DOI 10.5162/SMSI2023/D3.2

Raman Spectroscopy applied to detection of grapevine disease: the case of Esca detection

Camilla Baratto[1], Gina Ambrosio[1], Guido Faglia[2,1], Massimo Turina[3],
[1] CNR-INO, PRISM Lab, Via Branze 45, 25123 Brescia, Italy,
[2] Department of Information Engineering, University of Brescia, 25133 Brescia, Italy
[3] IPSP-CNR, Strada delle Cacce,Turin, 25133, Italy
camilla.baratto@ino.cnr.it

Summary:

We report on the use of Raman Spectroscopy (RS) and chemometric analysis as a non-invasive sensor to implement precision agriculture for early detection of the ESCA disease. Initial encouraging findings allow to identify symptomless leaves in ESCA-affected vines as belonging to a diseased vine through a change in spectroscopic properties measured by RS likely due to a change in carotenoids. To date, there is no report of RS for Esca disease detection.

Keywords: Raman Spectroscopy; Esca disease; precision agriculture; chemometric.

Introduction

Advanced detection of plant pathogens is fundamental to allow human intervention that can limit crop damages, with a proper pesticide use in sustainable crop management systems. When available, diagnostic techniques different than operator-dependent visual assessment must support the identification of pathogens. Esca disease has a tremendous economic impact [1] in all wine-producing countries: different fungi (*Phaeoacremonium aleophilum, Phaeomoniella chlamydospora, and Fomitiporia Mediterranea*) often associated to the disease, can be present also in healthy plants[2], and their inoculation often does not reproduce the disease [3]. Thus, it is difficult to support the diagnosis even with destructive techniques like Enzyme-Linked Immuno Sorbent Assay (ELISA) or Polymerase Chain Reaction (PCR), which remain inconclusive. Once symptoms of ESCA diseases are detected visually, it is already too late for a possible precision therapeutic intervention. It is thus of utmost importance to find a technique that allows for early monitoring of the disease.

Recently the applicability of Raman spectroscopy (RS) to precision agriculture has been demonstrated [4][5]. RS is a non-invasive technique that does not require sample preparation and could be readily available for in field monitoring. The advantages of Raman technique with respect to other more established optical techniques, such as reflectance spectroscopy [6], hyperspectral broad band remote sensing [7], chlorophyll fluorescence spectroscopy [8], relies on the capability of monitoring molecules associated with the plant health. The main concept of this work is to combine the sensing capabilities of the RS technique and chemometric analysis in order to develop a non-destructive analyzer for early detection of the esca disease that allows the correlation and prediction of the different symptomatic expressions of the disease (Brown Wood Streaking, Grapevine Leaf Stripe Disease and apoplexy). We demonstrated the proof of concept of disease detection by analyzing leaf samples from healthy plants and from symptomatic and symptomless shoots in diseased plants. The final symptomatic outcome of ESCA disease later in the season was used as the final benchmark.

Results

Leaves from diseased and healthy grapevines of Marzemino cultivar were sampled and analyzed 24 hrs after collection by Raman spectroscopy. Two types of spectroscopic system were used zfor investigation, a micro-Raman modular system by Horiba, with 532 nm excitation and 50x long working distance objective, and a portable Raman spectrometer (BWTek) equipped with 785 nm excitation and optical fiber attachment. The tested portable system can eventually be used for future applications in field. With both excitation sources, the fluorescence of chlorophylls is a competing signal to Raman spectroscopy which should be removed prior to signal analysis. For 532 nm light, a small detection time (2 s) was used to avoid leaves deterioration due to light absorption. For 785 nm excitation the detection time was increased to 10*s* as no deterioration of the leaves was noticed. The data were acquired from leaves taken from different vines that are healthy or are infected. From the infected plants, symptomatic and asymptomatic leaves were tested.

The visual inspection of leaves does not allow to distinguish healthy from infected/asymptomatic vines. The spectra of grapevine leaves (Fig. 1) showed vibrational bands assigned to carotenoids, polyphenols and chlorophylls [9][10]. The typical spectra are similar for different classes with only minimal changes, thus indicating that a data analysis is necessary to extract information from the data. For this purpose, spectral data were elaborated using a classification method, namely partial least squares (PLS) algorithm-discriminant analysis (PLS-DA), which combines dimensionality reduction and discriminant analysis into one algorithm and is especially applicable to modeling high dimensional (HD) and collinear data as produced by RS.

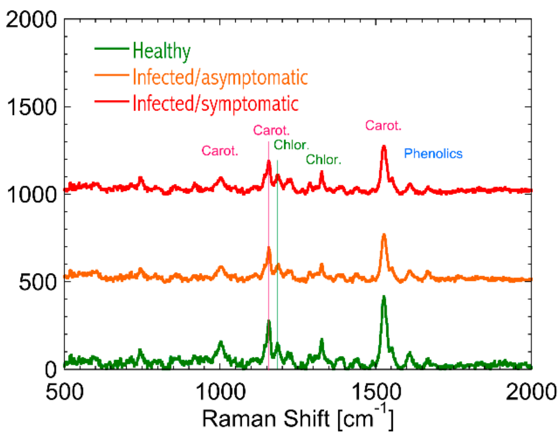

Fig. 1. Example of Raman spectra collected from healthy and Infected (symptomatic and asymptomatic) leaves.

Table 1 report the confusion matrix of the test dataset resulting from PLS-DA analyses when using 532nm laser is shown in Tab. 1. Regarding the prediction from the chemometrics analysis, each row corresponds to a predicted class, each column to an actual class. The classification accuracy (the ratio of correct predictions to total predictions made) is equal to 97%.

real/predicted	Infected	Healthy	not assigned
Infected	147	3	0
Healthy	4	96	0

Table 1: confusion matrix of the test dataset prediction resulting from PLS-DA.

Conclusions

The outcomes of this preliminary investigation showed that we can identify symptomless leaves in ESCA-affected vines as belonging to a diseased vine by measuring a change in spectroscopic properties by RS. A very good classification accuracy was obtained by preliminary tests.

References

[1] C. Bertsch *et al.*, *Plant Pathol.*, vol. 62, no. 2, pp. 243–265, 2013, doi: 10.1111/j.1365-3059.2012.02674.x.

[2] V. Hofstetter, B. Buyck, D. Croll, O. Viret, A. Couloux, and K. Gindro,*Fungal Divers.*, vol. 54, pp. 51–67, 2012, doi: 10.1007/s13225-012-0171-z.

[3] G. Surico, L. Mugnai, and G. Marchi, *Phytopathol. Mediterr.*, vol. 45, no. SUPPL. 1, pp. 68–86, 2006, doi: 10.14601/Phytopathol_Mediterr-1847.

[4] S. Yeturu, P. Vargas Jentzsch, V. CiobotĂ, R. Guerrero, P. Garrido, and L. A. Ramos, *Anal. Methods*, vol. 8, no. 17, pp. 3450–3457, 2016, doi: 10.1039/c6ay00381h.

[5] N. Altangerel *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, vol. 114, no. 13, pp. 3393–3396, 2017, doi: 10.1073/pnas.1701328114.

[6] A. A. Gitelson and M. N. Merzlyak, *J. Plant Physiol.*, vol. 148, no. 3–4, pp. 494–500, 1996, doi: 10.1016/S0176-1617(96)80284-7.

[7] A. H. Elmetwalli and A. N. Tyler, *Agric. Water Manag.*, vol. 242, no. March, p. 106413, 2020, doi: 10.1016/j.agwat.2020.106413.

[8] J. K. et al H. M. Kalaji, K. Bosa, *Omi. J. Integr. Biol.*, vol. 15, no. 12, pp. 925–934, 2011.

[9] C. Baratto, G. Ambrosio, G. Faglia, and M. Turina, *IEEE Sens. J.*, vol. PP, no. Xx, p. 1, 2022, doi: 10.1109/JSEN.2022.3211616.

[10] L. Mandrile, A. M. Giovannozzi, A. M. Rossi, G. Gambino, E. Noris, and A. M. Rossi, " vol. 13, no. June, 2022, doi: 10.3389/fpls.2022.917226.