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Development of a standardized methodology for phenotypical characterizations in apple

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Abstract

The description of phenotypic traits in apple cultivars is generally performed using internationally agreed descriptors such as UPOV guidelines, which defines for each trait several states of expression. However, it is not always possible to classify a cultivar unambiguously using those guidelines, because in practice the states are not clearly enough defined or the example varieties are not always available in the collections. This work presents the results of a harmonization project performed by the teams responsible of the main apple germplasm collections in Spain. The objective was to develop a standardized method for the 57 traits included in the TG/14/9 UPOV guidelines for apple characterization, defining their states of expression in a clear and unambiguous way for Spanish germplasm. Phenotypic data collected for more than 1,600 accessions from Spanish collections were used and the method to define each state depended on the type of expression. For quantitative traits the number of states and their limits were defined according to the variability that exists within and between accessions. For qualitative traits, high-resolution images clearly depicting each state were selected. A standardized characterization protocol for the 57 traits of apple germplasm has been provided, enabling to comparing properly the phenotypes of Spanish genetic resources.

Keywords: *Malus* x *domestica*, harmonization, quantitative, qualitative, traits

INTRODUCTION

The description of phenotypic traits in apple cultivars is generally performed using internationally agreed descriptors such as UPOV guidelines, which defines for each trait several states of expression. However, it is not always possible to classify a cultivar unambiguously using those guidelines, because in practice, the states of expression sometimes are not clearly defined, or the reference varieties used to illustrate the different states are not always available in the collections. The Spanish Program of Plant Genetic Resources integrates apple collections including mainly local cultivars from their respective regions. Though some previous studies about the genetic variability of apple genetics resources from Spain have been already performed, the methods, descriptors and markers

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used on each collection were not standardized. Thus, a complete harmonization work was needed in order to evaluate the complete diversity of *Malus* spp. in Spain. For doing that, the Spanish Government funded the project "Harmonization of the methodology of characterization, assessment of the genetic diversity and definition of the core collection of the apple germplasm conserved in Spanish genebanks". This paper presents the results on the phenotypical descriptor harmonization performed within the Project, made by the teams responsible of the main apple germplasm collections in Spain. The objective was to develop a standardized method for the 57 traits included in the TG/14/9 UPOV guidelines for apple characterization, defining their states of expression in a clear and unambiguous way for Spanish germplasm.

MATERIAL AND METHODS

Phenotypic data

Phenotypic data collected for about 1,600 apple accessions preserved in seven Spanish collections, covering most of the Spanish apple growing areas. The collections involved were those located at Public University of Navarre, SERIDA, Centro de Investigaciones Agrarias de Mabegondo, Cabildos (Tenerife, La Palma and Gran Canaria), University of Lleida, Estación Experimental de Aula Dei-CSIC and CITA of Aragon. Characterization data was available for the 57 descriptors compiled in UPOV guideline TG/14/9 (UPOV, 2005).

Standardization of the quantitative traits

The methodological assumptions in the standardization of the quantitative traits were that the number of the states of expression within a descriptor would be determined according to i) the real expression range observed within each character (RGa) in all accessions and, ii) the variability measured within each accession (within yearly repeats or within years). As a consequence, a wider expression range and lower variability within accessions would result in a greater number of descriptor states.

The discrimination unit (DU), used to define the size of the trait levels, corresponded to the lowest difference between two accessions that allow considering them as belonging to different states of expression and was calculated using the following expressions:

$$DU = Rg_{pob} + SD_{pob} \quad (1)$$

$$RG_{max} = RG_m + SD_m \quad (2)$$

where Rg_{pob} is the mean range for the accessions with $Rg_a < Rg_{max}$. SD_{pob} is the standard deviation of the accessions with $Rg_a < Rg_{max}$. Rg_a is the intra-accession range, that is, the difference between the max and min trait values within each accession, RG_m is the mean value of Rg_a in the set of accessions and SD_m is the standard deviation for Rg_a.

Once the DU was obtained, the number of expression levels for the trait was calculated using the frequency distribution of mean trait values measured on the set of accessions. The central level was centered on the median of the distribution, and the rest of levels placed at increases/decreases of 1 DU respect of central level.

Standardization of the qualitative traits

The methodological assumptions in the standardization of the qualitative traits were that the assignment to a trait level should be clear and unambiguous, so that the reference varieties should be avoided whenever possible and being replaced with figures or high-resolutions photographs.

The expression levels on each qualitative trait were defined taking into account the collective experience of the members of the Project, and previous standardization works

(Boré and Fleckinger, 1997; Dapena and Blázquez, 2009; Pereira et al., 2002; Royo et al., 2009; Szalatnay, 2006).

RESULTS AND DISCUSSION

The standardized characterization protocol applied on 20 quantitative traits allowed the definition of new levels for all the traits (Table 1). In most cases, the number of expression levels defined after the harmonization was different to the UPOV reference cultivar-defined ones, in 13 traits we defined more levels than UPOV, generally 1-2 more, and in 4 traits fewer levels could be observed. The greater changes were observed for the Leaf blade: length (UPOV 11), leaf blade: length/width ratio (UPOV 13), Fruit diameter, ratio height/diameter, width of stalk cavity and width of eye basin (UPOV 26, 27, 49 and 51, respectively) in which tree more classes could be defined, and Fruit: size, for which 3 less classes could be defined.

High-resolution characterization sheets were produced for the 37 qualitative traits in UPOV TG/14/9, describing unambiguously the trait levels. In 10 traits the number of expression levels defined after the harmonization was different to the UPOV defined ones (Table 2), generally obtaining a lower number of unambiguous levels, but for 2 traits (UPOV 14, intensity of green color of leaf blade and UPOV 56, time of harvest) more levels could be differentiated, which indicates the usefulness of using wide and diverse databases to define characterization protocols suitable for germplasm collections with old cultivars.

CONCLUSIONS

A standardized characterization protocol for the 57 traits of apple germplasm has been provided, enabling to compare properly the phenotypes of Spanish genetic resources. We believe that the methodology can significantly reduce the subjectivity of the classifications for all those who perform this kind of work. However, to reach a higher degree of generalization it would be convenient to use a methodology similar to that used in this work, but based on a wider database from as many international collections as possible.

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Tables

Table 1. Number of expression levels in UPOV TG/14/9 and defined after the harmonization for twenty quantitative traits.

UPOV character		nº of expression levels	
		UPOV	Harmonized
05	One-year-old shoot: thickness	4	4
06	One-year-old shoot: length of internode	4	4
09	One-year-old shoot: number of lenticels	3	4
11	Leaf blade: length	4	7
12	Leaf blade: width	3	5
13	Leaf blade: ratio length/width	3	6
17	Petiole: length	3	5
20	Flower: diameter with petals	4	5
24	Fruit: size	9	6
25	Fruit: height	3	5
26	Fruit: diameter	3	6
27	Fruit: ratio heigth/diameter	5	8
36	Fruit: relative area of over colour	5	3
41	Fruit: area of russet around stalk	3	3
46	Fruit: length of stalk	5	4
47	Fruit thickness of stalk	3	4
48	Fruit: depth of stalk cavity	3	5
49	Fruit: Width of stalk cavity	3	6
50	Fruit: depth of eye basin	3	5
51	Fruit: width of eye basin	3	6

Table 2. Number of expression levels in UPOV TG/14/9 and defined after the harmonization for ten qualitative traits.

UPOV character		nº of expression levels	
		UPOV	Harmonized
08	One-year-old shoot: pubescence	5	4
14	Leaf blade: intensity of green colour	3	4
15	Leaf blade: incisions of margin	5	4
19	Flower: predominant colour at balloon stage	7	6
28	Fruit: general shape	7	5
36	Fruit: relative area of over colour	5	3
39	Fruit: pattern of over colour	7	4
52	Fruit: firmness of flesh	5	3
56	Time for harvest	5	9
57	Time of eating maturity	9	5