Scientia Horticulturae 238 (2018) 107–115

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Variation of morphological descriptors for the evaluation of tomato germplasm and their stability across different growing conditions

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ARTICLE INFO

Keywords:
Characterization
Descriptors
Genotype × environment interaction
Germplasm
Heritability
Solanum lycopersicum

ABSTRACT

Germplasm and breeding materials are usually characterized using morphological and agronomic descriptors, which should have a high heritability. Despite the widespread use of tomato (*Solanum lycopersicum*) standardized descriptors, little information exists on environmental effects on descriptor values and their heritability. We have evaluated 12 tomato accessions from seven cultivar groups in three different environments (open-field conventional, open-field organic, and greenhouse) and characterized them with 36 descriptors. A wide range of variation was found for most descriptors, demonstrating their utility for describing tomato materials and their diversity and relationships. The analysis of descriptors variation reveals that while for some descriptors with a simple genetic control the accession effect accounts for 100% of the variation, for others like yield per plant only 10.83% of the variation observed is due to the accession effect. Although significant differences were found among environments for most descriptors, including a much higher yield in the open-field conventional environment than in the two others, the environmental effect was low for most traits. However, the genotype × environment effect generally had an important contribution to the structure of variation for many descriptors, and for three traits it had the highest contribution to the percentage of the sum of squares. As a result of the variation structure, the heritability values are high (> 0.7) for only 10 descriptors, while for five is low (< 0.3). Principal components analysis (PCA) reveals that projections in the PCA graph of a same accession grown in different environments plot together in the same area of the PCA graph. Although cultivar groups are generally clearly separated in the PCA graph, accessions from the same cultivar group in some cases are intermixed. These results have important implications for detecting tomato duplicates and establishing core collections, as well as for analyzing germplasm and breeding results, when using data sets containing data of accessions grown in different environments.

1. Introduction

Standardized descriptor lists for the characterization of germplasm collections and breeding stocks constitute an important tool for germplasm banks and breeders as they allow using an internationally agreed format, facilitating comparison of characterization data sets among germplasm banks and trials (Gotor et al., 2008). Up to now, Bioversity International has published descriptors lists for over 100 crops (Bioversity International, 2017). Also, the UPOV has descriptors lists for the characterization of new varieties in distinctness, uniformity and stability (DUS) tests (UPOV, 2017). The characterization and evaluation descriptors lists include morphological and agronomic traits that are of relevance for breeders. Depending on the trait, descriptors are metric, meristic, measured according to an arbitrary quantitative scale, or assigned to qualitative states (Grum and Atieno, 2007). Ideally, standardized descriptors should display a wide variation in the collections of materials characterized, as well as having a high heritability (Ortiz Ríos, 2015), which in turn requires a low environmental influence. Descriptors having these characteristics are highly informative. However many traits that are of interest for breeders, in particular those polygenic, are influenced by the environment (Annicchiarico, 2002). For example, yield is a typical example of an important trait highly affected by the environment (van Bueren et al., 2011; van Ittersum et al., 2013). A way to overcome the influence of the environment is using common controls in the trials, so that an estimate of the environment effect can be obtained allowing its removal in the comparisons of data sets from different environments (Ortiz Ríos, 2015). However, when important genotype × environment exists, the

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https://doi.org/10.1016/j.scienta.2018.04.039
Received 15 November 2017; Received in revised form 18 April 2018; Accepted 19 April 2018

comparisons of distinct materials grown in different environments are flawed, and this can lead to unreliable results (Annicchiarico, 2002). High genotype × environment interaction also represents a challenge for the morphological traits-based detection of duplicates in germplasm banks (Diederichsen, 2009).

Tomato (*Solanum lycopersicum* L.) is the most important vegetable crop, with over 75,000 accessions being conserved in germplasm banks (Robertson and Labate, 2006). Bioversity International standardized descriptors have been available for tomato for over two decades (IPGRI, 1996). Since then, Bioversity International descriptors for tomato have been widely used by germplasm banks and breeders (Mazzucato et al., 2008; Gonçalves et al., 2009; de Castro et al., 2010; Cebolla-Cornejo et al., 2013; Cortés-Olmos et al., 2015; Figàs et al., 2015; Parisi et al., 2016). These reports generally show that IPGRI (1996) descriptors display a large range of variation and are useful to distinguish among accessions and varietal groups. However, amazingly, in most of the cases where germplasm is characterized using IPGRI (1996) descriptors they contain data of a single location and year, and there are few works reporting data of several years or environments. One exception is the work done by Mazzucato et al. (2008), whom used 22 morpho-physiological traits, largely conforming with IPGRI (1996) tomato descriptors, in 61 tomato and wild relatives accessions grown in two locations. In this work, significant genotype × environment interaction was found for 21 out of the 22 descriptors, although the authors indicate that this interaction was mostly caused by the performance of a few genotypes for each trait (Mazzucato et al., 2008). In another work, Rao et al. (2006) used UPOV descriptors to evaluate 'San Marzano' accessions for three years. These authors found that some homogeneous accessions that matched the 'San Marzano' type in one year did not match it in other years. Overall, these data seem to indicate that, while IPGRI (1996) tomato descriptors are appropriate for describing the main morphological characteristics of tomato materials as well as for assessing variation in germplasm and breeding collections, their values and scores may be influenced by the environment and by genotype × environment interaction.

The lack of information on the stability of morphological descriptors in tomato in different environments contrasts with the large number of studies evaluating the effects of genotype × environment in tomato for agronomic and fruit quality traits (Ortiz et al., 2007; Cebolla-Cornejo et al., 2011; Adalid et al., 2012; Panthee et al., 2012, 2013). In general, these works reveal that there is a large genotype × environment interaction for yield and composition traits, and a moderate or low one for fruit shape traits. Given the importance of standardized descriptors, like those of IPGRI (1996), in tomato germplasm management and in breeding, it is necessary to have an assessment of the genotype × environment interaction of these widely used descriptors, in particular when comparing data from data sets from different environments or years,.

In this work we use a set of IPGRI (1996) descriptors to evaluate 12 tomato accessions in three different cultivation conditions (open-field conventional, open-field organic, and greenhouse conventional). The results will provide information on the stability of the different descriptors in different environmental conditions, and on the utility of the utilization of a multiple set of standardized descriptors for providing a characterization profile that allow differentiation among varieties grown in different environments. All this information will be relevant for tomato germplasm characterization and breeding.

2. Material and methods

2.1. Plant material

Twelve phenotypically diverse local varieties from the region of València (Spain) were used in the present study (Table 1; Fig. 1). The accessions belong to seven different cultivar groups of local Valencian varieties (Borseta, Cor, Penjar, Plana, Pruna, Redona, Valenciana) as described in Figàs et al. (2015). Four accessions belong to the Penjar group, which is characterized by the presence of the *alc* mutation, which confers a long shelf-life (Casals et al., 2012), and small or medium-sized fruits, three to the Plana group, characterized by large flattened fruits, and one accession to each of the groups Borseta (pyriform), Cor (slightly heart-shaped), Pruna (cylindrical), Redona (rounded), and Valenciana (heart-shaped) (Table 1).

Table 1
Local tomato varieties used for the present study including the varietal type (according to Figàs et al., 2015), and origin (municipality and province) within the Valencian Region (Spain).

Accession code	Varietal type	Predominant fruit shape	Origin	
			Municipality	Province
AX1	Penjar	Flattened	Alcalà de Xivert	Castelló
AX2	Penjar	Rounded	Alcalà de Xivert	Castelló
DA2	Cor	Slightly heart-shaped	Dos Aigües	València
FU1	Plana	Flattened	Fuenterrobles	València
MA2	Pruna	Cylindrical	Massalfassar	València
OR1	Borseta	Pyriform	Oriola	Alacant
OR3	Plana	Flattened	Oriola	Alacant
PI1	Valenciana	Heart-shaped	Picanya	València
RE2	Plana	Flattened	Requena	València
VI1	Penjar	Slightly flattened	Vinaròs	Castelló
VS1	Penjar	Flattened	Vistabella	Castelló
XA1	Redona	Rounded	Xàtiva	València

2.2. Cultivation conditions

All accessions were grown under three cultivation conditions: i) open field under conventional management (open-field conventional), ii) open field under organic management (open-field organic), and iii) greenhouse under conventional management (greenhouse conventional). Seeds for the conventional cultivation trials were disinfected with a 1:10 w/v solution of dodecahydrate trisodium phosphate ($Na_3PO_4 \cdot 12\ H_2O$) for 3 h and rinsed three times with distilled water for 15 min; subsequently, the seeds were subjected to an additional round of disinfection with commercial bleach (40 g/l of NaOCl) at 30% for 7 min and rinsed three times with distilled water for 7 min. After that, the seeds were left to dry for several weeks on filter paper and then subjected to thermotherapy at 80 °C for 24 h. The seeds for the organic cultivation conditions were subjected to the same treatments except that the trisodium phosphate disinfection was not performed. Seedling trays for 96 plants filled with Humin-substrat N3 (Klasmann-Deilmann, Germany) substrate for the conventional cultivation conditions or Natur Pots Premium (Projar) organic substrate for organic conditions were used for sowing the seeds. Seedling trays were kept in a climatic chamber with a 14 h light / 10 h dark photoperiod and a 25 °C (light) / 18 °C (dark) temperature regime. For the open-field conventional and organic trials, five plants per accession were grown, while for the greenhouse trial six plants per accession were grown. In all trials plants were transplanted on the 23rd of March of 2014, spaced 1.25 m among rows and 0.33 m within the row and distributed according to a completely randomized design.

The open-field conventional trial was located in La Pobla de Vallbona (Valencia, Spain; geographical coordinates: 39°34′33″ N, 0°33′13″ W, 90 m.a.s.l.). A background fertilization of 0.15 kg/m^2 of fertilizer containing 15% N, 15% P_2O_5, and 15% K_2O (NPK(S) 15-15-15 (20), Fertiberia, Madrid, Spain) was applied before transplant. An additional top-dressing fertilization at a dose of 0.05 kg/m^2 of the same fertilizer was applied three months after transplant. Flood irrigation was used for watering the plants, which were stacked with canes. Weeds were removed manually. Phytosanitary treatments against spider mites, aphids, caterpillars, and tomato leaf miner were performed using spinosad, emamectin, imidacloprid, and dimethoate. A