Phenomics of elite heirlooms of peppers (*Capsicum annuum* L.) from the Spanish centre of diversity: conventional and high-throughput digital tools towards varietal typification

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Abstract

Spain is a relevant secondary centre of diversity for *Capsicum annuum* peppers, especially for the bell types known as *Pimiento Morrón* or *Pimiento de Morro*. Thus, a myriad of highly regarded landraces adapted to a wide range of conditions can be found throughout the country, as a result of centuries of farmers breeding. Despite that, these materials lack of proper characterization, of paramount importance for farmers, breeders and germplasm management. In this regard, in addition to internationally accepted conventional descriptors, high-throughput digital phenotyping tools like Tomato Analyzer, a software originally developed to process scanned images of cut tomato fruits and to record a range of morphological parameters, may provide an important help towards exhaustive germplasm characterization. With this aim, 32 conventional and 35 Tomato Analyzer digital traits were used herein to characterize a large collection of *C. annuum* accessions from all Spanish regions, including Protected Designations of Origin and Protected Geographical Indications, with emphasis on *Morrón* peppers, in order to assess the diversity within Spanish elite germplasm and to test the efficiency of those methods to differentiate varietal types and closely related materials. A considerable amount of variation was found using both conventional and digital traits, even within *Morrón* pepper groups, reflecting the diversity of Spanish peppers in terms of plant and fruit morphology, essential for future breeding programs. Both conventional descriptors and digital parameters were able to distinguish varietal groups. However, on the whole, digital phenotyping was able to discriminate in a more accurate way. Most digital parameters were able to discriminate varietal groups into higher numbers of categories (≥4) than conventional traits (usually 2-4). In addition, the number of significant pairwise differences among varietal groups was considerably higher for digital parameters than for conventional descriptors, enabling a powerful separation, particularly relevant for closely related groups such as *Morrón* peppers. Likewise, as revealed by Principal Components Analysis, digital phenotyping allowed a more powerful intra-varietal separation compared to conventional descriptors. Finally, a subset of 4 conventional descriptors and 13 Tomato Analyzer traits were identified as the most discriminant to distinguish among closely related *C. annuum* accessions, explaining 81.81% of total variance found by Principal Components Analysis. Fruit traits explained the highest percentage of variance for our collection.

Keywords: *Capsicum annuum*, ecotypes, germplasm, protected designations of origin, IPGRI, Tomato Analyzer

1. Introduction

Native from America, peppers (*Capsicum* spp.) are one of the most popular vegetables, contributing with its flavour to a wide range of culinary specialities all around the world (Bosland and Votava, 2012). *Capsicum* is a small but genetically and morphologically diverse genus comprising five cultivated species and almost 40 wild species (Barboza et al., 2019; Carrizo García et al., 2016; Moscone et al., 2007). Among the cultivated species, *Capsicum annuum* L. (var. *annuum*) is the most diverse and economically important species, and its cultivars are grown in almost all temperate and tropical regions of the world (Bosland and Votava, 2012; FAO, 2019).

Spain is a highly relevant secondary centre of diversity for *C. annuum*, since its introduction from America in the late XVth century (Andrews, 1995; González-Pérez et al., 2014; Nuez et al., 2003). Five centuries of cultivation and breeding have led to the bearing of a plethora of Spanish ecotypes adapted to a wide range of agro-climatic conditions (González-Pérez et al., 2014; Rivera et al., 2016; Rodríguez-Burruezo et al., 2016). As a consequence, many landraces, grown since immemorial times, can still be found nowadays in all Spanish regions, particularly those from varietal types known as *Pimiento Morrón*, *Morrón de Cascos* or *Pimiento de Morro* (*i.e.* resembling the nose of a cow, *Morro* in Spanish), encompassing sweet bell peppers (from blocky to rectangular shapes) with medium-large sized pods, as well as their round/heart-shaped relatives called *Morrón de Bola* or *Morrón de Conserva* (Rodríguez-Burruezo et al., 2016).

Furthermore, peppers hold the highest number of Protected Designations of Origin (PDOs) and Protected Geographical Indications (PGIs) among vegetables and food derivatives in Spain (MAPA, 2019). Ecotypes such as *Padrón-Herbón* (Galicia), *Bola* (Murcia; for *Pimentón de Murcia*), *Bierzo* and *Morrón de Fresno-Benavente* (Castilla y León), *Piquillo de Lodosa* (Navarra), *Jaranda* (Extremadura; for *Pimentón de la Vera*), *Riojano* (La Rioja), and *Guindilla de Ibarra* and *Gernika* (Basque Country), among others, are highly considered among consumers (Rivera et al., 2016; Rodríguez-Burruezo et al., 2016).

However, not all Spanish landraces benefit from being recognized with protected designations. Those that are not included in such groups are in high risk of genetic erosion due to its substitution by F_1 cultivars of California Wonder and Lamuyo types, highly productive and resistant to several pathogens, but encompassing a narrow genetic diversity (Lanteri et al., 2003; Rivera et al., 2016; Rodríguez-Burruezo et al., 2016). Consequently, the abandonment of ancient materials seriously threatens agrodiversity (Brugarolas et al., 2009; Hammer, 2004; Hammer et al., 2003; Votava et al., 2005).

Fortunately, consumers are becoming increasingly interested in tastier foods produced in environmentally sustainable systems, and this situation offers a great opportunity for recovering the ancient cultivars while maintaining the farmer's source of income (Brugarolas et al., 2009; Casals et al., 2011; Hurtado et al., 2014; Parisi et al., 2017; Pérez-López et al., 2007; Rivera et al., 2016; Zonneveld et al., 2015). In fact, the demand for traditional varieties is increasing gradually and they even reach higher prices than those from modern varieties (Brugarolas et al., 2009; Casals et al., 2011). In this frame, it is essential to increase the added-value of landraces among consumers and to make efforts to characterize and to preserve such valuable resources *in situ*. Thus, an exhaustive characterization of cultivars is of paramount importance, especially for those which still lack typification (Lanteri et al., 2003; Parisi et al., 2017; Spataro and Negri, 2013). For instance, the popular term *Pimiento Valenciano* encompasses a wide range of relevant *Morrón* peppers from the Region of Valencia, but this denomination still lacks varietal typification (Rodríguez-Burruezo et al., 2016).

With this aim, morphological characterization based on standardized descriptors is an important practice for germplasm identification. The availability of an internationally recognized set of highly heritable descriptors throughout the scientific community enables the comparison of results as well as the characterization of cultivars (Bioversity International, 2019; Gotor et al., 2008; UPOV, 2019). However, these descriptors are sometimes tedious and often difficult to evaluate, particularly when differences between accessions are very subtle (Brewer et al., 2006; Costa et al., 2011; Figàs et al., 2018).

To this regard, Tomato Analyzer, a high-throughput phenomics software tool, provides fast, accurate and semi-automatic measurements of a large set of fruit traits that are otherwise impossible to obtain manually (Brewer et al., 2007, 2006; Darrigues et al., 2008; Gonzalo et al., 2009; Gonzalo and van der Knaap, 2008; Rodríguez et al., 2010a, 2010b). Despite being initially developed for tomato fruits characterization, it has been successfully used to characterize other crops (Darrigues et al., 2008; Hurtado et al., 2013; Naegele et al., 2016; Plazas et al., 2014).

On this matter, pepper germplasm still lacks large-scale phenomics characterization that could be used in parallel with the exponentially increasing available genomic information in order to fully exploit all the resources and unveil new favourable allelic combinations (Ashrafi et al., 2012; Hulse-Kemp et al., 2018, 2016; Kim et al., 2017, 2014; Malika et al., 2019; Park et al., 2012; Qin et al., 2014; Zonneveld et al., 2015). The use of both conventional and Tomato Analyzer descriptors might lead to a more detailed and powerful morphological characterization of pepper varieties resulting in a better separation of closely related materials.

Here we present the morphological characterization, using a set of conventional descriptors and Tomato Analyzer parameters, of a large collection of pepper accessions that includes a comprehensive representation of heirlooms and landraces from all the Spanish regions. To our knowledge, this is the first work to use conventional and phenomics tools to characterize such a large collection of peppers from the relevant Spanish centre of diversity. Our goals were: i) to assess the morphological diversity of the Spanish pepper landraces in order to contribute to varietal typification, promotion and preservation, and ii) to estimate the discrimination power of both conventional descriptors and phenomics software, separately and combined, particularly for highly close materials.

2. Material and methods

2.1 Plant material and growing conditions

A collection of 109 pepper accessions, encompassing 106 *C. annuum* accessions and other species from the *annuum* complex, i.e. *C. chinense* (2) and *C. frutescens* (1), was characterized (Table 1). This collection is representative of the most relevant heirlooms and landraces from the Spanish centre of diversity, with special emphasis on the highly appreciated *Morrón* peppers, as well as other foreign materials as controls. All the regions of Spain and 14 different countries were represented, as well as three varietal status (traditional, commercial and experimental lines) and nine main groups based on varietal assignment (Figure 1). These materials are maintained at the COMAV Germplasm Bank (Universitat Politècnica de

València) and at the COMAV *Capsicum* breeding group and are the result of several collection expeditions over the past four decades (Table 1).

Five plants per accession were grown under mesh greenhouse and open field conditions, during the spring-summer of 2015, at the COMAV experimental fields (UPV Vera Campus, GPS coordinates: 39°28'56.33"N; 0°20'10.88"W). Transplanting was done in May at the five leaves stage, and fruit harvest was carried-out from July to October. Plants were spaced 1 m between rows and 0.50 m within the row, following a completely randomized design. Individual plants were trained with vertical strings, drip irrigated and pruned accordingly to the standard local practices for this crop. Phytosanitary treatments against whiteflies, spider mites, aphids and caterpillars were applied accordingly to their population levels.

Table 1 - List of accessions and corresponding abbreviation, name, origin, and varietal status.

Abbreviation	Accession: Local name (germplasm bank code)	Origin	Varietal type
Group I: *Morrón de cascos* (MC) Pochard´s groups A and B – Blocky and rectangular shape, medium to large size			
MC-1	Pimiento morro de vaca (BGV-57)	Huesca, Spain	Traditional
MC-2	Pimiento cuatro cascos (BGV-637)	Granada, Spain	Traditional
MC-3	Pimiento morrón (BGV-1319)	Asturias, Spain	Traditional
MC-4	Pimiento morrón (BGV-1814)	Tarragona, Spain	Traditional
MC-5	Cuatro morros (BGV-1834)	Barcelona, Spain	Traditional
MC-6	Morro de Vedella (BGV-1844)	Cataluña, Spain	Traditional
MC-7	Largo de Reus (BGV-1862)	Barcelona, Spain	Traditional
MC-8	Pimiento gordo de asar (BGV-4036)	Cáceres, Spain	Traditional
MC-9	Pimiento grueso de Murcia (BGV-4322)	Murcia, Spain	Traditional
MC-10	Morro de vaca (BGV-4329)	Murcia, Spain	Traditional
MC-11	Pimiento trompa de vaca (BGV-4348)	Murcia, Spain	Traditional
MC-12	Pimiento morro de vaca (BGV-4349)	Murcia, Spain	Traditional
MC-13	Pimiento cuatro cantos (BGV-5035)	Valencia, Spain	Traditional
MC-14	Pimiento cuatro cantos (BGV-5057)	Castellón, Spain	Traditional
MC-15	Pimiento gordo (BGV-5083)	Castellón, Spain	Traditional
MC-16	Trompa de vaca (BGV-5109)	Alicante, Spain	Traditional
MC-17	Morrón cuatro cantos (BGV-5113-1)	Alicante, Spain	Traditional
MC-18	Pimiento de Infantes (BGV-10368)	Ciudad Real, Spain	Traditional
MC-19	Pimiento de casco (BGV-10540)	Albacete, Spain	Traditional
MC-20	Pimiento cuatro morros (BGV-10599)	León, Spain	Traditional
MC-21	Largo de Reus (BGV-10600)	Tarragona, Spain	Traditional
MC-22	Morrón de cuatro Picos (BGV-10946)	Asturias, Spain	Traditional
MC-23	Pimiento morro de vaca (BGV-11038)	Albacete, Spain	Traditional
MC-24	Pimiento de cuatro morros (BGV-11213)	Cantábria, Spain	Traditional
MC-25	Pimiento morrón largo (BGV-11267)	León, Spain	Traditional
MC-26	Morrón de Loyola cuatro cantos (BGV-11528)	Guipúzcoa, Spain	Traditional
MC-27	Pimiento gordo de ensalada (BGV-11558)	Cáceres, Spain	Traditional
MC-28	Pimiento morrón gordo (BGV-11630)	Vizcaya, Spain	Traditional
MC-29	Pimiento gordo morro de vaca (BGV-11751)	Huesca, Spain	Traditional
MC-30	Pimiento gordo (BGV-13636)	Salamanca, Spain	Traditional
MC-31	Pimiento gordo (BGV-13638)	Zamora, Spain	Traditional
MC-32	California Wonder (red)	COMAV, Valencia, Spain	Experimental line
MC-33	California Wonder (yellow)	COMAV, Valencia, Spain	Experimental line
MC-34	De Infantes	Ciudad Real, Spain	Traditional
MC-35	De Infantes	Mascarell Seeds, Spain	Commercial heirloom
MC-36	Largo de Reus	Barcelona, Spain	Traditional
MC-37	Largo de Reus	Mascarell Seeds, Spain	Commercial heirloom
MC-38	Morrón de Fresno de la Vega y Benavente P.G.I.	León, Spain	Traditional
MC-39	Pimento de assar	Aveiro, Portugal	Traditional
MC-40	Tendre de Châteaurenard	F. Jourdan, INRA-GEVES, France	Traditional
MC-41	Carmagnola giallo	Carmagnola, Pierdmont, Italy	Traditional
MC-42	Carmagnola rosso	Carmagnola, Pierdmont, Italy	Traditional
MC-43	Cuneo Giallo	Franchi Sementi, Italy	Commercial heirloom
MC-44	Giallo D'Asti	Franchi Sementi, Italy	Commercial heirloom
MC-45	Peperone Cuneo giallo	Cuneo, Italy	Traditional
MC-46	Atina	Serbia	Commercial heirloom
Group II: Valenciano (MV) Pochard´s groups B1 and B2 – Rectangular shape, large size			
MV-1	Valenciano (BGV-4331)	Murcia, Spain	Traditional
MV-2	Valenciano (BGV-5030)	Valencia, Spain	Traditional
MV-3	Valenciano (BGV-5103)	Valencia, Spain	Traditional
MV-4	Valenciano (BGV-5113 (2))	Alicante, Spain	Traditional
MV-5	Valenciano (BGV-5121)	Alicante, Spain	Traditional
MV-6	Valenciano (BGV-5126)	Alicante, Spain	Traditional
MV-7	Pimiento valenciano (BGV-10582)	Valencia, Spain	Traditional
MV-8	Valenciano	Valencia, Spain	Traditional
Group III: Morrón de bola/conserva (MB) Pochard´s groups F and P – round/heart shape, small to medium size			
MB-1	Pimiento morrón de bola (BGV-60)	Zaragoza, Spain	Traditional
MB-2	Pimiento morrón de conserva (BGV-614)	Jaén, Spain	Traditional
MB-3	Morrón de conserva (BGV-4335)	Murcia, Spain	Traditional
MB-4	Morrón de conserva (BGV-5041)	Valencia, Spain	Traditional
MB-5	Morrón de conserva (BGV-5114 (1))	Alicante, Spain	Traditional
MB-6	Morrón de conserva (BGV-5114 (2))	Alicante, Spain	Traditional
MB-7	Pimiento del País (BGV-10447)	Baleares, Spain	Traditional
MB-8	Lora (BGV-11500)	León, Spain	Traditional
MB-9	Pimiento morrón de conserva (BGV-11881)	Guadalajara, Spain	Traditional
MB-10	Calahorra	La Rioja, Spain	Traditional
MB-11	Bulgarski Ratund	Maritsa VCRI, Bulgaria	Traditional
MB-12	Topepo rosso	Italy	Traditional
MB-13	Topepo rosso	Franchi Sementi, Italy	Commercial heirloom

Table 1 (continuation) - List of accessions and corresponding abbreviation, name, origin, and varietal status.

Abbreviation	Accession: Local name (COMAV seedbank code)	Origin	Varietal type
Group IV: other thick flesh peppers Pochard´s groups C2 and C3 – Triangular shape, medium to large size			
IV-1	Pimiento grueso del país (BGV-4507)	Cantabria, Spain	Traditional
IV-2	Pimiento Najerano gordo (BGV-10451)	La Rioja, Spain	Traditional
IV-3	Pimiento gordo najerano (BGV-11092)	La Rioja, Spain	Traditional
IV-4	Pimiento de asar gordo najerano (BGV-13004)	Vizcaya, Spain	Traditional
IV-5	Pimiento de asar najerano (BGV-13009)	Vizcaya, Spain	Traditional
IV-6	Bierzo, Cons. Reg. P.G.I. Pimiento Asado Bierzo	León, Spain	Traditional
IV-7	Najerano	Ramiro Arnedo Seeds, Spain	Commercial heirloom
Group V: Ancho/Piquillo peppers Pochard´s group C4 – Triangular shape, small size			
V-1	Pimiento de Pico (BGV-10183)	Navarra, Spain	Traditional
V-2	Pimiento del Piquillo (BGV-10186)	Navarra, Spain	Traditional
V-3	Pimiento del Piquillo, Cons. Reg. P.D.O. Pimiento Piquillo de Lodosa	Navarra, Spain	Traditional
V-4	Ancho 101	Reimer Seeds, Mexico/USA	Commercial heirloom
V-5	Ancho mulato	Mexico	Traditional
Group VI: Cayenne/Guindilla			
VI-1	Guindilla (BGV-11531)	Guipúzcoa, Spain	Traditional
VI-2	Guindilla de Ibarra	S. Larregla, NEIKER, Spain	Traditional
VI-3	Torpedo de Bangalore	Bangalore, India	Traditional
VI-4	Chile de árbol	Mexico	Traditional
VI-5	Pasilla bajío	Mexico	Traditional
VI-6	Ka 2	Sri Lanka	Commercial heirloom
VI-7	Rashi Bonnet	Sri Lanka	Traditional
VI-8	Ací Sivri	Turkey	Traditional
Group VII: Numex and Padrón Pochard´s groups B2, B4 and C2 – Elongated (medium to large size) and rectangular (small size)			
VII-1	Pimiento de Padrón (BGV-10185)	La Coruña, Spain	Traditional
VII-2	Pimiento de Padrón (BGV-11205)	Navarra, Spain	Traditional
VII-3	Pimiento de Herbón-Padrón P.D.O.	Galicia, Spain	Traditional
VII-4	Arnoia, P.G.I. Pemento da Arnoia	Galicia, Spain	Traditional
VII-5	Gernika cv. Derio, P.G.I. Gernikako Piperra	S. Larregla, NEIKER, Spain	Traditional
VII-6	Kapiya UV	Maritsa VCRI, Bulgaria	Traditional
VII-7	Sivriya 600	Maritsa VCRI, Bulgaria	Traditional
VII-8	Espelette P.G.I.	F. Jourdan, INRA-GEVES, France	Traditional
VII-9	Pettit Marsellais	F. Jourdan, INRA-GEVES, France	Traditional
VII-10	Poivre rouge de Bresse	F. Jourdan, INRA-GEVES, France	Traditional
VII-11	Peperone di Senise P.G.I.	Potenza, Senise, Italy	Traditional
VII-12	Numex Conquistador	New Mexico, USA	Traditional
VII-13	Chimayó	P.W. Bosland, New Mexico, USA	Traditional
VII-14	Numex Big Jim	P.W. Bosland, New Mexico, USA	Traditional
Group VIII: Jalapeno			
VIII-1	Chile Serrano	Mexico	Traditional
VIII-2	Jalapeno Candelaria	P.W. Bosland, New Mexico, USA	Traditional
VIII-3	Jalapeno Espinalteco	P.W. Bosland, New Mexico, USA	Traditional
VIII-4	Jalapeno M	Reimer Seeds, Mexico/USA	Commercial heirloom
Group IX: Control/other Capsicums			
CON-1	Ají chirere (*C. frutescens*)	Venezuela	Traditional
CON-2	ECU-994 (*C. chinense*)	Equador	Traditional
CON-3	Habanero (*C. chinense*)	State College, Pennsylvania, USA	Traditional
CON-4	Pimiento de Bola, Cons. Reg. P.D.O. Pimentón Murcia	Murcia, Spain	Traditional

Figure 1 – Illustration of fruit diversity in the collection. Evaluated fruits and corresponding longitudinal cut indicate the common fruit morphology in each group: Group I (A – California Wonder (MC-33) and B – Morrón de cuatro cascos (MC-2)), Group II (C – Valenciano (MV-6)), Group III (D – Morrón de Conserva (MB-4)), Group IV (E – Najerano (IV-7) and F – Pimiento de asar mucha carne (IV-5)), Group V (G– Piquillo de Lodosa (V-3)), Group VI (H – Guindilla de Ibarra (VI-2), I – Chile de árbol (VI-4) and J – Pasilla bajío (VI-5)), Group VII (K - Pimiento de Padrón (VII-3), L – Gernika (VII-5) and M – Kapiya UV (VII-6)), Group VIII (N – Jalapeno M (VIII-4) and O – Chile Serrano (VIII-1)), and Group IX (P - Habanero (CON-3).

2.2 Conventional characterization

Five individual plants per genotype were characterized using 32 conventional morphological descriptors for *Capsicum* related to plant (9), inflorescence/flower (7), fruit (15) and agronomic (1) traits (Table 2). Most descriptors corresponded to *Capsicum* descriptors from Bioversity International (IPGRI, 1995). In addition, other commonly used traits for germplasm characterization such as plant height, fruit weight, fruit colour, fruit cross-sectional shape, and yield were also considered. To avoid tedious reading of the manuscript as well as to make it more visual, all descriptors and traits were presented with abbreviations (Table 2).

Fruit colour was measured using a Minolta CR-300 colorimeter (Minolta Corporation, Osaka, Japan) and expressed accordingly to CIE L*a*b* 1976 colour space at the two commercial ripening stages of pepper, i.e. unripe and fully ripe. Two measures per fruit were taken at opposite sides of the equatorial region of the fruit. Colour parameters Chroma and HUE angle were then calculated as reported by Rodríguez-Burruezo et al. (2005). For descriptors involving measurements, four representative leaves (for mature leaf length and mature leaf width descriptors) and four representative fruits (for L*a*b* colour space coordinates) per plant were measured and values obtained for individual fruits were used to calculate the average value for each plant. Finally, yield per plant and average fruit weight were estimated at the end of the experiment considering all the commercial fruits per plant (Table 2).

Table 2 – List of conventional descriptors measured, corresponding abbreviation, IPGRI descriptor number, and units/scale.

Descriptor	Abbreviation	IPGRI Number	Units/scale
Plant descriptors			
Plant height	**PH**	-	cm
Growth habit	**GH**	7.1.2.7	3=Prostrate, 5=Intermediate (compact), 7=Erect
Nodal anthocyanin	**NA**	7.1.2.3	1=Green, 3=Light purple, 5=Purple, 7=Dark purple
Stem length	**SL**	7.1.2.9	cm
Branching habit	**BH**	7.1.2.11	3=Sparse, 5=Intermediate, 7=Dense
Leaf density	**LD**	7.1.2.13	3=Sparse, 5=Intermediate, 7=Dense
Leaf shape	**LS**	7.1.2.15	1=Deltoid, 2=Ovate, 3=Lanceolate
Mature leaf length	**MLL**	7.1.2.18	cm
Mature leaf width	**MLW**	7.1.2.19	cm
Inflorescence/Flower descriptors			
Number of flowers per axil	**FA**	7.2.1.2	1=One, 2=Two, 3=Three or more, 4=Many in bunches
Corolla colour	**CC**	7.2.1.4	1=White, 2=Light yellow, 3=Yellow, 4=yellow-green
Corolla spot colour	**CSC**	7.2.1.5	0=Absent, 1=White, 2=Yellow, 3=Green yellow, 4=Green, 5=purple
Anther colour	**AC**	7.2.1.8	1=White, 2=Yellow, 3=Light blue, 4=Blue, 5=Purple, 6=Dark purple
Flower position	**FP**	7.2.1.3	3=Pendant, 5=Intermediate, 7=Erect
Calyx margin	**CM**	7.2.1.15	1=Entire, 2=Intermediate, 3=Dentate
Calyx annular constriction	**CAC**	7.2.1.16	0=Absent, 1=Present
Fruit descriptors			
Fruit set	**FS**	7.2.2.4	3=Low, 5=Intermediate, 7=High
Fruit weight	**FW**	-	g
Fruit shape	**FSH**	7.2.2.7	1=Elongate, 2=Almost round, 3=Triangular, 4=Campanulate, 5=Blocky
Fruit surface	**FSUR**	7.2.2.19	1=Smooth, 2=semi wrinkled, 3=Wrinkled
Fruit cross-sectional shape	**FCSC**	-	1=Elliptic, 2=Rounded, 3=Quadrangular, 4=Triangular, 5=Irregular
Ripe fruit pungency (tasting)	**CAPS**	-	0=Absent, 1=Present
Anthocyanin spots or stripes	**AS**	7.2.2.2	0=Absent, 1=Present
Fruit shape at pedicel attachment	**FSPA**	7.2.2.13	1=Acute, 2=Obtuse, 3=Truncate, 4=Cordate, 5=Lobate
Fruit shape at blossom end	**FSBE**	7.2.2.15	1=Pointed, 2=Blunt, 3=Sunken, 4=Sunken and pointed
Exterior fruit colour lightness (unripe)	**Lu**	-	0=Black to 100=White
Exterior fruit colour Chroma (unripe)	**CHRu**	-	0=completely unsaturated to 100=fully saturated
Exterior fruit colour HUE (unripe)	**HUEu**	-	0°=red, 90°=yellow, 180°=green, 270°=blue
Exterior fruit colour lightness (ripe)	**Lr**	-	0=Black to 100=White
Exterior fruit colour Chroma (ripe)	**CHRr**	-	0=completely unsaturated to 100=fully saturated
Exterior fruit colour HUE (ripe)	**HUEr**	-	0°=red, 90°=yellow, 180°=green, 270°=blue
Agronomic descriptors			
Yield per plant	**Y**	-	g

2.3 Digital characterization

Four representative samples of genotype and commercially viable fruits per plant were longitudinally cut and scanned at a resolution of 300 dpi (dots per inch) with an Epson Expression 1640XL G650C scanner (Seiko Epson Corp., Japan). Stored images (TIF format) were then analysed using the Tomato Analyzer software (version 3.0) for 35 quantitative traits (Brewer et al., 2006; Darrigues et al., 2008; Rodríguez et al., 2010a). Tomato Analyzer is a software, originally developed for tomato fruit, that measures a range of two-dimensional parameters related to fruit shape in a semi-automatic reproducible way, of particular interest for measuring angles and distal and proximal ends of fruits and indentation areas (Rodríguez et al., 2010a). The descriptors measured herein included basic (7), fruit shape (3), blockiness (3), homogeneity (3), proximal fruit end shape (4), distal fruit end shape (4), asymmetry (6) and internal eccentricity (5) traits (Table 3). For blockiness, proximal fruit end shape and distal fruit end shape descriptors default settings were used (Rodríguez et al., 2010a). Individual measures of each fruit were then used to obtain an average value for the corresponding plant (Table 3). As for the conventional descriptors and traits, all digital parameters were also given abbreviations.

Table 3 – List of digital phenotyping traits and parameters considered, and corresponding abbreviation, units, and brief description.

Trait	Abbreviation	Units	Description*
Basic			
Perimeter	**P**	cm	Fruit perimeter length.
Area	**AR**	cm²	Fruit area.
Width Mid-height	**WMH**	cm	Measured at 1/2 of the fruit's height.
Maximum Width	**MW**	cm	Maximum horizontal distance of the fruit.
Height Mid-Width	**HMW**	cm	Measured at 1/2 of the fruit's width.
Maximum Height	**MH**	cm	Maximum vertical distance of the fruit.
Curved Height	**CH**	cm	Measured along a curved line through the fruit.
Fruit shape index			
Fruit Shape Index External I	**FSIE.I**	-	The ratio of maximum height to maximum width.
Fruit Shape Index External II	**FSIE.II**	-	The ratio of height mid-width to width mid-height.
Curved Fruit Shape Index	**CFSI**	-	The ratio of curved height to the width of the fruit at mid-curved-height.
Blockiness			
Proximal Fruit Blockiness	**PFB**	-	The ratio of the width at the upper blockiness position to width mid-height.
Distal Fruit Blockiness	**DFB**	-	The ratio of the width at the lower blockiness position to width mid-height.
Fruit Shape Triangle	**FST**	-	The ratio of the width at the upper blockiness position to the width at the lower blockiness position.
Homogeneity			
Ellipsoid	**ELL**	-	The ratio of the error resulting from a best-fit ellipse to the area of the fruit. Smaller values indicate more ellipsoid.
Circular	**CIR**	-	The ratio of the error resulting from a best-fit circle to the area of the fruit. Smaller values indicate more circular.
Rectangular	**RECT**	-	The ratio of the area of the rectangle bounding the fruit to the area of the rectangle bounded by the fruit.
Proximal fruit end shape			
Shoulder Height	**SH**	-	The ratio of the average height of the shoulder points above the proximal end point to maximum height.
Proximal Angle Micro	**PAMi**	degrees	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal end point.
Proximal Angle Macro	**PAMa**	degrees	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal end point.
Proximal Indentation Area	**PIA**	-	The ratio of the area of the proximal indentation to the total area of the fruit multiplied by 10.
Distal fruit end shape			
Distal Angle Micro	**DAMi**	degrees	The angle between best-fit lines drawn through the fruit perimeter on either side of the proximal end point.
Distal Angle Macro	**DAMa**	degrees	The angle between best-fit lines drawn through the fruit perimeter on either side of the distal end point.
Distal Indentation Area	**DIA**	-	The ratio of the area of the distal indentation to the total area of the fruit multiplied by 10.
Distal End Protrusion	**DEP**	-	The ratio of the area of the distal protrusion to the total area of the fruit multiplied by 10.
Asymmetry			
Obovoid	**OB**	-	Calculated as described by Rodríguez et al. 2010a.
Ovoid	**OV**	-	Calculated as described by Rodríguez et al. 2010a.
V. Asymmetry	**VA**	-	Average distance between a vertical line through the fruit at mid-width and the midpoint of the fruit's width at each height.
H. Asymmetry.ob	**HAOb**	-	Average distance between a horizontal line at mid-height and the midpoint of the fruit's height at each width.
H. Asymmetry.ov	**HAOv**	-	Average distance between a horizontal line at mid-height and the midpoint of the fruit's height at each width.
Width Widest Position	**WWP**	-	The ratio of the height at which the maximum width occurs to the maximum height.
Internal eccentricity			
Eccentricity	**ECC**	-	The ratio of the height of the internal ellipse to the maximum height.
Proximal Eccentricity	**PE**	-	The ratio of the height of the internal ellipse to the distance between the bottom of the ellipse and the top of the fruit.
Distal Eccentricity	**DE**	-	The ratio of the height of the internal ellipse to the distance between the top of the ellipse and the bottom of the fruit.
Fruit Shape Index Internal	**FSII**	-	The ratio of the internal ellipse's height to its width.
Eccentricity Area Index	**EAI**	-	The ratio of the area of the fruit outside the ellipse to the total area of the fruit.

* For more detailed information about the descriptors check Rodríguez et al. (2010a).

2.4 Data analyses

Analysis of variance (ANOVA) was performed using individual plant values (*n*=5) to identify significant differences among the means of the accessions as well as among the means of varietal groups (*n* depending on the number of accessions per group) for both conventional and digital traits and parameters. To avoid scaling effects the ANOVA was performed using log transformed data (Figàs et al., 2014; Hills and Jackson, 1978). Student-Newman-Keuls post-hoc multiple range test was used to assess significant differences among varietal groups. Means and standard deviations for each trait and accession were estimated and reported in the supplementary data (Supplementary data 1 and 2). In addition, Principal Component Analysis (PCA) was carried out using Euclidean pairwise distances among accessions, first considering separately both conventional and digital characterizations and finally considering both sets of traits together. Statistics were carried out using STATGRAPHICS software (Statgraphics Centurion XVI, StatPoint Technologies, Warrenton, VA, USA) and plotted using the R package ggplot2 v2.2.1 (R Development Core Team, 2009; Wickham, 2016).

3. Results and discussion

3.1 Study of variation for the whole collection

3.1.1 Conventional descriptors

Highly significant differences (P<0.001 and P<0.01) were found among accessions for all conventional descriptors, with the only exception of corolla spot colour which was null for all accessions (Table 4). This was due to the fact that corolla spot colour is mainly used for species identification since it is present in *C. baccatum*, and other wild relatives, and consequently not useful for differentiation within *C. annuum* or related species, i.e. *C. chinense* and *C. frutescens* (DeWitt and Bosland, 1996; Moscone et al., 2007).

Most conventional descriptors showed considerable variation as can be observed in plant height (24-207 cm), stem length (5-60.20 cm), mature leaf length (7.10-25 cm), mature leaf width (2.30-14.60 cm), fruit weight (0.54-353.67 g), exterior fruit colour lightness (unripe) (33.00-74.89), exterior fruit colour Chroma (unripe) (9.85-36.74), exterior fruit colour HUE (unripe) (99.76-148.75º), exterior fruit colour lightness (ripe) (12.49-68.76), exterior fruit colour Chroma (ripe) (11.60-45.36), exterior fruit colour HUE (ripe) (7.89-108.06º) or yield (127-3872 g) (Table 4). By contrast, other traits (mainly qualitative) showed a limited variation despite being polymorphic. This was particularly obvious for those traits with mean values close to one of the extremes of the range of variation, indicating that most accessions fit one of the categories of the descriptor (Table 4 and Supplementary Data 1). This was the case of number of flowers per axil and corolla colour (range of 1-4, but average close to 1), and ripe fruit pungency and anthocyanin spots or stripes (usually absent). Such results were mainly due to the fact that most accessions belong to *C. annuum* and bell pepper (*Morrón*) type, and they usually present a single flower per axil, white corolla, and sweet fruits. Moreover, anthocyanin spots or stripes is an unwanted trait in ripe fruits, particularly for bell peppers, and farmers and breeders have usually performed selection against it. The low frequency of anthocyanin spots in the fruits was also observed in another report regarding pepper landraces from Northern Spain (Rivera et al., 2016).

In terms of variation, our findings are in agreement with the high diversity reported for *C. annuum* (Bosland and Votava, 2012). In addition to the huge range of fruit shapes, a remarkable variation for several traits that could have an important impact in future pepper breeding programs was also found (Table 4). For example, the accumulation of carotenoids in the fruits is one of the most important traits of pepper, since they are an important component in the human diet (Fiedor and Burda, 2014; Rodríguez-Burruezo et al., 2009), and a considerable variation for this trait was found among our accessions in terms of fruit colour parameters (Table 4). Finally, high diversity for both fruit weight and yield was observed, which have paramount importance for the acceptance of the variety among farmers, retailers and consumers (Costa et al., 2011).

3.1.2 Digital phenotyping

Regarding the characterization based on phenomics tool, 33 traits showed highly significant differences ($P<0.001$) and two showed significant differences ($P<0.05$) for the whole collection. A wide range of variance was detected for most traits, with the most variable traits being perimeter (8.53-53.63 cm), area (3.19-129.51 cm^2), proximal angle micro (0.40-358.19º), proximal angle macro (1.09-352.04º), distal angle micro (0.55-358.33º) and distal angle macro (0.28-330.55º) (Table 4). Furthermore, mean values in most descriptors were close to the mid-value of the corresponding range, suggesting a wide diversity among the accessions evaluated (Table 4 and Supplementary Data 2). Only a few traits such as fruit shape indexes (fruit shape index external I, fruit shape index external II and curved fruit shape index), distal fruit end shape descriptors (distal end protrusion), some asymmetry descriptors (V. asymmetry, H. asymmetry.ob and H. asymmetry.ov) and one Internal eccentricity descriptor (fruit shape index internal) showed mean values close to the lowest value of the corresponding range, indicating that a high number of accessions fit those values and therefore a low diversity among them. As found for conventional traits these findings were mainly due to limited variation among *Morrón de Cascos* and closely related types such as *Valenciano* and *Morrón de Bola* (Table 4).

These findings revealed an interesting level of diversity in our collection for both fruit size and shape and suggest a range of different shapes within *C. annuum* and even within the *Morrón* types which could have a direct application in future breeding programs in order to enhance the uses of these varieties. Size and shape of the fruits have a huge impact in several aspects, from farmers to consumer's preferences, in fact, they are the main attributes determining the packaging method and the acceptance of the variety (Costa et al., 2011).

Table 4 – Global means, ranges, and statistical significances of the conventional and digital traits and parameters considering the whole collection.

Conventional	Global mean/range [a]	Digital	Global mean/range [a]
Plant		***Basic***	
		P	33.20/8.53 - 53.63***
PH	103.84/24.00 - 207***	**AR**	52.06/3.19 - 129.51***
GH	5.09/3.00 - 7.00***	**WMH**	6.25/0.84 - 14.62***
NA	4.98/1.00 - 7.00***	**MW**	7.10/1.26 - 14.85***
SL	20.81/5.00 - 60.20***	**HMW**	8.16/1.25 - 17.13***
BH	4.61/3.00 - 7.00***	**MH**	10.17/2.93 - 18.70***
LD	4.83/3.00 - 7.00***	**CH**	10.91/3.05 - 20.47***
LS	1.63/1.00 - 3.00***	***Fruit shape index***	
MLL	14.90/7.10 - 25.00***	**FSIE.I**	1.76/0.45 - 9.95***
MLW	7.88/2.30 - 14.60***	**FSIE.II**	1.93/0.28 - 14.17***
		CFSI	2.56/0.51 - 16.88***
Flower		***Blockiness***	
		PFB	1.01/0.22 - 1.80***
FA	1.06/1.00 - 3.00***	**DFB**	0.61/0.25 - 1.09***
CC	1.08/1.00 - 4.00***	**FST**	1.79/0.30 - 4.19***
CSC	0.00/0.00 - 0.00 ns	***Homogeneity***	
AC	4.99/1.00 - 6.00***	**ELL**	0.11/0.05 - 0.23***
FP	6.27/3.00 - 7.00***	**CIR**	0.22/0.08 - 0.48***
CM	2.80/2.00 - 3.00***	**RECT**	0.49/0.09 - 0.68***
CAC	0.72/0.00 - 1.00***	***Proximal fruit end shape***	
		SH	0.08/0.00 - 0.19***
Fruit		**PAMi**	213.93/0.40 - 358.19***
		PAMa	217.28/1.09 - 352.04***
FS	3.44/3.00 - 7.00***	**PIA**	0.30/0.00 - 0.87***
FW	98.15/0.54 - 353.67***	***Distal fruit end shape***	
FSH	3.79/1.00 - 5.00***	**DAMi**	140.76/0.55 - 358.33***
FSUR	1.34/1.00 - 3.00***	**DAMa**	116.87/0.28 - 330.55***
FCSC	3.81/1.00 - 5.00***	**DIA**	0.00/0.00 - 0.39*
CAPS	0.20/0.00 - 1.00***	**DEP**	0.04/0.00 - 0.69***
AS	0.07/0.00 - 1.00***	***Asymmetry***	
FSPA	3.75/1.00 - 5.00***	**OB**	0.00/-0.21 - 0.22***
FSBE	2.16/1.00 - 4.00***	**OV**	0.37/0.00 - 0.89***
Lu	50.51/33.00 - 74.89***	**VA**	0.38/0.01 - 1.57***
CHRu	26.49/9.85 - 36.74***	**HAOb**	0.03/0.00 - 1.13***
HUEu	125.60/99.76 - 148.75**	**HAOv**	1.16/0.00 - 6.46***
Lr	41.35/12.49 - 68.76***	**WWP**	0.28/0.04 - 0.67***
CHRr	27.15/11.60 - 45.36***	***Internal eccentricity***	
HUEr	46.06/7.89 - 108.06***	**ECC**	0.64/0.10 - 0.82***
		PE	0.89/0.12 - 1.37***
Agronomic		**DE**	0.86/-4.37 - 2.45*
		FSII	1.96/0.29 - 14.34***
Y	1183.18/127 - 3872***	**EAI**	0.53/0.38 - 0.93***

[a] ***, **, *, ns indicate respectively significant for $P<0.001$, $P<0.01$, $P<0.05$, and non-significant differences among accessions´ means ($n=5$) obtained after the analysis of variance (ANOVA) (i.e. significance of the accession factor in the analysis of variance).

3.2 Study of variation between and within varietal groups

3.2.1 Conventional descriptors

Considering varietal groups means, significant differences were found for 30 of the 32 studied conventional descriptors (Table 5). Corolla spot colour and anther colour showed no significant differences among the nine varietal groups. As explained previously, corolla spot colour is not a useful trait to differentiate materials within the *annuum* complex. Anther colour may be a discriminating trait for collections encompassing different morphologies, but not for the varietal groups considered in our collection. Regarding anther colour, the *annuum* complex usually presents purple anthers but in some cases it also shows shades of blue to violet, as in our collection, while yellow or pale anthers are very unusual (DeWitt and Bosland, 1996; Russo, 2012).

For most traits with significant differences among varietal groups, groups fell into different categories, showing a clear separation (Table 5). Fruit traits fruit weight, fruit shape and fruit shape at pedicel attachment, and agronomic trait yield were able to separate varietal groups into several categories and were therefore the most discriminant. On the other hand, for some traits significant differences among groups were only due to one varietal group that differed from the others. Thus, leaf density and flower position enabled to differentiate group VIII from the other groups. The same happened for corolla colour and group IX, and finally fruit set for group IV. Ripe fruit pungency separated the first four groups from the others, where some (or even all) accessions were pungent (Table 5).

Intra-group variability was also observed for several conventional descriptors. Based on the standard deviations, descriptors such as nodal anthocyanin and leaf shape (plant), calyx annular constriction (flower), fruit weight, ripe fruit pungency, anthocyanin spots or stripes (fruit), and, as expected (Soares et al., 2019) yield (agronomic) were the ones responsible for most of the observed intra-group variation, including *Morrón* types (Table 5). In the case of ripe fruit pungency and anthocyanin spots or stripes, intra-variability was only observed in the more heterogeneous groups (V, VII and IX), where some accessions differed for these traits. The intra-varietal diversity may have an important impact in future pepper breeding programs for these materials by providing the opportunity to select those traits that have a major acceptance by consumers and producers (Costa et al., 2011; Parisi et al., 2017).

Table 5 – Means (± standard deviation) for conventional descriptors corresponding to the varietal groups considered.

Descriptor	Group I (MC)	Group II (MV)	Group III (MB)	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX (CON)
PH	93.95 ± 20.80 a ǂ	102.29 ± 22.78 ab	96.85 ± 14.47 ab	97.32 ± 15.00 ab	133.15 ± 31.38 c	113.16 ± 21.70 b	108.27 ± 26.05 ab	140.62 ± 56.46 c	146.19 ± 26.81 c
GH	4.91 ± 0.41 a	5.00 ± 0.00 ab	4.85 ± 0.54 a	5.29 ± 0.71 ab	5.40 ± 0.82 b	5.25 ± 0.67 ab	5.14 ± 0.92 ab	6.00 ± 1.03 c	6.00 ± 1.03 c
NA	4.76 ± 2.41 bc	4.18 ± 2.52 ab	5.15 ± 2.30 bc	4.71 ± 2.29 ab	5.40 ± 2.01 bc	6.00 ± 1.76 c	5.71 ± 1.96 bc	6.00 ± 1.79 c	3.00 ± 2.53 a
SL	20.00 ± 4.27 ab	20.70 ± 3.03 ab	19.88 ± 6.43 ab	22.84 ± 7.06 ab	21.26 ± 5.17 ab	21.53 ± 4.95 ab	18.45 ± 5.60 a	34.27 ± 15.26 c	22.62 ± 3.65 b
BH	4.60 ± 1.08 bc	4.76 ± 1.18 bc	4.08 ± 1.28 ab	4.71 ± 0.71 bc	5.00 ± 0.00 c	5.50 ± 1.68 c	3.86 ± 1.00 a	5.50 ± 1.71 c	5.50 ± 0.89 c
LD	4.89 ± 1.05 ab	4.41 ± 1.35 a	4.69 ± 1.08 ab	5.00 ± 1.09 ab	5.40 ± 0.82 b	4.50 ± 0.88 a	4.57 ± 1.36 a	6.00 ± 1.03 c	5.00 ± 0.00 ab
LS	1.42 ± 0.54 ab	1.71 ± 0.46 bcd	1.62 ± 0.49 bc	1.14 ± 0.36 a	1.40 ± 0.50 ab	2.88 ± 0.34 e	1.93 ± 0.71 cd	2.00 ± 0.73 d	1.25 ± 0.45 a
MLL	16.03 ± 2.59 d	15.98 ± 3.06 d	16.27 ± 2.70 d	16.29 ± 3.53 d	15.17 ± 2.08 d	10.21 ± 2.05 ab	13.90 ± 2.58 c	10.71 ± 2.35 b	9.49 ± 1.27 a
MLW	8.61 ± 1.43 d	8.28 ± 1.73 d	9.03 ± 1.66 d	8.71 ± 2.01 d	8.39 ± 1.41 d	4.32 ± 0.88 a	7.12 ± 1.53 c	5.27 ± 1.20 b	5.42 ± 0.76 b
FA	1.00 ± 0.00 a	1.00 ± 0.00 a	1.08 ± 0.27 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.25 ± 0.67 b	1.00 ± 0.00 a	1.00 ± 0.00 a	1.75 ± 0.45 c
CC	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.00 ± 0.00 a	3.25 ± 1.34 b
CSC	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
AC	4.91 ± 0.59 a	5.00 ± 0.00 a	5.08 ± 0.27 a	5.14 ± 0.36 a	5.20 ± 0.41 a	5.00 ± 0.00 a	5.00 ± 0.38 a	5.00 ± 0.00 a	5.00 ± 0.00 a
FP	6.56 ± 1.02 d	7.00 ± 0.00 d	6.69 ± 1.08 d	6.43 ± 1.43 cd	5.40 ± 1.54 b	5.25 ± 1.22 b	5.86 ± 1.47 bc	3.50 ± 0.89 a	7.00 ± 0.00 d
CM	2.87 ± 0.34 cd	3.00 ± 0.00 d	2.77 ± 0.43 cd	2.86 ± 0.36 cd	2.80 ± 0.41 cd	3.00 ± 0.00 d	2.64 ± 0.48 bc	2.50 ± 0.52 b	2.00 ± 0.00 a
CAC	0.91 ± 0.28 d	0.88 ± 0.34 d	0.62 ± 0.49 c	1.00 ± 0.00 d	1.00 ± 0.00 d	0.12 ± 0.34 a	0.36 ± 0.48 b	0.25 ± 0.45 ab	0.75 ± 0.45 cd
FS	3.53 ± 1.22 a	3.00 ± 0.00 a	3.31 ± 0.73 a	4.43 ± 1.79 b	3.80 ± 1.64 a	3.00 ± 0.00 a	3.29 ± 1.04 a	3.00 ± 0.00 a	3.50 ± 0.89 a
FW	142.78 ± 48.8 gh	160.52 ± 46.29 h	95.35 ± 26.27 f	111.44 ± 21.14 fg	41.17 ± 11.14 e	8.10 ± 6.61 b	33.22 ± 19.56 d	12.48 ± 7.21 c	8.01 ± 6.77 a
FSH	5.00 ± 0.00 e	5.00 ± 0.00 e	2.69 ± 1.28 c	3.57 ± 0.92 d	3.00 ± 0.00 d	1.00 ± 0.00 a	3.00 ± 1.87 c	2.00 ± 1.03 b	2.50 ± 1.15 c
FSUR	1.09 ± 0.28 a	1.00 ± 0.00 a	1.00 ± 0.00 a	1.14 ± 0.36 a	1.20 ± 0.41 a	3.00 ± 0.00 c	1.79 ± 0.41 b	1.00 ± 0.00 a	2.00 ± 1.03 b
FCSC	4.67 ± 0.80 d	4.88 ± 0.48 d	2.46 ± 1.09 ab	4.57 ± 0.50 d	3.00 ± 1.72 bc	2.00 ± 0.00 a	3.21 ± 1.84 c	2.00 ± 0.73 a	3.25 ± 1.34 c
CAPS	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.40 ± 0.50 b	1.00 ± 0.00 d	0.36 ± 0.48 b	1.00 ± 0.00 d	0.75 ± 0.45 c
AS	0.11 ± 0.31 ab	0.24 ± 0.43 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.25 ± 0.45 b	0.00 ± 0.00 a
FSPA	4.51 ± 0.58 f	4.24 ± 0.43 f	3.38 ± 0.49 d	4.14 ± 0.36 f	3.80 ± 0.77 e	1.00 ± 0.00 a	3.57 ± 0.83 de	2.00 ± 0.73 b	2.50 ± 0.89 c
FSBE	2.76 ± 0.64 c	2.53 ± 0.71 c	2.08 ± 0.27 b	2.00 ± 1.22 b	1.00 ± 0.00 a	1.00 ± 0.00 a	1.79 ± 0.87 b	1.00 ± 0.00 a	1.25 ± 0.45 a
Lu	49.03 ± 5.34 ab	51.36 ± 8.77 ab	47.47 ± 6.58 ab	46.08 ± 4.20 a	50.49 ± 4.90 b	55.68 ± 6.21 c	55.93 ± 6.91 c	54.73 ± 7.34 c	49.72 ± 5.52 ab
CHRu	25.58 ± 3.20 bc	26.16 ± 3.55 bc	23.50 ± 4.44 a	24.83 ± 2.31 b	28.75 ± 2.29 d	29.65 ± 3.63 d	29.19 ± 2.83 d	27.75 ± 3.32 cd	30.22 ± 3.75 d
HUEu	125.30 ± 4.51 ab	125.26 ± 6.98 b	126.24 ± 4.93 b	128.62 ± 3.22 b	125.96 ± 2.98 b	124.92 ± 5.23 ab	125.09 ± 3.55 ab	122.81 ± 5.25 a	127.84 ± 2.59 bc
Lr	42.26 ± 7.87 b	43.93 ± 5.39 b	40.67 ± 5.68 b	38.51 ± 4.83 b	40.59 ± 2.15 b	38.22 ± 4.13 b	43.41 ± 9.20 b	40.30 ± 5.13 b	33.72 ± 6.09 a
CHRr	24.96 ± 4.67 a	24.73 ± 4.90 a	24.75 ± 5.69 a	25.29 ± 5.02 a	28.52 ± 4.56 b	32.98 ± 5.47 c	31.54 ± 3.48 b	30.96 ± 1.55 c	35.54 ± 6.84 c
HUEr	51.54 ± 20.34 b	51.77 ± 10.95 b	45.80 ± 10.90 b	41.74 ± 7.37 b	40.75 ± 4.17 a	32.92 ± 10.37 b	42.82 ± 13.12 b	42.27 ± 7.77 b	28.14 ± 5.35 a
Y	1250.12 ± 525.04 e	1725.94 ± 568.50 cd	1298.46 ± 646.28 cd	1352.96 ± 446.24 d	1123.15 ± 278.35 cd	644.66 ± 262.03 a	982.02 ± 399.41 bc	733.25 ± 470.27 a	902.75 ± 455.85 ab

ǂ Different letters indicate significant differences among varietal groups for the corresponding descriptor, according to Student-Newman-Keuls post-hoc test for P<0.05.

3.2.2 Digital phenotyping

Significant differences among varietal groups were detected for 30 of the 35 digital traits and parameters. Traits distal indentation area, distal end protrusion, obovoid, H. asymmetry.ob, and distal eccentricity showed no significant differences among varietal groups.

A considerable diversity was found among varietal groups. Most traits, 29 out of 30, separated the groups into several categories, with the only exception of proximal eccentricity, for which significant variability was only found for group VI. On the whole, digital phenotyping separated varietal groups into more categories than those observed for conventional descriptors, suggesting a higher capability to differentiate among varietal pepper types. Basic descriptors like area, width mid-height, maximum height, and curved height were the ones with higher discriminating ability. Even more, digital phenotyping was able to separate morphologically close varietal groups such as *Morrón de Cascos* (group I), *Valenciano* (group II), *Morrón de Bola* (group III), and group IV. Our results are similar to others reported for a collection of tomato landraces, where digital phenotyping detected a higher number of differences among closely related accessions (Figàs et al., 2014).

Furthermore, a considerable intra-group variation, indicated by standard deviation values, was also detected by the digital traits and parameters. Fruit shape index trait fruit shape index external I, proximal fruit end shape traits shoulder height, proximal angle macro and proximal indentation area, distal end fruit shape traits distal angle micro and distal angle macro, asymmetry traits V. asymmetry, H. asymmetry.ov and width widest position, and finally internal eccentricity trait fruit shape index internal were the parameters associated to higher variance levels within varietal groups. These findings are consistent to others from a recent work encompassing several *Capsicum* species which also reported a remarkable variation for these traits (Nankar et al., 2020; Tripodi and Greco, 2018). In our work this ability to detect variation has been extended to closely related materials within specific varietal pepper types, which is of paramount importance for registration and typification purposes.

Table 6 – Means (± standard deviation) for digital traits corresponding to the varietal groups considered.

Descriptor	Group I (MC)	Group II (MV)	Group III (MB)	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX (CON)
P	39.08 ± 6.57 d[ǂ]	44.95 ± 3.86 e	26.39 ± 2.68 c	41.09 ± 5.04 de	27.31 ± 3.23 c	30.20 ± 9.61 c	30.94 ± 7.74 c	15.30 ± 4.10 b	13.43 ± 2.59 a
AR	71.33 ± 18.80 e	90.67 ± 12.19 f	39.82 ± 7.95 d	71.78 ± 15.39 e	36.54 ± 10.23 d	17.31 ± 9.23 c	36.38 ± 16.31 d	12.68 ± 5.18 b	8.82 ± 3.42 a
WMH	8.53 ± 1.72 f	7.32 ± 1.11 f	8.08 ± 0.64 f	6.15 ± 1.12 e	4.65 ± 1.44 d	1.42 ± 0.44 a	3.60 ± 1.07 c	2.46 ± 0.58 b	2.65 ± 1.52 b
MW	9.23 ± 1.55 e	8.78 ± 0.53 e	8.34 ± 0.60 e	7.87 ± 1.18 e	5.66 ± 1.29 c	3.15 ± 1.51 a	4.61 ± 1.00 b	2.73 ± 0.71 a	3.01 ± 1.41 a
HMW	7.93 ± 2.72 c	12.05 ± 2.87 d	5.02 ± 1.15 b	11.73 ± 1.82 c	8.21 ± 0.78 c	9.34 ± 4.89 d	9.98 ± 3.49 cd	5.38 ± 1.45 b	3.87 ± 0.98 a
MH	10.6 ± 2.54 cd	14.39 ± 2.39 f	6.19 ± 1.12 b	13.69 ± 1.78 ef	9.33 ± 0.98 c	12.75 ± 4.11 de	11.54 ± 3.55 d	5.69 ± 1.58 b	4.37 ± 1.21 a
CH	11.92 ± 2.68 e	15.01 ± 1.90 g	6.52 ± 1.20 c	14.19 ± 1.87 fg	9.63 ± 1.18 d	13.47 ± 4.24 ef	11.75 ± 3.50 e	5.79 ± 1.56 b	4.55 ± 1.24 a
FSIE I	1.18 ± 0.36 b	1.65 ± 0.32 c	0.74 ± 0.13 a	1.78 ± 0.34 c	1.73 ± 0.40 c	4.73 ± 2.24 e	2.55 ± 0.75 d	2.12 ± 0.42 d	1.84 ± 0.94 c
FSIE II	1.00 ± 0.50 b	1.76 ± 0.68 c	0.63 ± 0.16 a	1.99 ± 0.51 c	2.01 ± 0.82 c	6.76 ± 3.43 e	2.94 ± 1.15 d	2.23 ± 0.53 de	2.06 ± 1.20 c
CFSI	1.48 ± 0.52 b	2.24 ± 0.73 c	0.81 ± 0.16 a	2.54 ± 0.62 c	2.30 ± 0.87 c	9.77 ± 2.52 e	3.56 ± 1.31 d	2.40 ± 0.50 c	2.46 ± 1.52 c
PFB	0.93 ± 0.18 b	1.03 ± 0.14 bcd	0.82 ± 0.19 a	1.20 ± 0.23 cd	1.17 ± 0.32 bcd	1.12 ± 0.21 bcd	1.23 ± 0.19 d	0.96 ± 0.15 bc	0.99 ± 0.19 bc
DFB	0.64 ± 0.14 cd	0.72 ± 0.10 d	0.61 ± 0.10 bc	0.55 ± 0.10 bc	0.46 ± 0.10 a	0.64 ± 0.21 cd	0.58 ± 0.15 bc	0.56 ± 0.07 bc	0.53 ± 0.11 ab
FST	1.56 ± 0.50 a	1.50 ± 0.36 a	1.42 ± 0.47 a	2.24 ± 0.58 cd	2.59 ± 0.66 d	2.00 ± 0.80 bc	2.23 ± 0.69 cd	1.76 ± 0.40 ab	1.97 ± 0.62 bc
ELL	0.11 ± 0.02 b	0.11 ± 0.01 b	0.08 ± 0.02 a	0.12 ± 0.02 b	0.11 ± 0.02 b	0.17 ± 0.05 c	0.12 ± 0.02 b	0.07 ± 0.01 a	0.08 ± 0.02 a
CIR	0.17 ± 0.04 a	0.21 ± 0.05 b	0.16 ± 0.06 a	0.24 ± 0.05 b	0.21 ± 0.07 b	0.45 ± 0.02 d	0.31 ± 0.08 c	0.24 ± 0.05 b	0.24 ± 0.11 b
RECT	0.55 ± 0.06 c	0.52 ± 0.07 c	0.55 ± 0.04 c	0.43 ± 0.07 b	0.45 ± 0.07 b	0.28 ± 0.10 a	0.44 ± 0.09 b	0.51 ± 0.06 c	0.45 ± 0.09 b
SH	0.10 ± 0.04 e	0.08 ± 0.02 de	0.09 ± 0.04 e	0.07 ± 0.03 cd	0.05 ± 0.03 bc	0.05 ± 0.06 bc	0.06 ± 0.04 cd	0.02 ± 0.01 a	0.03 ± 0.03 ab
PAMi	229.81 ± 32.06 c	246.20 ± 21.71 c	205.26 ± 13.63 bc	240.06 ± 24.74 c	229.03 ± 16.79 c	58.07 ± 29.62 a	235.20 ± 56.82 bc	207.91 ± 49.06 bc	201.10 ± 69.87 b
PAMa	259.53 ± 38.28 d	236.21 ± 50.66 cd	252.92 ± 16.26 d	234.19 ± 32.09 cd	227.09 ± 17.68 cd	32.81 ± 24.48 a	193.18 ± 82.71 c	139.76 ± 62.94 b	162.35 ± 47.94 cd
PIA	0.37 ± 0.20 c	0.36 ± 0.14 c	0.33 ± 0.14 c	0.35 ± 0.20 c	0.22 ± 0.12 b	0.00 ± 0.01 a	0.35 ± 0.23 c	0.08 ± 0.06 a	0.10 ± 0.10 a
DAMi	190.50 ± 87.80 c	160.86 ± 64.37 c	164.33 ± 39.11 c	109.17 ± 29.47 c	86.82 ± 30.51 ab	25.61 ± 14.01 a	101.56 ± 76.00 ab	101.54 ± 29.18 ab	80.96 ± 52.20 b
DAMa	171.88 ± 79.28 c	109.96 ± 53.92 bc	145.85 ± 43.55 c	62.02 ± 22.76 b	80.34 ± 28.31 bc	16.10 ± 9.92 a	60.18 ± 41.43 b	71.98 ± 19.18 bc	86.66 ± 52.75 bc
DIA	0.01 ± 0.04 a	0.00 ± 0.00 a	0.00 ± 0.01 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
DEP	0.04 ± 0.11 a	0.01 ± 0.03 a	0.00 ± 0.02 a	0.05 ± 0.16 a	0.06 ± 0.07 a	0.10 ± 0.18 a	0.09 ± 0.19 a	0.00 ± 0.00 a	0.01 ± 0.03 a
OB	0.00 ± 0.04 a	0.00 ± 0.01 a	0.02 ± 0.05 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
OV	0.30 ± 0.15 ab	0.40 ± 0.08 c	0.23 ± 0.12 a	0.52 ± 0.16 de	0.56 ± 0.13 e	0.44 ± 0.15 cd	0.52 ± 0.17 de	0.37 ± 0.13 bc	0.39 ± 0.13 c
VA	0.44 ± 0.22 cd	0.54 ± 0.29 de	0.23 ± 0.13 ab	0.48 ± 0.38 d	0.21 ± 0.13 ab	0.65 ± 0.41 e	0.31 ± 0.21 bc	0.11 ± 0.13 a	0.15 ± 0.14 ab
HAOb	0.05 ± 0.20 a	0.02 ± 0.08 a	0.03 ± 0.09 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
HAOv	0.98 ± 0.71 b	1.59 ± 0.63 cd	0.43 ± 0.24 a	1.91 ± 0.52 de	1.33 ± 0.45 c	2.21 ± 1.01 e	1.60 ± 0.80 cd	0.49 ± 0.30 a	0.52 ± 0.41 a
WWP	0.32 ± 0.12 d	0.21 ± 0.05 ab	0.40 ± 0.08 e	0.16 ± 0.03 a	0.18 ± 0.08 a	0.23 ± 0.13 abc	0.20 ± 0.11 a	0.29 ± 0.10 cd	0.27 ± 0.10 bcd
ECC	0.59 ± 0.10 ab	0.65 ± 0.07 bc	0.65 ± 0.06 bc	0.68 ± 0.06 c	0.70 ± 0.03 cd	0.56 ± 0.22 a	0.70 ± 0.05 cd	0.77 ± 0.02 d	0.70 ± 0.15 cd
PE	0.90 ± 0.05 b	0.89 ± 0.02 b	0.90 ± 0.03 b	0.89 ± 0.02 b	0.89 ± 0.02 b	0.79 ± 0.34 a	0.89 ± 0.04 b	0.89 ± 0.02 b	0.89 ± 0.02 b
DE	0.87 ± 0.06 a	0.88 ± 0.04 a	0.89 ± 0.01 a	0.88 ± 0.07 a	0.87 ± 0.03 a	0.65 ± 1.25 a	0.90 ± 0.06 a	0.90 ± 0.05 a	0.86 ± 0.15 a
FSII	1.03 ± 0.50 b	1.78 ± 0.68 a	0.64 ± 0.18 a	2.09 ± 0.52 b	2.05 ± 0.83 b	6.70 ± 3.51 c	3.04 ± 1.16 d	2.26 ± 0.52 b	2.00 ± 1.22 b
EAI	0.54 ± 0.07 cd	0.56 ± 0.07 d	0.50 ± 0.04 bc	0.52 ± 0.07 bcd	0.50 ± 0.04 bc	0.61 ± 0.15 e	0.49 ± 0.05 bc	0.44 ± 0.03 a	0.48 ± 0.12 b

[ǂ] Different letters indicate significant differences among groups for the corresponding descriptor according to Student-Newman-Keuls post-hoc test for P<0.05.

3.2.3 Pairwise differences among varietal groups

The number of pairwise significantly different traits can illustrate how varietal groups differed from each other depending on the method of phenomics used (Table 7). Thus, considering conventional descriptors, groups VIII and IX encompassed the highest mean number of significantly different traits (19 and 20, respectively) to other groups, whereas the groups corresponding to *Morrón* peppers were the ones with least significant differences (12-13), followed by groups IV and V (13-14) (Table 7). Group IX included four accessions belonging to three different species with several traits that make them unique within the collection. On the other hand, group I included a large cluster of accessions of *Morrón de Cascos* type with several plant, flower, and fruit traits common to other varietal groups. Thus, *Morrón de Cascos*, *Valenciano*, *Morrón de Bola*, and thick fleshed peppers (IV) groups share many traits, and therefore showed a relative low number of pairwise differences among them (ranging from 1 to 8) (Table 7). This indicates a close relationship for these groups which was also confirmed by DNA polymorphisms in a recent work (Pereira-Dias et al., 2019).

By contrast, for digital phenotyping, the number of pairwise significant differences was generally higher in comparison to the conventional traits, particularly for the *Morrón* peppers groups. On the whole, group VI was the one with the highest mean number of significantly different descriptors (23), followed by *Morrón de Bola* (20), while group IV showed the lowest number of pairwise significant differences (15) (Table 7). Also, in contrast to the findings with conventional descriptors, groups VIII and IX (18-19) were not the ones with the highest number of differences, despite considering three different species in the case of group IX (Table 1). In fact, the number of pairwise differences was similar or lower than observed for conventional characterization. As Tomato Analyzer only takes into account fruit traits, these results suggest that the traits that made these groups unique in the conventional characterization were particularly plant traits. Thus, the group IX includes a round shaped *C. annuum* accession

(*Pimiento de Bola*) which shares many traits with the *Morrón* groups, especially with round-shaped fruits of group III. The other three accessions of this group are Ají chirere (*C. frutescens*), ECU-994 and Habanero (*C. chinense*), and the first shares resemblance to a short cayenne or a Serrano (group VIII), the second has triangular shape with pointed end without shoulders, similar to the groups VI and VIII, and finally the third has irregular round shape and slightly pointy distal end which could be the middle ground between a *Morrón de Bola* and a Jalapeno.

In addition, with similar number of traits and parameters as the conventional characterization, digital phenotyping enabled to detect a higher mean number of differences between varietal groups (Table 7). Thus, as an example, conventional descriptors were only able to detect 1, 8, and 4 significantly different traits between *Morrón de Cascos* and the three closest varietal groups *Valenciano*, *Morrón de Bola* and thick fleshed peppers from group IV, respectively, whereas considering the same pairwise comparisons digital phenotyping increased to 13, 14, and 16 parameters with significant differences among the mentioned varietal groups (Table 7). Regardless this phenomics tool only analyses fruit parameters, it provides higher discrimination power, essential for germplasm characterization and typification (Figàs et al., 2014; Hurtado et al., 2013; Nankar et al., 2020).

Table 7 – Number of pairwise significantly different (P<0.05) for varietal groups for conventional descriptors (above the diagonal and highlighted in blue) and for digital traits and parameters (below the diagonal and highlighted in grey). The average number of significant of differences for each group is also provided.

Varietal groups	Group I (MC)	Group II (MV)	Group III (MB)	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX (CON)	Mean of conventional
Group I (MC)		1	8	4	13	18	15	18	21	**12.25**
Group II (MV)	13		8	6	12	19	17	19	21	**12.88**
Group III (MB)	14	17		7	11	17	11	21	21	**13.00**
Group IV	16	8	21		12	19	16	23	21	**13.50**
Group V	20	17	21	11		17	13	20	16	**14.25**
Group VI	25	24	28	21	25		16	15	19	**17.50**
Group VII	19	18	21	12	10	19		18	22	**16.00**
Group VIII	22	21	19	21	20	21	22		21	**19.38**
Group IX (CON)	24	19	20	16	14	23	19	10		**20.25**
Mean of digital	**19.13**	**17.13**	**20.13**	**15.75**	**17.25**	**23.25**	**17.50**	**19.50**	**18.13**	

3.3 Principal components analysis (PCA)

3.3.1 PCA for conventional descriptors

Only conventional descriptors which provided significant differences: i) among accessions considering the whole collection and ii) among varietal groups were considered to perform PCA. The first two principal components explained 35.90% of total variance (Figure 2). PC_1 explained 27.03% of total variance and was positively correlated with fruit traits ripe fruit pungency, fruit surface and exterior fruit colour Chroma (ripe), and negatively to fruit traits fruit weight, fruit shape at pedicel attachment, fruit shape and plant traits mature leaf width and mature leaf length (Table 8). In addition, PC_2 accounted for 8.87% of variation and was positively correlated with fruit traits exterior fruit colour lightness (unripe) and exterior fruit colour Chroma (unripe), and to plant descriptors leaf shape and nodal anthocyanin, whereas flower descriptors corolla colour and number of flowers per axil, fruit descriptor exterior fruit colour HUE (unripe), and plant trait plant height were negatively correlated with PC_2 (Figure 2). Therefore, fruit traits were responsible for most of the explained variance (Table 8).

As a result, pungent, wrinkled and less saturated (lighter) red colour fruits like the ones included in group VI and some accessions of groups VIII and IX appeared in the positive side of PC_1 (Figure 2). By contrast, most accessions with big fruits, lobate pedicel attachment, and big leaves, such as the *Morrón* groups and fleshy peppers (groups I to IV), appeared in the negative side of PC_1. Likewise, accessions with lighter colour immature fruits, ovate or lanceolate leaves, and higher content of anthocyanin in the nodes were located on the top while accessions with yellow-greenish corollas, two flowers per axil, dark green immature fruits and taller plants were placed on the bottom of the graph (Figure 2).

The use of standardized descriptors is an important practice for germplasm identification (Bioversity International, 2019; Gotor et al., 2008; UPOV, 2019). However, its discrimination power is sometimes limited, especially when differences among materials are very subtle fruit traits (Brewer et al., 2006; Costa et al., 2011; Figàs et al., 2014). The set of 31 conventional descriptors was able to separate clearly distinct materials such as group VI and the *C. chinense* and *C. frutescens* accessions. In the same way, in another report, for a remarkably diverse collection encompassing nine species, leaf shape, nodal anthocyanin, and several flower traits were the most informative descriptors, indicating their usefulness when working with interspecific collections (Tripodi and Greco, 2018). Unfortunately, for closely related materials, particularly the ones included in our collection, separation was not so satisfactory (Figure 2).

Thus, fruit traits explained the highest percentage of variance for this collection. However, conventional descriptors lack of detail to be fully descriptive of the subtle differences between these accessions. In this regard, other works also reported an insufficient resolution of conventional descriptors and that fruit traits explain most of the variance (Costa et al., 2011; Figàs et al., 2014; Hurtado et al., 2013). Thus, as found in other crops, pepper varietal types are displayed mainly based on fruit shape, colour and flesh culinary properties (i.e. to eat fresh, fried, dry, roasted, as dip, etc.) which is in agreement with the fact that fruit traits explain a higher percentage of the variability (Bosland and Votava, 2012; Rivera et al., 2016; Tripodi and Greco, 2018).

3.3.2 PCA for digital phenotyping

Based on Tomato Analyzer traits and parameters the first two principal components explained 53.17% of total variance for our collection, 35.29 and 17.88% corresponding to PC_1 and PC_2, respectively, which was considerably higher than those recorded with convention descriptors. PC_1 was positively correlated with fruit shape index descriptors curved fruit shape index, fruit shape index external I, fruit shape index external I, internal eccentricity descriptor fruit shape index internal, and homogeneity descriptor circular, while on the other hand, it was negatively correlated with homogeneity descriptor rectangular, basic descriptors width mid-height and maximum width, proximal end shape descriptor proximal angle macro, and distal end shape descriptor distal angle macro (Table 8). PC_2 was positively correlated with basic descriptors perimeter, curved height, maximum height, area, height mid-width and maximum width, while it was negatively correlated to asymmetry descriptor width widest position (Table 8).

Thus, accessions bearing fruits with high height/width ratios, between internal eccentricity and width, were located in the positive side of PC_1, whereas wider and rectangular fruits and wider proximal and distal ends appeared on the negative side (Figure 2). In this way, accessions with elongated shape, as the cayenne from group VI, were on the opposite side to blocky and fleshy peppers from *Morrón* groups I to III and group IV. In addition, PC_2 separated those accessions with bigger fruit sizes, height and width to the upper part of the PCA, while those accessions with higher ratio between height at maximum width and maximum height appeared mainly on the bottom of the PCA (Figure 2).

Despite considering only fruit traits and parameters, digital phenotyping provided a good separation among accessions. A more detailed insight into the PCA clusters shows a better separation of accessions than that observed with conventional descriptors (Figure 2). Varietal groups were discriminated into several sub-clusters, e.g. three sub-groups of different cayenne size appeared from group VI. Likewise group VII accessions were divided into roughly three sub-clusters based on fruit shape. In addition, the phenomics tool was close to separate in the PCA groups I to IV, particularly *Valenciano* from *Morrón de Bola*. This four groups share many characteristics for both plant and fruit, resulting almost impossible to separate them based on conventional descriptors. Digital phenotyping demonstrated a higher accuracy to identify subtle differences in pepper fruits and to separate morphologically close materials, which is in agreement with the higher contribution to variation found for PC_1 and PC_2 with this tool (Figure

2). In other words, our results suggest that the higher percentage of variance detected by digital phenotyping enabled a better separation of the materials along both coordinates, indicating a more efficient differentiation of phenotypes based on a lower number of scored traits. Our results are in agreement with other works in tomato, where digital phenotyping explained a higher percentage of variability than conventional descriptors and therefore that fruit morphology is essential to assess variation among cultivars and to varietal typification (Figàs et al., 2014).

Nonetheless, not all digital traits contribute similarly to differentiate accessions or varietal groups, as it is highly dependent on the accessions considered. However, there are a set of descriptors that, regardless of the considered collection, explain a high level of diversity. According to our findings, basic and fruit shape index descriptors are usually the most informative, a pattern also reported in several other species (Figàs et al., 2014; Hurtado et al., 2013).

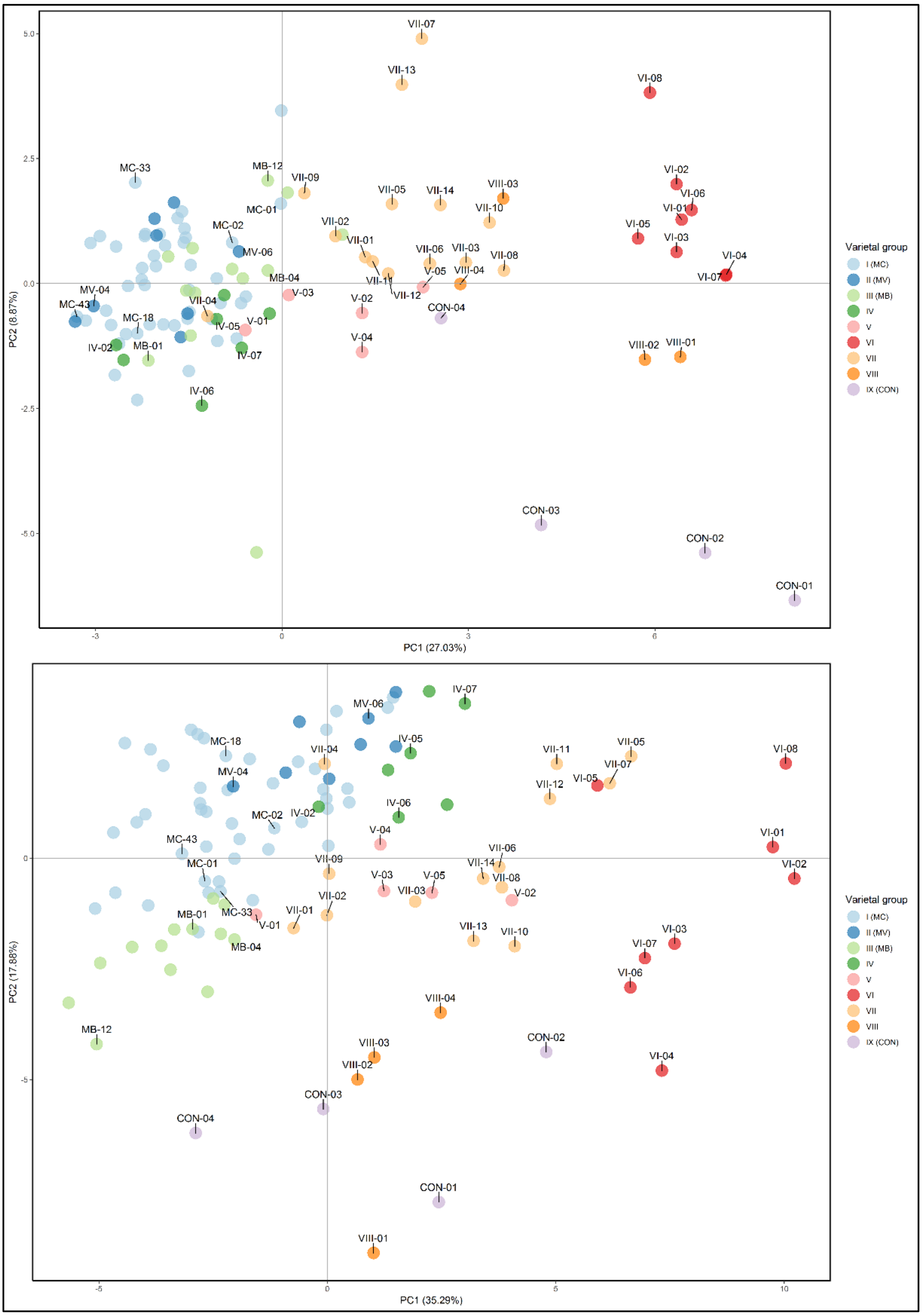

Figure 2 – First and second principal components for the studied pepper accessions using selected conventional descriptors (31; top) and digital traits and parameters (35; bottom). To avoid plot saturation, labels were displayed for accessions from groups V to IX and for only a few representatives from groups I to IV for orientation (i.e. Figure 1 fruits).

3.3.3 PCA combining conventional descriptors and digital phenotyping

After the comparison of both types of descriptors, we proceeded to check how they would behave when considered together. For this, non-significant descriptors were discarded. Thus, 66 traits and descriptors (31 conventional and 35 digital) were considered. The PCA explained 41.02% of total variance in our collection (Figure 3). PC_1 explained 28.34% of variance and was positively correlated with Tomato Analyzer fruit shape index descriptors curved fruit shape index, fruit shape index external I, fruit shape index external II and internal eccentricity descriptor fruit shape index internal. Also, it was negatively correlated with Tomato Analyzer basic traits width mid-height and maximum width, and with conventional fruit descriptor fruit weight (Table 8). PC_2 accounted for 12.68% of total variance and was positively correlated with Tomato Analyzer basic descriptors maximum height, curved height, height mid-width, perimeter, area, and asymmetry descriptor H. asymmetry.ov, while it was negatively correlated to Tomato Analyzer asymmetry descriptor width widest position and to IPGRI inflorescence/flower descriptors corolla colour and number of flowers per axil (Table 8).

This enabled the separation of accessions with higher height/width ratio (elongated and thinner fruits) to the positive side of the first component from those accessions with bigger and heavier fruits that located to the left side of the axis. Thus, *cayenne*/*guindilla* and the blocky/*Morrón de Cascos* types appeared in opposite sides of the axis (Figure 3). PC_2 grouped tall, bulky, and accessions with larger proximal area than distal area at the top of the component axis, whereas accessions with higher height at maximum width/total height ratio and non-white corolla and more than one flower per axil were located at the bottom of the plot (Figure 3).

The combination of both sets of traits and descriptors explained an intermediate level of variation than that considering these sets separately, it also provides a detailed separation of accessions and corresponding varietal groups (Figure 3). In any case, the use of both sets of descriptors is admissible in order to get as many morphological differences as possible. In this study, *Morrón de Cascos* group was not separated into a clearly distinct cluster but into a continuum that connects the group III to the groups II and IV. This indicates a relatively wide range of morphologies within this varietal group, where some accessions are closer to the bell peppers with round shape from group II, some others with intermediate form, and the rest with close resemblance to the big, rectangular and triangular, thick fleshed peppers from *Morrón Valenciano* and group IV (Figure 3). Such findings indicate a considerable intra-varietal diversity within *Morrón* peppers, similarly to the reports from other varietal types (Parisi et al., 2017; Rivera et al., 2016).

For groups Ancho/Piquillo and Numex/Padrón peppers (groups V and VII), accessions were distributed along both axis (Figure 3). Thus, group VII accessions 5, 7, 11, and 12 with similar horn shape clustered together. Accessions from group VI formed two clusters by fruit size. On one hand, accessions VI-1, 2, 5, and 8 formed one cluster, while accessions 3, 4, 6, and 7 formed another. Group VIII Jalapeno peppers clustered relatively near, although variation among them accessions was detected. Accessions from group IX were very different among them but they shared the reduced size fruits so they positioned in the same quadrant of the plot and closer to the most similar fruits. Finally, in the middle of the plot, remaining accessions from groups V and VII clustered together (Figure 3). These accessions present a triangular or slightly triangular shape, with little to no shoulders and roughly the same size.

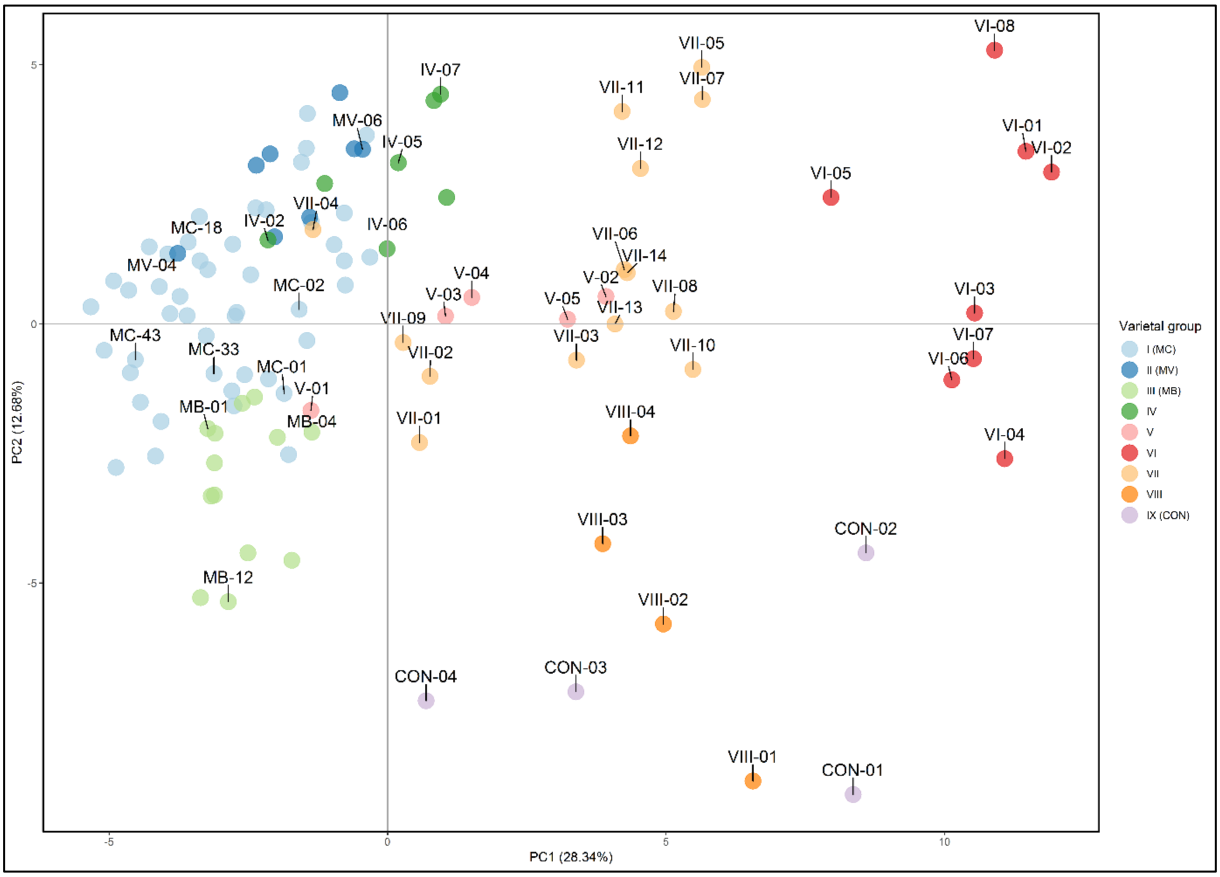

Figure 3 – First and second principal components for the studied pepper accessions using selected conventional descriptors and digital traits and parameters. To avoid plot saturation, labels were displayed for accessions from groups V to IX and for only a few representatives from groups I to IV for orientation (i.e. Figure 1 fruits).

Our findings indicate a good performance of both methods when used together. This was already tested by other authors with good results (Figàs et al., 2014; Tripodi and Greco, 2018), although it has been now implemented in Spanish pepper landraces, including both very different varietal types and closely related materials sharing similar morphological traits. In addition, we report here a considerable amount of diversity within *C. annuum* and especially within blocky peppers for several morphological and agronomic traits. The exploitation of these resources in future pepper breeding programmes in collaboration with farmers and local communities could translate into the development of highly adapted and highly productive varieties that correspond to the consumer demand (Egea-Fernández et al., 2018; Hurtado et al., 2014; Parisi et al., 2017; Zonneveld et al., 2015).

Table 8 - Correlation coefficients between significant conventional descriptors and digital traits and the first two principal components when used separately and in combination.

		Conventional		Digital		Combination	
Descriptor/trait	Type	PC_1	PC_2	PC_1	PC_2	PC_1	PC_2
PH	Plant	0.16	-0.25			0.09	-0.08
GH	Plant	0.12	-0.21			0.07	-0.06
NA	Plant	0.05	0.23			0.04	0.06
SL	Plant	0.06	-0.16			0.03	-0.04
BH	Plant	0.07	-0.18			0.04	-0.02
LD	Plant	-0.01	-0.24			-0.01	-0.03
LS	Plant	0.15	0.28			0.11	0.04
MLL	Plant	-0.26	-0.05			-0.15	0.11
MLW	Plant	-0.27	-0.10			-0.17	0.07
FA	Infl./flower	0.14	-0.30			0.07	-0.13
CC	Infl./flower	0.13	-0.34			0.06	-0.14
AC	Infl./flower	0.01	0.00			0.00	-0.03
FP	Infl./flower	-0.16	0.09			-0.10	0.07
CM	Infl./flower	-0.08	0.21			-0.04	0.13
CAC	Infl./flower	-0.21	-0.14			-0.12	0.06
FS	Fruit	-0.05	-0.05			-0.04	-0.04
FW	Fruit	-0.32	0.05			-0.20	0.14
FSH	Fruit	-0.26	-0.04			-0.17	0.05
FSUR	Fruit	0.25	0.09			0.18	0.03
FCSC	Fruit	-0.20	-0.09			-0.13	0.08
CAPS	Fruit	0.29	-0.04			0.18	-0.09
AS	Fruit	-0.03	-0.01			-0.02	0.03
FSPA	Fruit	-0.30	-0.04			-0.19	0.08
FSBE	Fruit	-0.24	0.07			-0.17	0.02
Lu	Fruit	0.12	0.35			0.08	-0.01
CHRu	Fruit	0.16	0.23			0.10	0.00
HUEu	Fruit	-0.03	-0.28			-0.01	0.02
Lr	Fruit	-0.09	0.22			-0.05	0.03
CHRr	Fruit	0.21	0.07			0.12	-0.07
HUEr	Fruit	-0.18	0.12			-0.12	0.04
Y	Agronomic	-0.20	0.01			-0.11	0.11
P	Basic			-0.03	0.39	-0.11	0.29
AR	Basic			-0.12	0.34	-0.17	0.22
WMH	Basic			-0.24	0.19	-0.22	0.04
MW	Basic			-0.21	0.25	-0.21	0.11
HMW	Basic			0.13	0.29	0.02	0.30
MH	Basic			0.11	0.35	0.01	0.33
CH	Basic			0.09	0.37	-0.01	0.33
FSIE.I	Fruit shape index			0.27	0.02	0.20	0.14
FSIE.II	Fruit shape index			0.27	0.01	0.20	0.13
CFSI	Fruit shape index			0.27	0.04	0.20	0.16
PFB	Blockiness			0.18	0.08	0.10	0.15
DFB	Blockiness			-0.06	0.04	-0.05	0.00
FST	Blockiness			0.17	0.04	0.11	0.12
ELL	Homogeneity			0.13	0.19	0.07	0.19
CIR	Homogeneity			0.24	-0.02	0.19	0.09
RECT	Homogeneity			-0.25	0.00	-0.18	-0.11
SH	Prox. fruit end shape			-0.17	0.17	-0.15	0.04
PAMi	Prox. fruit end shape			-0.15	0.10	-0.15	0.01
PAMa	Prox. fruit end shape			-0.21	0.10	-0.18	-0.02
PIA	Prox. fruit end shape			-0.15	0.19	-0.15	0.07
DAMi	Dist. fruit end shape			-0.18	0.05	-0.15	-0.03
DAMa	Dist. fruit end shape			-0.21	0.03	-0.17	-0.07
DIA	Dist. fruit end shape			-0.06	-0.02	-0.04	-0.04
DEP	Dist. fruit end shape			0.10	0.08	0.05	0.11
OB	Asymmetry			-0.07	-0.07	-0.04	-0.08
OV	Asymmetry			0.20	0.08	0.12	0.16
VA	Asymmetry			0.05	0.23	0.00	0.19
HAOb	Asymmetry			-0.07	0.07	-0.06	0.01
HAOv	Asymmetry			0.21	0.21	0.11	0.26
WWP	Asymmetry			-0.19	-0.13	-0.10	-0.19
ECC	Internal eccentricity			0.08	-0.09	0.06	-0.02
PE	Internal eccentricity			-0.08	0.07	-0.08	0.01
DE	Internal eccentricity			-0.04	0.00	-0.04	-0.01
FSII	Internal eccentricity			0.26	0.00	0.20	0.12
EAI	Internal eccentricity			0.01	0.12	0.00	0.08

3.4 Identification of highly discriminating descriptors

The classification of germplasm based on morphological standardized descriptors is a well-extended practice. However, it is a tedious and time-consuming task that requires a minimum level of expertise and well-defined descriptors. Even when these conditions are met, the classification and differentiation among materials may be compromised due to the close relationship among materials and to the limited ability of most descriptors to find differences (Brewer et al., 2006; Costa et al., 2011; Figàs et al., 2014). Herein, it is reported the utility of combining conventional and digital descriptors (66 in total) in order to successfully discriminate closely related materials. Notwithstanding, not all descriptors and parameters significantly contributed to the differentiation of materials so it is pertinent to select a reduced subset of descriptors in order to capture the maximum diversity while reducing the data collecting labour.

Our collection included a comprehensive representation of landraces and heirlooms from the relevant Spanish centre of diversity as well as other peppers from different countries and three different species (Table 1). Most of these materials were closely related and presented similar morphological traits for plant, flower and fruit (Table 7) (Pereira-Dias et al., 2019). It was observed that the 17 most descriptive descriptors could explain 81.81% of total variability while successfully discriminating the closely related groups *Morrón de Cascos*, *Valenciano*, *Morrón de Bola*, and thick-fleshed peppers of the group IV, as well as the rest of groups, without losing discrimination power (Figure 4). For this we used four conventional descriptors for flower (2) and fruit (2) (number of flowers per axil, corolla colour, fruit weight, and ripe fruit pungency, respectively) and 13 Tomato Analyzer fruit traits corresponding to basic measurements (7), fruit shape index (3), asymmetry (2), and internal eccentricity (1) (perimeter, area, width mid-height, maximum width, height mid-width, maximum height, curved height, fruit shape index external I, fruit shape index external II, curved fruit shape index, H. asymmetry.ov, width widest position, and fruit shape index internal, respectively).

As mentioned before, fruit traits explain the majority of the variance for our collection. This is probably linked to the fact that pepper varietal types are set based mainly on fruit shape, colour and culinary uses (Bosland and Votava, 2012; Rivera et al., 2016; Tripodi and Greco, 2018).

Figure 4 - First and second principal components for studied pepper accessions using 17 most discriminating descriptors and traits of both conventional (4) and digital phenotyping (13) methodologies. PCA explained 81.81% of total variance with a similar level of detail then when considering 66 descriptors and traits. To avoid plot saturation, labels were displayed for accessions from groups V to IX and for only a few representatives from groups I to IV for orientation (i.e. Figure 1 fruits).

4. Conclusions

Thanks to an in-depth characterization based in 67 conventional and digital descriptors and parameters, a considerable inter and intra-varietal variation has been found in the most valued peppers of the Spanish centre of diversity. This characterization has also enabled the identification of a reduced set of descriptors and parameters which can accurately separate varietal groups, as well as accessions within varietal types, even when considering closely related cultivars. Finally, digital phenotyping of fruits based on Tomato Analyzer software results as a fast and efficient tool to complement varietal characterization and typification of *C. annuum* peppers. These findings will be very useful to farmers and breeders devoted to breeding and recovery of heirloom peppers and will boost germplasm characterization and management in seed banks.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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