Agromorphological genetic diversity of Spanish traditional melons

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Abstract

The variability of sixty-two Spanish landraces (and two hybrids used as reference) was described by analysing thirty-nine morphological traits and eight SSR makers. Results showed that 81 % of the examined genetic pool belonged to the *inodorus* type. Spanish traditional melons presented fruits from flattened and globular shapes to elliptical. Rind colour varied from pale green, almost white and yellow to dark green and almost black. Rind texture varied from smooth to intensely wrinkled. Spanish landraces also had larger fruits (average fruit weight ~ 2.6 kg) and longer vegetative cycles (117 days to maturity) compared to landraces from other geographical origins. Farmers seem to have focused on selection towards large fruits, which usually requires the longest production cycles. Fruit colour, size and shape seemed to have been determinant in varietal selection. Hierarchical clustering resulted in two main groups (climacteric and non-climacteric). The largest group was composed of 60 accessions of non-climacteric types, which includes the most demanded by national markets, 'Piel de Sapo', which fruits were characterized by an ovate or elliptical shape, a green rind, big spots and stains

distributed over the whole fruit, a rounded blossom end shape and a very pointed stem end shape. The study demonstrates that the Spanish genetic pool is much more diverse. Wide variability was found in a geographical area with vast historical importance in melon farming. These evaluation has allowed the identification of several uniform groups of non-climacteric cultivars (‘Piel de Sapo’, ‘Mochuelo’, ‘Tendral’, Yellow/White, Winter and Black groups) and a set of highly variable climacteric ones. However, many accessions with singular properties remain unclassified, demonstrating the morphological variability of the studied collection. Melons in Spain have wide variability together with a vast historical importance on farms. Some fruit types, or at least some morphological characters reflected by painters during centuries, have reached the present.

The present study confirmed the need to preserve these irreplaceable genetic resources and continue their study and evaluation for valuable traits which could enhance farmer´s opportunities for entering new markets.

Introduction

Melon (*Cucumis melo* L.) is a cross-pollinated annual crop that has been grown in Spain since at least Roman times. It was first cultivated in Asia (Paris et al. 2012), and intensive trade and dispersion led to a rapid species diversification (Kerje and Grum 2000; Pitrat 2008; Esteras et al. 2011). As a result of this expansion, melon is adapted to several growing areas, and then, secondary centres of diversity in countries such as China, Korea, Portugal and Spain have been originated (McCreight et al. 1993).

Different sources provide information about the arrival of melon to the Iberian Peninsula. Columella, who was born in Southern Spain, mentioned *cogombro* in his *De Re Rustica* (*On Agriculture*), published between 61 and 64 AD (Alvarez de Sotomayor 1824). This *cogombro,* also referred to as *cohombro, culebrino* or snake-shape *cohombro*, is probably a type of *Cucumis melo* belonging to the currently recognized botanical variety *flexuosus (*L.*) Naud.* (Hammer and Gladis 2014), which develops extremely long snake-shaped non sweet fruits (Rodriguez-Mohedano and Rodriguez-Mohedano 1781). The introduction of sweet melons, consumed when fully ripe, seems to have occurred later. Paris et al. (2012) documented the introduction of large sweet melons from Central

Asia on Southern and Central Iberian Peninsula, during the Islamic conquest in the Middle Age. Ibn al-Awwam (also known by his contemporary Christians as Abu Zacarias) reported in 1180 the existence in that region of six types of melons, some of which were sweet and aromatic (Cubero 2001). Andrés Laguna, born in Segovia (central Spain) in the 16th century, translated the Dioscorides' *Materia Medica* into Spanish and referred to Plinio (Gaius Plinius Secundus (AD 23 – August 25, AD 79) who described a kind of big, round, yellow and aromatic melon (Laguna 1991) [1555]). Gabriel Alonso de Herrera (1470–1539), born in Talavera de la Reina (Central Spain) described several types of melons in his Treatise on Agriculture, published in 1513 -consulted edition of 1777 (Herrera 1777)-. Among them, he reported one with a thick hard rind and green flesh and another one with a yellow rind that continued ripening after harvest, spreading a nice strong aroma. The characteristics reported in these sources fit both, casaba types (non-climacteric, non-aromatic, usually of very sweet, big fruits) and muskmelons and cantaloupes (climacteric and aromatic melons), currently included in the botanical varieties *inodorus* H. Jacq. and *cantalupensis* Naud. respectively.

Due to such a long crop history, the preferences of Spanish farmers and consumers during centuries favoured the selection of a large number of varieties adapted to the diverse agro-climatic conditions of this country. Some of the historical paintings, painted by Spanish artists throughout four centuries, portray what those ancient melons looked like. Such agro-biodiversity probably reached the 20th century, when genetic erosion started as a consequence of worldwide varietal replacement and other global changes. Since then, collecting missions have been conducted, and a large number of local varieties or landraces is currently being conserved in different genebanks. The Spanish National Inventory of Plant Genetic Resources (PGR) reports 1,109 different Spanish accessions conserved in several Plant Germplasm Collections. Spanish genetic diversity, together with commercial cultivars, supports one of the greatest melon productions in the world, and the highest melon trade mark (FAO 2013).

Although the concept of traditional variety, also called native, local or landrace, is difficult to define, most definitions focus on the variability or genetic diversity of such old varieties and the lack of intensive genetic improvement processes (Negri et al. 2000; Camacho-Villa et al. 2005). Local varieties are currently the subject of increasing interest. They have valuable traits in terms of agro-ecological adaptation, consumer preference and sensory quality (Escribano and Lázaro 2012). This interest, along with policies for plant genetic resources conservation, requires a greater understanding of the genetic diversity contained in the collections of traditional varieties. This knowledge is important, not only for germplasm management and crop breeding, but also for the identification and protection of these varieties. Spanish melon landraces mainly belong to the botanical varieties *cantalupensis* and *inodorus* defined by Pitrat (2008). They are genetically different from those produced in other areas (Staub et al. 2000; López-Sesé et al. 2003; Esteras et al. 2013). Previous studies have focused on the morphological description and/or the genetic evaluation of some Spanish melon landraces (Staub et al. 2000; López-Sesé et al. 2003; Escribano and Lázaro 2009; Escribano et al. 2012; Esteras et al. 2013). However, this work includes material from the three largest Spanish germplasm collections.

In the present work, we have carried out a detailed morphological description, complemented with a molecular evaluation of a collection of varieties which represents all the morphotypes found in Spanish melon landraces. Our objectives have been: (1) to assess the genetic diversity of Spanish melons from different geographical origins and; (2) to provide a more comprehensive understanding of melon diversification patterns in Spain, based on this information along with historical and cultural evidence.

Material and Methods

Plant material

Sixty-two landraces were analysed, and two commercial hybrids (‘Sancho’ and ‘Amarillo Canario’) were used as reference accessions. These landraces were selected from three largest Spanish melon collections, maintained at the Institute for the Conservation and Breeding of Agro-Biodiversity at the Polytechnic University of Valencia (COMAV), at the Institute for Subtropical and Mediterranean Horticulture La Mayora (IHSM-LaMayora, CSIC-UMA) in Málaga, and at the Madrilean Institute of Rural, Agrarian and Alimentary Research and Development (IMIDRA), Madrid. Preliminary data from previous studies (López-Sesé et al. 2003; Escribano and Lázaro 2009; Blanca et al. 2012; Esteras et al. 2013) were used to select the set of accessions that best represent the genetic, morphological, and geographic diversity of Spanish melon landraces. Geographic origin and other Passport data of the landraces selected for this study are shown in Table 1.

Evaluation trials

Twenty plants per accession were cultivated in three replicates (three years) at the IMIDRA experimental station 'La Isla', (40º 18.75' N; 3º 29.89' W; 528 m.asl). Plants were cultivated in an open-air farming system using traditional cultural practices from May to October. Fertilization consisted of 10,000 kg/ha of organic manure two weeks before sowing, 300 kg/ha of 15-15-15 mineral fertilizers and 500 kg/ha of 9-18-27 during the plant growth period. Experimental evaluation plots were designed with a distance of 1.2 m between rows and 0.5 m between plants. Mulching was used to avoid weed growth although plots were hand-weeded when necessary. Drip irrigation was applied and no chemicals were ever sprayed on the plots. Temperatures in the melon growing season ranged from 4ºC to 39.5ºC. Soils belong to the alluvial terrace type, order Alfisol, Suborder Xeralf, Group Haploxeralf (USDA classification).

Agro-morphological evaluation

Each year the accessions were characterized for a set of 39 quantitative, qualitative and phenological characters, by evaluating leaves, flowers, immature and mature fruits and seeds. These characters were evaluated following IPGRI descriptors (ECPGR Working Group on Cucurbits 2008) with minor modifications for some traits. The nomenclature of the mature fruit morphology of the melon accessions used herein was articulated by Esquinas-Alcázar and Gulik (1983) and later used in melon landrace evaluations by López-Sesé et al. (2003). Quantitative and qualitative traits were measured in ten to twenty plants/fruits per accession. Categories for qualitative traits are detailed in Table 2. The quantitative traits included fruit length, diameter and weight, blossom scar size, flesh and rind thickness and 100-seed weight.. The phenological characters, days to flowering and maturity, were scored when 50% of the plants/fruits reached these phenological stages.

Morphological data analysis

Mean, standard deviations and range were calculated for quantitative data in the three years. Intra-accession variability was estimated by calculating the coefficient of variation (CV) of each quantitative trait in each accession. The CV is defined as the ratio of the standard deviation to the mean. For qualitative traits, the frequency of each category was considered, and the phenotypic heterozygosity was also estimated ($1-\sum freq^2$). Effects of variety (V), repetition (R) and environment (E) were expressed as percentages of total sums of squares type III. Analysis of variance (ANOVA) was conducted along with a a Duncan test of means comparison ($P < 0.05$). To perform a combined multivariate analysis using both the qualitative and quantitative variables, values from the qualitative traits were transformed based on a quantitative scale with the highest value representing the highest intensity of the character. Colour traits were codified according to the Royal Horticultural Colour Chart.

A Principal Component Analysis (PCA) was used to reduce the multidimensional data set to less dimensions still containing most of the information from the original set. Total explained variation (TEV) was calculated as the sum of extracted Eigenvalues. Correlation coefficients between the first three Principal Components (Fi) and the morphological variables were estimated and then used to calculate the correlation index between traits. The standardized original data-matrix with the averages of the three years was used for cluster analysis. A dendrogram was constructed using Euclidean distances between accessions and the Ward's grouping method. ANOVA, PCA and Ward's analyses were performed using XLSTAT statistics software (Addinsoft© 2010.3.09).

DNA extraction

DNA was extracted from 15 plants per accession. Individual DNA was quantified and diluted to 20 ng/µl. Subsequently, DNA was bulked such that each bulk contained five individuals whose final DNA concentration was adjusted to 5 ng/µl. Three DNA pools of each accession were used in the molecular analysis.

SSR amplification

Eight SSR markers associated to valuable melon characters (Tomason et al. 2013) were employed to genotype the three DNA pools per accession (Table 3). Amplification reactions were performed using the polymerase chain reaction (PCR) with a range of melting temperatures (Tm 53.3ºC-56.2ºC). Loading buffer containing xylene cyanol was mixed with the PCR products and then subjected to agarose gel electrophoresis using 3% (w/v) Metaphor agarose (Lonza, NJ) stained with midori green which fluoresces under ultraviolet (UV) light. Once the electrophoresis was completed, the gel was viewed with an UV transilluminator and photographed using GelExpert Software (NucleoTech Corporation, 1996, San Mateo, Calif.). The amplifications allowed the identification of consistent and non-redundant banding patterns, where band sizes were defined in reference to a standard 100 bp DNA ladder.

Molecular data analysis

A data matrix of putative allele sizes was constructed according to their migration distances after electrophoresis. Allelic frequency matrix was then used in a Principal Coordinates Analysis (PCoA) by employing GENALEX software (Version 6.5). This analysis identified the most informative SSR primers and provided a three-dimensional graphic for visual inspection of genetic relationships.

Genetic distances (GD) were calculated using Nei´s (Nei 1973) genetic distance coefficients with POPGENE software (Version 1.31). The unweighted pair-group method using an arithmetic average cluster analysis (UPGMA) included in the NTSYSpc software (Version 2.2)was employed to provide a graphic visualization of the molecular relationships among accessions.

The population structure of germplasm was investigated using POPGENE , which estimates the observed number of alleles per locus, unique alleles, degree of heterogeneity using Shannon's Information Index (SI), and percentage of polymorphic loci (Lewontin 1972). Nei diversity index (DI) was also calculated.

Historical and cultural evidences. Sources of the illustrations

Historical melon records were compiled from pictures available on the web sites of the Prado Museum, the Art Institute of Chicago, the National Gallery of Scotland and the Bayerische Staatsgemäldesammlungen.

Results

Morphological variation of melon landraces

The entire morphological description of each landrace is available on-line (Lázaro et al. 2010). The scores of some quantitative traits are summarized in Table 4. ANOVA results found significant differences ($P \leq 0.05$) between accessions (V effect) and years (E effect) and a significant V x E interaction for all the quantitative traits. Only fruit diameter and weight showed a significant repetition effect. Certain intra-cultivar variability was detected. The least variable character was fruit diameter (average CV per accession ~ 9%), and scar size was the most variable (average CV per accession ~ 38%).

Table 5 shows the frequency distribution of some qualitative traits and, based on phenotypic frequencies, the total heterozygosity (Ht) and the average of expected heterozygosity within accessions (Hs). Fruits varied in shape from flattened and globular to elongate, with different rind colours (from pale green, almost white or yellow, to dark green or almost black) and rind textures (from smooth to deeply wrinkled and with varying degrees of corking/netting). Despite this variation, the main characteristics of Spanish landraces were the ovate to elliptical shapes and the green rind with a secondary colour pattern (spots and stains). Most of the studied samples (81%) do not spread aroma when ripen. Among the qualitative traits, those related to the intensity of dotted and longitudinal corking varied considerably within accessions (average Hs ~ 0.47), whereas netting, which was less frequent, was quite stable. The remaining qualitative traits showed lower intra-accession heterozigosity (average Hs ~ 0.19).

Classification of melon landraces in morphotypes

A PCA was performed with all the studied morphological and phenological traits. This first analysis grouped the accessions along three axes, which explained the 38.97% of total variability. To better understand the observed morphological variation, and to increase de variability explained by model, PCA was recalculated only with the most discriminating fruit and seed traits (Table 6, Figure 1). Then, the cumulative contribution of the three main axes reached 51.14%. The first axis explained 24.55% of the total variation with quantitative (fruit length and weight rind and flesh thickness, and wrinkling intensity) and phenological traits (Days to flowering and days to maturity) as the major contributing variables, which indicates that this axis separates accessions with long growing cycles producing long big melons with thick wrinkle skins from those earlier, producing smaller fruits with smooth skins. The 2nd factorial axis accounted 15.33% of total variation and flesh colours and the presence of aroma were the main contributing variables. The 3rd factorial axis explained 11.26% of total variation and was mainly defined by rind colour, secondary colour distribution and netting density. Therefore, variation in flesh and rind properties (colour, aroma and texture) explains the accession distribution along these two axes.

Figure 1 shows the scatter diagram of the first two principal components, calculated with the 17 most discriminating traits (indicated in Table 6). PCA allow us to see a main group of accessions in the middle of the diagram, and a few accessions at the periphery. These accessions that were located out of the main group showed some singular values of different traits. For instance, the extremely big and long melons COMAV11 ('Blanco largo') and BGCM121 ('Melón de Invierno') are clearly separated from the round-flat and small fruit accessions COMAV16 ('Tempranillo') and COMAV17 ('Escrito oloroso'). First have extremely large fruits (average weight 5.28 kg) with an elongated shape and a thick wrinkly rind. The second axis separated those very netted with white flesh like COMAV19 ('de Calamonte') from those smooth and striped and with aromatic orange flesh like C-250 ('Ardales-1'). Most of the Spanish melon genepool showed a continuous variation between these extremes.

Searching groups differentiation, a Ward's dendrogram based on the entire morphological data set was constructed (Figure 2). The most divergent accessions did spread aroma when ripen (climacteric group in Figure 2). The melons within the other main group (non-aromatic melons) could be differentiated mainly by phenology, and by fruit size, shape and colour. Among them, some morphotypes (accessions sharing common characteristics) could be distinguished. For instance, four accessions that clustered together ('Puchero'-BGCM4; 'Verde Blanco'-COMAV9; 'Melón de Invierno'-BGCM121 and 'Blanco Largo'-COMAV11), shared their extremely large and elongated fruits (average weight 4.89 kg) and a thick wrinkly rind (3.52 cm average), but were variable in rind colour. Their vegetative cycle was long (average 138 days to maturity). As the thickness of their rind preserves them until winter, they have been traditionally referred to as 'Winter' melons (Figure 2). 'Tendral' melons also appeared quite homogeneous ('Tendral negro'–COMAV8 and C-2; 'Tendral verde'-C-308; 'Verrugoso'–COMAV-18 and C-344 and 'Mollerusa-1'-C-69). They are defined by their wrinkled, dark green and thick rind, their long growth cycle (average 136 days to maturity) and a long shelf life.

Nowadays, the most consumed melon at Spanish market is ‘Piel de Sapo’ type, which is represented herein by the reference accession T2. Some of the studied landraces grouped with T2 and seemed to be morphologically quite similar (‘Piel de Sapo tradicional’ (BGCM126); ‘Piñonet’ (COMAV22); ‘Pinta sapo’ (C-19) and others) (Figure 2). This morphotype has medium sized fruits (average weight 2.61 kg), ovate, elliptical or elongated, with a green to yellowish rind colour, pronounced spots and stains distributed over the whole fruit, a rounded blossom end shape and a pointed stem end shape (Figure 2). Their fruits also showed superficial wrinkling and longitudinal fine corking. A group of melon landraces that did not fit the ‘Piel de Sapo’ morphotype grouped close to the ‘Piel de Sapo’ accessions. Some of these landraces belonged to the ‘Mochuelo’ morphotype (COMAV5, BGCM2, BGCM125), along with some ‘Hilo Carrete’, (C-53) that shares some characteristics with ‘Piel de Sapo’ (such as green rind colour, secondary colour presence or white flesh), but differ from this group in few characters. For instance ‘Mochuelo’ has a rounded fruit shape and lightly spotted and smooth rind, while ‘Hilo carrete’ only differs from ‘Piel de Sapo’ in yellow main rind colour.

The second most consumed melon type in Spain is the Yellow morphotype (melons with yellow rinds), herein represented by the reference accession T1 ‘Amarillo Canario’. The cluster analysis grouped most of the yellow accessions (COMAV13, COMAV23, C-110, COMAV20, BGCM5, C-10, etc), along with a few landraces with white fruits (C-326 ‘Ardales-9’ and COMAV12 ‘Blanco redondo’). Two accessions belonging to a different morphotype, the Black melons (BGCM1 ‘Largo negro’ and BGCM69 ‘Largo negro escrito’), appeared close to them (Figure 1).

Molecular analysis: Intra-accession and intra-group genetic diversity

Three pools of 5 plants per accession were analysed with SSRs. Table 7 shows the number of alleles and the genetic diversity detected with each of the SSRs. Most of accessions were molecularly uniform, but SSRs detected intra-accession variability in COMAV9, COMAV10, COMAV15, COMAV16, COMAV19 and COMAV20 (‘Verde

Blanco Largo', 'Bolas', 'Coca', 'Tempranillo', 'De Calamonte', 'Amarillo Manchado'). Nei´s genetic diversity index (Nei 1973), DI, from 0.056 to 0.063), BGCM1, BGCM6, BGCM7, BGCM9, BGCM69, BGCM70, BGCM124 ('Largo negro escrito', 'Pata negra', 'Felipe', 'Reyes', 'Largo', 'Tradicional de Villaconejos', 'Moscatel', DI= from 0.035 to 0.12), and C19, C55, C58, C98, C319, C333, C420 ('Pinta sapo', 'Del pais', 'Maduro negro', CA-101084, AN-C-42, 'Ardales', 'Melón', DI= from 0.063 to 0.13). Some of these accessions were traditional 'Piel de Sapo', 'Mochuelo', Black and Winter types, and despite they were quite uniform morphologically, still retain some molecular diversity. The other variable accessions belonged to heterogeneous groups, i.e. intermediate between 'Piel de Sapo' and 'Mochuelo', Yellow and other singular types, some aromatic and climacteric that share characteristics with traditional melons from other countries.

The genetic diversity was also studied within the groups established in the morphological analysis (Figure 2). No variability was found within the 'Tendral' group (DI=0), and the other morphologically homogeneous groups, Winter and 'Piel de Sapo' had only certain genetic variability (DI=0.023, 0.064). Consistently with their higher morphological diversity the 'Mochuelo' and Black had higher genetic diversity (DI=0.126 and 0.163, respectively). Groups that were morphologically heterogeneous (the group of Yellow/White, and the singular aromatic and climacteric types) also were molecularly more variable (DI= 0.151 and 0.163, respectively).

Molecular relationships among accessions

SSR analysis provided enough polymorphism to cluster most landraces into two major groups (Figure 3). The largest one included most of the landraces that were morphologically classified in the 'Piel de Sapo', 'Tendral' and Winter groups. Similar SSRs profiles were found in the accessions within each group of melons, which is in accordance with the low morphological diversity found within them. In this large group of melons are also interspersed some landraces that were morphologically classified in the group of Yellow/White (COMAV12, COMAV13, COMAV23, BGMC5, C-326, C-278). Despite their Yellow or White rind colour, these accessions share some fruit characteristics with 'Tendral' melons.

The second major group includes most of the landraces of the Black and ‘Mochuelo’ type. Also a second group of Yellow/White landraces are in this cluster (COMAV24, C-98, C-420). The remainder landraces of the Yellow/White group were more diverse and molecularly different from the accessions of the two main groups. Accessions with climacteric behaviour were also highly molecularly variable. Some of them were quite distant from the two main molecular groups and other interspersed within the main cluster (Figure 3). The PCoA confirms these relationships. Figure 4 shows the accessions of the casaba types (all Piel de Sapo’, ’Winter’ and ’Tendral’ melons) clustered accessions in the left part of the PCoA according to the first component (that explained 41.90% of the total variation). A group of Yellow, White ‘Mochuelo’ and Climacteric landraces were close to them, although were more variable. In fact accession of these three variable groups, along with all ’Black’ melons were scattered in the right part of the PCoA, being also variable according to the second component (that explained 17.1% of the total variation).

Spanish melons in historical art and literature

The morphological characterization allowed us to compare the currently existing melon landraces with those represented or mentioned in historical sources. One of the melon fruits reported by Alonso de Herrera in 1513 (Herrera 1777) had ‘yellow skin that ripens after harvest, spreading a nice strong smell’. This description matches with the fruits of BGCM3 named ‘Tempranillo’ from Madrid, as well as some other climacteric accessions such as ‘Coca’, or ‘Bola’ types (COMAV10 and COMAV15). The green rind colour in melons was depicted in 1602 by Juan Sánchez Cotán (born in Toledo, Central Spain) (Figure 5 A). The greenish skin with some characteristic light stripes was also identified in one of the studied accession collected in the same region, ‘Tendral verde’ from Central Spain (COMAV7) (Figure 5 B). Despite this accession is named ‘Tendral’, it belongs to the ‘Mochuelo’ group, according to its morphological and molecular characteristics. Yellow melons were represented in the artwork of Murillo (1617-1682) and Velázquez (1599-1660) (Fig. 5 C and 5 E). Both are famous 17th century painters, and they painted fruits with yellow wrinkled rind and white flesh (Fig. 5 E), similar to Yellow melons of the collection (‘Caña Dulce’ COMAV13) and also to one Winter melons with yellow skin (‘Melon de invierno’ BGCM121). The landrace ‘Rochet’ (COMAV6) (Fig. 5 H), collected in Toledo (Central Spain), has fruits similar to the one painted by Luis E. Meléndez in 1764 (Fig. 5 G). In his painting, the artist presented an ovate melon with a dark green rough, thick rind, ready to be hung, since several types of melons were usually hung for winter conservation. In 1513, Alonso Herrera described this thick-hard skin types, when he reported melon typologies in that period (Herrera 1777). Also several accessions of the 'Piel de Sapo' group greatly resembled those represented in historical paintings. The accession 'Pata negra' (BGCM6), with the smallest fruits, displayed ovate fruits similar to the melon fruit that appears in the art of José López Enguindanos (1751-1812) who painted his 'still life' around Toledo (Castilla-la Mancha, Central Spain) (Fig. 5 I J). The accession 'Pipa de oro' (COMAV1), with fruits with typical large dark green spots, resembles the fruit painted by Benito Lleonart y Senent (1860) who worked in Valencia. In the same painting (Figure 5 K), other melon fruits were also represented. One of them looks like the accession locally-called 'Loperos' (C-53), with the morphological typology 'Hilo Carrete' (Fig 5 K L).

Discussion

Diversity of Spanish melons has been previously studied by using both morphological and molecular characterization (Staub et al. 2000; López-Sesé et al. 2003; Escribano and Lázaro 2009; Escribano et al. 2012; Esteras et al. 2013). These studies characterized different sets of accessions representative of the diversity of Spanish landraces from specific regions. . The present study widens the collections by selecting a great diverse melon set representative of the variation maintained in the three main Spanish melon collections. This study also tries to provide a more comprehensive understanding of melon diversification patterns in Spain, based on the analysis of historical and cultural evidences. Sixty-two Spanish landraces and two commercial varieties were described herein by analysing thirty-nine morphological traits and eight SSR makers.

Agronomic results pointed out a notable phenotypic variability among melon accessions. Most of the studied samples are non-aromatic, non-climacteric melons that belong to the *inodorus* botanical variety, like most of Spanish melon types (Fernandez-Trujillo et al. 2012), but we also found a set of climacteric melons within this collection Most of the analysed accessions had green rinds. This was probably a character demanded by consumers and farmers throughout the history of Spanish melons. In our collection, fruit size and phenological traits were quite variable, but most genotypes had longer cycles and larger fruits than modern cultivars. Although our study did not detect significant differences in rind thickness between old landraces and modern cultivars the genetic pool examined herein generally showed quite thick rinds, many of which were thicker than 1.3 cm. Spanish farmers seem to have focussed selection towards large fruits adapted to long production cycles resulting in high sugar accumulation. This phenotype has successfully reached current markets as the Spanish consumer usually prefers not only *inodorus* types, but specifically large green melon fruits.

Results of PCA provided information of the morphological traits more suitable for varietal discrimination. Apart from the phenological traits and fruit size and shape, rind colour patterns and texture have been determinant in Spanish varietal differentiation. The studied Spanish landraces were mainly green when ripe, but approximately the 30% presented yellow or white rinds. Some combinations of primary fruit colour and secondary patterns were distinctive among varietal groups. For example, fruits of some accessions showed dark green spots on a yellowish green skin, typical of 'Piel de Sapo' types, or on a yellow rind, such as the 'Hilo carrete' pattern. Also yellow dots on green rind are commonly observed in the 'Mochuelo' type. A singular Spanish type is the White with smooth and solid white rind.

Despite the high variability found among accessions, Cluster analysis allowed the identification of seven morphological groups sharing common characteristics. The 'Piel de Sapo' group is the best known and the one that is more commercially important nowadays, having trait combinations that have been co-selected for centuries. Although the 'Piel de Sapo' is the most consumed morphotype in Spanish markets, this study shows that the Spanish *inodorus* genetic pool is much more diverse. Also commercially relevant is the group of Yellow that includes some high quality landraces highly appreciated in international markets, such as the Brazilian. Other groups are less known as they are not usually found in markets. For example the landraces found in the group generically named 'Mochuelo' (name given by the donor farmers to many of the landraces of this group) (Escribano and Lázaro 2009). These have round, medium-sized, smooth, yellowish to green fruits, with a very particular golden speckled secondary colour and a transversal corking distribution around the peduncle area that gives them a mark of quality required by traditional farmers. The 'Tendral' morphotype is also a traditional Spanish type, described in previous studies (López-Sesé et al. 2003), with a long shelf life that has been exploited through centuries by using a traditional practice of hanging melons until ripeness at home in fresh and airy rooms. The Winter melons share thick rinds, long growing cycles and long shelf life with ‘Tendrals’. Also the Black melons have their specific characteristics that make them singular.

The Spanish melon genetic pool shows traits that are different from landraces of other European countries. (Staub et al. 2004; Lotti et al. 2008; Roy et al. 2012; Gao et al. 2012). Lotti et al. (2008) found that 30% of the evaluated varieties from Italy and Albania had a climacteric ripening pattern, with fruits with a tropical aroma when ripe. The percentage of the Spanish accessions with climacteric fruits was lower (19%), which may reflect farmers and consumer preferences. In their study, Lotti et al. (2008) found that the fruit weight of the Italian and Albanian genetic pools ranged from 1.1 to 2 kg in both *inodorus* and *cantalupensis* varieties. Most of the studied climacteric Spanish varieties have fruits ranging from 1.1 to 2 kg , but 18% of the climacteric accessions produced melons with larger fruits, ranging from 3.1 to 4 kg. The Spanish *inodorus* melons mostly varied from of 2.1 to 3 kg. These results indicated that the Spanish melons are generally larger than those selected in other countries in the South of Europe. The germplasm from Hungary and Turkey is mainly represented by fruits with a yellow skin colour (65.5%), and only 19% of the varieties have green fruits (Szamosi et al. 2010). In the Greek germplasm described by Staub et al. (2004), the total absence of green melon varieties was also remarkable.

Despite these differences, some studies suggest similarities of different European melon sets with Spanish landraces. For example Tzitzikas et al. (2009) described some cultigens form Greece and Cyprus similar to ‘Piel de Sapo’. Sensoy et al. (2007) also found similarities between some Turkish *inodorus* melons and ‘Piel de Sapo’. Moreover, the thick wrinkled rind of ‘Tendral’ Spanish melons seemed like the ‘Yuva’ Turkish one. The stains of ‘Piel de Sapo’ or ‘Hilo carrete’ remind those found in the ‘Kirkagaç’ melon type. Those similarities could indicate a common diversification or domestication route.

The eight SSR markers used herein are associated to valuable melon’s characters (Tomason et al. 2013), specifically soluble solids content, fruit shape, and length, traits of high importance in varietal differentiation. Despite the low number, they could identify molecular variation within this melon set. Molecular results agree with morphological results, showing, for example, a low level of diversity within the ‘Tendral’, ‘Piel the Sapo’, and Winter groups, the most morphologically uniform groups of landraces. However, SSRs failed to clearly distinguish these three groups, suggesting a common origin, Winter, ‘Tendral’ and ‘Piel de Sapo’ melons are typical *inodorus* Spanish landraces characterized by medium to large fruits, with firm and sweet white flesh, thick rinds, usually green, and by long growing cycles and extended shelf life. However, each group exhibited differential phenological and morphological characteristic, very large fruits in Winter melons, long shelf life and dark green wrinkle rinds in ‘Tendral’ and typical ‘Piel de Sapo’ features, respectively, whereas SSRs indicate genetic similarity among them. Likely, it would be necessary to increase the number of SSRs to molecularly distinguish these three types of melons. Some of the Winter types retain different levels of genetic diversity, and have alleles different to those of the main market class of ‘Piel de Sapo’ melons. Also the ‘Mochuelo’ types, that were morphologically similar to the green melons groups (mainly to the ‘Piel de Sapo’), were quite molecularly variable, thus being a promising source of interesting traits for breeding new melon cultivars.

The molecular pattern of the second main group from which modern commercial cultivars have been developed, the Yellow/White group, was quite different to that of ‘Piel de Sapo’, with a higher genetic diversity that can be used for breeding commercial melons of the Yellow market class. This group that was quite morphologically homogeneous was molecularly divided in three set of accessions, a set similar to the Green melons (‘Piel de Sapo’, ‘Tendral’ and Winter) a second one interspersed with ‘Mochuelo’ types and a group of a few additional accessions much more diverse.

Finally the singularity of some aromatic and climacteric landraces, that are less common in Spain, but still conserved for local uses, is confirmed at the molecular level. These seem to be more variables and molecularly different from the other *inodorus* like Spanish landraces, and might represent later independent introductions from melons adapted in European and Asian countries (*ameri, cantalupensis* etc) and/or neglected varieties due to the poor post-harvest conservation in our climatic conditions. These results are consistent with previous molecular analyses. Esteras et al. (2013) analysed the population structure of melons, using a representative germplasm collection, including a reference set of Spanish landraces. Some Spanish landraces are clearly different from other European melons (‘Piel de Sapo’, ‘Mochuelo’, ‘Tendral’ and Yellow types), whereas some singular types (‘Escrito oloroso’, ‘Amarillo Manchado’, ‘Erizo’, included in the set of climacteric landraces analysed in the present study) showed similarities with melon populations from Asia, Europe and Africa.

This study shows the high morphological variability of this melon collection which is representative of the current diversity of Spanish landraces. As previously stated, some of this diversity was described by early sources. This confirms that melon diversification was quite high in those times and that representatives of this variation have reached our days. In fact, most of the melons represented in historical pictures are quite similar to some of the currently-preserved landraces. However, some current variants seem to be new or at least seem less frequent in the past. Since Velazquez and Murillo were born in Andalusia (southern Spain), they probably reflected the melon varieties from their region. In fact, 50% of the yellow fruit accessions studied were collected in that region. Nowadays, some melon fruits could have a similar appearance, at least in some characters, to the fruits painted four centuries ago. Results showed that the accessions of the ‘Piel de Sapo’ group, the most commercially important and locally appreciated in Spain, started to be noticed by José López Enguindanos (1751-1812), and the typical feature of green spots on the fruit rind of ‘Piel de Sapo’ first appeared in the artwork of Benito Lleonart y Senent (1860). This late registration in artistic references might suggest a late development or introduction of this morphotype. This late selection is also consistent with the narrow genetic basis found in ‘Piel de Sapo’ melons (Blanca et al. 2012; Esteras et al. 2013), which currently limits breeding programmes in this highly valuable market class. However, in all the consulted paintings since the 16th century, green types were represented along with some yellow ones, and this diversity is maintained nowadays. This preference could have guided farmers to select these morphotypes for centuries, until the occurrence of the ‘Piel de Sapo’ melon type some time before the 19th century, as it was painted by Benito Senent in 1860.

Today, the genetic control of the traits fruit shape and fruit colour in melons is quite well known (Fernandez-Trujillo et al. 2012). A few genes or genomic regions are involved in these traits. Melon is easily self -and cross-pollinated manually, and it behaves like an out-crossing plant in open fields. The ideal types pursued by farmers have been changing, and the existing variability could have been combined, both artificially or naturally. Thus, new combinations of some of the traits represented in farmers' selections of the 15th-17th centuries could have reached the 20th century. Despite the variation in colour and shape shown by these early sources, all the paintings generally represent large fruits with a thick rind, which is a very useful character for fruit conservation. Furthermore, some of them appeared ready to be hung, a traditional practice for storing some types of melons until their consumption. Aromatic melons turning to yellow after harvest has been reported in medieval sources, which suggests that these are quite old varieties. However, they are less represented in paintings, indicating a preference in Spain for the non-climacteric melons that have reached the present.

These old landraces, selected by farmers during generations, are sometimes cultivated nowadays because of their preference in local markets (Escribano and Lázaro 2012). The evaluation of their genetic diversity seems essential for their conservation and protection to keep them for future breeding programs to broad the genetic basis of cultivated varieties, promoting a sustainable agriculture (Spataro and Negri 2013). Additionally, our experience shows that today consumers, especially those living in European cities, are strongly demanding food quality, flavours and textures from old varieties. Flavours, quality and diversity are still in the folk´s memory (Lázaro et al. 2016).

Conclusions

In the present work, a multidisciplinary approach was carried out for assessing the variability within a collection representative of the variation of melon landraces from Spain. The results revealed differences among all studied samples at the phenotypic and genotypic levels. These evaluation and analyses has allowed the identification of several uniform groups of non-climacteric cultivars ('Piel de Sapo', 'Mochuelo', 'Tendral', Yellow/White, Winter and Black groups) and a set of highly variable climacteric ones. However, many accessions with singular properties remain unclassified, demonstrating the morphological variability of the studied collection.

Melons in Spain have wide variability together with a vast historical importance on farms. Some fruit types, or at least some morphological characters reflected by painters during centuries, have reached the present.

The present study confirmed the need to preserve these irreplaceable genetic resources and continue their study and evaluation for valuable traits which could enhance farmer´s opportunities for entering new markets.

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Table 1. Melon (*Cucumis melo* L.) landraces analysed in the present study. Seed collectors and other passport information were found in the Spanish National Inventory of Plant Genetic Resources at http://wwwx.inia.es/webcrf/CRFesp/Paginaprincipal.asp. The Spanish varieties (hybrids) T1 = Amarillo Canario and T2 = Sancho were included as reference varieties.

ID	National Inventory ID	Accession Name	Collection	Collection site	Latitude	Longitude	Altitude	Collection date	Cited[1]
BGCM1	NC083952	Largo negro escrito	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B
BGCM2	NC083953	Mochuelo	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B; D
BGCM3	NC074569	Tempranillo	IMIDRA	Chinchón (Madrid)	400833N	0032526W	753	2000	B; D
BGCM4	NC083954	Puchero	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B; D
BGCM5	NC083955	Amarillo de Villaconejos	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B; D
BGCM6	NC083956	Pata negra	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B
BGCM7	NC083957	Felipe	IMIDRA	Alcalá de Henares (Madrid)	402853N	0032205W	587	-	B
BCCM8	NC083958	Alfonso	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B
BGCM9	NC083959	Reyes	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	-	B
BGCM69	NC037303	Largo	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	1984	B; C; D
BGCM70	NC006910	Tradicional de Villaconejos	IMIDRA	Alcalá de Henares (Madrid)	402853N	0032205W	587	1971	B
BGCM121	NC079132	De invierno	IMIDRA	Patones (Madrid)	405222N	0032928W	832	2003	B; D
BGCM124	NC036026	Moscatel normal	IMIDRA	Pedrezuela (Madrid)	404448N	0033607W	800	1987	B; D
BGCM125	NC086532	Mochuelo tradicional	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	2006	B
BGCM126	NC086533	Piel de sapo tradicional	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	2006	B
BGCM153	NC086543	Azul	IMIDRA	Villaconejos (Madrid)	400618N	0032904W	650	2007	B; D
COMAV1	NC082731	Pipa de oro	COMAV	Membrilla (Ciudad Real)	385826N	0032038W	670	1985	D
COMAV2	NC036234	Negro de invierno	COMAV	Sotos (Cuenca)	401147N	0020945W	991	1985	D
COMAV3	NC037040	Piñoncillo	COMAV	Alborea (Albacete)	391646N	0012344W	700	1985	D
COMAV4	NC037299	Verde cuarenteno	COMAV	Alcolea de Calatrava (Ciudad Real)	385917N	0040653W	652	1984	
COMAV5	NC037300	Mochuelo	COMAV	Rielves (Toledo)	395746N	0041128W	495	1984	D
COMAV6	NC036233	Rochet	COMAV	Aldea Nueva (Toledo)	393820N	0050644W	573	1984	
COMAV7	NC037302	Tendral verde	COMAV	Daimiel (Ciudad Real)	390407N	0033637W	620	1984	
COMAV8	NC073474	Tendral negro	COMAV	Cilanco (Albacete)	392012N	0011822W	485	1985	
COMAV9	NC037053	Verde blanco largo	COMAV	Madrigueras (Albacete)	391417N	0014754W	700	1985	
COMAV10	NC038016	Bolas	COMAV	Belvis de la Jara (Toledo)	394540N	0045654W	449	1984	
COMAV11	NC100540	Blanco largo	COMAV	Retamoso (Toledo)	394439N	0044514W	607	1984	D
COMAV12	NC037046	Blanco redondo	COMAV	Montalvos (Albacete)	391006N	0020135W	700	1985	D
COMAV13	NC036020	Caña dulce	COMAV	Torredonjimeno (Jaen)	374608N	0035724W	594	1981	C; D
COMAV14	NC100391	Blanco escrito	COMAV	Rota (Cádiz)	363717N	0062142W	48	1987	D
COMAV15	NC036933	Coca	COMAV	Alcalá la Real (Jaen)	372740N	0035541W	1033	1984	C; D

COMAV16	NC036013	Tempranillo	COMAV	Canena (Jaen)	380300N	0032849W	545	1984	D
COMAV17	NC036950	Escrito oloroso	COMAV	Arcos de la frontera (Cádiz)	364458N	0054818W	218	1984	C; D
COMAV18	NC036223	Verrugoso	COMAV	Santa Fe (Granada)	371126N	0034304W	571	1985	
COMAV19	NC100491	De Calamonte	COMAV	Jarandilla de la vera (Cáceres)	400737N	0053934W	585	1984	D
COMAV20	NC043144	Amarillo manchado	COMAV	Campanario (Badajoz)	385153N	0053659W	398	1984	D
COMAV21	NC043153	Verde pinto	COMAV	La Codosera (Badajoz)	391226N	0071020W	330	1984	C; D
COMAV22	NC037376	Piñonet	COMAV	Ademuz (Valencia)	400345N	0011708W	741	1989	
COMAV23	NC036223	Amarillo oro	COMAV	Torrepacheco (Murcia)	374439N	0005708W	46	1984	C; D
COMAV24	NC100323	Común	COMAV	Alacon (Teruel)	410128N	0004143W	702	1984	
COMAV25	NC037356	Erizo	COMAV	Manacor (Mallorca)	393412N	0031238E	80	1984	C; D
C-1	NC073464	Verde gordo	CSIC	(Córdoba)	-	-	-	-	
C-2	NC073465	Tendral negro	CSIC	(Córdoba)	-	-	-	-	D
C-10	NC075083	Amarillo alargado	CSIC	(Badajoz)	-	-	-	-	
C-19	NC036048	Pinta sapo	CSIC	Cartagena (Murcia)	373605N	0005851W	68	-	
C-53	NC058572	Lopero	CSIC	S. Sebastian Ballesteros(Córdoba)	373921N	0044926W	312	-	D
C-55	NC036049	Del pais	CSIC	S. Sebastian Ballesteros(Córdoba)	373921N	0044926W	312	-	D
C-58	NC098570	Maduro negro	CSIC	(Badajoz)	-	-	-	-	D
C-65	NC036055	Franceset	CSIC	Valencia (Valencia)	392820N	0002229W	13	-	
C-69	NC073468	Mollerusa-1	CSIC	(Lerida)	-	-	-	-	D
C-70	NC098571	Mollerusa-2	CSIC	(Lerida)	-	-	-	-	
C-98	NC050248	CA-101084-1-C	CSIC	San Enrique de Guadairo (Cádiz)	361817N	0051733W	14	-	A
C-110	NC098602	Loperano	CSIC	Alcaudete (Jaen)	373500N	0040600W	676	-	D
C-250	NC050624	Ardales-1	CSIC	Ardales (Málaga)	365240N	0045039W	453	1984	A
C-278	NC036911	Melón	CSIC	San Enrique de Guadairo (Cádiz)	361817N	0051733W	14	1984	A
C-308	NC037154	Tendral verde	CSIC	San Fulgencio (Alicante)	380648N	0004303W	4	1984	
C-319	NC050216	ANC-42	CSIC	Ardales (Málaga)	365240N	0045039W	453	1984	A
C-326	NC050627	Ardales-9	CSIC	Ardales (Málaga)	365240N	0045039W	453	1984	
C-333	NC100367	Ardales-16	CSIC	Ardales (Málaga)	365240N	0045039W	453	1984	
C-344	NC036214	Verrugoso	CSIC	Santa Fé (Granada)	371126N	0034304W	579	1985	
C-420	NC100385	Melón	CSIC	Benaocaz (Cádiz)	364205N	0052512W	800	1984	
C-426	NC100457	Melón	CSIC	Chulilla (Valencia)	393925N	0005331W	322	1984	

[1] Accession previously studied in A) López-Sesé et al. 2003; B) Escribano and Lázaro 2009; C) Blanca et al. 2012; D) Esteras et al. 2013;

Table 2. Qualitative traits analysed and considered categories.

	Trait	Categories
Flower	Ovary pubescence	1.Weak; 2. Intermediate; 3. Strong
Leaf	Green colour intensity	1.Pale; 2. Medium; 3. Strong
	Lobe development	1.Shallow; 2. Intermediate; 3. Deep
	Margin dentition	1.Weak; 2. Intermediate; 3. Strong
	Margin undulation	1.Weak; 2. Intermediate; 3. Strong
	Petiole attitude	1.Erect; 2. Semi-erect; 3. Horizontal
Immature fruit	Rind colour	1 White; 2. Yellow; 3. Green; 4. Greyed green
	Rind colour intensity	1.Pale; 2. Medium; 3. Strong
Ripe fruit	Shape	1.Flattened; 2. Globular; 3. Ovate; 4. Elliptical; 5. Elongate
	Maximum width position	1. Close to blossom; 2. Centre; 3. Close to peduncle
	Rind colour	1. Dark green; 2. Greyed green; 3. Green; 4. Pale green; 5. Yellowish green; 6. Light yellow; 7. Yellow; 8. Light orange
	Colour intensity	1.Pale; 2. Medium; 3. Strong
	Secondary colour presence	1. Absent; 2. Present
	Secondary colour distribution	1.Absent; 2. Spots; 3. Stains; 4. Spots and stains
	Spot density	1.Weak; 2. Intermediate; 3. Strong
	Stain density	1.Weak; 2. Intermediate; 3. Strong
	Blossom end shape	1.Pointed; 2. Rounded; 3. Flattened
	Stem end shape	1.Pointed; 2. Rounded; 3. Flattened
	Wrinkling intensity	1.Absent/Very superficial; 2.Superficial 3.Intermediate; 4.Pronounced; 5.Very pronounced
	Corking/netting pattern	1.Absent; 2.Low dotted; 3.Dotted; 4. Longitudinal; 5. Netted; 6. High netted
	Corking/netting density	1.Absent; 2.Very superficial; 3.Superficial 4.Intermediate; 5.Pronounced; 6.Very pronounced
	Striped bands presence	1. Absent; 2. Present
	Striped bands colour	1.Absent; 2.Blackish green; 3. Dark green; 4. Greyed green; 5. Yellowish green; 6. Greenish yellow; 7.Light yellow; 8.Yellow; 9.Ligth orange; 10. Grey
	Striped bands colour intensity	1.Pale; 2. Medium; 3. Strong
	Flesh colour	1. White; 2. Yellow; 3. Cream; 4. Pale green; 5. Green; 6. Pale orange; 7. Orange (yellow-red); 8. Salmon (pink-red); 9. Others
	Flesh colour intensity	1.Pale; 2. Medium; 3. Strong
	Flesh outer layer colour	1. White; 2. Yellow; 3. Cream; 4. Pale green; 5. Green; 6. Pale orange; 7. Orange (yellow-red); 8. Salmon (pink-red); 9. Others
	Internal aroma	1. Absent; 2. Present
Seed	*Hilum* end shape	1.Sharply pointed; 2.Bluntly pointed
	Coat colour	1.White; 2.Pale cream; 3. Dark cream

Table 3. Simple sequence repeat markers selected to assess the genetic variability among melon genotypes

SSR	Linkage group	FORWARD (5'-3')	REVERSE (5'-3')	Tm (°C)
MU7161	VI	TTG CCG ATG AAC TCA AGG AT	TGT TAC AAC ACA CCC TTG GAA	54,4
CMBR104	IV	CAA AAG GAA AAG AAA AAG ACC AAA	GGT ATT ATT TGC CCC CAC CT	54,8
CMBR105	III	TGG TAA GCA TTT TGA AAT CAC TTT T	TTT GTA TGG TTG GAG GGG AA	53,7
CMBR024	VIII	TGG GGT TGT CAA TAC AGC AA	AAA ATG AAT GGG AGT GTG TGG	53,4
CMBR001	III	AGA TGA CCA AAC CAA ACC CA	CAA CGT TAT GGG GAT GAA GG	53,3
TJ30	III	TTA GGG AAG GCA ATC AAT CG	AGG AGG AGG GAA TGC TTT GT	56,2
CMN06_19	IV	GCT CTC CCA AGC CTT CTC TT	CAG ACC AAC AAT AGA ATG CAC A	53,3
ECM56	IX	CCC GGA AAA TCT GAG ATC G	CCA CCC ACT AAT TCC AGC AT	54,9

Table 4. Variability (Average, standard deviation SD, range and coefficient of variation CV) of each quantitative trait in studied plant material. Effects of variety (V), repetition (R) and environment (E) were expressed as percentages of total sums of squares type III. Analysis of variance (ANOVA) was executed and a Duncan was conducted to provide significant differences ($P < 0.05$).

Trait	Average	SD	Total CV	Average CV per accession	Range	V effect (%)	R effect (%)	E effect (%)	V x E
Fruit length (cm)	22.83	5.02	21.99	10.68	(10.6 - 44.5)	99.15*	0.30	0.55*	*
Fruit diameter (cm)	15.46	2.13	13.78	9.03	(10 - 26)	95.99*	2.51*	1.50*	*
Fruit weight (kg)	2.61	1.01	38.70	22.23	(0.69 – 8.84)	97.24*	1.30*	1.46*	*
Fruit: flesh width (cm)	3.21	0.59	18.38	15.07	(1.7 – 6)	85.37*	3.19	11.44*	*
Scar size (cm)	1.26	0.61	47.94	37.76	(0 – 10.5)	94.61*	1.02	4.36*	*
Fruit: rind thickness (cm)	1.13	0.23	20.35	16.94	(0.4 – 2)	78.86*	0.83	20.31*	*
100seeds weight (g)	4.40	1.05	-	-	(1.05 – 7.15)	-	-	-	
Days to flowering (50%)	50.85	2.66	-	-	(43 – 58)	48.96	-	51.04	
Days to maturity (50%)	116.69	15.20	-	-	(78 – 150)	82.68*	-	61.70*	

*significant differences ($p<0.05$)

Table 5. Frequency distribution of some qualitative traits. Based on phenotypic frequencies, total heterozygosity (Ht) and the average of expected heterozygosity within accessions (Hs) were calculated. Estimated inter-accession heterozygosity was calculated as (Ht – Hs).

Trait	Categories	Frequency	Ht	Hs	Ht - Hs
Ripe fruit shape	Flattened	0.01	0.70	0.39	0.31
	Globular	0.11			
	Ovate	0.44			
	Elliptical	0.25			
	Elongate	0.19			
Ripe fruit rind colour	Dark green	0.07	0.44	0.18	0.26
	Greyed green	0.01			
	Green	0.48			
	Pale green	0.03			
	Yellowish green	0.01			
	Light yellow	0.17			
	Yellow	0.20			
	Light orange	0.03			
Ripe fruit secondary colour distribution	Absent	0.32	0.60	0.28	0.32
	Spots	0.04			
	Stains	0.11			
	Spots and stains	0.53			
Fruit: blossom end shape	Pointed	0.17	0.39	0.26	0.14
	Rounded	0.76			
	Flattened	0.07			
Fruit: stem end shape	Pointed	0.56	0.58	0.30	0.27
	Rounded	0.31			
	Flattened	0.13			
Fruit: wrinkling intensity	Absent/Very superficial	0.46	0.68	0.36	0.32
	Superficial	0.27			
	Intermediate	0.15			
	Pronounced	0.11			
	Very pronounced	0.01			
Fruit: corking/netting density	Absent	0.28	0.68	0.47	0.21
	Superficial	0.47			
	Perceptible	0.14			
	Intermediate	0.03			
	Pronounced	0.04			
	Very pronounced	0.04			
Fruit: corking/netting pattern	Absent	0.28	0.74	0.43	0.31
	Low dotted	0.23			
	Dotted	0.08			
	Longitudinal	0.30			
	Netted	0.03			
	High netted	0.08			

Table 6. Correlation coefficients between the first three Principal Components (Fi) and some morphological characters. Square cosines. Those traits marked in bold are the most contributing traits to each factor and were used to construct the PCA shown in Figure 1

		F1	F2	F3
	Cumulative contribution	24.552	39.880	51.136
Ripe fruit	Length	**0.707**	0.000	0.113
	Diameter	0.252	**0.334**	0.057
	Weight	**0.690**	0.156	0.003
	Shape	**0.317**	0.076	0.194
	Rind colour	0.000	0.252	**0.297**
	Secondary colour distribution	0.012	0.052	**0.359**
	Wrinkling intensity	**0.215**	0.074	0.139
	Corking/netting density	0.080	0.026	**0.353**
	Striped bands presence	0.030	**0.161**	0.020
	Flesh width	**0.317**	0.026	0.030
	Rind thickness	**0.433**	0.147	0.000
	Flesh colour	0.130	**0.303**	0.002
	Flesh outer layer colour	0.021	**0.284**	0.000
	Internal aroma	0.161	**0.470**	0.001
Seeds	100-seed weight	0.000	0.037	**0.219**
Phenology	Days to flowering (50%)	**0.289**	0.069	0.031
	Days to maturity (50%)	**0.520**	0.137	0.096

Values in bold are the highest values of each variable

Table 7. Allele number and genetic diversity detected with eight SSR *loci* in the collection of Spanish melon landraces

Locus	na*	ne*	I*	Obs_Het	Exp_Het*	Nei**	Fis	Fit	Fst	Nm*
CMBR104*	5	1.2782	0.5064	0.0727	0.2183	0.2177	-1.0000	0.7099	0.8550	0.0424
CMBR001	4	1.1816	0.3523	0.0545	0.1541	0.1537	-0.5000	0.6752	0.7835	0.0691
ECM56	2	1.0370	0.0909	0.0242	0.0358	0.0357	-0.7143	0.5890	0.7602	0.0789
TJ30	3	1.0372	0.1024	0.0121	0.0360	0.0358	-0.5000	0.6624	0.7750	0.0726
CMBR105	3	1.7271	0.7338	0.1697	0.4223	0.4210	-0.6471	0.6373	0.7798	0.0706
CMN06_19	2	1.0754	0.1562	0.0485	0.0703	0.0701	-0.3770	0.2465	0.4528	0.3021
CMBR024	3	1.0629	0.1562	0.0242	0.0594	0.0592	-0.5000	0.7417	0.8278	0.0520
MU7161	3	1.0246	0.0739	0.0000	0.0241	0.0240	****	1.0000	1.0000	0.0000
Mean	3.1250	1.1780	0.2715	0.0508	0.1275	0.1271	-0.6191	0.6455	0.7811	0.0701
St. Dev	0.9910	0.2386	0.2397	0.0536	0.1369	0.1365				

*Details about the SSR loci are in Table 3

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]
* Expected heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity DI
The number of polymorphic loci is : 8
The percentage of polymorphic loci is : 100.00 %
* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

Fig. 2. Cluster analysis of studied accessions (see Table 1) by Ward's grouping method and using Euclidean distances.

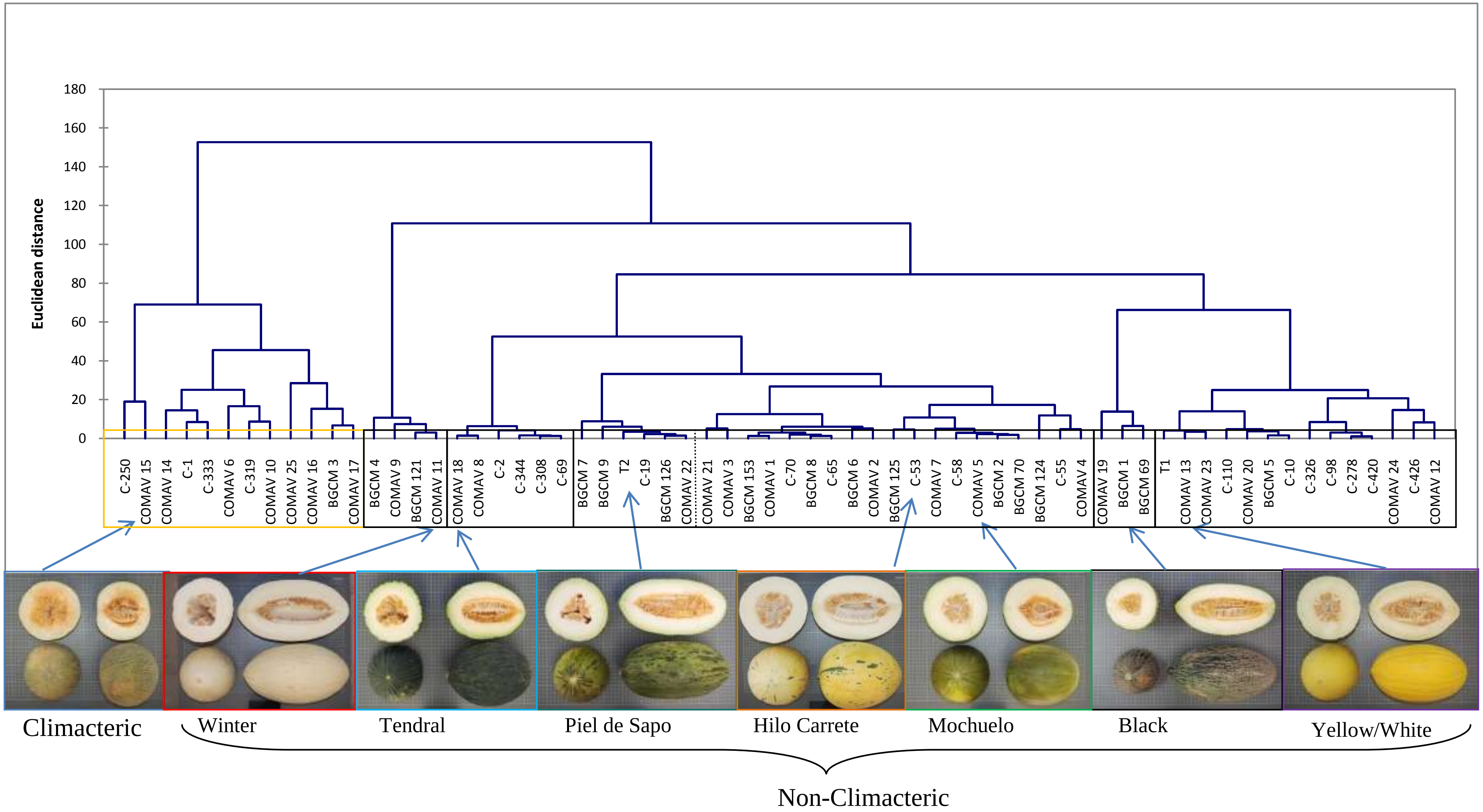
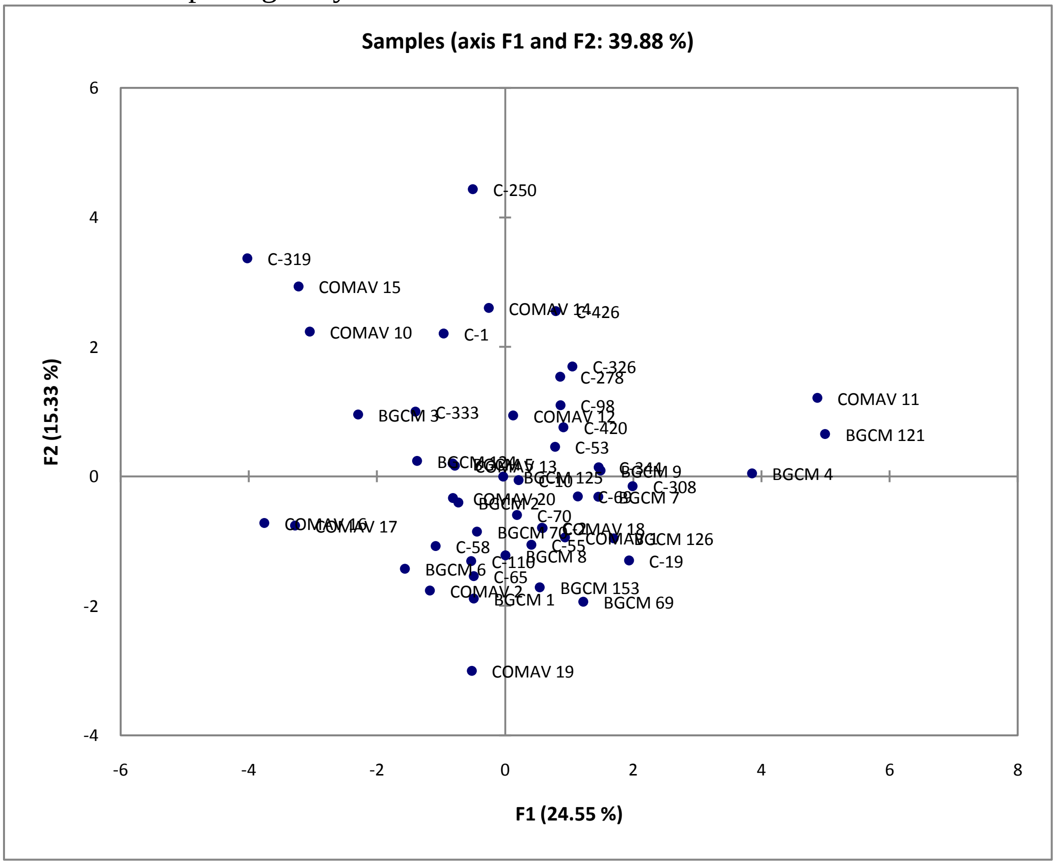


Fig. 1. PCA results. Scatter diagram of the first two principal components for the melon landraces morphologically characterized with the 17 most discriminant traits



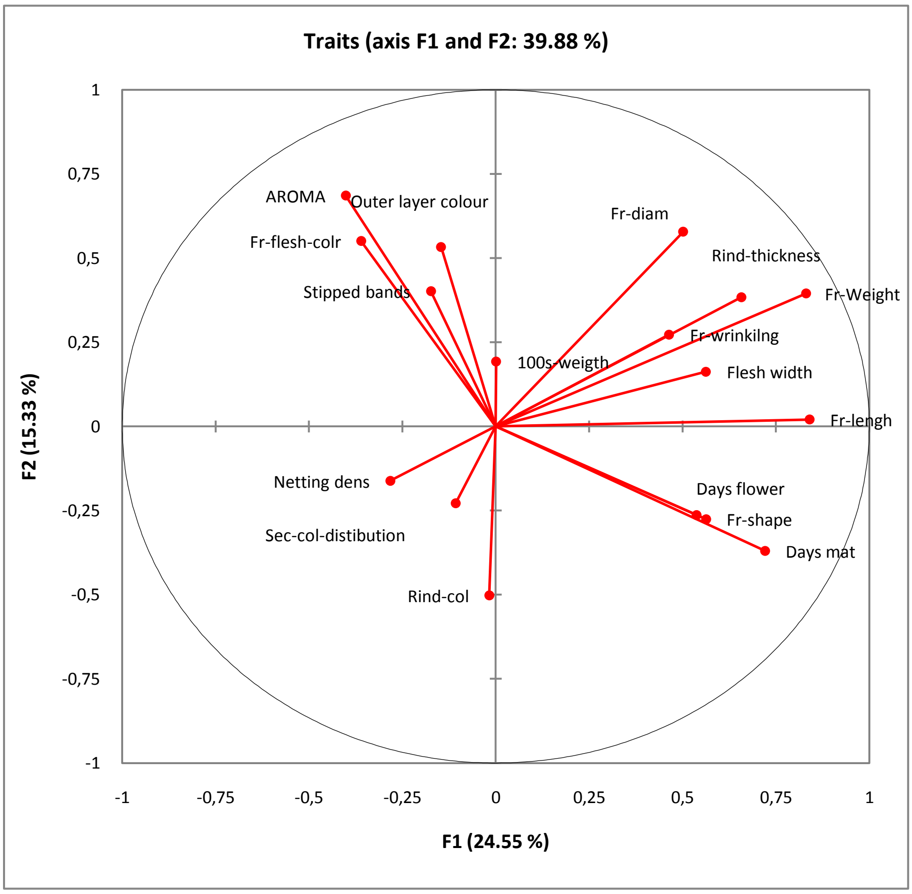


Fig 3. Cluster analysis showing the molecular relationships among the accessions. Nei´s genetic distance (1973) and UPGMA were used

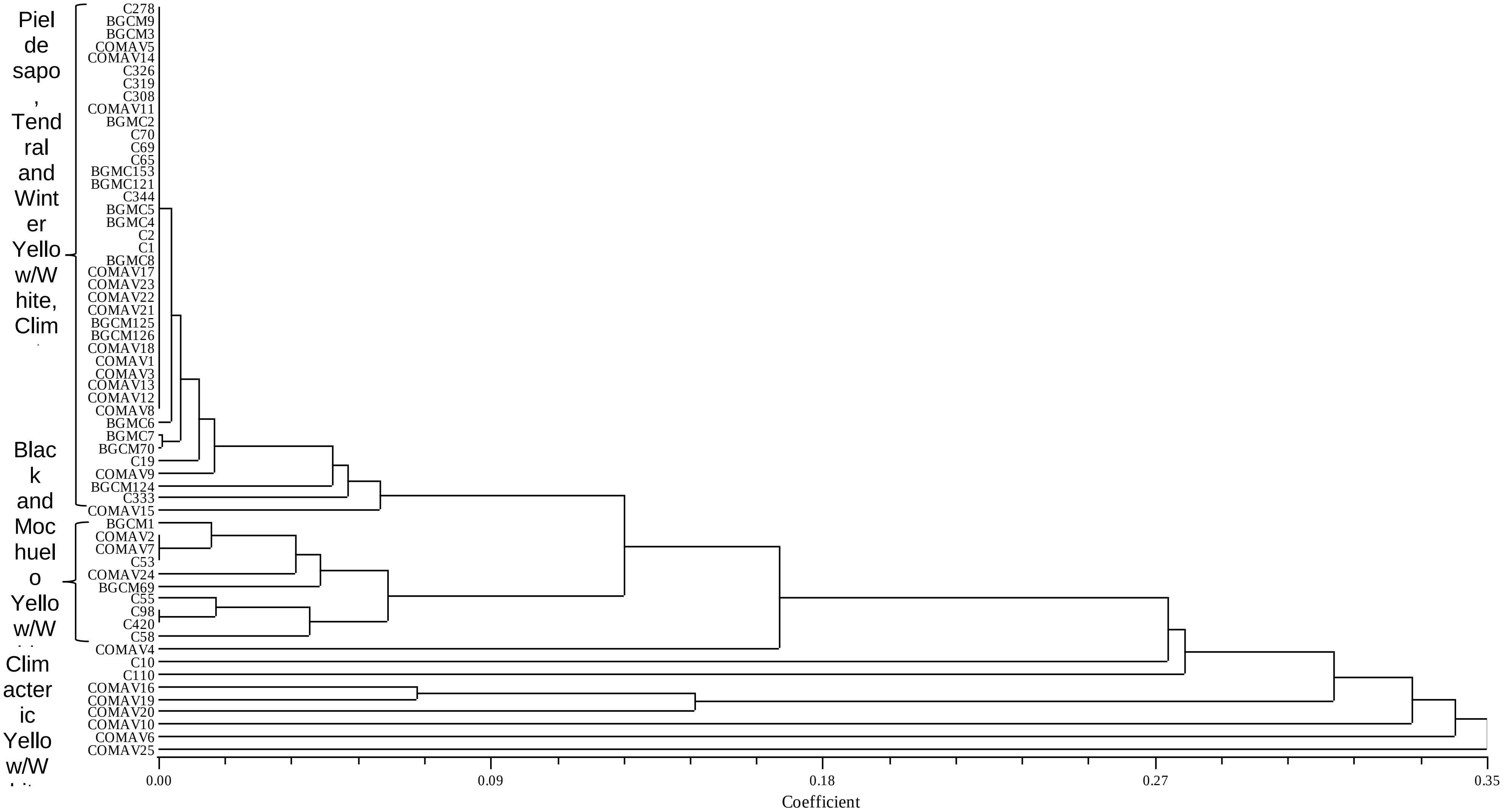
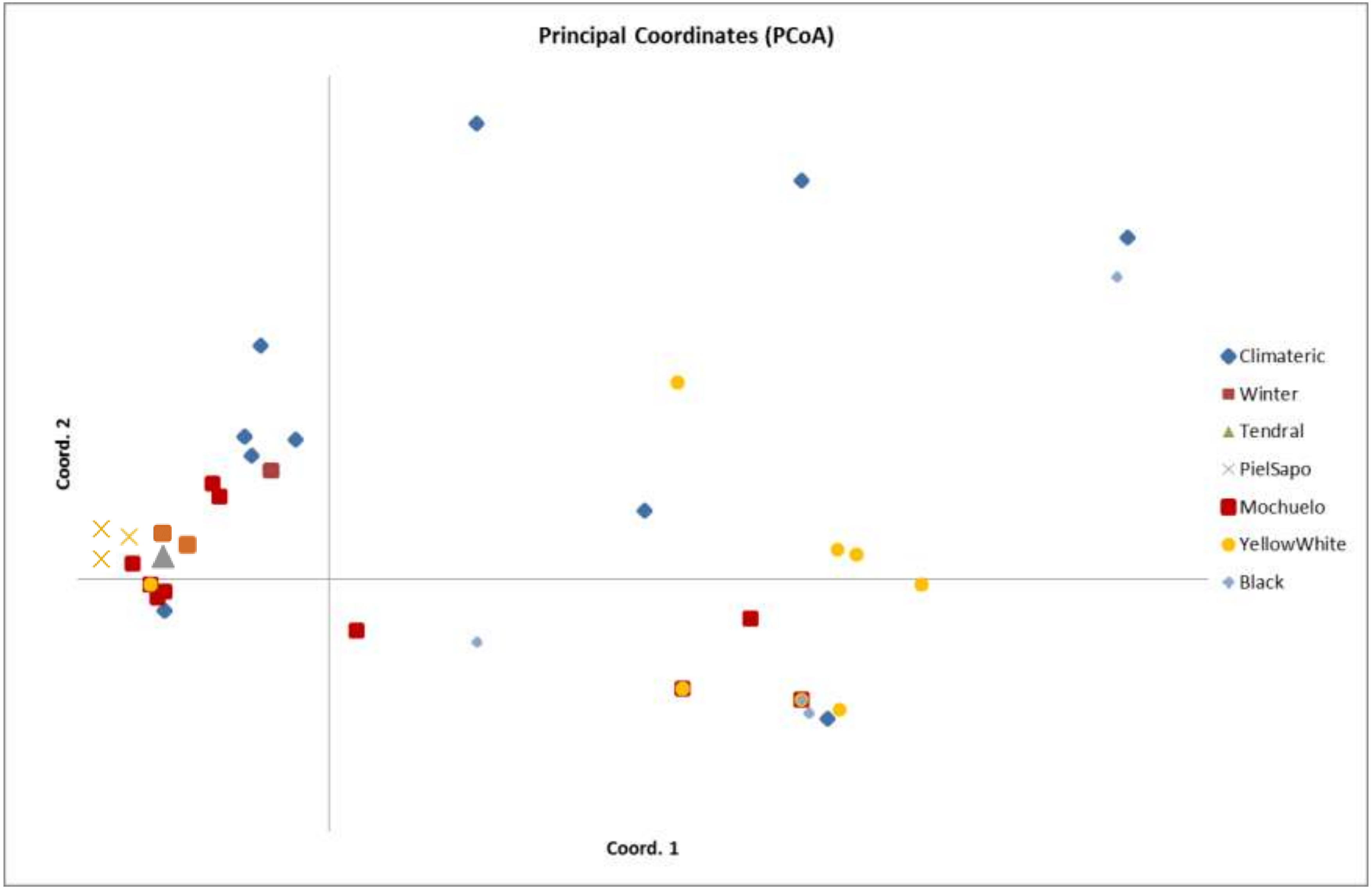


Fig 4. PCoA showing the molecular relationships of the different accessions. The first and second component explained a 41.90 and 17.07 % of the total variation



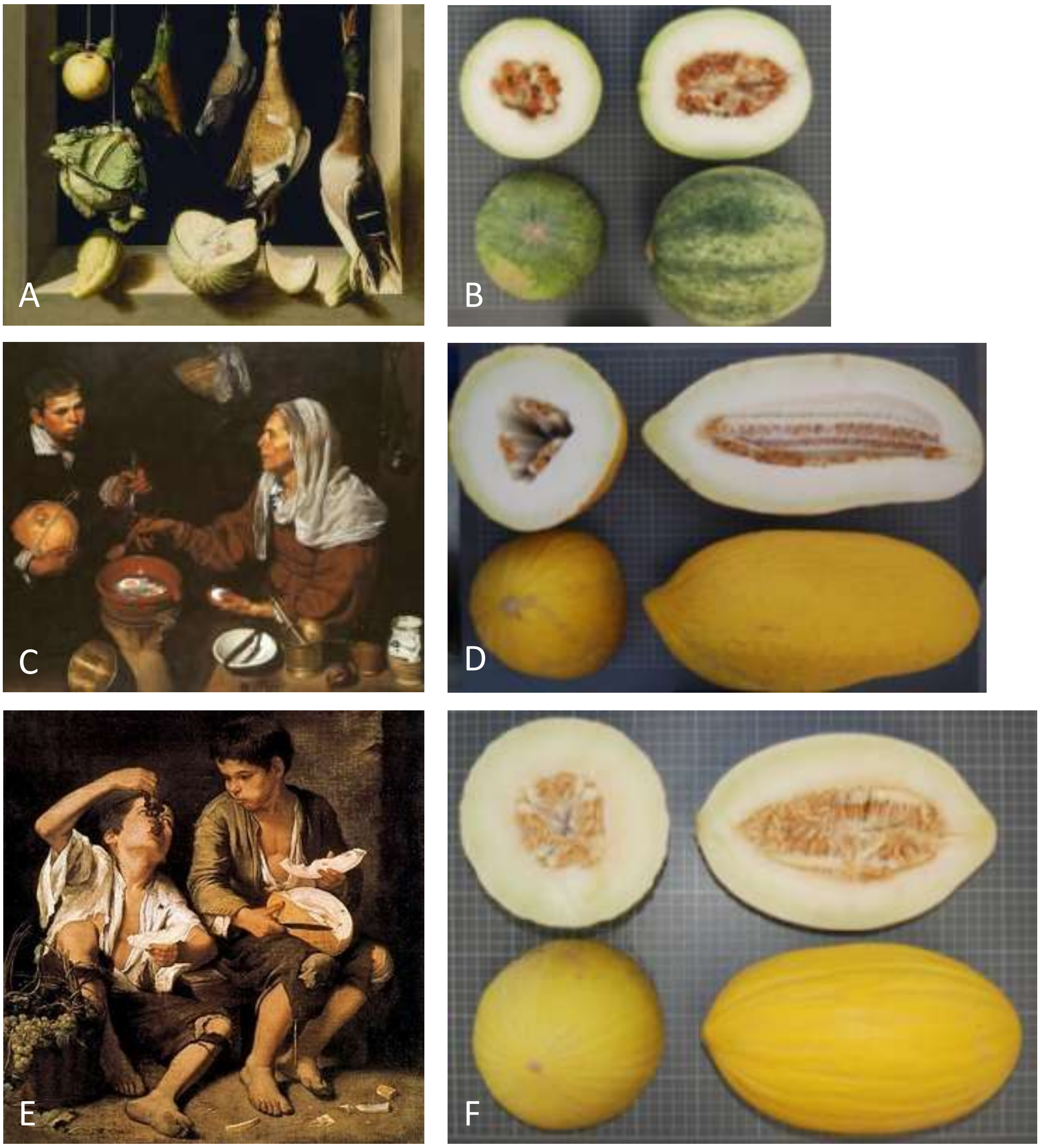


Fig. 5. Six images of Spanish melons. A) **Juan Sánchez Cotán (1560-1627)**: *Still-Life with Game Fowl*. Oil on canvas 67,8 x 88,7 cm. Chicago, The Art Institute. B) Accession COMAV7. C) **Diego Velázquez:** *Old Woman Frying Eggs.* (1618). Oil on canvas 100, 5 x 119, 5 cm. Edinburgh, The National Gallery of Scotland. D) Accession BGCM121 . E) **Bartolomé Esteban Murillo** *Children eating melon and grapes (*1650). Oil on canvas, 146 x 104 cm. Munich, Bayerische Staatsgemäldesammlungen, Alte Pinakothek . F) Accession COMAV13

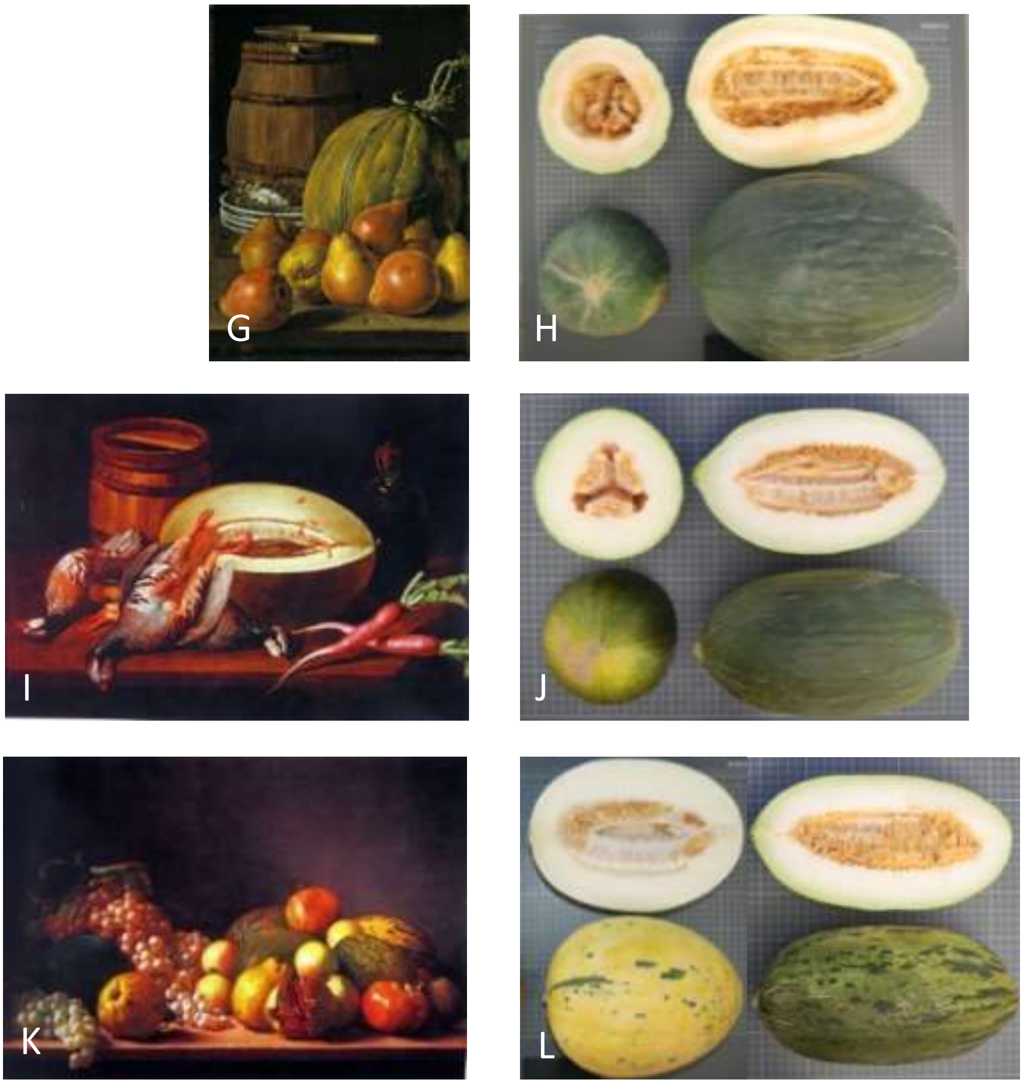


Fig. 5. (Continuation) Six images of Spanish melons. G) **Luis Egidio Meléndez:** *Still-Life with pears, melons and barrel for marinading.* (1764). Oil on canvas, 48 x 35 cm. Madrid, Museo Nacional del Prado. H) Accession COMAV6. I) **José López Enguídanos (1751-1812)**: *Still Life With a Melon, Radishes, Partridges, a Small Barrel and a Bottle of Wine on a Wooden Table*. Oil on canvas, 50 x 69 cm. Madrid, private collection. J) Accession BGCM6. K) **Benito Ll. Senent (1860):** *Still-Life with melons, grapes, quinces and pomegranates*. Oil/Canvas, 56 x 84 cm. Madrid, private collection. L) Accessions C-53 and COMAV1