medium (5), strong (7), very strong (9)
Habit	UPOV 2	UPOV 2		upright (1), semi-upright (2), spreading (3), drooping (4)
Yield				
Size				assessed in mm
Size	UPOV 20	UPOV27	IBPGR 6.2.3.	very small (1), small (3), medium (5), large (7), very large (9)
Weight				assessed in g
Fruit - outer quality traits				
Shape	UPOV 21			cordate (1), reniform (2), oblate (3), circular (4), elliptic (5)
Shape in ventral view		UPOV 27		reniform (1), oblate (2), circular (3), elliptic (4)
Color of skin	UPOV 27			yellow (1), yellow with blush (2), orange red (3), light red (4), red (5), brown red (6), dark red (7), blackish (8)
Color of skin		UPOV 36		orange red (1), light red (2), medium red (3), dark red (4), brown red (5), blackish (6)
Length of stalk				assessed in mm
Length of stalk	UPOV 24			very short (1), short (3), medium (5), long (7), very long (9)
Fruit - inner quality traits				
Color of flesh	UPOV 31			cream white (1), yellow (2), pink (3), red (4), dark red (5)
Color of flesh		UPOV 37		yellowish (1), pink (2), medium red (3), dark red (4)
Color of juice	UPOV 32			colorless (1), cream yellow (2), pink (3), red (4), purple (5)
Color of juice		UPOV 38		colorless (1), light yellow (2), pink (3), medium red (4), dark red (5)
Firmness	UPOV 33	UPOV 39		soft (3), medium (5), firm (7), very firm (9)
Juiciness	UPOV 36	UPOV 42		weak (1), medium (5), strong (7)
sweet/acid balance	UPOV 35/ 34	UPOV 41/ 40		very sweet (1), sweetish (3), balanced (5), acidulated (7), very acid (9)
Stone shape	UPOV 38			medium elliptic (1), broad elliptic (2), circular (3)
Taste/eating quality			IBPGR 6.2.7.	bad (3), fair (5) good (7)
Phenology				
Time of beginning of flowering	UPOV 40	UPOV 46		very early (1), early (3), medium (5), late (7), very late (9)
Date of full flowering			IBPGR 6.2.1.	early (3), mid-season (5), late (7), extremely late (9)
Time of beginning of ripening (maturity)	UPOV 41	UPOV 47		very early (1), early (3), medium (5), late (7), very late (9)
Harvest maturity			IBPGR 4.2.2.	early (3), mid-season (5), late (7), extremely late (9)

out of 84 sweet cherry and 14 out of 64 sour/cherry descriptors used to characterize cherry collection in Europe are MU (Table 2). Their UPOV/ Bioversity codes and scales are reported in table 3, grouped in the respective categories of descriptors.

Tree

Tree vigor was assessed by 17 respondents in sweet cherry and 10 in sour/duke cherry, according to the following UPOV and Bioversity categories and scores: very weak (1), weak (3), medium (5), strong (7), very strong (9). Categories and scores for tree habit changed significantly from the UPOV DLs published in 1995 and those published in 2006, resulting in variability in their choice among respondents. However, 70% of respondents, both for sweet cherry and sour/duke cherry, use the most recent (2006) scales: upright (1), semi-upright (2), spreading (3), drooping (4).

Yield

Fruit size, a trait markedly affecting the yield, was routinely measured by nearly all curators. Fruit size is generally assessed by measuring the weight of a 10-fruit sample (in g) or the diameter of the fruit (in mm). Only few of the respondents measure the length, the diameter and the thickness. The categories and scores used conform to the descriptor 6.2.3 proposed by Bioversity in 1985 and the UPOV descriptors 20 and 27 proposed in the DLs published in 2006.

Fruit - outer quality

In both crops, over two-thirds of the respondents routinely assessed fruit shape and color; in sweet cherry, most partners also measured (mm) or rated (1-9) the length of the stalk. The scale used to assess fruit shape changed dramatically from the UPOV DLs published in 1995 as compared to the UPOV DLs published in 2006, leading to variability of the scoring method observed in the survey. However, in sweet cherry, 71% of the respondents used the new UPOV scores, five used the old ones and one partner used their own scale; in sour/duke cherry, 75% of the curators used the new scores, two the old ones and one partner used their own scale. For the skin color, the new scores were applied by 90% of the cases in sweet and 69% in sour/duke cherry; two partners used the Bioversity descriptor 4.2.3 in the DLs published in 1985.

Fruit - inner quality

The internal quality of the fruit is assessed by the majority of the respondents by rating the flesh and juice color, firmness, juiciness and sweet/acid balance in both species, the stone shape only in sweet cherry and the eating quality only in sour/duke cherry. The flesh and the juice color are described by 70% of sweet cherry and 94% of sour cherry curators by using the 2006 UPOV categories and scales. Three partners measured the anthocyanin content (g/l): AU, Denmark; KGZS-Zavod GO, Slovenia, and LSIFG, Latvia. Nearly all curators estimated sensorially the juiciness and the firmness

according to the most recent UPOV scales, only 6 of them assessed the flesh firmness instrumentally (by penetrometers). Although most respondents assessed the sweetness/acidity balance of the fruit, the methodology used is variable: i) scoring the sweetness and acidity separately according to the new UPOV DLs, and assessing their balance indirectly; ii) scoring directly the sugar/acid ratio from 1 to 9, using the Bioversity 6.2.8. descriptor; iii) measuring the soluble solids concentration (°Brix) and the titratable acidity (g l^{-1} or meq l^{-1}) and then assessing the balance indirectly basing on analyses. The taste/eating quality was evaluated by a large number of sour/duke cherry curators using the IBGRI guidelines, ranking poor (3), fair (5) and good (7). No equivalent descriptor in the UPOV exists.

Phenology data

The flowering and ripening time of accessions was routinely recorded by nearly all respondents. Most referred to the phenological stages encoded by Meier, et al.: 10% open flowers (BBCH61), 50% open flowers (BBCH65), end of flowering (BBCH67) for the blooming period [27]. Similarly, the ripening was determined by the time where 10%, 50% and/or 100% of fruits are ripe. The data records were expressed i) as day of the year (DOY), ii) days before or after the reference cvs. Burlat or Kordia or iii) scaled very early (1), early (3), medium (5), late (7), to very late (9). The UPOV descriptors 40/46 and UPOV 41/47 conform to the Bioversity descriptors 6.2.1. and 4.2.2. (IPBGR/85/37) but in different phenological stages.

Compared to the recommended list of primary characterization descriptors mentioned for cherry by Zanetto, et al. [28], the newly developed list of MU descriptors is more comprehensive for the fruit morphological traits and could be considered as representative for descriptors that characterize the sweet and sour/duke cherries for the European market because all important traits are considered [29].

Conclusion

The genetic diversity maintained at germplasm collections is the base material for breeders to address current and future challenges of climatic change and the need for adaptation of cultivated crops [11]. Detailed and accurate description of PGRs is a prerequisite when aiming to identify accessions endowed with traits with potential for further improvement by breeders.

A more organized, cost-efficient system for the conservation and exploitation of germplasm conserved in Europe is a priority target of the ECPGR network, as well as the establishment of a European Collection, conceived as a virtual genebank consisting of valuable and genetically unique European Accessions managed according to agreed technical standards. As harmonization of C&E descriptors and protocols is needed when aiming to compare C&E data records from different plant collections, the *Prunus* WG of ECPGR in the recent years has embarked on the initiative of selecting, for each crop species, a set of descriptors to prioritize in the characterization of the accessions of *Prunus* spp.

The survey carried out in 2013 in the framework of the COST Action FA1104 'Sustainable production of high-quality cherries for the European market', involving numerous sweet cherry and sour/duke cherry curators from 20 EU countries, has highlighted a set of 15 UPOV/IPGRI descriptors for sweet and 14 descriptors for sour/duke cherry largely used by the respondents. As these descriptors are considered by the curators as the most effective in describing cherry accessions, this list of most used descriptors, relating phenology of blooming and harvesting, tree habit and vigor, fruit size, morphology and internal quality, is now under consideration by the *Prunus* WG for inclusion in the FPD list recommended for the characterization of the ECPGR sweet and sour cherry resources. Based on the results presented the objectives of the survey were fulfilled.

Broad use of the FPDs in the ECPGR community will push forward the process of standardization of the documentation on the genetic resources and boost their exchange and use. The minimum descriptors list could be also very useful for the researchers participating in collecting missions and working on farm. A quick description of *Prunus* accessions found is needed because of the limited time in nature.

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