# Seed Morphology in the Vitaceae Based on Geometric Models

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**Abstract:** Morphometric methods based on artificial vision algorithms provide measurements for magnitudes descriptive of seed images (i.e., the length, width, area, and surface circularity index). Nevertheless, their results frequently omit the resemblance of the images to geometric figures that may be used as models. A complementary method based on the comparison of seed images with geometric models is applied to seeds of *Vitis* spp. The J index gives the percentage of similarity between a seed image and the model. Seven new geometric models are described based on the heart-shaped and piriform curves. Seeds of different species, subspecies and cultivars of *Vitis* adjust to different models. Models 1 and 3, the heart curve and the water drop, adjust better to seeds of *V. amurensis*, *V. labrusca* and *V. rupestris* than to *V. vinifera*. Model 6, the Fibonacci's pear, adjusts well to seeds of *V. vinifera*, in general, and better to *V. vinifera* ssp. *vinifera* than to *V. vinifera* ssp. *sylvestris*. Seed morphology in species of *Cissus* and *Parthenocissus*, two relatives of *Vitis* in the Vitaceae, is also analysed. Geometric models are a tool for the description and identification of species and lower taxonomic levels complementing the results of morphometric analysis.

**Keywords:** geometric curves; grapes; heart-shaped; image analysis; J index; morphology; pips; shape; *Vitis*

## 1. Introduction

Grapevines belong to the genus *Vitis* L., which comprises about seventy species of lianes in the family Vitaceae Juss. Grape (*Vitis vinifera* L. ssp. *vinifera*) is one of the most ancient fruit-bearing plants in the Old World, and together with the olive, fig, pomegranate and date palm, it comprises the oldest groups of domesticated fruit trees [1–3]. Today, it is widely accepted that the modern cultivars of *V. vinifera* are the result of the domestication of *V. vinifera* ssp. *sylvestris* (C.C.Gmel.) Hegi (wild grape) [4–6]. The main feature used to distinguish between wild and cultivated *Vitis* is the reproductive

system: the wild grape is dioecious, whereas the cultivated grapevine has hermaphroditic flowers; however, a small percentage of hermaphroditism also exists in the wild grape populations [7]. The domestication process, likely involving vegetative propagation of clones by growing cuttings, induced important changes in the reproductive biology of the grape cultivars, resulting in different properties; both physical (size, color), nutritional (sugar, carbohydrate and phenolic compounds), as well as in seed shape diversification. Ampelography, the botanical discipline dedicated to the identification of grapevines, has been traditionally focused on the shape of vine leaves and grape berries, and it has more recently been focused on DNA fingerprinting [8]. The seeds of grape, often called pips, are highly polymorphic and have an important role for the taxonomic study within the genus *Vitis* [9–15]. The description of seed shape proposed in this work is complementary to morphometric analysis and can be useful in Ampelography.

In early morphological studies with grape seeds, two morphotypes were defined consisting of pear-shaped or oblong to ovoid seeds, that corresponded respectively to the taxonomic groups Euvitis and Muscadinia [9,10]. The seeds of cultivated species (*V. vinifera*, *V. labrusca* L., *V. aestivalis* Michx.) are larger and their dimensions are more variable than those of wild species [9–11]. Stummer's index was proposed to discriminate between cultivars and wild forms, defining the "wild syndrome" for those seeds having values of breadth/length above 0.8 and corresponding to wild extra-European *Vitis* species [9,12]. The seeds of cultivated grapevines have, in general, elongated, pear-shaped seeds, with a long beak, while the wild forms have shorter seeds, although caution must be taken in the identification of the latter, because of the difficulty in differentiating wild forms and feral cultivars [9].

Seed morphology departs from two points: first, it requires a concise description, based in qualitative aspects; and, second but not less important, metrology, i.e., the set of magnitudes that provide the dimensions of a seed. Metrology may also confirm, define and expand information about the adjectives used in the former, qualitative, description. Adjectives used to describe seed shape in *Vitis* include some already mentioned, like oblong, ovoid or pear-shaped (pyriform) and others like cordate, globose, oval, rounded, squat or triangular [10,11,13]. Magnitudes used to measure seed shape include seed length, seed breadth, chalazal length, chalazal position and others describing primary aspects of the morphology of pips, as indicated by Jaquat and Martinolli [1] and Mangafa and Kotsakis [13]. Modern methods of automated vision are based on the coordinates of the points in a bi-dimensional image and permit new approaches to seed morphology. Digital image analysis coupled with morphometric studies of grape seeds is a relatively new application in the plant research field. By using a set of high-resolution digital images, it is possible to obtain measurements of morphometric, colorimetric and textural features, describing the shape, size, color and texture of seeds, and this quantitative information can be correlated with various qualitative aspects of the seeds. The data obtained, corresponding to magnitudes such as area, perimeter, circularity and roundness [13–17], can be combined and subjected to analysis based on diverse algorithms, for example, elliptic Fourier (EFD) and Aralik descriptors. Finally, in all cases, the data are collected in a matrix and can be submitted to Linear Discriminant Analysis (LDA), to classify the seeds in groups [9,14,15,18,19–21].

There is an interest, both theoretical and applied, in joining both aspects of morphology: the descriptive adjectives and the measurements with the objective of testing if the groups defined by artificial vision and statistical analysis correspond to well defined geometric figures. This objective, that we may define as obtaining new measurable adjectives, can be reached by comparing the seed images with geometric models based on mathematical curves. This way, we obtain the J index that gives the percent of similarity between the two images (the bi-dimensional image of the seed and the geometric model). High values of J index were obtained when comparing images of seeds with the cardioid or modified cardioids in the model plant *Arabidopsis thaliana* (L.) Heynh [22], as well as in the model legumes, *Lotus japonicus* L. and *Medicago truncatula* Gaertn. [23], also in *Capparis spinosa* L. [24], and in species of the Papaveraceae and Malvaceae [25,26].

Other geometric models include the ovoid and the ellipse, that give high values of J index with seeds of species in the Cucurbitaceae [27] and in the Euphorbiaceae, such as *Ricinus* L. and *Jatropha*

L. [28,29]. Seed shape in wheat kernels was described based on three geometric figures: an ellipse of aspect ratio (AR) = 1.8 fitting the "round varieties" (*T. aestivum* L. ssp. *aestivum* cv. Zebra and Torka), 2), a lens of AR = 3.2 for the elongated kernels (*T. monococcum* L.), and 3) an ellipse of AR = 2.4 that adjusted well the kernels of intermediate-shaped varieties such as *T. durum* Desf. cv. Floradur [30]. This latter work, demonstrating the adjustment of seed shape to geometrical forms, is useful to complement the results of morphometric analysis as those reported in *Vitis* sp. [9,14,15,18,19–21].

Our objective was to describe seed shape in species, subspecies and cultivars of *Vitis* by a combined geometric and statistical approach. The images of grape pips belonging to different genotypes were compared with geometrical models derived from mathematical curves that represent diverse heart shapes [31,32] or the pyriform curve [33]. This allows the description of seed shape with adjectives that may be measurable. For example, cordate means heart-like, thus defining a family of curves, responding to different equations that include different models. Among the cordate figures (heart-shaped), the cardioid is the curve defined by the trajectory of the point in a circle rolling around another circle of the same radius. This curve responds to an algebraic equation and is reproducible, thus, for any given two-dimensional figure well defined by a profile, we may calculate the percentage similarity to the cardioid. News models based on geometrical figures are defined for seed shape quantification in the species and cultivars of *Vitis*. In addition, the overall morphology of *Cissus* and *Parthenocissus*, genus related to *Vitis* in the Vitaceae [34] is described, presenting an overview of seed shape in the Vitaceae.

## 2. Materials and Methods

### 2.1. Species and Varieties Used

The study included seeds of four species of *Vitis* (*V. amurensis* Rupr., *V. labrusca*, *V. rupestris* Scheele and *V. vinifera*), as well as seeds of *Cissus verticillata* (L.) Nicolson and C. E. Jarvis and *Parthenocissus tricuspidata* (Siebold and Zucc.) Planch. *V. vinifera* is represented by two subspecies: *V. vinifera* ssp. *vinifera* and *V. vinifera* ssp. *sylvestris.* In addition, six cultivars of *V. vinifera* ssp. *vinifera* were included: Camarate, Carignano, Cercial, Malvasía, Meserguera and Morenillo. Seeds of *V. amurensis*, *V. labrusca*, *V. rupestris* and *V. vinifera* ssp. *vinifera* cultivars Camarate, Cercial, Morenillo and Meserguera proceed from "Colección de Vides del Encín" (IMIDRA, Comunidad de Madrid). Morenillo and Meserguera are Spanish varieties, while Cercial and Camarate have their origin in Portugal; *V. vinifera* ssp. *sylvestris* and *V. vinifera* ssp. *vinifera* cultivars Carignano, Malvasía proceed from Sardinia. Morenillo, Camarate and Carignano cultivars have black berries, and Cercial, Malvasía and Meserguera show white berries. All seeds were obtained from Sardinia Germplasm Bank (BG-SAR) of Hortus Botanicus Karalitanus (HBK), Università degli Studi di Cagliari (Italy).

Seeds of *C. verticillata* were collected at Pastaza (Ecuador) in disturbed areas and, after being photographed, the seeds were returned into the wild. Seeds of *P. tricuspidata* were collected at the Faculty of Pharmacy, University of Salamanca.

### 2.2. Seed Images

The seeds were oriented with their chalaza downwards, such as to expose the ribs upwards (ventral orientation; Figure 1). This offers an advantage in relation to the dorsal orientation, because the seed has a more plane surface of sustentation. In the dorsal orientation, the seed rests on top of the rib and the seed is skewed. Photographs containing between 33 and 122 seeds per accession were taken with a digital camera Sony ILCE 5100, with an AF-S Micro NIKKOR 60 mm. 1:2.8 G ED objective (Nikon).

The photographs were obtained with a single-leg tripod, putting the seeds on a glass and illuminating them above and below it to avoid shadows. The resolution of the images is 13.8 pixels per mm. Image data have been deposited in Zenodo: https://zenodo.org/record/3786726#.XrVL0agzaM8

**Figure 1.** Seeds of *V. vinifera* ssp. *sylvestris* in three orientations: dorsal (D), lateral (L) and ventral (V). Ventral views are used in all the images, because the seeds are more stable and the orientation occurs in a plane perpendicular to the line of vision. In the dorsal view, the seed tends to oscillate resting on the central rib and a flank, resulting in more variable sub-lateral orientations.

### 2.3. General Morphological Description by Image Analysis

Photographs were used to obtain data of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R). All these magnitudes were calculated with the Image J program [35]. Length and width are the Feret diameters of the seed images. Feret, or calliper diameters, are the distance between two parallel planes restricting the object. The seeds where oriented vertically, so length and width correspond respectively with the vertical and horizontal Feret diameters. The models were adjusted to fit the size of the seed images. Circularity Iindex and roundness were calculated as described [36–38].

### 2.4. Comparison with Geometric Models

The morphological description of seed shape in *Vitis* accessions is based on the comparison with geometric figures used as models. The seven models are original from this work. They are shown in Figure 2 and described below.
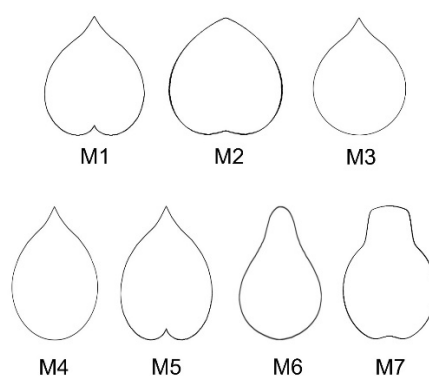


**Figure 2.** Geometric models for the quantification of shape in seeds of the Vitaceae: M1) The fourth heart curve of Weisstein [31]. M2) A rounded heart curve modified from M1. M3) The water drop, obtained from M1, adapting the basis in the heart curve to a circumference overlapping the maximum width of the curve. M4) Obtained by scalling M3 in the y-axis by a factor of 123/100. M5) Obtained by scalling M1 in the y-axis by a factor of 123/100. M6) Fibonacci's pear, obtained by scalling the pear curve of Weisstein [30] with $r = 2$ in the y-axis by a factor equal to the Golden Ratio $\Phi$. M7) Derived from an ellipse.

2.4.1. Description of the Geometric Models Used

Model 1 (M1 in Figure 2) has been obtained inverting the heart curve in Weisstein [31]; then, it is defined by the equation:

$$x^2 + \left(y + \frac{2(x^2 + |x| - 6)}{3(x^2 + |x| + 2)}\right)^2 = 36. \tag{1}$$

Model 2 (a rounded heart curve, M2 in Figure 2) results from a slight modification of equation (1):

$$x^2 + \left(y + \frac{2(x^2 + |x| - 6)}{12(x^2 + |x| + 2)}\right)^2 = 3. \tag{2}$$

Model 3 (M3 in Figure 2; the water drop) is obtained from M1, adapting the basis in the heart curve to a circumference overlapping the maximum width of the curve. It is the joint graphical representation in the interval [–6,6] of the function $f$ defined by:

$$f(x) = -\frac{2}{3} + \sqrt{36 - x^2} + \frac{16}{3(2 + x^2 + |x|)} \tag{3}$$

and $g$, derived from the circumference with centre (0,-6/11) and radius 6:

$$g(x) = -\frac{6}{11} - \sqrt{36 - x^2} \tag{4}$$

Model 4 (M4 in Figure 2) results from the elongation of M3 in the y-axis by a factor of 123/100; then, it corresponds to the joint graphical representation of y=123/100 f(x) and y=123/100 g(x), with f and g defined in (3) and (4), respectively.

Model 5 (M5 in Figure 2) results from the elongation of M1 in the y-axis by a factor of 123/100; then, it is determined by the equation:

$$1681x^2 + \left(\frac{100}{9}y + \frac{10086x^2 + 41|x| - 2}{3(45387x^2 + 123|x| + 2)}\right)^2 = 4 \tag{5}$$

To obtain Model 6 (M6 in Figure 2; the Fibonnacci's pear), we shrink the pear curve with $r = 2$ in [30], so that it adjusts to a Fibonacci's rectangle. The proposed curve is given by the equation:

$$(49x^2 + 25y^2)\big(117649x^6 + 15625(y^3 - 2y^2 + y - 1)^2 + 60025x^4(3y^2 - 4y + 2) \\ + 30625x^2(3y^4 - 8y^3 + 8y^2 + 2y - 3)\big) = 1562500 \tag{6}$$

Model 7 (M7 in Figure 2) derives from the modification of an ellipse of equation:

$$\left(\frac{x}{22}\right)^2 + \left(\frac{y}{25}\right)^2 = \frac{3}{44} \tag{7}$$

It is given by the combined representation of the functions:

$$f(x) = \frac{25}{22}\sqrt{33 - x^2} + \frac{125}{550\left(\frac{x^2}{10}\right)^{10} + 33} \ ; \ g(x) = -\frac{25}{22}\sqrt{33 - x^2} + \frac{50}{11(x^4 + x^2 + |x| + 5)} \tag{8}$$

2.4.2. Calculation of the J Index

Figure 3 shows examples of the adjustment between seeds and models and Figure 4 two examples for the calculation of the J index.

Images containing the seed photographs and the geometric model were composed with Corel PHOTO-PAINT X7.

Areas of seed images were calculated with ImageJ program. To obtain the J index, the areas in two regions were obtained: the region shared by the model and the seed image (common region, C) and the region not shared between both areas (D). The J index is defined by:

$$J = (\text{area C})/(\text{area C} + \text{area D}) \times 100 \qquad [22,32]$$

where C represents the common region and D the regions not shared (Figure 4). Note that J is a measure of seed shape, not of its area. It ranges between 0 and 100 decreasing when the size of the not-shared region grows and equals 100, when the geometric model and the seed image areas coincide; that is, when area (D) is zero. Similarity was considered when J index values were over 90.
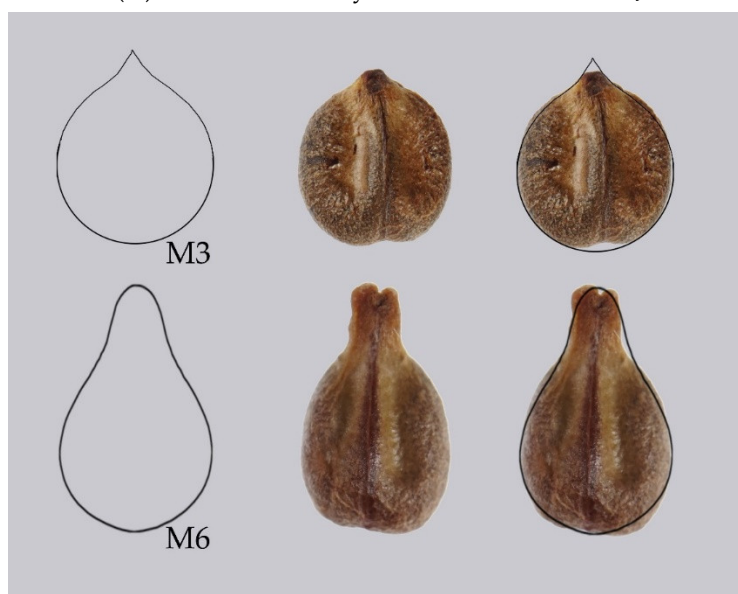


**Figure 3.** The application of geometric models to seeds of the Vitaceae for the calculation of the J index. Left: two of the models used. Above: seeds of *V. amurensis* adjust well to M3. Below: Seeds of *V. vinifera* ssp. *vinifera* cultivar Morenillo adjust well to M6.



**Figure 4.** Method for obtaining the J index (percentage of similarity between two images; the geometric figure and the seed image). Left: the seed (*V. amurensis*) and the geometric model (M3). The

composed image, in the center contains both the seed and the model. Right: total surface occupied by both figures (top) and shared surface (bottom). The surface comprised in the white perimeter is the common region, C; while total area (C+D) is obtained from the image in the right top figure. The J index is the ratio between the shared and the total area x 100. The bar is equal to 1 cm.

### 2.5. Statistical Analysis

One way ANOVA was used to show significant differences between populations for the measured variables, followed by Scheffé or Tukey's tests to provide specific information on which means were significantly different from one another. The former is used if the cases have different number of samples and the latter if the cases have the same or similar number of samples. The analysis was done with software IBM SPSS statistics v25.

## 3. Results

### 3.1. General Morphological Description and Comparison of Vitis Species, Subspecies and Cultivars

### 3.1.1. Comparison of *Vitis* Species and Subspecies

Table 1 shows the mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R) obtained for the images of the four species of *Vitis* analysed.

**Table 1.** Mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R) in the seeds of four species of *Vitis* analyzed.

| | N | A | P | L | W | AR | C | R |
|---|---|---|---|---|---|---|---|---|
| *V. amurensis* | 44 | 0.14[a] (0.01) | 1.48[a] (0.06) | 0.49[a] (0.03) | 0.38[b] (0.02) | 1.22[a] (0.07) | 0.80[d] (0.03) | 0.82[d] (0.05) |
| *V. labrusca* | 41 | 0.27[c] (0.02) | 2.13[c] (0.06) | 0.71[c] (0.03) | 0.55[d] (0.02) | 1.23[a] (0.08) | 0.76[b,c] (0.03) | 0.81[c,d] (0.06) |
| *V. rupestris* | 33 | 0.16[b] (0.02) | 1.60[b] (0.11) | 0.55[b] (0.04) | 0.41[c] (0.02) | 1.29[a] (0.07) | 0.78[c,d] (0.02) | 0.78[c] (0.04) |
| *V. vinifera* ssp. *sylvestris* | 122 | 0.16[b] (0.03) | 1.66[b] (0.24) | 0.58[b] (0.08) | 0.39[b] (0.03) | 1.49[b] (0.26) | 0.73[b] (0.06) | 0.69[b] (0.09) |
| *V. vinifera* ssp. *vinifera* | 301 | 0.15[a,b] (0.03) | 1.64[b] (0.21) | 0.58[b] (0.07) | 0.36[a] (0.04) | 1.71[c] (0.21) | 0.68[a] (0.06) | 0.60[a] (0.07) |
| *Vitis amurensis* | 44 | 0.14[a] (0.01) | 1.48[a] (0.06) | 0.49[a] (0.03) | 0.38[b] (0.02) | 1.22[a] (0.07) | 0.80[d] (0.03) | 0.82[d] (0.05) |
| *Vitis labrusca* | 41 | 0.27[c] (0.02) | 2.13[c] (0.06) | 0.71[c] (0.03) | 0.55[d] (0.02) | 1.23[a] (0.08) | 0.76[b,c] (0.03) | 0.81[c,d] (0.06) |
| *Vitis rupestris* | 33 | 0.16[b] (0.02) | 1.60[b] (0.11) | 0.55[b] (0.04) | 0.41[c] (0.02) | 1.29[a] (0.07) | 0.78[c,d] (0.02) | 0.78[c] (0.04) |
| *Vitis vinifera* ssp. *sylvestris* | 122 | 0.16[b] (0.03) | 1.66[b] (0.24) | 0.58[b] (0.08) | 0.39[b] (0.03) | 1.49[b] (0.26) | 0.73[b] (0.06) | 0.69[b] (0.09) |
| *Vitis vinifera* ssp. *vinifera* | 301 | 0.15[a,b] (0.03) | 1.64[b] (0.21) | 0.58[b] (0.07) | 0.36[a] (0.04) | 1.71[c] (0.21) | 0.68[a] (0.06) | 0.60[a] (0.07) |

The values of A are given in $cm^2$; P, L and W, in cm. Standard deviation is given between parentheses. 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 used.

In relation to seed size (area of images), *V. labrusca* seeds were larger and a group composed by *V. amurensis* and *V. vinifera* ssp. *vinifera*, smaller. The seeds of *V. rupestris* and *V. vinifera* ssp. *sylvestris* had intermediate values.

The seeds of *V. vinifera* ssp. *vinifera* had higher AR values, corresponding to lower circularity and roundness, characteristics of the elongated or piriform seed morphology [5]. The seeds of *V.*

*vinifera* ssp. *sylvestris* had intermediate values of AR. The seeds of *V. amurensis, V. labrusca* and *V. rupestris* had lower values of AR and higher values of roundness, corresponding to their more circular morphology. Smaller differences between circularity and roundness are due to the irregularity of the perimeter of the images.

### 3.1.2. Comparison of *Vitis vinifera* Cultivars

Table 2 presents the mean values of the morphometric magnitudes measured in six cultivars of *V. vinifera* ssp. *vinifera*.

**Table 2.** Mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R), obtained for the images of the seeds of six cultivars of *V. vinifera* ssp. *vinifera*).

|  | N | A | P | L | W | AR | C | R |
|---|---|---|---|---|---|---|---|---|
| Camarate | 73 | 0.13[a,b] | 1.54[a] | 0.55[a,b] | 0.34[a,b] | 1.70[b,c] | 0.69[b] | 0.59[b,c] |
|  |  | (0.02) | (0.11) | (0.04) | (0.02) | (0.12) | (0.04) | (0.04) |
| Carignano | 52 | 0.15[c] | 1.76[b] | 0.64[c] | 0.35[b] | 1.95[d] | 0.62[a] | 0.51[a] |
|  |  | (0.02) | (0.16) | (0.04) | (0.03) | (0.12) | (0.06) | (0.03) |
| Cercial | 58 | 0.12[a] | 1.46[a] | 0.52[a] | 0.32[a] | 1.75[c] | 0.69[b] | 0.58[b] |
|  |  | (0.02) | (0.10) | (0.03) | (0.03) | (0.13) | (0.04) | (0.04) |
| Malvasia | 33 | 0.17[d] | 1.75[b] | 0.61[c] | 0.39[d] | 1.63[b] | 0.70[b] | 0.62[c,d] |
|  |  | (0.02) | (0.11) | (0.05) | (0.02) | (0.15) | (0.04) | (0.06) |
| Meserguera | 39 | 0.14[b,c] | 1.57[a] | 0.56[b] | 0.36[c] | 1.51[a] | 0.73[c] | 0.67[e] |
|  |  | (0.02) | (0.09) | (0.03) | (0.03) | (0.12) | (0.03) | (0.05) |
| Morenillo | 46 | 0.18[d] | 1.84[b] | 0.65[c] | 0.40[d] | 1.63[b,c] | 0.68[b] | 0.63[d] |
|  |  | (0.04) | (0.32) | (0.11) | (0.03) | (0.30) | (0.06) | (0.07) |

Values of A are given in cm²; P, L and W, in cm. Standard deviation is given between parentheses. The values marked with the same letter in each column do not differ significantly at $p < 0.05$ (Scheffe's test).

Three different groups of increasing seed area include: 1) Cercial, 2) Carignano, and 3) Malvasía and Morenillo. Camarate and Meserguera are of intermediate size between Cercial and Carignano. Aspect ratio is smaller in Meserguera, then Malvasía, Cercial and Carignano. Morenillo and Camarate have intermediate values between Malvasía and Cercial. When the cultivars are classified by their values of roundness, the order is inverse to the classification by aspect ratio. Smaller differences between circularity and roundness in some cultivars are due to the irregularity of the perimeter of the seed images.

### 3.2. *Morphological Description of* Vitis *Species and Cultivars by Similarity with Geometric Models. Values of J Index*

### 3.2.1. Comparison of *Vitis* Species and Subspecies

The seeds of *V. amurensis, V. labrusca* and *V. rupestris* adjusted better to M1 than the seeds of *V. vinifera* ssp. *sylvestris* and *Vitis vinifera* ssp. *vinifera*. *V. amurensis* and *V. rupestris* adjusted better to M3 than the other species, with values of J index of 90.6 and 90.0, respectively (Table 3; Figure 5). Seeds of *V. vinifera* ssp. *sylvestris* and *V. vinifera* ssp. *vinifera* adjusted better to M6 and M7 than the seeds of the other species. *V. vinifera* ssp. *sylvestris* gave higher values than *V. vinifera* ssp. *vinifera* with M3, while the reverse was observed with M6 (Table 3; Figure 5).

**Table 3.** Mean values of the J index in seeds of four species of *Vitis*, with four of the models used.

| | N | J Index M1 | J Index M3 | J Index M6 | J Index M7 |
|---|---|---|---|---|---|
| *Vitis amurensis* | 20 | 86.8 [b] (2.65) | 90.6[c] (2.16) | 68.4[a] (2.77) | 79.1[a] (3.02) |
| *Vitis labrusca* | 20 | 89.6 [b] (2.48) | 88.3[b,c] (2.08) | 74.3[b] (3.78) | 81.5[a,b] (4.35) |
| *Vitis rupestris* | 20 | 87.4 [b] (2.74) | 90.0[c] (2.03) | 76.3[b] (2.50) | 83.7[b] (2.38) |
| *Vitis vinifera* ssp. *sylvestris* | 20 | 78.6 [a] (5.88) | 86.3[b] (3.82) | 80. 7[c] (4.83) | 88.4[c] (3.41) |
| *Vitis vinifera* ssp. *vinifera* | 120 | 75.8 [a] (5.32) | 77.6[a] (4.19) | 87. 5[d] (3.10) | 88.9[c] (3.35) |

Standard deviation is given between parentheses. Values marked with the same letter in each column do not differ significantly at *p* < 0.05 (Scheffe's test).



**Figure 5.** From left to right in all rows: Three of the models used (M3, the water drop is applied in rows 1 and 3; M1, the heart curve is applied in row 2; and M7 is applied in rows 5 and 6; a figure composed with the silhouettes of 20 seed images (120 for *V. vinifera* ssp. *vinifera* in row 5)), and six representative images of seeds of the following species (top to bottom): *V. amurensis* (row 1), *V. labrusca* (row 2), *V. rupestris* (row 3), *V. vinifera* ssp. *sylvestris* (row 4), *V. vinifera* ssp. *vinifera* (row 5; the second image contains the composed silhouettes of all six varieties). The bar is equal to 1 cm.

### 3.2.2. Comparison of *Vitis vinifera* Cultivars

In general, the seeds of all cultivars of *V. vinifera* ssp. *vinifera* tested adjusted better to Models 5, 6 and 7 than to Models 1, 2, 3 or 4 (not shown). Model 5, the elongated heart curve, gave the highest values of J index with Meserguera (Table 4, Figure 6). The Fibonnacci's pear, M6, gave similar high values with all cultivars tested, being the model that better adjusted the shape of the seeds of Carignano (Table 4, Figure 6). Differences with M7 were observed between Carignano and the other cultivars, with lower values of J index in the former (Table 4, Figure 6).

**Table 4.** Mean values of the J index in six cultivars of *Vitis vinifera* ssp. *vinifera*, with the models used (M5, M6 and M7).

| Cultivar | N | J index M5 | J index M6 | J index M7 |
|---|---|---|---|---|
| Camarate | 20 | 84.5[b,c] (2.45) | 88.5[a] (2.33) | 89.9[b,c] (2.18) |
| Carignano | 20 | 79.7[a] (2.08) | 86.8[a] (2.91) | 83.9[a] (2.39) |
| Cercial | 20 | 83.4[b] (2.44) | 88.4[a] (1.83) | 90.1[b,c] (2.50) |
| Malvasia | 20 | 85.9[c,d] (3.74) | 86.3[a] (2.44) | 91.7[c] (3.26) |
| Meserguera | 20 | 90.4[e] (1.80) | 86.1[a] (4.35) | 88.6[b] (1.93) |
| Morenillo | 20 | 88.0[d] (2.11) | 88.6[a] (3.21) | 89.4[b] (1.35) |

Standard deviation is given between parentheses. Values marked with the same letter in each column do not differ significantly at *p* < 0.05 (Tukey's test).
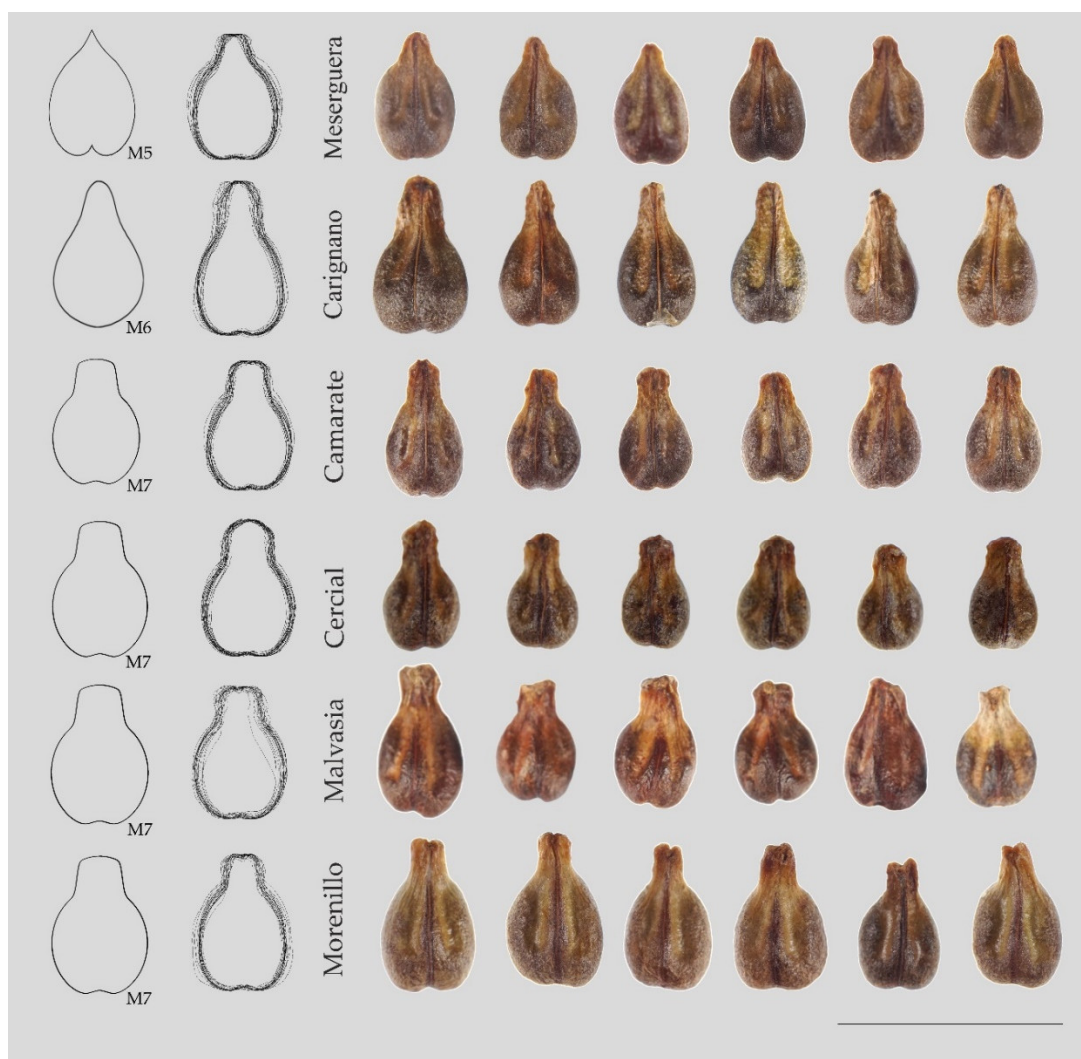


**Figure 6.** From left to right in all rows: Models used (M5, the elongated heart curve is applied in row 1; M6, the Fibonnacci's pear is applied in row 2; and M7 is applied in rows 3 to 6); a figure composed of silhouettes of 20 seed images; representative images of seeds of the six *V. vinifera* ssp. *vinifera* cultivars analyzed. From top to bottom: Meserguera (row 1), Carignano (row 2), Camarate (row 3), Cercial (row 4), Malvasia (row 5) and Morenillo (row 6). The bar is equal to 1 cm.

### 3.3. Seed Size and Shape of Cissus and Parthenocissus, Two Relatives of Vitis in the Vitaceae

*C. verticillata* seeds are smaller and more elongated than *P. tricuspidata*, having lower values of circularity (C) and roundness (R) (Table 5; Figures 7 and 8). Seeds of both *C. verticillata* and *P. tricuspidata* are contained in berry fruits. While in *C. verticillata* there is only one seed per fruit, in *P. tricuspidata* the number of seeds oscilates between one and three. Figure 8 contains representative images of seeds obtained from berries having one, two and three seeds obtained from the same plant.

Seeds of *C. verticillata* adjust well to M4 (mean J index values = 91.2 with *N* = 28), while seeds of P. tricuspidata adjust to M2 with different values of J index, depending on the number of seeds per fruit (Table 6, Figure 7).

**Table 5.** Mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R) for seeds in the species *C. verticillata* and *P. tricuspidata*.

|  | N | A | P | L | W | AR | C | R |
|---|---|---|---|---|---|---|---|---|
| *C. verticillata* | 28 | 1.15[a] | 1.43[a] | 0.50[b] | 0.35[a] | 1.40[b] | 0.71[a] | 0.72[a] |
|  |  | (0.07) | (0.05) | (0.02) | (0.01) | (0.05) | (0.03) | (0.02) |
| *P. tricuspidata* | 36 | 1.40[b] | 1.45[a] | 0.45[a] | 0.41[b] | 1.10[a] | 0.83[b] | 0.93[b] |
|  |  | (0.17) | (0.08) | (0.03) | (0.03) | (0.08) | (0.03) | (0.06) |

Values of A are given in cm$^2$; P, L and W, in cm. Standard deviation is given between parentheses. Values marked with the same letter in each column do not differ significantly at $p < 0.05$ (Scheffe's test).



**Figure 7.** From left to right: Model 4 (an elongated water drop), a figure composed with the silhouettes of 20 seed images, and five representative images of seeds of *C. verticillata*. The bar is equal to 1 cm.

**Table 6.** Mean values of the area (A), perimeter (P), length of the major axis (L), length of the minor axis (W), aspect ratio (AR is the ratio L/W), circularity (C) and roundness (R) and J index values in seeds of *P. tricuspidata* (Siebold and Zucc.) Planch has one, two or three seeds per fruit. J index values were obtained with M4 (an elongated water drop).

| Seeds/fruit | N | A | P | L | W | AR | C | R | J index (M2) |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 12 | 1.25[a] | 1.40[a] | 0.42[a] | 0.40[a] | 1.03[a] | 0.82[a] | 0.97[b] | 93.7[b] |
|  |  | (0.10) | (0.06) | (0.02) | (0.02) | (0.02) | (0.03) | (0.02) | (1.29) |
| *2* | 12 | 1.52[c] | 1.50[c] | 0.47[b] | 0.43[b] | 1.05[a] | 0.84[a] | 0.95[b] | 91.8[b] |
|  |  | (0.13) | (0.07) | (0.02) | (0.02) | (0.02) | (0.03) | (0.02) | (1.47) |
| *3* | 12 | 1.38[b] | 1.40[b] | 0.47[b] | 0.40[a] | 1.16[b] | 0.83[a] | 0.86[a] | 88.6[a] |
|  |  | (0.14) | (0.07) | (0.02) | (0.03) | (0.08) | (0.02) | (0.06) | (3.71) |

Values of A are given in cm$^2$; P, L and W, in cm. Standard deviation is given between parentheses. Values marked with the same letter in each column do not differ significantly at $p < 0.05$ (Scheffe's test).
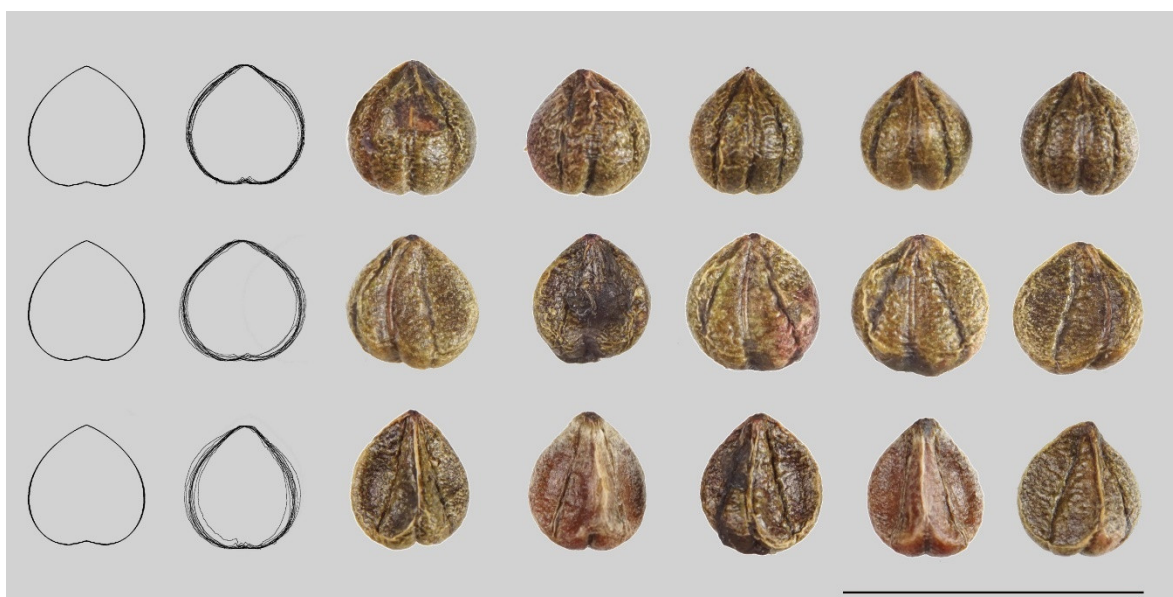
**Figure 8.** From left to right: Model 2 (three rounded heart curves), three figures composed with the silhouettes of 20 seed images, and five representative images of seeds of *P. tricuspidata*. The first row contains seeds from one-seed fruits; the second from two-seed fruits and the third, from three seed-fruits. The bar is equal to 1 cm.

## 4. Discussion

Seven geometric models have been described for the first time and applied to the morphological description of seeds in the Vitaceae. M1, a heart curve defined by Weinstein [31], is useful for the quantification of seed shape in species of *Vitis* and for the differentiation between the species *V. amurensis*, *V. labrusca* and *V. rupestris,* in one side, and *V. vinifera*, in the other. M1 adjusts better to seeds of *V. amurensis, V. labrusca* and *V. rupestris* than *V. vinifera.* M6, the Fibonacci's pear, adjusts well to seeds of *V. vinifera,* in general, and better to *V. vinifera* ssp. *vinifera* than to *V. vinifera* ssp. *sylvestris.* This way, *V. vinifera* ssp. *sylvestris* can be differentiated from *V. vinifera* ssp. *vinifera*, because the values of J index obtained with M6 (the Fibonacci's pear) are higher in the former. The water drop, a rounded heart curve (M3) corresponds better to the rounded seeds of species *V. amurensis* and *V. rupestris* than those of *V. labrusca*, also giving high values of J index with the seeds of *Vitis vinifera* ssp. *sylvestris.* Thus, M3 permits to differentiate between *V. vinifera* ssp. *vinifera* and *V. vinifera* ssp. *sylvestris,* the former giving higher values of J index with this model. The combination of M3, M6 and M7 is useful for the differentiation of *V. vinifera* ssp. *sylvestris* from other species of *Vitis*.

The models proposed are related to previous seed shape descriptions of the Euvitis and Muscadinia taxonomic groups. Models 1, 2 and 3 resemble the Euvitis type, while Models 4, 5, 6 and 7 fit better the Muscadinia shape [9,10]. The seeds of wild species are smaller, robust and with a rounded outline resulting in a heart-like shape, often termed as "cordate", with short stalks and a flat ventral side with sharp angles and a strongly developed chalaza, while those of cultivars are large, elongated, oval or pyriform, with an elongated stalk [9].

The identification of geometric curves resembling the outline of seed images complements the results of morphometric analysis and allows more exactitude in the description of species and cultivars and for their comparison. Among the two subspecies of grape, subspecies *sylvestris* adjusts better to M3, while subspecies *vinifera* adjusts better to M6, thus concerning seed shape, *V. vinifera* ssp*. sylvestris* resembles more other species of *Vitis* than *V. vinifera* ssp. *vinifera*. This demonstrates a great capacity of variation in the shape of seeds of cultivars, where significant changes in seed shape are due to selection during cultivation [28].

Concerning cultivars, Meserguera gave higher values with the elongated heart curve (M5), while Malvasia seeds adjusted better to M7. New models based on geometrical curves can be useful for the description of seed shape in other cultivars.

Seeds of *Cissus verticillata* adjust well to M2, while seeds of the species *Parthenocissus tricuspidata* adjust better to M4. The quantification of seed shape with M4 shows differences between seeds depending on the number of seeds per fruit. Fruits with one or two seeds have their seeds more rounded and with higher values of J index than the seeds from fruits with three seeds per fruit.

Both *C. verticillata* and *P. tricuspidata* seeds resemble more the seeds of *V. amurensis*, *V. labrusca* and *V. rupestris* than those of cultivars of *V. vinifera*, suggesting that the primitive seed type, associated with a reduced number of seed per fruit, resembles more Model 1.

The seeds in the Vitaceae present a very peculiar shape, for which there is no adaptation to a single geometric model, like the ellipse, the ovoid or the cardioid [30,32], but to more complex figures. This is in contrast with other families, where the ellipse, the ovoid or the cardioid are the models defining seed shape for most species and genera [22–30,32].

The magnitudes used in morphology have different information about the shape of a plane figure. Magnitudes such as the area, perimeter, length or width do not give much information on shape, and thus, are of little value on their own to discriminate between cultivars or species. Other measures, such as circularity or roundness are more informative when they are close to the unity, but their usefulness depends on the similarity of the figure with the circle. If the figure is different to a circle, low values are of no informative value. In contrast, J index compares the image of a plane figure with a similar geometric figure selected as a model. In the case of grape seeds, J index is a way to provide valuable information in a single measure and, in consequence, useful in ampelography studies.

The current edition of the OIV Descriptor List for Grape Varieties and *Vitis* species [39] concerning seeds includes only three variables: length, weight and the presence of transversal ridges on dorsal side of seeds. J index is a new way of describing seeds that allows one to relate the variety studied to its genetic origin. The longer seed varieties are of eastern origin and generally table grapes, as seen in the descriptor, and the varieties of western origin are shorter and rounded linked to the origin of the domestication of most wild vines, as, for example, varieties of French and Spanish origin [40,41]. The work described in this article allows an approximation to elucidate if the variety is of eastern or western origin. Intermediate varieties such as Pinot and Merlot are of mixed origin. Further study of Spanish varieties where there is an Arabic influence would allow us to test this methodology.

The description of seed images by comparison with geometric figures will contribute to the validation of results obtained by automated artificial vision methods, improving the description and identification of species and varieties. It is a new, low-cost and relatively simple technique that can help growers, producers or breeders in seed identification.

## 5. Conclusions

New geometric models for seed shape quantification in the Vitaceae are described. Model 1, a heart curve, adjusts well the shape of the seeds of species *V. amurensis*, *V. labrusca* and *V. rupestris.* Model 2, a rounded heart curve, defines well the shape of the seeds of *Parthenocissus tricuspidata.* Model 3, the water drop, adjusts well the shape of the seeds of *Vitis amurensis* and *Vitis rupestris.* Model 4 gives a good adjustment with the seeds of *Cissus verticillata*. A combination of the models is useful for the differentiation of the seeds of cultivars of *Vitis vinifera,* ssp. *vinifera*. Geometric models provide a new method for the description and classification of grape species and cultivars. Future studies will be directed to the identification of cultivar-specific models.

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