Article

Seed Morphology in Key Spanish Grapevine Cultivars

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Abstract: Ampelography, the botanical discipline dedicated to the identification and classification of grapevine cultivars, was grounded on the description of morphological characters and more recently is based on the application of DNA polymorphisms. New methods of image analysis may help to optimize morphological approaches in ampelography. The objective of this study was the classification of representative cultivars of *Vitis vinifera* conserved in the Spanish collection of IMIDRA according to seed shape. Thirty eight cultivars representing the diversity of this collection were analyzed. A consensus seed silhouette was defined for each cultivar representing the geometric figure that better adjusted to their seed shape. All the cultivars tested were classified in ten morphological groups, each corresponding to a new model. The models are geometric figures defined by equations and similarity to each model is evaluated by quantification of percent of the area shared by the two figures, the seed and the model (*J* index). The comparison of seed images with geometric models is a rapid and convenient method to classify cultivars. A large proportion of the collection may be classified according to the new models described and the method permits to find new models according to seed shape in other cultivars.

Keywords: ampelography; geometry; grapevine; morphology; peduncle; shape; taxonomy

1. Introduction

The genus *Vitis* L. (Vitaceae) contains more than 79 accepted species [1], with *Vitis vinifera* L. being extensively used in viticulture worldwide. Two forms of *Vitis vinifera* co-exist in Eurasia and North Africa: the cultivated form, *V. vinifera* ssp. *Vinifera* and the wild form *V. vinifera* ssp. *Sylvestris* (Gmel.) Beger [2]. Thousands of cultivars of this species are grown today in viticulture worldwide receiving different denominations in many languages [3–6]. The description, classification and phylogenetic relationships of grape cultivars were traditionally based on different methods. For centuries, starting with the *Agricultura General* of Gabriel Alonso de Herrera (1470-1539), first published in Madrid in 1513 [7], and following with the classical treatise of Clemente (1777-1827), published in French and entitled *Essay sur les varietés de la vigne qui végètent en Andalousie* (1826) [8], the cultivars were described and classified based on morphological characteristics. Ampelography, the botanical discipline dedicated to the identification and classification of the varieties of grapevines, was traditionally based on the description and comparison of the shape and color of the vine leaves, grape clusters and berries [9]. More recently, from the last decades of the 20th century, molecular techniques based on the detection of both chloroplast and nuclear polymorphisms (DNA fingerprinting) by Simple Sequence Repeats (SSRs) have been widely used for the identification of cultivars as well as in the description of samples collected in the field [10-15]. Like in other disciplines of Biology, molecular approaches have become the main trend in recent decades, while morphology

was relegated to a second term, but now due to increased possibilities of obtention, storage and analysis of high-resolution images, morphometric approaches have witnessed interesting developments [16].

The seeds of grape, often called pips, present a high degree of variation, that is still higher in cultivars than in wild species [17]. In general, the seeds of cultivated varieties are more elongated than those of wild species. Thus, Planchon [18] defined two subgenera: *Euvitis* as those species with pear-shaped seeds, while species in subgen. *Muscadinia* had more rounded seeds, oblong to ovoid. The geometrical analysis of seed shape based on elliptical Fourier transform method has revealed useful in the discrimination of wild grapes and cultivars in agreement with results from microsatellites (SSRs), as well as to associate archaeological seeds with wild forms or other morpho-clades [19,20,21]. New developments of these techniques have included 3D scanning technology [22] allowing to discriminate between cultivars classified as identical by their SSR profiles, and the geo-chronological dynamics of the cultivars used in viticulture [23]. The groups obtained in these analyses may be associated with geometric figures representing graphically the morphological diversity of grape seeds and their relatedness to geometric forms.

A new method to describe and quantify seed shape in the Vitaceae including *Vitis* species based on geometric shapes was recently published. The seeds of different *Vitis vinifera* cultivars were described based on the comparison of their images with different geometric models [24]. The models were based on the heart and piriform curves [25,26], as well as the graphic representation of an equation composed by two parts representing a modified ellipse (Model 7 in [24]). The protocol is based on a parameter (*J* index) that quantifies the degree of similarity between seed images and geometric figures [27,28]. *J* index was first applied to the model plant *Arabidopsis thaliana* [29,30], then to the model legumes *Lotus japonicus* and *Medicago truncatula* [31] and to other species belonging to a variety of plant families [32-38], including the description of seed morphology in the complex genus *Silene* L, in the family Caryophyllaceae [39].

The first approximation to seed shape in the Vitaceae by this method involved a reduced number of cultivars (Camarate, Cariñena, Cercial, Malvasía, Merseguera and Morenillo), but enough to detect differences in shape associating each cultivar to a different geometric model [24]. While cultivar Merseguera gave better results with Model 5, an elongated heart curve, Model 7 resembled more closely seed shape in a majority of cultivars tested (Camarate, Cercial, Malvasía and Morenillo) [24]. Model 7 has revealed interesting because changes in the corresponding equation result in diverse curves that reproduce well the outline of seed shape of many cultivars, that subsequently may be grouped according to the different models.

The objective of this work is to compare selected cultivars in the Core germplasm collection of *Vitis* maintained in "El Encín" [40] with nine new geometric models obtained from the algebraic modification of Model 7 and a new model derived from Model 6 [24]. A representative sample of thirty eight cultivars were chosen among the total of germplasm entries of the collection. The criteria for selection included their antiquity, historical relevance and broad international application in viticulture, as well as their diversity in shape, covering an important proportion of this aspect in the collection.

2. Materials and Methods

2.1. Plant material

The thirty eight cultivars used in this study are described in Appendix A. Table A1.

2.2. Seed images

Photographs were taken with a camera Nikon D80 of 10,2 megapixels. The seeds (30 per cultivar) were oriented with their chalaza downwards, such as to expose the ribs upwards (ventral orientation) and straight in the middle to provide each seed image a maximum of symmetry (Figure 1). Features observed in the elaboration of the models include the line at the top (flat, round or sharp), the curves defined by the edges of the peduncle and the curvature at the basis. Composed images containing 30 seeds per ac-

cession were prepared with Corel Photo Paint and are stored in: https://zenodo.org/record/4433813#.X_7hiOhKh9A

Figure 1. Seeds of the cultivars Albillo Real (left) and Airén (right) showing the regions observed for modelling. The variable regions are indicated with rectangles and correspond to the upper and lateral edges of the peduncle, and to the curvature at the base. The red lines show the variations in these regions corresponding to a flat peduncle in Albillo Real, in contrast to a rounded top in Airén. The peduncle appears more differentiated from the rest of the seed in Albillo Real, while there is more continuity in Airén. The discontinuity at the base is more pronounced in the Airén seed, on the right.

2.3. General Morphological Description by Image Analysis

The area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio length/width), circularity (C) and roundness (R) were measured for each seed image and the calculations were done for all 30 seeds in each composed figure with ImageJ [41]. Length and width are the Feret diameters of the seed images. Feret, or caliper diameter, is the distance between two parallel planes restricting the object. All the seeds were vertically oriented, so length and width correspond respectively with the vertical and horizontal Feret diameters. Circularity Index and Roundness were calculated as described [42]. Circularity is the ratio $4\pi A/P^2$, while Roundness is $4A/(\pi L^2)$, thus Circularity values decrease with surface irregularities, while Roundness values are insensitive to them.

2.4. Obtention of the new models

New models were obtained by modifications in the equations corresponding to Models 6 and 7 previously described [24]. The objective was to search for a maximum similarity between the seed images of each cultivar and the graphic representation of the equations. The 38 cultivars under study were classified in ten groups defined by their similarity to each of the respective models.

2.4.1. Obtention of an average silhouette for each cultivar

The average silhouette is a representative image of seed shape for each cultivar. It was obtained in Corel Photo Paint, by the following protocol (a detailed video is available at Zenodo: https://zenodo.org/record/4478344#.YBPOguhKiM8):

The layers containing the seeds are superimposed and the opacity is given a value of 3 in all layers. All the layers are combined, and the brightness is adjusted to a minimum value. From this image we are interested in the inner region representing the area where most of the seeds coincide, which is the darkest area. To select it, we use the magic wand tool and with tolerance equal to 10, this selection is copied and pasted as a new layer.

2.4.2. New models

Ten new geometric models were obtained from the modification of previously described equations, whose geometric representation gives figures resembling the silhouette of *Vitis* seeds. The equations were modified searching for the similarity of their representation to seed shape of the different cultivars. Of the new models, nine were obtained from Model 7 [24], and derive from the representation of two functions, one corresponding to the upper part of the seed containing the peduncle and the other to the lower part. Both functions were combined in a single equation. The remaining tenth model (de Cuerno) was derived from Model 6, the Fibonnacci's pear [24]. The models are described in section 3.1.

2.5. Quantification of similarity between the seeds and the models: J index

J index is the percent of similarity between two plane figures: The seed image and the geometric model. To obtain *J* index the model is superimposed to each seed image in the groups of 30 seeds per cultivar with Corel PhotoPaint. Images are kept for each group of 30 seeds with the models superimposed (PSD). Once the 30 models are adjusted, two documents are kept: A JPEG file with the models in black and another with the models in white. Both are successively opened with Image J, and the corresponding areas quantified. The figure with the models in black gives the total area (T), while the figure with the models in white gives the values of shared area between the model and the seed image (S). All images used in the calculations are stored in Zenodo (https://zenodo.org/deposit/4478301). *J* index is the ratio S/Tx100 and has a maximum value of 100 when both areas coincide (Figure 2). A video showing the process has been uploaded in Zenodo: https://zenodo.org/record/4478315#.YBPKFuhKiM8).

Figure 2. Schematic representation of the method used to obtain *J* index. *J* index is the ratio between shared and total area in two images. Left: a seed from the cultivar Albillo Real. Middle: The same seed with the model superimposed; above, with the model in black; and below, the model in white. Right: Reed zones show the areas quantified in each of the figures. ImageJ gives the total area for the seed with the model in black, while shared area is obtained with the white model.

3. Results

3.1. New models

The Mathematica code for the ten new models described in this work is stored in Zenodo: (https://zenodo.org/record/4478500#.YBPetOhKiM8). The following nine models were obtained from modifications in Model 7 [24] (between parenthesis the cultivars to which the model applies):

Model Listán Prieto (Listán Prieto and Tortozona Tinta):

$$\left(\frac{2}{17}\left(\sqrt{3300-90x^2}-\frac{400}{24+5x^2}\right)+y\right)\left(\frac{25}{187}\left(-\sqrt{3300-90x^2}-\frac{1200}{60+x^6}\right)+y\right)=0;$$

Model Sylvestris (wild varieties):

$$\left(\sqrt{33 - x^2} + \frac{50}{17 + (10x^{18})^{1/5}} - \frac{9y}{10}\right)\left(\frac{25}{22}\left(-\sqrt{33 - x^2} + \frac{4}{5 + x^2 + x^4}\right) - \frac{9y}{10}\right) = 0;$$

Model Albillo Real (Alarije, Albillo Real, Cayetana Blanca, Graciano, Juan García and Tempranillo):

$$\left(y - \frac{25\sqrt{33 - x^2}}{22} - \frac{125}{33 + \frac{11x^8}{200}}\right)\left(y + \frac{25}{22}\sqrt{33 - x^2} - \frac{50}{11(5 + x^2 + x^4)}\right) = 0;$$

Model Hebén (Hebén, Macabeo and Zalema):

$$\left(\frac{9y}{10} + \sqrt{33 - x^2} - \frac{25}{33 + 55x^2}\right)\left(\frac{9y}{10} - \frac{25}{22}\left(\sqrt{33 - x^2} + \frac{20}{6 + x^4}\right)\right) = 0;$$

Model Moscatel (Beba, Bruñal, Caíño Tinto, Castellana Blanca, Garnacha Tinta, Gewürztraminer, Malvasía Aromática, Mollar Cano, Moscatel de Alejandría, Moscatel de Grano Menudo, Palomino Fino, Prieto Picudo):

$$\left(\frac{9y}{10} + \frac{25}{22}\left(\sqrt{33 - x^2} - \frac{4}{5 + x^2 + x^4}\right)\right)\left(\frac{9y}{10} - \frac{25}{22}\left(\sqrt{33 - x^2} + \frac{2000}{600 + x^8}\right)\right) = 0;$$

Model Tortozón (Imperial and Tortozón):

$$\left(\frac{17y}{20} + \sqrt{33 - x^2} - \frac{25}{33 + 55x^2}\right)\left(\frac{17y}{20} - \frac{25}{22}\left(\sqrt{33 - x^2} + \frac{20}{6 + x^4}\right)\right) = 0$$

Model Teta de Vaca (cultivars Teta de Vaca Dominga, and Verdejo):

$$\left(\frac{4y}{5} - \sqrt{33 - x^2} - \frac{10000}{3400 + 317x^{18/5}}\right)\left(\frac{4y}{5} + \frac{25}{22}\left(\sqrt{33 - x^2} - \frac{4}{5 + x^2 + x^4}\right)\right) = 0;$$

Model Doña Blanca (Doña Blanca, Monastrell and Pedro Ximénez):

$$\left(\sqrt{33 - x^2} - \frac{4}{5 + x^2 + x^4} + \frac{22}{25}y\right)\left(\sqrt{33 - x^2} + \frac{2000}{600 + x^8} - \frac{88y}{125}\right) = 0;$$

Model Airén (Airén, Bobal and Mazuela):

$$\left(\sqrt{33 - x^2} - \frac{16}{12 + 17x^2} + y\right)\left(-\frac{25}{22}\sqrt{33 - x^2} - \frac{17600}{2640 + 17\sqrt{5}\,|x|^5} + y\right) = 0;$$

In contrast with the previous models that derive from Model 7, model De Cuerno was obtained from the modification of Model 6, the Fibonnacci's pear [24]:

Model De Cuerno (De Cuerno)

It results from the graphic representation of the implicit equation:

$$(49x^2 + 25y^2)(117649x^6 + 15625(y^3 - 2y^2 + y - 1)^2 + 60025x^4(3y^2 - 4y + 2) + 30625x^2(3y^4 - 8y^3 + 8y^2 + y + 4) = 962500;$$

The models obtained from the graphic representation of these functions are shown in Figure 3 superimposed to the average silhouettes of the cultivars that gave higher values with each model as indicated in Table 1.
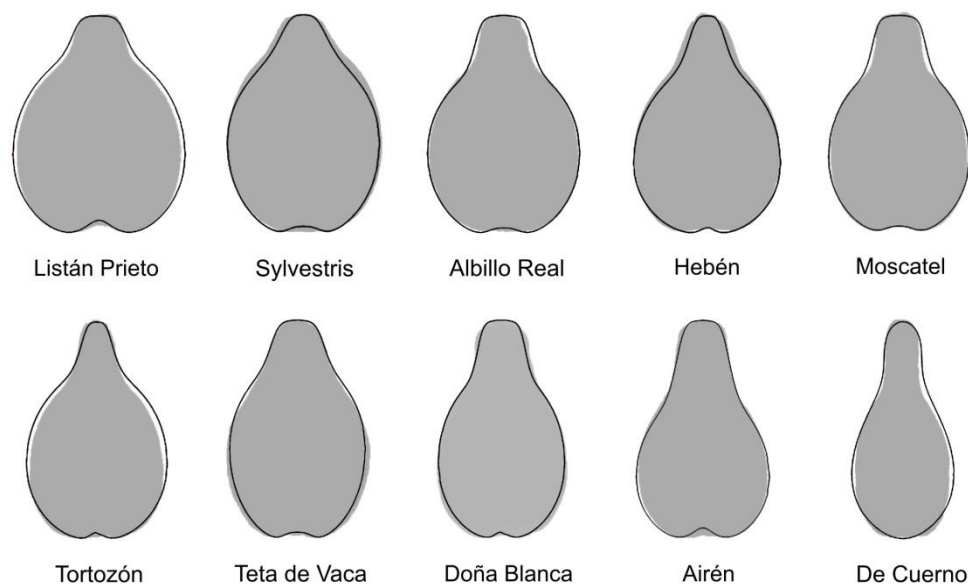
Figure 3. Models used in the classification of 38 representative cultivars of *Vitis vinifera* L. maintained in El Encín and the respective average silhouettes of some of the cultivars. The average silhouettes of the varieties that best fitted each model are represented by a gray area, and the models by a continuous line.

3.2. General morphological description and comparison between the seeds of different groups

Table 2 presents the mean values for area, perimeter, length, width, aspect ratio (AR), circularity and roundness for the groups of cultivars and the results of ANOVA.

Table 2. Mean values for morphological characters in the groups corresponding to the different-models. Groups are denominated by the corresponding model and appear ordered by increasing values of aspect ratio (AR). The mean values marked with the same letter in each column do not differ significantly at p<0.05 (Scheffe's test). N is the number of seeds analysed.

Model	N	Area	Perimeter	Length	Width	AR	Circularity	Roundness
Listan Prieto	60	17.2[d]	16.8[e]	5.4[f]	4.0[b]	1.3[f]	0.76[a]	0.75[a]
Sylvestris	90	13.8[e]	14.8[f]	5.0[g]	3.5[e]	1.4[e]	0.78[a]	0.70[b]
Albillo Real	180	18.7[c]	17.8[d]	5.8d[e]	4.1[b]	1.4[e]	0.73[b]	0.70[b]
Hebén	90	17.3[d]	17.3[e]	5.8[e]	3.8[cd]	1.5[d]	0.72[c]	0.66[c]
Moscatel	356	16.8[d]	17.3[e]	5.8[e]	3.7[d]	1.6[c]	0.70[d]	0.64[d]
Tortozon	60	17.6[cd]	18.5[c]	6.0[d]	3.7[cd]	1.6[c]	0.66[e]	0.63[d]
Teta de Vaca	90	23.2[b]	20.1[b]	6.8[b]	4.3[a]	1.6[c]	0.72[bc]	0.63[d]
Doña Blanca	90	18.7[c]	18.4[cd]	6.4[c]	3.7[cd]	1.7[b]	0.70[d]	0.59[e]
Airén	90	17.5[d]	18.1[cd]	6.2[c]	3.6[e]	1.7[b]	0.67[e]	0.57[e]
De Cuerno	20	29.1[a]	25.9[a]	9.4[a]	3.9[bc]	2.4[a]	0.55[f]	0.42[f]

The values of aspect ratio (AR) follow an opposite trend to those of circularity and roundness. Differences between the later are due to surface irregularities, that result in decreased roundness. Values of area were comprised between 13.8 mm^2 (Sylvestris) and 29.1 mm^2 (De Cuerno). The minimum values of perimeter (14. 8 mm), length (5.0 mm) and width (3.5 mm) corresponded to the wild varieties (Sylvestris) which had also low aspect ratio values. Listán Prieto was the unique group with higher values of roundness and lower values of aspect ratio than the group containing the sylvestris cultivars.

Table 3 presents a list of the groups formed by the cultivars resembling each model. Underlined are the cultivars with highest values of *J* index within each.

Table 3. Mean *J* index values in the cultivars with their respective models. Underlined: cultivars that gave highest values of *J* index with each of the models.

Model	Cultivars (*J* index values)
Listán Prieto	Listán Prieto (90.7), Tortozona Tinta (92.6)
Sylvestris	CA 13.4 (90.7), CA 13.6 (89.3), SE 2.1 (90.4)
Albillo Real	Alarije (90.8), Albillo Real (91.5), Cayetana Blanca (90.0), Graciano (91.1), Juan García (90.0), Tempranillo (90.9)
Hebén	Hebén (90,0), Macabeo (90.8), Zalema (91.6)
Moscatel	Beba (91.2), Bruñal (92.2), Caíño Tinto (90.6), Castellana Blanca (91.4), Garnacha Tínta (88.8), Gewürztraminer (90.8), Malvasía Aromática (90.3), Mollar Cano (90.8), Moscatel de Alejandría (90.5), Moscatel de Grano Menudo (90.4), Palomino Fino (91.0), Prieto Picudo (90.9)
Tortozón	Imperial (88.9), Tortozón (89.9)
Teta de Vaca	Dominga (90.4), Teta de Vaca (90.4), Verdejo (90.3)
Doña Blanca	Doña Blanca (91.0), Monastrell (89.4), Pedro Ximénez (90.5)
Airén	Airén (90.4), Bobal (90.3), Mazuela (90.1)
De Cuerno	De Cuerno (88.8)

3.3. Groups of cultivars for each of the models

3.3.1. Model Listán Prieto

Among the cultivars characterized by a peduncle plane at the top, Listán Prieto is the one with higher roundness (lower Aspect Ratio). Cultivars Listán Prieto and Tortozona Tinta gave high values of *J* index with Model Listán Prieto (Figure 4; Table 4).

Figure 4. Model Listán Prieto. From left to right: An image with the sum of the silhouettes of 30 seeds, average silhouette obtained from the first image, the model Listán Prieto, and four representative seeds of this cultivar. Bar represents 1 cm.

Table 4. Values of area and *J* index in the cultivars of Group Listán Prieto. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Listán Prieto	30	19.2[a] (1.31)	16.5	21.4	90.7[b] (1.32)	87.7	92.9
TortozonaTinta	30	15.2[b] (1.21)	12.1	17.1	92.6[a] (1.42)	89.5	94.6

Figure 5 contains the Box-plot representations of the values of *J* index for the varieties Listán Prieto and Tortozona Tinta with model Listán Prieto.
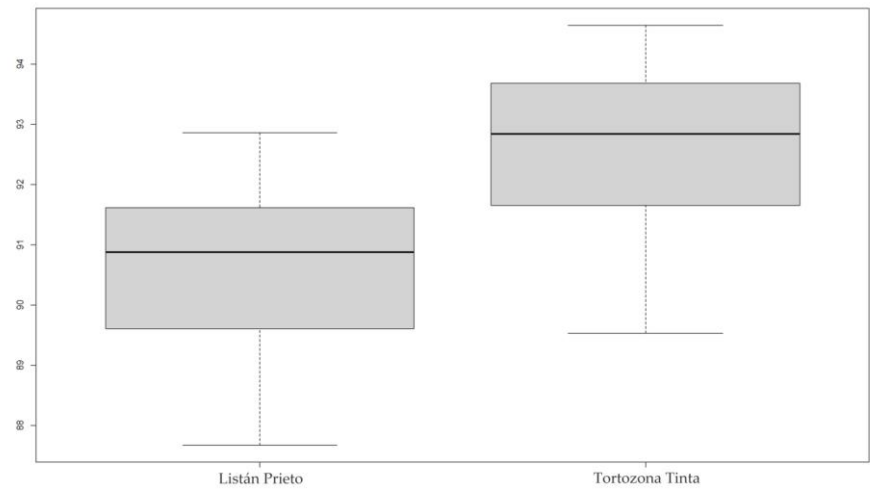
Figure 5. Box-plot representations showing the distribution of *J* index values in cultivars Listán Prieto and Tortozona Tinta with model Listán Prieto.

3.2.2. Model Sylvestris

Three cultivars gave high values of *J* index with the model Sylvestris (Figure 6, Table 5). These are CA 13.4, CA 13.6 and SE 2.1. Scheffé test indicates that here is a difference between the mean *J* index values of CA 13.4 and CA 13.6.

Figure 6. Model Sylvestris . From left to right: An image with the sum of the silhouettes of 30 seeds of CA 13.4, average silhouette obtained from the first image, the model Sylvestris, and four representative seeds of CA 13.4. Bar represents 1 cm.

Table 5. Values of area and *J* index in the three cultivars of Group Sylvestris . Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min.	Max.	Mean (std dev)	Min.	Max.
CA 13.4	20	13.7[b] (1.24)	11.5	15.7	90.7[a] (1.15)	89.2	92.8
CA 13.6	20	14.8[a] (1.56)	12.9	17.9	89.3[b] (1.57)	86.8	91.7
SE 2.1	20	12.6[c] (0.99)	10.1	14.2	90.4[ab] (1.67)	86.6	92.8

Figure 7 contains the Box-plot representations of *J* index values for the varieties CA 13-4, CA 13-6 and SE 2-1.
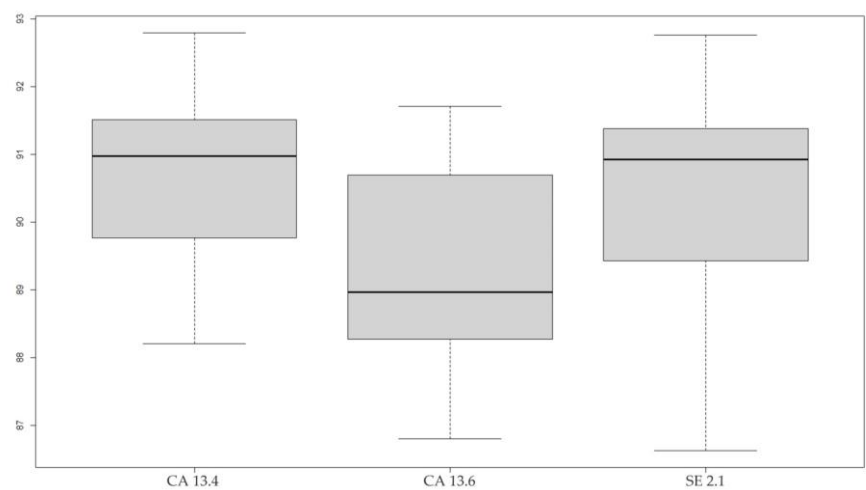
Figure 7. Box-plot representations showing the distribution of *J* index values in cultivars CA 13-4, CA 13-6 and SE 2-1 with model Sylvestris .

3.2.3. Model Albillo Real

Cultivars Alarije, Albillo Real, Juan García and Tempranillo gave values of *J* index higher than 90 with the model Albillo Real. Figure 8 shows the sum of the silhouettes, a representative silhouette, the model Albillo Real and the photographs of four seeds representative of this cultivar. Table 6 presents the values of área and *J* index in these cultivars and the results of the ANOVA test. Several different groups were found in seed size (area), but only Albillo Real and Juan Garcia can be considered different in *J* index values.

Figure 8. Albillo Real. From left to right: A sum of the silhouettes of 30 seeds of cultivar Albillo Real, a silhouette obtained by the pixel profile of the first image, the model Albillo Real, and four representative seeds of this cultivar. Bar represents 1 cm.

Table 6. Values of area and *J* index in the cultivars of Group Albillo Real. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min.	Max.	Mean (std dev)	Min.	Max.
Alarije	30	18.0^{bc} (1.59)	15.2	21.2	90.8^{ab} (2.10)	83.8	93.2
Albillo Real	30	16.2^{d} (1.17)	13.2	17.9	91.5^{a} (2.06)	85.4	94.5
Cayetana Blanca	30	18.6^{b} (1.43)	15.8	22.7	91.0^{ab} (4.84)	85.4	93.6
Graciano	30	17.3^{c} (1.22)	14.5	20.1	91.1^{ab} (1.62)	87.0	93.5
Juan García	30	22.9^{a} (1.68)	20.2	26.0	90.0^{b} (1.74)	83.0	92.0
Tempranillo	30	18.9^{b} (0.99)	16.4	20.6	90.9^{ab} (1.28)	88.1	93.1

Figure 9 contains the Box-plot representations with values of *J* index for varieties Alarije, Albillo Real, Cayetana Blanca, Graciano, Juan García and Tempranillo.
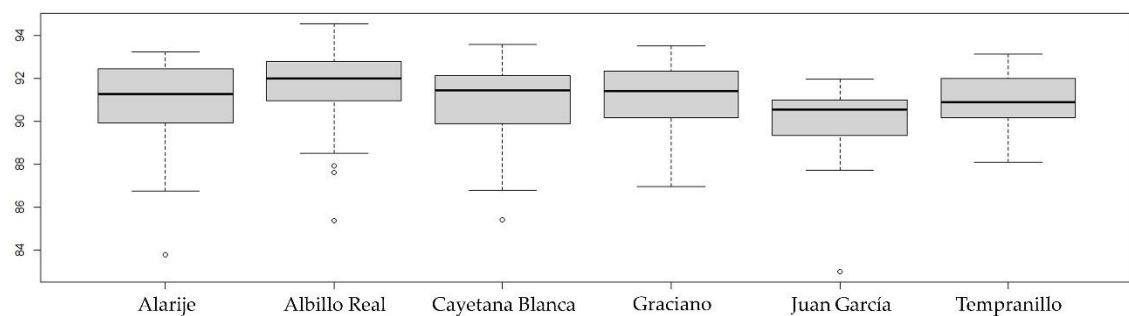
Figure 9. Box-plot representations showing the distribution of *J* index values in cultivars Alarije, Albillo Real, Graciano, Juan García and Tempranillo with model Albillo Real.

3.2.4. Model Hebén

Three cultivars gave high values of *J* index with model Hebén. These are: Hebén, Macabeo and Zalema. Figure 10 shows the Model Hebén, with representative seeds of this cultivar. Table 7 presents the values of *J* index obtained with the Model Macabeo in the five cultivars indicated.

Figure 10. Model Hebén. From left to right: A sum of the silhouettes of 30 seeds, a silhouette obtained by the pixel profile of the first image, the model Hebén, and four representative seeds of this cultivar. Bar represents 1 cm.

Table 7. Values of area and *J* index in the cultivars of Group Hebén. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Hebén	30	21.2[a] (1.68)	18.3	24.8	90.0[b] (1.72)	85.7	91.9
Macabeo	30	16.1[b] (0.83)	13.9	18.1	90.8[a,b] (1.70)	86.1	93.1
Zalema	30	14.6[c] (0.99)	12.7	17.2	91.6[a] ((1.26)	89.1	94.0

Figure 11 contains the Box-plot representations of the values of *J* index for the varieties Hebén, Macabeo and Zalema with model Hebén.
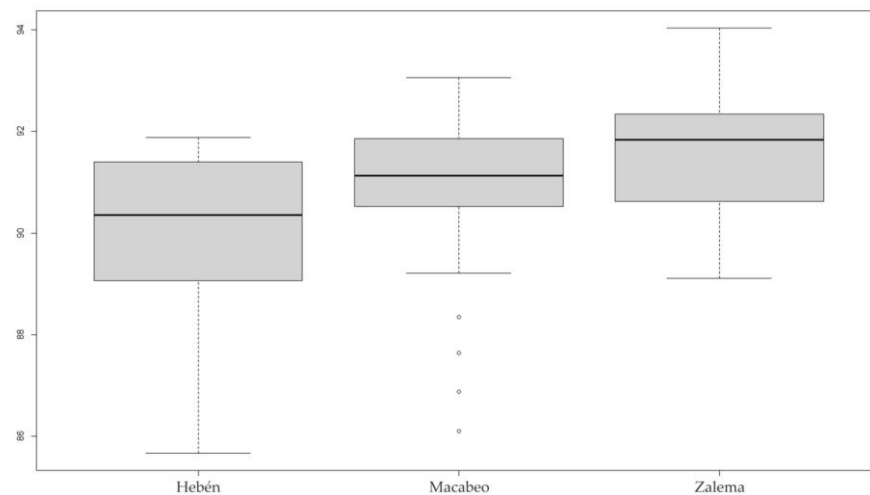
Figure 11. Box-plot representations showing the distribution of *J* index values in cultivars Hebén, Macabeo and Zalema with Model model Heben.

3.2.5. Model Moscatel

Cultivars Beba, Bruñal, Caíño Tinto, Castellana Blanca, Garnacha Tinta, Gewürztraminer, Malvasía Aromática, Mollar Cano, Moscatel de Alejandría, Moscatel de Grano Menudo, Palomino Fino and Prieto Picudo gave high values of *J* index with model Moscatel (Figure 12; Table 8).

Figure 12. Model Moscatel. From left to right: A sum of the silhouettes of 30 seeds of cultivar Mollar Cano, average silhouette for this cultivar obtained by the pixel profile of the first image, the model Moscatel, and four representative seeds of cultivar Mollar Cano. Bar represents 1 cm.

Table 8. Values of area and *J* index in the cultivars of Group Moscatel. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index **Moscatel**		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Beba	30	14.7[f] (0.82)	13.2	16.3	91.2[ab] (1.43)	88.1	93.3
Bruñal	30	14.6[fg] (0.93)	13.0	16.5	92.2[a] (1.12)	89.7	94.4
Caíño Tinto	30	23.7[a] (1.85)	20.2	27.8	90.6[ab] (2.99)	82.7	94.3
Castellana Blanca	30	20.1[b] (1.14)	18.0	23.3	91.4[ab] (0.91)	89.6	93.7
Garnacha Tinta	30	13.9[gf] ((0.94)	12.1	15.9	88.8[c] (3.03)	81.6	92.7
Gewürztraminer	30	17.2[de] (0.79)	15.1	18.5	90.8[ab] (2.43)	83.1	93.7
Malvasía Aromática	30	17.5[d] (1.02)	15.0	19.9	90.3[bc] (1.55)	85.4	92.5
Mollar Cano	30	18.5[c] (0.77)	16.9	20.1	90.8[ab] (1.68)	87.6	94.0
Moscatel de Alejandría	30	17.2[de] (1.54)	14.5	20.2	90.5[ab] (2.53)	83.2	93.2
Moscatel de Grano Menudo	30	13.8[g] (0.84)	11.9	15.4	90.4[bc] (2.12)	84.3	93.1

Palomino Fino	30	14.5[fg] (072)	12.9	16.2	91.0[ab] (1.69)	86.7	93.8
Prieto Picudo	26	16.4[e] (0.84)	14.2	17.5	90.9[ab] (2.07)	84.3	93.3

Figure 13 contains the Box-plot representations with values of J index for the varieties Beba, Bruñal, Caíño Tinto, Castellana Blanca, Garnacha Tinta, Gewürztraminer, Malvasía Aromática, Mollar Cano, Moscatel de Alejandría, Moscatel de Grano Menudo, Palomino Fino, Prieto Picudo.
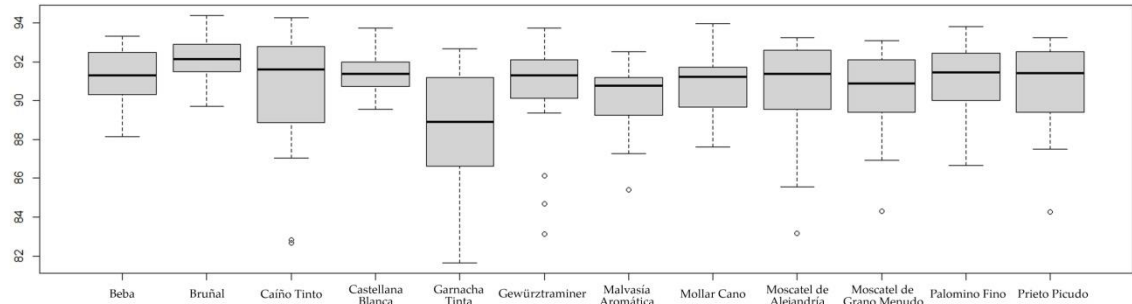
Figure 13. Box-plot representations showing the distribution of J index values in cultivars Beba, Bruñal, Caíño Tinto, Castellana Blanca, Garnacha Tinta, Gewürztraminer, Malvasía Aromática, Mollar Cano, Moscatel de Alejandría, Moscatel de Grano Menudo, Palomino Fino, Prieto Picudo with model Moscatel.

3.2.6. Model Tortozón

The seeds of cultivars Imperial and Tortozón resemble Model Hebén but are smaller and with the peduncle thinner. Change in size does not imply a change in shape, but the thickness of the peduncle, does. Both cultivars gave high values with model Tortozón and there was no difference in area between them. Figure 14 shows the Model Tortozón, with representative seeds of this cultivar. Table 9 presents the values of J index obtained with the Model Tortozón in the cultivars Imperial and Tortozón.

Figure 14. Tortozón. From left to right: A sum of the silhouettes of 30 seeds, a silhouette obtained by the pixel profile of the first image, the model Tortozón, and four representative seeds of this cultivar. Bar represents 1 cm.

Table 9. Values of J index in cultivars cultivars Imperial and Tortozón. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at $p<0.05$. N is the number of seeds analyzed.

Cultivar	N	Area			J index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Imperial	30	17.5[a] (1.18)	15.6	20.2	88.9[b] (2.24)	82.9	92.1
Tortozón	30	17.7[a] (0.93)	16.0	20.1	89.9[a] (1.52)	84.9	92.3

Figure 15 contains the Box-plot representations of the values of J index for the varieties Imperial and Tortozón with model Tortozón.
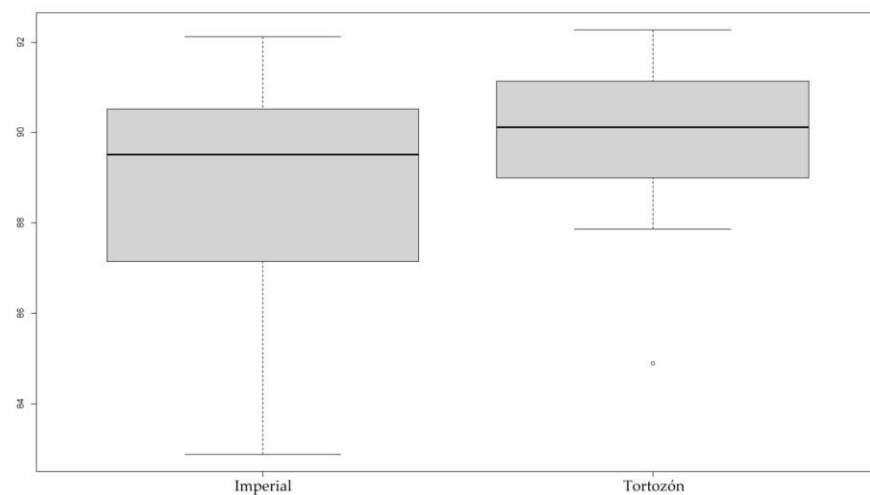
Figure 15. Box-plot representations showing the distribution of *J* index values in cultivars Imperial and Tortozón with Model Tortozón.

3.2.7. Model Teta de Vaca

Three cultivars gave high values of *J* index with the model Teta de Vaca (Figure 16, Table 10). These are Dominga, Teta de Vaca and Verdejo. There is no difference in *J* index values between these three varieties that are only distinguishable by their differences in area, the seeds of Teta de Vaca being larger, Verdejo, smaller and Dominga, with intermediate values.

Figure 16. Model Teta de Vaca. From left to right: A sum of the silhouettes of 30 seeds of cultivar Teta de Vaca, a silhouette obtained by the pixel profile of the first image, the model Teta de Vaca, and four representative seeds of this cultivar. Bar represents 1 cm.

Table 10. Values of area and *J* index in the two cultivars of Group Teta de Vaca. Superscript letters indicate the results of Scheffé test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Dominga	30	23.8[b] (1.50)	20.7	23.8	90.4[a](2.60)	79.3	93.7
Teta de Vaca	30	25.8[a] (1.73)	20.3	29.1	90.4[a](1.81)	86.0	93.8
Verdejo	30	20.0[c] (0.87)	17.8	21.3	90.3[a](1.09)	87.8	92.3

Figure 17 contains the Box-plot representations with Values of *J* index for the varieties Dominga, Teta de Vaca and Verdejo with model Teta de Vaca.
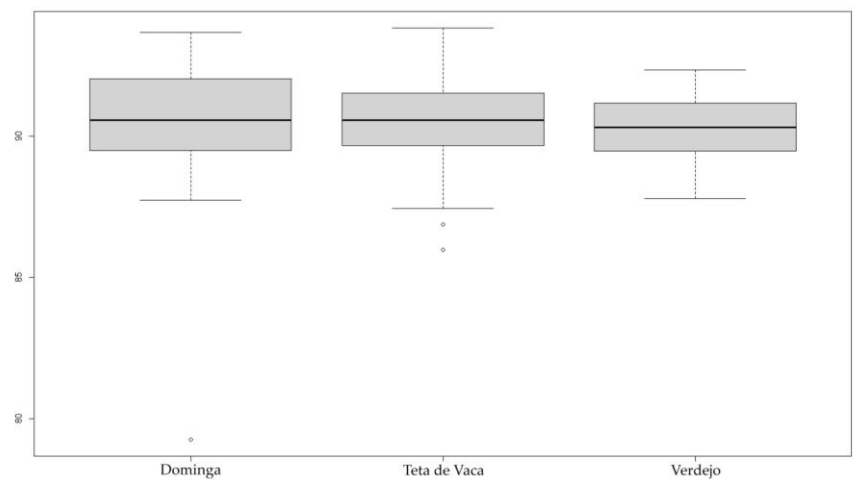
Figure 17. Box-plot representations showing the distribution of *J* index values in cultivars Dominga, Teta de Vaca and Verdejo with model Teta de Vaca.

3.2.8. Model Doña Blanca

The seeds of cultivars Pedro Ximénez, Doña Blanca and Monastrell resemble Model Moscatel, but are more elongated. Figure 18 shows the Model Doña Blanca, with the average silhouette and representative seeds of this cultivar. Table 11 presents the values of area and *J* index obtained with the Model Doña Blanca in the cultivars Doña Blanca, Monastrell and Pedro Ximénez. The values of *J* index obtained with this model are higher in Doña Blanca than in Monastrell. Area values are higher in Monastrell and smaller in Pedro Ximénez.

Figure 18. From left to right: A sum of the silhouettes of 30 seeds, a silhouette obtained by the pixel profile of the first image, the model Doña Blanca , and four representative seeds of Doña Blanca. Bar represents 1 cm.

Table 11. Values of area and *J* index in the cultivars of Group Doña Blanca. Superscript letters indicate the results of Tukey test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

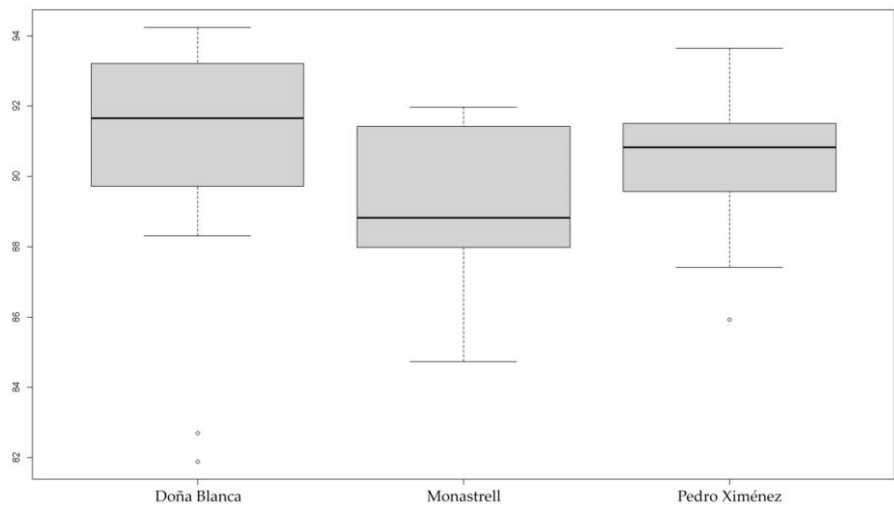| Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Doña Blanca	30	18.3[b] (1.17)	15.9	20.9	91.0[a] (2.93)	81.9	94.2
Monastrell	30	20.4[a] (1.46)	17.4	23.1	89.4[b] (2.09)	84.7	92.0
Pedro Ximénez	30	17.4[c] (0.87)	15.1	19.0	90.5[ab] (1.73)	85.9	93.7

Figure 19. Box-plot representations showing the distribution of *J* index values in cultivars Doña Blanca, Monastrell and Pedro Ximénez with model Doña Blanca.

3.2.9. Model Airén

The group defined by model Airén (Figure 20) has three cultivars with *J* index values superior to 90. These are Airén, Bobal and Mazuela. They differ in size, with the seeds of Mazuela being smaller than the other two cultivars (Table 12, Figure 21). On the other hand, there are no differences between these three cultivars with respecto to their J indices.

Figure 20. From left to right: A sum of the silhouettes of 30 seeds, a silhouette obtained by the pixel profile of the first image, the model Airén, and four representative seeds of Airén. Bar represents 1 cm.

Table 12. Values of area and *J* index in the cultivars of Group Airen. Superscript letters indicate the results of Tukey test: the mean values marked with the same letter in each column do not differ significantly at p<0.05. N is the number of seeds analyzed.

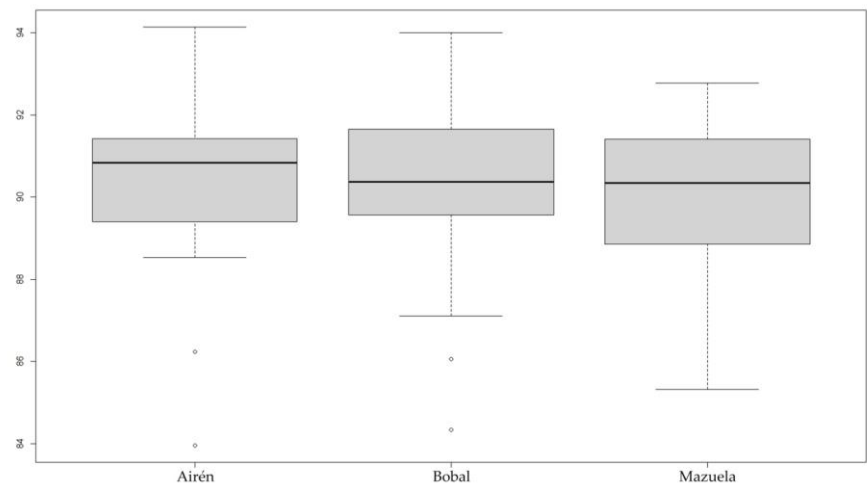| Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
Airen	30	17.7[a] (1.21)	15.5	19.7	90.4[a] (2.09)	83.9	94.1
Bobal	30	18.3[a] (1.30)	15.7	20.8	90.3[a](2.02)	84.3	94.0
Mazuela	30	16.4[b] (0.96)	14.5	18.3	90.1[a](1.93)	85.3	92.8

Figure 21. Box-plot representations showing the distribution of *J* index values in cultivars Airén, Bobal and Mazuela with Model Airén.

3.2.10. Model De Cuerno

Model De Cuerno is the most elongated of all ten models and defines seed shape of variety De Cuerno (Figure 22, Table 13).
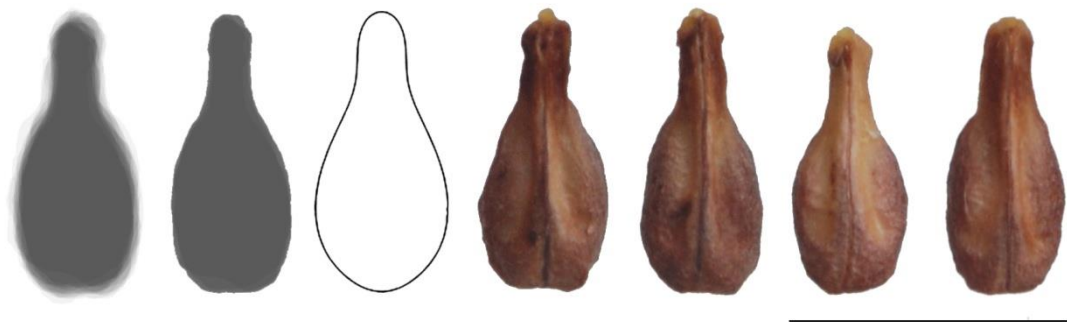

Figure 22. From left to right: A sum of the silhouettes of 30 seeds, a silhouette obtained by the pixel profile of the first image, the model De Cuerno, and four representative seeds of De Cuerno. Bar represents 1 cm.

Table 13. Values of area and *J* index in the cultivar De Cuerno.

Cultivar	N	Area			*J* index		
		Mean (std dev)	Min	Max	Mean (std dev)	Min	Max
De Cuerno	20	29.1 (1.46)	26.0	31.1	88.8 (1.63)	85.8	91.6

4. Discussion

The seeds of the Vitaceae have peculiar shapes different from those predominant in the Angiosperms that resemble the ellipse, ovoid and cardioid figures [27,42]. A first study showed similarity to geometric figures derived from the heart and pear curves of Weisstein [24-26]. Seven models termed M1 to M7 were proposed to describe seed shape of species belonging to three genera in this family (*Cissus verticillata*, *Parthenocissus tricuspidata*, diverse *Vitis* species and six cultivars of *Vitis vinifera* subsp. *Vinifera*) [24]. In the comparisons between cultivars, Merseguera gave higher values with the elongated heart curve (M5), while seeds of other cultivars, including Malvasia adjusted better to M7 [24].

In this work we have investigated seed shape in thirty eight cultivars of *Vitis vinifera* conserved in the germplasm collection of El Encín. The collection has a total of 3699 en-

tries, of which 241 will be the subject of a first approach to seed morphology. The culti-vars used in this work were selected on the basis of their historical relevance. Many of them were reported in the first treatises of viticulture [7,8,44-47] (Table A1), others have been used as parentals in crosses [48] and most of them are still used broadly today. In addition, the cultivars of this work represent a large proportion of the variation of seed shape in the collection. Based on the morphological diversity in this set of 38 representa-tive cultivars, ten new models were developed, nine based on variations of M7 and one based on the piriform curve (M6), both M6 and M7 described before [24]. The protocol included the obtention of a mean seed image for each cultivar, a step that was helpful for the identification and design of the models. The cultivars were classified according to their similarity to the models and *J* index, representing the percent area shared by each seed and the respective model, was obtained for 30 seeds in each of 38 cultivars.

In an attempt to define and describe seed shape, three aspects were considered in the seed images for the obtention of the models: the surface on top of the peduncle that may be plane, rounded or acute, the lateral inclination of the peduncle, that may be more or less pronounced and sometimes takes the aspect of a shoulder, and the structure of the curve that forms the basis of the seed. Approximately half of the cultivars have the top of the peduncle flat, while in the others it is rounded or acute. The cultivars grouped around models Listán Prieto, Albillo Real, Moscatel, Airén and Doña Blanca have the top of the peduncle plane, while in the remaining of the groups it is rounded or acute. The bio-chemical and structural properties associated with these types remain to be investigated. Considering that lignin is an important component of the cell walls it may be interesting to explore the possibility of changes in lignin composition in both types. Pedicel thick-ening in grape berries results by increased the number of secondary xylem cells and their lignification [49]. It may be interesting to check whether there are differences in these aspects among the cultivars.

Ten groups were formed representative of the variation in seed shape in the 38 cul-tivars. Each group is based on the similarity of the cultivars to one of the models. The groups are heterogenous in size as well as in fidelity of their cultivars to their respective model. Some of them include a high number of cultivars, others a reduced number. In most of the cultivars adscribed to each group, the values of *J* index with the correspond-ing model are superior to 90, representative of high similarity [39]. Exceptionally, culti-vars ascribed to a group had their *J* index value lower than 90 with the corresponding model. This was due to either a high morphological variation in the samples of some cultivars (De Cuerno) or to the possibility of existence of two or more seed types in a cultivar (Airén, Garnacha Tinta). The largest group is the one formed around Model Moscatel, containing 12 cultivars. Although most of them have high values of *J* index with this model, based on the average silhouette it may be possible to obtain new models that define sub-groups or varieties inside this group, as well as some in of the other groups. Thus, it is expected that this group will be split in two or more when the analysis is done in other cultivars. The second group in size is the formed around Albillo Real, with six cultivars, all with *J* index values superior to 90. The existence of differences in *J* index values is not restricted to the large groups and indicates the possibility of obtaining more specific models for some of the cultivars.

The values of *J* index obtained have improved with respect to the already reported [24]. In *Vitis vinifera* subsp. *Vinifera*, a mean value of 88.9 was reported with M7, while in this work the values corresponding to the three cultivars of this subspecies with the re-spective model were of 90.7, 90.4 and 89.6 for CA 13.4 SE 2.1 and CA 13.6. Considering the six cultivars tested in the previous article, the maximum values obtained of 90.1 and 91.7 for Cercial and Malvasia with Model 7 and 90.4 with Model 5 for Merseguera are comparable to those obtained with the majority of cultivars in this work. In future tests, Cercial and Malvasia may give higher values with model Albillo Real, while Merseguera may be included in the group of Hebén or Tortozón. The other three varieties tested may also be included in some of the groups here described, for example, Camarate in Moscatel or Doña Blanca. Cariñena may give high *J* index values with model Hebén or Tortozón,

or a similar, but more elongated model. The work is open to the addition of new models and testing with new cultivars.

Hebén is an interesting cultivar that played a fundamental role in the current varietal map of Spain and Portugal [48]. Possibly originating from North Africa, it is a female variety whose reproduction by seed between the 9th and 12th centuries has given rise to a large number of descendants. Many of the cultivars currently grown on the Iberian Peninsula are the result of a natural cross between Hebén and other grapes mentioned by Alonso de Herrera in his treatise. Among the parentals are Teta de vaca, Monastrell and Tortozón. Crosees of Tortozón with Heben gave Alarije, Malvar and Merseguera [48]. Although in the group Hebén there is a reduced number of cultivars (3), it may be possible to obtain more specific models adapted to their shape.

This work confirms the possibility of a classification of grapes based on the similarity of seed shape with geometric models, a method that is complementary to the classifications based on molecular protocols such as SSRs and particularly of those based on elliptical Fourier transform [19,20], contributing to give them a visual support. Similar to the traditional classification of Planchon in short and elongated seeds, our method is based in seed shape, but this is a complex character. Morphological aspects of seed shape such as peduncle length, structure and thickness of the in the apex may be evaluated by magnitudes. In addition to distance, curvature values may provide new descriptions.

5. Conclusions

Seeds of 38 cultivars of *Vitis vinifera* subsp. *Vinifera* representing the biodiversity in the germplasm collection of El Encín were classified according to their similarity to geometric figures resulting from the bi-dimensional representation of algebraic equations.

Based on morphological aspects of the seeds, ten models were designed that define the groups containing all the cultivars. Similarity of seed images to the geometric models was described and quantified by J index, measuring the percent similarity between seeds and models. The analysis has given ten new models able to describe and classify seed shape in 38 cultivars representative of the variability in shape on the seeds of *Vitis vinifera* conserved in the germplasm collection of El Encín (Madrid). More cultivars will be tested to find new models, confirm and expand these groups.

Appendix A. Table A1

Table A1. List of cultivars of of *Vitis vinifera* L. used in this work, indicating the origin of the culti-
vars and their first mention in the literature [6-8,22-26]. CA 13.4 and CA 13.6 were obtained from
the terrains close to the stream Nateruela in Cadiz. Se 2.1 was obtained in Ribera del Huéznar,
Sevilla

N	Main name	First description	Synonyms	Origin	Group
1	Airén	[8]	Blancón, Lairén, Manchega, Valde-peñas,	Castilla-La Mancha	Airén
2	Alarije	[7]	Aris, Malvasía Riojana, Rojal, Subi-rat Parent, Torrontés.	Extremadura	Albillo Real
3	Albillo Real	[7]	Albillo de Madrid, Albillo de Toro, Temprana.	Castilla y León	Albillo Real
4	Beba	[43]	Calop Blanco, Chelva, Lairén, Va-lencí, Mantua, Uva Rey.	Andalucía	Moscatel
5	Bobal	[43]	Moravia, Morenillo, Pansa negra, Provechón.	Valencia	Bobal
6	Bruñal	[44]	Albarín Negro, Baboso Negro, Al-frocheiro Preto (Portugal).	Castilla y León	Moscatel
7	Caiño Tinto	[45]	Caíño del País, Caíño Gordo, Caíño, Tinta Femia.	Galicia	Moscatel
8	Castellana Blanca	[7]	Without synonyms.	Castilla y León	Moscatel
9	Cayetana Blanca	[7]	Baladí, Cagazal, Calagraño, Jaén, Robal, Verdoncho.	Andalucía	Albillo Real
10	De Cuerno	[8]	Dedos de doncella, Cornichón Blanc, Pizzutelllo bianco.	Andalucía	De Cuerno
11	Dominga	[50]	Gloria, Murciana Blanca y Uva Verde de Alhama.	Murcia	Teta de vaca
12	Doña Blanca	[7]	Cigüente, Malvasía Castellana, Valenciana.	Extremadura	Airén
13	Garnacha Tinta	[43]	Aragonés, Lladoner Negre, Grena-che (Francia).	Aragón	Moscatel
14	Gewürztraminer	[46]	Savagnin Rosa Aromática (Francia).	Germany	Moscatel
15	Graciano	[8]	Morrastel en Francia, Tinta Miuda (Portugal).	La Rioja	Albillo Real
16	Hebén	[7,47]	Ben, Evén, Laco Blanco, Panseras, Pansero.	Castilla-La Mancha	Hebén
17	Imperial	[38]	Without synonyms.	Unknown	Tortozón
18	Juan García	[45]	Negrón de Aldán, Mouratón, Ne-greda, Tinto Madrid.	Castilla y León	Albillo Real
19	Listán Prieto	[7]	Mission (California); País (Chile), Hariri (Morocco).	Castilla-La Mancha	Listán Prieto
20	Macabeo	[43]	Blanco de Daroca, Viura.	Aragón	Hebén
21	Malvasía Aromática	[7]	Malvasía de Sitges; Malvasía de Lipari (Italy).	Greece	Moscatel
22	Mazuela	[43]	Cariñena, Concejón, Samsó, Carig-nan (France).	Aragón	Bobal
23	Mollar Cano	[43]	Mulata Negra, Moll, Negra Moll, Negra Mole (Portugal).	Andalucía	Moscatel
24	Monastrell	[43]	Alcayata, Garrut, Mandó, Mourvèdre (Francia).	Valencia	Airén
25	Moscatel de Alejandría	[43]	Moscatel de Grano Gordo, Romé, Zibibbo (Italy).	Egypt	Moscatel
26	Moscatel de grano menudo	[7]	Moscatel Almizclero, Moscatel Morisco, Moscatel Romano.	Valencia	Moscatel
27	Palomino Fino	[51]	Jerezana fina, Listán Blanco, Listán	Andalucía	Moscatel

			Gacho.		
28	Pedro Ximénez	[43]	Chironés, Chironet.	Andalucía	Airén
29	Prieto Picudo	[52]	Verdejo negro	Castilla y León	Moscatel
30	Sylvestris (CA 13.4)	[53]	Without synonyms	Andalucía	Sylvestris
31	Sylvestris (CA 13.6)	[53]	Without synonyms	Andalucía	Sylvestris
32	Sylvestris (SE 2.1)	[53]	Without synonyms	Andalucía	Sylvestris
33	Tempranillo	[7]	Cencibel, Tinta de Toro, Tinta Roriz (Portugal).	La Rioja	Albillo Real
34	Teta de vaca	[54]	Royal Gordo, Rojal Gordo, Ahmeur bou Ahmeur.	Egypt	Teta de vaca
35	Tortozón	[7]	Prieto Picudo Blanco, Folha de Figuera (Portugal)	Castilla-La Mancha	Tortozón
36	Tortozona Tinta	[7]	Without synonyms	Castilla-La Mancha	Listán Prieto
37	Verdejo	[45]	Without synonyms	Castilla y León	Teta de vaca
38	Zalema	[8]	Mantúo Perruno, Perruna, Torron-tés de Montilla.	Andalucía	Hebén

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