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Identification of grapevine varieties using leaf spectroscopy and partial least squares

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ABSTRACT

Grapevine variety identification is a matter of great interest in viticulture, which is currently addressed by visual ampelometry or wet chemistry genetic analysis. This paper reports the development of a simple and automatic method of classification of grapevine varieties from leaf spectroscopy. The method consists of a classifier based on partial least squares that discriminates among grapevine varieties using a hyperspectral image of a leaf measured in reflectance mode. Hyperspectral imaging was conducted with a camera with 1040 wavelength bands operating between 380 nm and 1028 nm. The classifier was created using 300 leaves, 100 of each of the varieties *Vitis vinifera* L., Tempranillo, Grenache and Cabernet Sauvignon. Monte-Carlo cross-validation confirmed the classifier's performance for the three varieties, which exceeded 92% in all cases. The proposed method has proven to satisfactory classify among grape varieties, but certainly a wider range of grapevine cultivars should be tested before it gets implemented for local sensing with the aim of providing the wine industry with a fast, automatic, environmentally friendly and accurate tool for grapevine variety identification.

1. Introduction

The great economical and social importance of the viticulture and wine industry worldwide encourages the development and application of innovative technologies aimed at objective monitoring the vineyard to improve grape and wine quality.

There are several thousand *Vitis vinifera* L. grapevine varieties available worldwide (Galet, 1979) and its cultivation, wine quality potential and the price paid for their grapes is variety dependent (Clarke and Rand, 2010). Therefore, there is an increasing interest for the accurate identification of grapevine varieties: in order to ensure the trueness-to-type of nursery's cuttings; to identify grapevines of forbidden varieties in certain appellation areas worldwide; or to estimate the supply and demand figures and prices in a given grape growing region.

Nowadays, the current techniques to discriminate between grapevine varieties include ampelometry (Galet, 1979), wet chemistry methods based on isoenzymes (Altube et al., 1991), or DNA analysis (Sefc et al., 2001; Borrego et al., 2002; Pelsy et al., 2010). Ampelometry uses morphological differences between varieties for their recognition. One of its disadvantages is that identification is visual and must be done by an expert with extensive training. Software has been developed to help in ampelographic measurements (Soldavini et al., 2009), with a correct classification percentage of 81% for 1100 leaves. However, the main drawback of this software is that only experts can use it correctly, as the measurement points are selected by the user. On the other hand, isoenzyme or DNA based methods, even though accurate, share the same disadvantage with ampelography, as they can only be applied by specifically-trained people. Moreover, these wet chemistry techniques are time-consuming and labour-demanding and cannot provide a truthful identification of thousands of grapevines in a fast way. Hence, the development of an environmentally-friendly (solvent-free), automatic and fast method for grapevine variety identification could be very valuable for the grape and wine industry, including nurseries, wineries, and grape growers and suppliers.

Spectroscopy is the analysis of how electromagnetic radiation interacts with matter at different wavelengths. The use of local spectroscopy for plant variety determination has been applied to several crops, such as tomato (Xu et al., 2009) and bayberry (Li et al., 2007) using partial least squares and neural networks as discrimination methods, respectively. However, in grapevines, local spectroscopy has been seldom applied for variety differentiation (Lacar et al., 2001b). These authors demonstrated that there were significant statistical differences between the mean reflectance spectra of four different grapevine varieties, but, they did not provide a method to classify their individual spectra.

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Airborne spectroscopic imaging, which measures both spectral and spatial information, has also been attempted for grapevine variety classification (Lacar et al., 2001a; Ferreiro-Arman et al., 2006). Airborne imaging and partial least squares discriminant analysis have also been used for tree species discrimination (Peerbhay et al., 2013). Airborne imaging captures information under natural field conditions concerning the various parts of the plant and, potentially, spurious information regarding the soil. In the works of Lacar et al. (2001a) and Ferreiro-Arman et al. (2006) imaging was done at grapevine scale with a spatial resolution of 0.6 m that prevented the classification of individual grapevines because the grapevines could not be spatially resolved. In the present work, imaging was done under controlled laboratory conditions. In addition, the spatial resolution used in the present article, which was significantly better than leaf scale, allowed the classification of individual grapevines based solely on leaf spectral information.

Hyperspectral imaging is a spectroscopic technique that measures hundreds of narrow wavelength bands and spatial positions. The present work aims at developing a method based on local hyperspectral imaging (Gowen et al., 2007) combined with partial least squares to classify grapevine varieties using individual leaves.

2. Materials and methods

2.1. Leaf samples

Leaves of grapevines of *Vitis vinifera* L. cv. Tempranillo, Grenache, and Cabernet Sauvignon were collected in October 2011. For each variety a number of 100 leaves (from 48 different vines) at various maturity stages (i.e. From different node positions along the shoots), of 4 clones (12 vines per clone) per variety (25 leaves per clone) were collected in the field (Vitis Navarra nursery, Tafalla (Navarra, Spain) and CIDA, Gobierno de La Rioja (La Rioja, Spain)). The clones were RJ24, RJ26, RJ43 and RJ75 for Tempranillo, RJ11, RJ26, ARA4 and ARA24 for Grenache and 15, 169, 685 and R5 for Cabernet Sauvignon. After being collected, the leaves were immediately submerged in deionized water at 10 °C. This was intended to avoid desiccation as well as to bring the leaves of all varieties to a similar water status (well hydrated leaf, 100% relative water content), in order to minimize the influence of differential water contents in the leaves in the NIR region of the spectrum acquired with the hyperspectral camera (i.e. 700–1028 nm). The leaves were then transported in portable refrigerators to the University of Rioja and kept in cold water for 18 h. After this time, each leaf was blotted dried and a disc of 2 cm diameter was cut in the apical part of the main lobe. Hyperspectral images of the leaves discs were taken on the adaxial side of the fully hydrated leaves discs.

2.2. Experimental setup and image acquisition

The experimental setup, depicted in Fig. 1, was composed of a hyperspectral camera and lighting. The hyperspectral camera comprised the Specim Imspector V10E (Specim, Oulu, Finland) spectrograph and a JAI Pulnix (JAI, Yokohama, Japan) black and white camera. The spectrograph decomposes the light in its composing wavelengths like an optical prism and the black and white camera registers the image formed by the spectrograph. The hyperspectral images had 1040 × 1392 pixels, with the 1040 pixels corresponding to the same number of wavelength bands between 380 and 1028 nm with a resolution of approximately 0.6 nm. The 1392 pixels corresponded to the spatial dimension over the sample and presented a spatial resolution of 79 μm. Prior to imaging the leaves, the camera underwent spectral calibration following the procedure provided by the camera manufacturer (EHE, 2006). The length of the imaged line over the sample, 110 mm, depended on the distance between the hyperspectral camera and the sample to be imaged, which was 420 mm. This distance allowed to have a perfectly focused image and was defined using a black and white target. The camera was operated using Coyote software (Version 2.2.0, JAI, Japan) and acquired eight images per second. The lighting comprised four 20 W, 12 V halogen lamps and two 40 W, 220 V reflector lamps (Spotline, Philips, Eindhoven, Nederlands) in both cases powered by continuous current supplies to avoid light flickering. The spotline lamps were used to provide a better illumination in the near infrared region and were powered at 110 V to reduce lighting intensity. The lamps were positioned over a holder with 300 × 300 × 175 mm^3 (length × width × height). Measurements were done at 22 °C in a dark room to prevent the presence of ambient light.

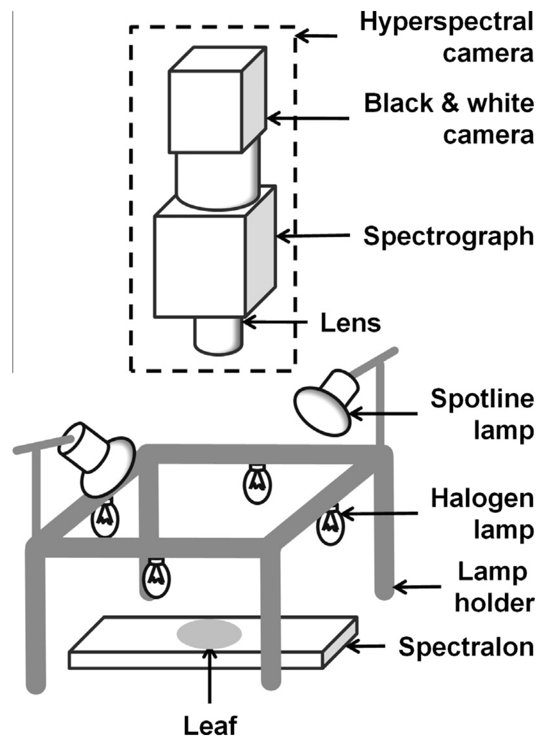

Fig. 1. Experimental setup.

2.3. Reflectance

The experimental setup allowed to determining the samples' reflectance (R), which measures the percentage of light intensity coming from the sample relative to total intensity of light incident on the sample. Reflectance (R) for a certain position x and wavelength λ is determined according to equation 1:

$$R(x,\lambda) = \frac{Li(x,\lambda) - D(x,\lambda)}{SI(x,\lambda) - D(x,\lambda)} \quad (1)$$

where $Li(x,\lambda)$ is the intensity of the light reflected by the leaf, and $SI(x,\lambda)$ is the intensity of light coming from a white reference made of a material called Spectralon (Specim, Oulu, Finland), which has the highest diffuse reflectance (99.9%) of any known material or coating over the ultraviolet, visible, and near-infrared regions of the spectrum, allowing to normalize for incident light on the leaf surface. $D(x,\lambda)$ is the camera's dark current which was measured with the camera lens covered. Its value was subtracted from $Li(x,\lambda)$ and $SI(x,\lambda)$ because it corresponded to the camera electronic noise which was independent from the light coming from the leaves or the Spectralon. If $D(x,\lambda)$ was not subtracted it would distort the