A new method for assessment of bunch compactness using automated image analysis

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Abstract

Background and Aims: Bunch compactness is a key feature determining grape and wine composition because tight bunches show a less homogeneous ripening, and are prone to greater fungal disease incidence. The Organisation Internationale de la Vigne et du Vin descriptor, the most recent method for the assessment of bunch compactness, requires visual inspection and trained evaluators, and provides subjective and qualitative values. The aim of this work was to develop a methodology based on image analysis to determine bunch compactness in a non-invasive, objective and quantitative way.

Methods and Results: Ninety bunches of nine different red cultivars of *Vitis vinifera* L. were photographed with a colour camera, and their bunch compactness was determined by visual inspection. A predictive partial least squares (PLS) model was developed in order to estimate bunch compactness from the morphological features extracted by automated image analysis, after the supervised segmentation of the images. The PLS model showed a capability of 85.3% for predicting correctly the rating of bunch compactness. The most discriminant variables of the model were highly correlated with the tightness of the berries in the bunch (proportion of visibility of berries, rachis and holes) and with the shape of the bunch (roundness, compactness shape factor and aspect ratio).

Conclusions: The non-invasive, image analysis methodology presented here enables the quantitative assessment of bunch compactness, thereby providing precise objective information for this key parameter.

Significance of the Study: A quantitative, objective and accurate system based on image analysis was developed as an alternative to current visual methods for the estimation of bunch compactness. This novel method could be applied to the classification of table grapes and/or at the receival point of wineries for sorting and assessment of wine grapes before vinification.

Keywords: *bunch architecture, bunch morphology, computer vision, multivariate analysis, PLS model*

Introduction

Bunch compactness on grapevines is a key feature determining grape and wine quality (Hed et al. 2009, Molitor et al. 2012, Tello and Ibáñez 2014). It can be defined by the degree of aggregation of the berries within the bunch, which denotes the density of berry distribution, their mobility and deformation, as well as the visibility of pedicels (Organisation Internationale de la Vigne et du Vin 2007). Following the Organisation Internationale de la Vigne et du Vin (OIV) criteria for compactness, grape bunches can be classified from loose to dense. In compact bunches, berries are packed in such a way that they touch each other in many areas of the bunch, some of them lose their spherical shape, and there are several berries hidden in interior layers, where air circulation is limited and ripening is frequently compromised because of their lack of exposure to sunlight (Vail and Marois 1991, Molitor et al. 2012). As a consequence of these morphological features, compact bunches are more susceptible to fungal diseases (Molitor et al. 2012), especially *Botrytis cinerea* (Vail and Marois 1991) and powdery mildew (Austin and Wilcox 2012). In addition, the heterogeneous ripening occurring between the inner and outer berries in compact bunches can have a detrimental effect on wine quality (Figueiredo-González et al. 2013). Therefore, winemakers aim to obtain loose bunches that are considered to be of higher quality (Vail and Marois 1991).

Many studies (Hed et al. 2009, Palliotti et al. 2011, Tardaguila et al. 2012) have estimated bunch compactness according to the visual descriptor scale proposed by the OIV (Organisation Internationale de la Vigne et du Vin 2007) or other visual systems specifically developed for the evaluation of this trait in certain grapevine cultivars (Zabadal and Bukovac 2006, Evers et al. 2010). The subjectivity linked to these visual methods, however, makes them impracticable for certain types of studies that require objective and quantitative measurements of the trait (Tello and Ibáñez 2014). As a result, there is a need to create methods capable of estimating the

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compactness of a grape bunch in an accurate, objective and quantitative way. The OIV method for bunch compactness assessment (Organisation Internationale de la Vigne et du Vin 2007) is the method that is most widely used by the grape and wine industry around the world, but it is a visual and subjective system that requires a panel with trained experts to be able to use it. Likewise, different indexes based on the architecture of the bunch can be found in the literature for the evaluation of bunch compactness. Recently, Tello and Ibáñez (2014) tested 19 of these indexes in 110 grape bunches with a large degree of morphological variability, and most of them provided a low estimation of this trait in a general framework. In this study, the morphological features of the bunch were evaluated manually, which hinders its industrial application. Given that bunch compactness is a key feature for the quality of table and wine grapes, both industries would benefit from the development of a quantitative, rapid and objective method to evaluate this viticultural parameter.

Computer vision is widely used to inspect vegetable production. This technology allows the creation of systems capable of estimating or predicting certain features of the inspected objects in a fast, repeatable and accurate way without the need for contact (Lorente et al. 2013, Vidal et al. 2013). Most applications are related to the measurement of external properties, such as colour, size, shape or detection of defects (Cubero et al. 2011). The morphological features most commonly used to characterise the shape of an object are the area, the perimeter, the length of the major and minor axes, and also the aspect ratio (Costa et al. 2011). The use of ratios has the advantage of allowing comparisons to be made among fruits of different size. Accordingly, Venora et al. (2009) used the length/circumference ratio for beans, and Wang and Nguang (2007) used length/diameter ratios in order to extract fruit volume of axi-symmetric agricultural products. Other authors used several ratios to estimate volume in bell peppers (Ngouajio et al. 2003) or for in-line sorting of mandarins (Blasco et al. 2009a).

Image processing has been used in viticulture to assess key canopy features, such as yield (Dunn and Martin 2004, Diago et al. 2012) and leaf area (Tardaguila et al. 2012). Recently, Herzog et al. (2014) have shown some initial results on the application of image analysis for high-throughput phenotyping in vineyards. Several attempts have been made for the determination of bunch morphology using image analysis. Wycislo et al. (2008) used different ratios, such as the major/minor axis ratio, shape factor, and compactness shape value, to estimate the shape of table grapes. Chen et al. (2010) configured an automated inspection system for grading grape bunches on the basis of their colour, size and shape. The shape features were calculated from the projection area and pixel accumulation curve. In order to conduct geometric measurements of grape fruits dynamically, Miao et al. (2012) used a snake-based model after image segmentation with the aim of discriminating each grape in the bunch and obtaining some descriptors of the individual grapes. Image analysis has been applied for the evaluation of different bunch and berry traits, including the number of berries per bunch, bunch mass, and berry size (Kicherer et al. 2013, Cubero et al. 2014, Diago et al. 2014b, Roscher et al. 2014). Recently, a new method for the assessment of the number of flowers per inflorescence by means of image analysis has also been successfully applied (Diago et al. 2014a).

An image represents a bi-dimensional projection of the imaged sample, and thus volumetric information is lost. Bunch compactness is a three-dimensional (3D) feature, which makes the development of automated inspection systems based on images specifically adapted to this task challenging, and it may only be achieved from the indirect measures of some morphological descriptors. For example, Schöler and Steinhase (2012) included automated 3D reconstruction of bunch architecture based on the analysis of rachises for the potential evaluation of bunch compactness.

The examples presented demonstrate that many morphological features of the bunch can be determined by image analysis. Despite the widespread use of machine vision in viticulture, the estimation of the compactness of bunches has still not been explored using this technique. This work is the first attempt to create an automated method to achieve quantitative, accurate and objective assessments of bunch compactness on grapevines using image analysis techniques.

Material and methods

Assessment of plant material and bunch yield components

The experiments were carried out with 90 randomly selected bunches of grapes from nine red cultivars of *Vitis vinifera* L. (10 bunches per cultivar). The cultivars under study were Aramon, Bobal, Cabernet Franc, Cinsaut, Danugue, Derechero de Muniesa, Monastrell, Moravia Agria and Ruby Seedless, which presented bunches of various size, shape, and density in order to ensure a large degree of variability between bunch morphology and compactness. The bunches of grapes were carefully handpicked in October 2011 at the Rioja Regional Government's Experimental Vineyard (Agoncillo, La Rioja, Spain), transported to the Instituto de Ciencias de la Vid y del Vino (ICVV, Logroño, La Rioja, Spain) and kept under refrigeration (4°C) until image acquisition. For each cultivar, 10 bunches showing the general features of the cultivar were selected, and their mass was recorded with a set of scales (Blauscal AC-5000, Barcelona, Spain).

Image acquisition

Images of the grape bunches were acquired under laboratory conditions at 24°C. To acquire the images, the bunches were placed in front of a camera (EOS 550D, Canon Inc., Tokyo, Japan) at a focal distance of 55 mm, the rachis hanging from a clamp so as not to distort the shape of the bunch and using a uniform background in order to facilitate later image segmentation by increasing the contrast between the berries and the background. The camera was placed inside an inspection chamber with the inner walls covered by a tissue diffuser. The lighting system was composed of four lamps placed on the sides of the inspection chamber, oriented 45° to the samples, and separated 30-cm from the bunch to be photographed, each lamp consisting of two fluorescent tubes (Biolux L18W/965, 6500 K, Osram AG, Munich, Germany) powered by high-frequency electronic ballasts to avoid the flicker effect.

The captured images had a resolution of 0.12 mm/pixel and were stored in Tagged Image File (TIF) format. The four sides of each bunch were imaged after three consecutive rotations of 90° in order to capture as much of the shape of the bunch as possible, thus resulting in a database of 360 images.

Evaluation of bunch compactness by visual inspection

The data on bunch compactness obtained with the developed image analysis system were compared with the routine visual ratings achieved following the OIV standard code 204 (Organisation Internationale de la Vigne et du Vin 2007) by 14 trained experts who characterised the compactness of each bunch following the OIV criteria. The final compactness rating assigned to each bunch was the average of the 14 scores. The OIV descriptor classified the bunch compactness using scores

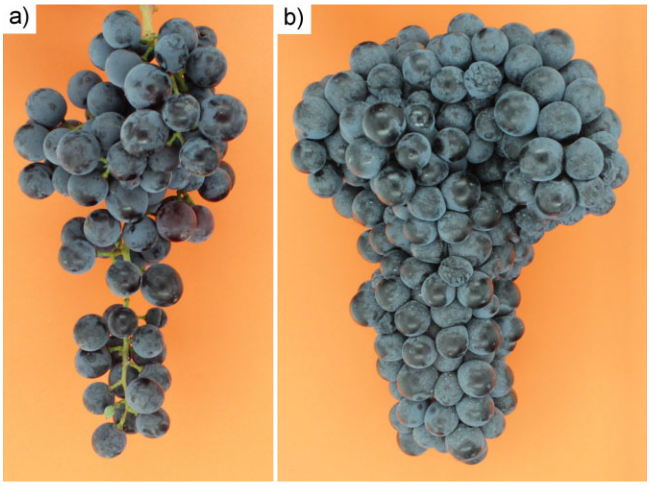

Figure 1. Examples of (a) loose bunch and (b) compact bunch.

from 1 to 9, depending on the visibility of the pedicels, the difficulty involved in moving the berries and the presence of deformed berries because of the pressure. Of these values, 1 is assigned to the loosest bunches (Figure 1a), while 9 is assigned to the most compact ones (Figure 1b).

Bunch morphological assessment by image analysis

The image analysis was designed to estimate the following bunch variables: area (A), perimeter (P), ratio between area and perimeter (AP), length (L), maximum width (MW), aspect ratio (AS), compactness shape factor (CSF) and roundness (RD) of the bunch, as well as width at 25% (W25), 50% (W50), and 75% (W75) of the length of the main axis, and proportion of the area corresponding to berries (AB), rachis (AR), and holes (AH) in the bunch. The influence on the statistical models of other bunch variables, such as the number of berries per bunch or the mass of the berries estimated by image processing techniques (Diago et al. 2014b) was analysed previously, but after preliminary tests it was concluded that their impact on the estimation of the compactness was slight, and hence they were not included for building the model.

The first step in the morphological analysis to extract the main features of each bunch was to carry out a supervised segmentation of the images in order to determine whether the pixels belonged to the class background (orange background), berry, or rachis. To train the segmentation model, one representative set of pixels from each class was manually selected by an operator using a training set of images composed of one image of each cultivar. These images were only used to train the system and were excluded from the validation set used to obtain the results. After this operation, a labelled dataset consisting of the colour values of the pixels, red (R), green (G), and blue (B), and the class they belonged to were obtained. This training dataset was used as input for a Bayesian discriminant analysis performed to obtain classification functions for each class in the problem. These functions had the three colour (R, G, and B) values of one pixel as input, the output being the probability of belonging to a given class. Hence, during the process of segmenting the images in the validation dataset, all the pixels in the images were classified using these classification functions in one of the three classes: background, berry, or rachis, and were assigned to the class with the highest probability. All this process was carried out with the software Food-ColorInspector (free available at http://www.cofilab.com).

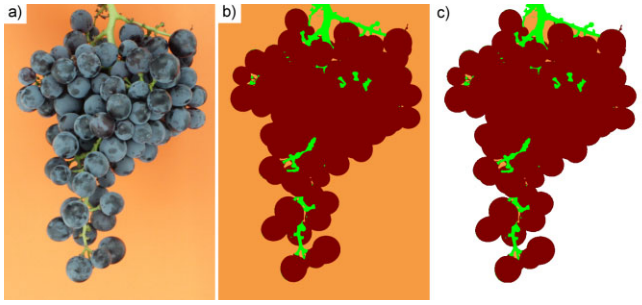

Figure 2. Process followed to segment the bunch image in the four classes: background, berries, rachis and holes: (a) original image, (b) segmented image, (c) watershed algorithm and detection of internal holes.

Then, filtering was undertaken to reduce the noise because of errors produced by the pixel classification model, and to smooth the contour in order to facilitate the later extraction of certain variables from the perimeter of the bunch. Each pixel was replaced with the result of applying a mode filter in the neighbourhood of 3×3. The images obtained after this operation contained four regions of interest, namely, background, berries, rachis, and holes inside the bunch (Figure 2b). The area of the bunch was estimated as the sum of the pixels belonging to berries and rachis. The proportion of pixels in the bunch corresponding to berries, rachis, and holes was calculated in order to include these values in the model. This was carried out under the premise that the number of holes and the visibility of the rachis could be related with compactness, since in a compact bunch they would be hardly observable, while they would be far more visible in a loose one. The background and the holes, however, had the same orange colour and both were sorted as background, so further operations were needed to discriminate between these two classes. To solve this problem, an algorithm based on the Watershed strategy (Beucher and Lantuéjoul 1979) was developed. The algorithm set four starting points (seeds) as the true background in the four corners of the image. In the next step, all the neighbouring pixels of these seeds that belonged to the background class were also labelled as true background. This process continued iteratively by labelling in each step all the background pixels that were adjacent to any other pixel already labelled as true background, thus flooding the outside of the bunch (Figure 2c). When the algorithm ended, all pixels still labelled as background were supposed to be inside the bunch and hence considered and labelled as holes.

The segmented image was converted into a binary image containing two regions of interest – background and bunch – that was used to extract the contour of the bunch using a chain code (Freeman 1961). The main morphological features were then obtained by analysing the perimeter of the bunch. The mass centre and the main axis of inertia were extracted from the contour coordinates. From the main axis of inertia, the width was calculated at 25, 50 and 75% of the length of the axis with the aim of studying whether the distribution of the berries along the length of the bunch was related with the compactness (Figure 3). To make the algorithm more robust against sharp variations in shape, these values were calculated as the average width at the selected lengths (25, 50 and 75% of the main axis) ± 5%. Finally, the A/P ratio and the additional features related to bunch morphology shown below were calculated as follows (Davies 2000, Burger and Burge 2009):

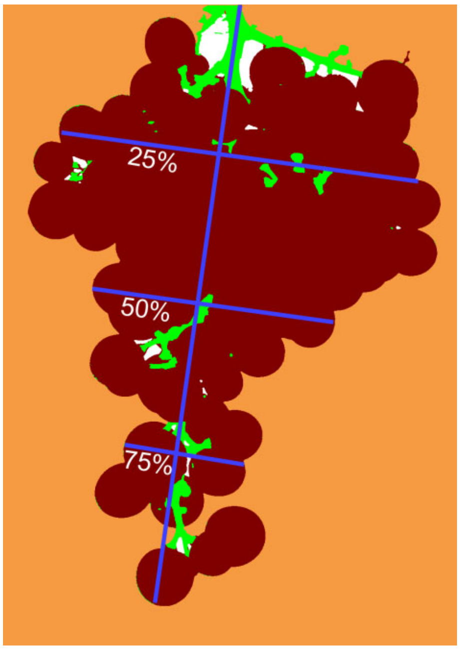

Figure 3. Main axis of inertia and width at 25, 50 and 75% of the main axis length in a grapevine bunch.

$$AS = MW/L \tag{1}$$

$$CSF = (P^2/A) \tag{2}$$

$$RD = (4.0 * \pi * A)/P^2 \tag{3}$$

where L, MW, P and A refer to the bunch under consideration. The variable AS is the ratio between the maximum width and the length, the objects with lower ratios being more elongated. The CSF is a relation between the area and the perimeter that considers that, given objects with the same perimeter, those with a lower perimeter will be more compact, regardless of the size of the object. Finally, RD measures how the shape of any object is related to the shape of a circle.

As four images were acquired of the four sides of each bunch, the morphological features included in the statistical analysis were the average of the ones obtained for each bunch.

Statistical analysis

A predictive multivariate statistical projection model was developed in order to estimate the compactness of grape bunches objectively and accurately from the morphological features extracted by the automated image analysis, as well as to find out whenever differences in compactness among cultivars occurred, and to select those morphological features with a statistically relevant predictive capability. Three out of the 10 bunches per cultivar were extracted to build a validation set. The remaining samples were used to calibrate a partial least squares (PLS) model (Geladi and Kowalski 1986). Partial least squares is a projection to latent structure (or latent based) multivariate statistical model used when a variable (such as OIV compactness) has to be predicted from a set of variables (morphological features extracted from images). Therefore, it is a suitable model when prediction of a qualitative variable is the final goal, as is the case here. Hence, by using PLS it becomes possible to deal with hidden or internal relationships between the different morphological features and the grape cultivar that show higher covariance with the compactness in a better way than by using principal components analysis plus any regression technique. Principal components analysis focuses on maximising the variance in the set of the extracted variables, which might not necessarily be related to the desired predicted variable, that is, compactness, in this study.

Since these models are usually built from a large number of X variables, in order to extract some of the variability in the predictions introduced by those features that do not correlate to the Y variable, one recommended approach (Martens and Naes 1989) is to build these models in the following sequential fashion:

1. Fit the PLS model to the dataset.
2. Search for outliers both in the latent space by inspecting Hotelling's T^2 and in the residuals by inspecting the square prediction error (SPE) or the distance to the model. These two statistics differ in their conceptual meaning. Hotelling's T^2 represents the projection of each bunch onto the reduced subspace defined by the PLS model. Thus, values over the confidence limits indicate that the corresponding bunches present extreme values of the features extracted by the vision system, even though the internal correlation structure is maintained in the model. In contrast, the distance to the model represents a measure of the squared Euclidean distance of each bunch to this subspace (Prats-Montalbán and Ferrer 2007), and those values over the confidence limits are related to bunches that do not behave in the same way as the ones used to create the model. Therefore, the prediction provided by the model should not be taken into account. In our case, all bunches fit the model quite well.
3. Look for those variables whose coefficients' confidence intervals pass through zero (i.e. they are not statistically significant). This means that they may possibly have little influence on the predicting model.
4. Eliminate those variables with clearly non-statistical significance from the model, and go to 1 until all variables show statistically significant coefficients.

From the morphological features extracted using image analysis and the continuous compactness rated by the judges, a PLS model was built following the procedure described above. Therefore, only the final results of the PLS model are shown. All calculations were performed using SIMCA P+12 (Umetrics Inc., Umeå, Sweden).

Results and discussion

Table 1 summarises the average ± standard error confidence intervals for the set of variables directly measured in bunches and for the set of variables estimated using image analysis, in the nine grape cultivars under study. Analyses of variance of each variable with respect to the cultivar (results not shown) have yielded a statistically significant difference among cultivars ($P < 0.001$) in all cases. Since these differences depended on the variable analysed, and no clear clustering could be performed because of the number of cultivars studied, no class labelling is reported. Nevertheless, it was not the objective of this work to analyse differences between cultivars for any of the variables computed, but to build some inferential model capable of predicting compactness from the image analysis variables. In order to deal with these issues, a PLS model capable of taking advantage of the potential internal correlation structure between the whole set of image analysis variables as well as the cultivars was built in order to create an inferential model with respect to bunch compactness.

Table 1. Average values of the different bunch features from the nine cultivars under study.

Variable	 Aramon	 Bobal	 Cabernet Franc	 Cinsaut	 Danugue	 Derechero de Muniesa	 Monastrell	 Moravia Agria	 Ruby Seedless
Directed measured variables									
Bunch mass (g)	132.3 ± 27.6	344.4 ± 101.3	107.3 ± 34.9	245.6 ± 54.3	347.7 ± 106.2	177.3 ± 52.3	211.6 ± 45.7	165.1 ± 31.4	411.3 ± 14.4
Berry number per bunch	98.2 ± 17.0	165.3 ± 52.1	133.0 ± 31.0	89.4 ± 14.8	167.3 ± 65.0	118.7 ± 39.5	124.3 ± 34.7	158.7 ± 26.1	271.1 ± 73.7
Mass of berries per bunch (g)	118.0 ± 28.7	317.3 ± 86.7	97.4 ± 34.5	225.3 ± 46.7	315.9 ± 99.5	166.1 ± 47.1	198.7 ± 43.2	151.8 ± 28.5	382.3 ± 98.0
OIV compactness rating	3.4 ± 0.9	8.3 ± 0.6	5.2 ± 1.2	6.9 ± 0.8	6.9 ± 1.2	4.5 ± 1.4	7.4 ± 0.7	6.5 ± 1.0	3.1 ± 0.2
Image analysis variables									
A (mm^2)	7058 ± 899	10175 ± 2370	6890 ± 1571	8840 ± 1291	12505 ± 3050	8399 ± 1602	7528 ± 1271	7507 ± 870	16065 ± 2328
P (mm)	476.0 ± 53.9	456.4 ± 63.0	456.3 ± 77.5	452.2 ± 39.4	579.1 ± 93.3	540.1 ± 61.1	400.2 ± 42.5	490.9 ± 70.1	785.3 ± 49.7
L (mm)	165.5 ± 16.3	163.5 ± 22.1	142.8 ± 16.3	157.2 ± 13.9	192.9 ± 34.7	166.3 ± 17.7	138.4 ± 17.4	163.6 ± 21.0	270.2 ± 14.2
MW (mm)	76.34 ± 6.42	98.99 ± 13.01	87.92 ± 12.85	92.77 ± 8.70	113.1 ± 16.93	96.06 ± 10.98	87.48 ± 8.43	88.99 ± 7.36	111.32 ± 8.96
AP	14.91 ± 1.67	22.07 ± 2.13	15.06 ± 1.80	19.55 ± 1.79	21.36 ± 2.01	15.52 ± 1.85	18.73 ± 1.26	15.44 ± 1.56	20.40 ± 2.07
W25 (mm)	65.36 ± 90.50	86.83 ± 13.34	76.36 ± 11.61	80.22 ± 8.09	98.84 ± 16.46	81.70 ± 11.05	73.39 ± 6.79	70.76 ± 8.07	94.38 ± 8.51
W50 (mm)	44.05 ± 5.01	74.51 ± 7.65	51.62 ± 13.53	65.55 ± 6.38	73.13 ± 10.27	58.38 ± 9.14	69.24 ± 7.91	65.51 ± 10.00	80.13 ± 20.42
W75 (mm)	34.12 ± 5.13	54.80 ± 5.65	36.04 ± 5.09	49.73 ± 7.28	50.18 ± 4.76	37.40 ± 4.36	47.47 ± 4.12	40.61 ± 2.64	39.67 ± 7.69
AS	0.47 ± 0.05	0.61 ± 0.05	0.62 ± 0.05	0.60 ± 0.06	0.60 ± 0.10	0.58 ± 0.05	0.64 ± 0.06	0.55 ± 0.07	0.41 ± 0.03
CSF	3.76 ± 0.73	2.39 ± 0.18	3.55 ± 0.70	2.70 ± 0.30	3.13 ± 0.30	4.08 ± 0.60	2.47 ± 0.16	3.75 ± 0.80	4.49 ± 0.39
RD	0.28 ± 0.05	0.42 ± 0.03	0.29 ± 0.06	0.38 ± 0.04	0.32 ± 0.03	0.25 ± 0.03	0.41 ± 0.03	0.28 ± 0.06	0.23 ± 0.02
AB (%)	93.15 ± 2.53	99.46 ± 0.13	94.92 ± 2.89	98.99 ± 0.47	98.72 ± 0.35	94.59 ± 2.93	98.78 ± 0.52	95.99 ± 1.45	93.32 ± 1.69
AR (%)	5.38 ± 1.74	0.52 ± 0.12	3.92 ± 1.99	0.93 ± 0.40	1.13 ± 0.33	4.44 ± 2.21	1.13 ± 0.45	3.82 ± 1.32	5.62 ± 1.41
AH (%)	1.47 ± 0.87	0.02 ± 0.01	1.16 ± 1.05	0.08 ± 0.08	0.16 ± 0.07	0.97 ± 0.76	0.09 ± 0.09	0.19 ± 0.15	1.04 ± 0.45

A, bunch area; AB, AH, AR, proportion of the bunch area corresponding to berries, holes and rachis, respectively. AP: ratio between bunch area and bunch perimeter; AS, aspect ratio; CSF, compactness shape factor; L, bunch length, MW, maximum bunch width; OIV, Organisation Internationale de la Vigne et du Vin; P, bunch perimeter; RD, bunch roundness; W25, W50, W75, bunch width at 25, 50, and 75%, respectively of the bunch main axis length.

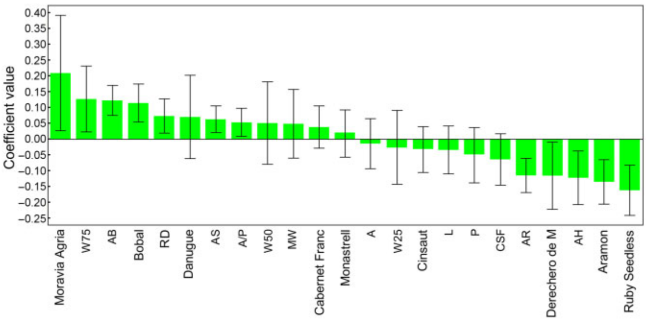

Figure 4. Plot of the coefficients with 95% confidence intervals. The Y axis corresponds to the value of the coefficient of each feature, and the X axis is related to the cultivar and image analysis variables (A, bunch area; P, bunch perimeter; L, bunch length, MW, maximum bunch width; AP, ratio between bunch area and bunch perimeter; W25, W50, W75: bunch width at 25, 50 and 75%, respectively, of the length of the main axis of the bunch; AS, aspect ratio; CSF, compactness shape factor; RD, bunch roundness; AB, AH, AR, proportion of the bunch area corresponding to berries, holes and rachis, respectively).

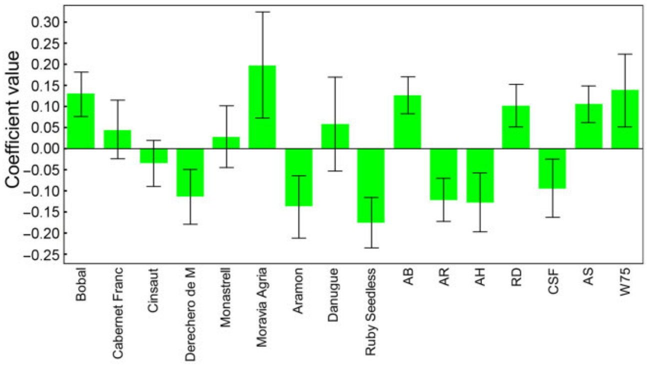

Figure 5. Plot of the coefficients for the final partial least squares model with 95% confidence intervals. The Y axis corresponds to the value of the coefficient of each feature and the X axis is related to the cultivar and image analysis variables (W75, bunch width at 75% of the length of the main axis of the bunch; AS, aspect ratio; CSF, compactness shape factor; RD, bunch roundness; AB, AH, AR, proportion of the bunch area corresponding to berries, holes and rachis, respectively).

Features were auto-scaled (mean centred, afterwards divided by their corresponding standard deviations) to give them all the same opportunity to contribute to the model. Figure 4 shows the coefficients plot with 95% confidence intervals for the initial set of variables. It can be observed that some variables, such as W50, MW, A, L or P, did not show a significant effect on the OIV compactness, since the 95% confidence intervals included the value zero. Furthermore, cultivars Cabernet Franc, Monastrell, Cinsaut and Danugue did not show any extra influence on the compactness either. As a result, the final PLS model included all the cultivars (depending on the cultivar, there was a higher, equal or lower compactness value with respect to the other cultivars in the study) and the seven variables AB, AR, AH, RD, CSF, AS and W75. It must be stressed that this procedure is conducted from a conservative point of view, that is, leaving those variables that are close to significance (and close to non-significance) in the model at each iterative step, hence looking for a reliable final model.

The PLS model, built using the selected features, showed a predictive capability of 85.3% for bunch compactness (OIV compactness rating). No outliers within the model or with respect to the model (high distance to the model) were detected. The nine cultivars and the seven features with statistically significant coefficients are shown in Figure 5. Thus, the PLS model reveals the higher role played by the variables AS, W75, AB, RD CSF, AR and AH in the compactness of the bunch.

Figure 6 depicts the Score Plot of all the individual bunches for the two components of the PLS model. The first component of the model is mainly related to the extracted morphological features shown in Figure 5, and explained 82.0% of bunch compactness (in predictive capability terms); whereas there was an additional 3.3% that was mainly related to the cultivar. Although some overlapping exists, cultivars are arranged on the two-dimensional score plot following a clear trend, with the looser cultivars, for example Ruby Seedless, Aramon and Derechero de Muniesa, in the negative part of component 1 and the more compact, for example Bobal and Monastrell, on the positive side. The clustering of the observations regarding the cultivars is in accordance with the position of their corresponding weights in the plot for the two components of the model (Figure 7), thereby revealing the capacity of the PLS model to

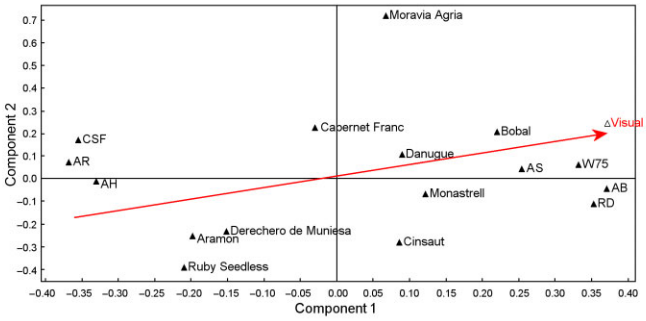

Figure 6. Score plot of the individual bunches of nine cultivars (seven bunches per cultivar) for the two components of the partial least squares model.

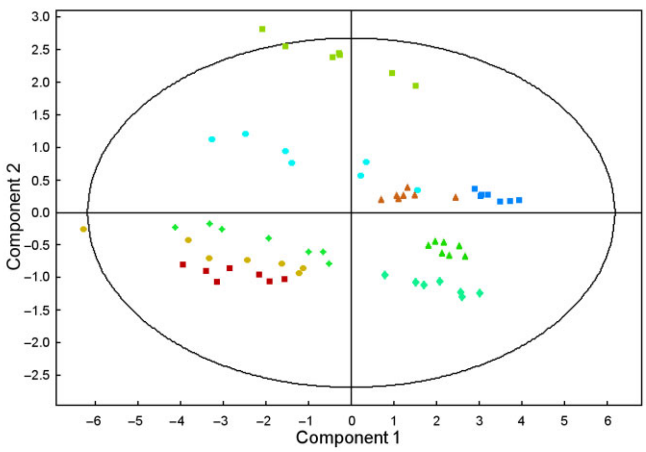

Figure 7. Plot of weights for components 1 and 2 of the partial least squares model for the cultivars Bobal (■), Cabernet Franc (●), Cinsaut (◆), Derechero de Muniesa (◆), Monastrell (▲), Aramon (●), Danugue (▲), Ruby Seedless (■) and Moravia Agria (■).

discriminate between the distinct values of compactness of the different cultivars under study.

The goodness of the PLS model was tested with the validation set of samples. Figure 8 shows the prediction of the validation and the training sets with respect to their observed

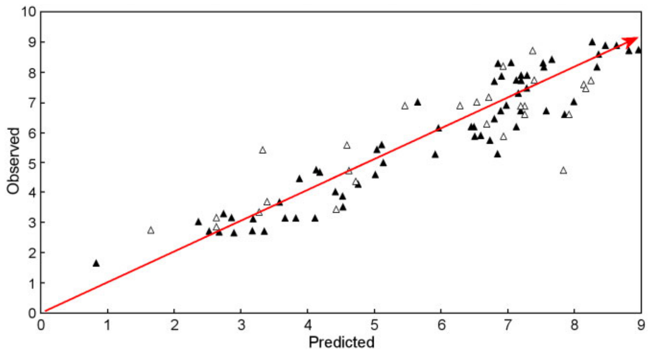

Figure 8. Observed (Organisation Internationale de la Vigne et du Vin compactness rating) versus predicted values of partial least squares model for bunch compactness, for the training set (▲) and test set (△). The predictive capability is 85%, and the accuracy of the model (root mean square prediction error in training set) is 1.03.

values. It can be seen how well the model performed with the test set over the training set with a root mean square prediction error (RMSEP) of 1.03. This RMSEP indicates the accuracy of the model. From the bulk data in the validation set, that is, the compactness values assessed for each bunch by a total of 14 inspectors, the global standard deviation equivalent to RMSEP (Bro et al. 2005) was 1.13. The similarity in the results obtained from the two methodologies allows greater confidence about the creation of an objective automated methodology for assessing the compactness of bunches of grapes. This can also be derived from the fact that the residual variance of the validation data was null. This was calculated from the difference between the variance components leading the model residuals ($RMSEP^2$) and the variance obtained from the analytical evaluation of the bunches by the evaluation panel, assuming that both were independent (Mortensen and Bro 2006).

As stated above, the PLS model revealed a close relationship between bunch compactness and seven variables (AS, W75, AB, RD CSF, AR and AH), which exhibited the larger absolute coefficients in the first component, accounting for 82.0% of the dependent variable (Figure 7). These shape features have the particularity that they are invariant with the size, while other variables whose measurement depends strongly on the size of the bunch, such as the area, the perimeter, the length or the width, have been discarded, since they have little influence on the prediction capability of the model. Of these, the variables AS, W75, RD and AB showed higher values in bunches with greater compactness. Thus, compact bunches are likely to have a larger proportion of area covered by berries (AB), and had a more rounded shape (RD), caused by a bigger area-to-perimeter ratio. This shape probably also gave rise to a higher width-to-length ratio (AS). Accordingly, Molitor et al. (2012) showed that the elongation of the bunch (i.e. a lower AS ratio) caused by the application of gibberellic acid led to a significant reduction in the bunch compactness. It is interesting to note that compact bunches also showed a higher width at 75% of the main axis (W75).

In contrast, the variables CSF, AR and AH showed a negative coefficient in the first component, being linked to the grapevine cultivars with a looser shape (Figure 7). Variables AR and AH are related to the proportion of the total area of the bunch image not covered by berries, and bunches in which parts of the rachis and holes are visible are expected to be looser. In fact, this is similar to one of the criteria used in the OIV descriptor for bunch compactness, that is, the visibility of pedicels (Organisation Internationale de la Vigne et du Vin 2007). Compactness shape factor has an inverse relationship with RD, and rises with perimeter. A higher perimeter is a consequence of the irregular arrangement of the berries in looser bunches, in which they are sparsely distributed all along the stem (Hed et al. 2009). The bunch perimeter also rises with the length of the first ramifications, which have been proven to correlate significantly and negatively with bunch compactness (Tello and Ibáñez 2014).

Most of the variables selected by the PLS model are difficult to measure directly in the bunch, but they can easily be extracted by automated image analysis. The exceptions are AS (which involves the length and the maximum width of the bunch) and W75, variables that can easily be measured by traditional systems. Nevertheless, the remaining important variables extracted by the model cannot be evaluated directly, and they require the quantitative measurement of more complex characteristics of the bunch, such as their total area and perimeter (needed for the calculation of RD and CSF), as well as the area of the bunch covered by the berries, holes and rachis (AB, AH and AR, respectively), which can be determined accurately only by image analysis. Therefore, the development of image processing approaches allows the measurement of new features that can be used for the construction of models for the objective and precise evaluation of bunch compactness.

The PLS model proposed here explains up to 85.3% of the variability of the trait (in predictive capability terms), which is an important figure considering the complexity of the trait. In a recent study conducted to test 19 compactness indexes, the best one was calculated on the basis of six characteristics of the architecture of the bunch (bunch mass, number of berries per bunch, number of seeds per berry, bunch length, first ramification length and number of ramifications per bunch) measured in 11 grapevine cultivars that included the nine red cultivars used in this work; a direct correlation value of $\tau_b = 0.556$ was obtained (Tello and Ibáñez 2014). Thus, the application of image-based technologies combined with multivariate analysis allows the quantitative, objective and rapid measurement of different bunch features that cannot be obtained by traditional direct systems.

In this work, the average values of all features from the four images of each bunch have been used to obtain a more robust model. Tentative attempts, however, at building PLS models have also been made using only one random image of each bunch. The results obtained (data not shown) were similar to those achieved using four images from the predictive capability point of view, as well as from the statistical significance of the selected features. Hence, from a practical approach, the analysis of only one image of the bunch could be enough to estimate compactness accurately.

Bunch compactness has never been estimated before using image analysis. The results obtained in this work indicate that bunch compactness may be assessed using image analysis-based methodologies, which is a sensible, objective and reproducible alternative to human-based inspection.

Moreover, the development of this image-based technology may allow phenotyping of this trait to be performed in a large number of samples in a short period of time, thus making it a potential tool for high-throughput studies in terms of accuracy and effectiveness. This is essential for breeding, where many individuals have to be phenotyped in a short time, and also for genetic studies. Currently, the availability of high-throughput genome sequencing platforms allows genotypic data to be produced quickly, while obtaining high-quality phenotypic data has become the bottleneck hindering the progress of many genetic surveys (Martínez-Zapater et al. 2010, Herzog et al. 2014). Consequently, the application of the image-based technology proposed in this work will accelerate the acquisition of high-quality phenotypic data, allowing a more suitable application of such genetic approaches for the study of this complex trait.

Most of the relevant bunch characteristics selected by the model can be measured accurately using only automated image analysis, which led to the development of a new tool that avoids the subjectivity introduced by different human judges, and providing quantitative data (instead of qualitative ordinal data), characteristics which are essential for the high throughput and accurate phenotyping of grape bunches.

At the commercial level, this new tool could potentially be applied to evaluate bunch compactness as a new indicator of winegrape quality in the assessment of vineyards conducted under field conditions, but a stronger segmentation algorithm robust against the changing conditions of the natural lighting and the presence of a complex background would be necessary. In contrast, the proposed method could be implemented on mobile devices to offer winegrowers a new practical and objective tool to evaluate the compactness in the field. Moreover, the novel method could be applied to the automatic classification of tablegrapes prior to commercialisation and at the reception point in wineries for sorting and quality assessment of winegrapes before crushing and vinification. This could be achieved by using a conveyor belt to transport the bunches under the camera and a backlighting illumination system to capture images in which the contrast between the bunches and the background is high, thus making easier the segmentation process and the extraction of the shape features. In addition, it would be convenient to individualise the bunches in a prior step to prevent the confusion of the shape analysis algorithms. Otherwise, additional image-processing steps should be developed instead to detect and separate the individual bunches in the images. To allow the physical classification and separation of the bunches depending on the compactness, a synchronisation mechanism should be included (Blasco et al. 2009a,b). In the case of working with other cultivars different from the ones in this study, particular models have to be created following the proposed methodology.

Conclusions

In this work, we present a practical step forward in the search for a method with which to automate the assessment of compactness from images of bunches using image processing and multivariate analyses. The PLS model built from different bunch features showed a predictive capability of 85.3%, which can be considered a good result taking into account the complexity of the trait. The most discriminant variables of the model were highly correlated with the tightness of the berries in the bunch (proportion of visibility of berries, rachis and holes) and with the shape of the bunch (roundness, compactness shape factor and aspect ratio).

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