Investigating biotic and abiotic stresses on green-leaf plants by means of hand-held Raman spectroscopy

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Summary:
Raman spectroscopy is a non-invasive technique that can be adopted for the in-field monitoring of crops and early detection of plant pathogens. Combining its sensing capabilities with chemometric analysis, it is possible to classify plants as healthy or infected by evaluating the leaf pigment content. The method is currently under investigation to characterize both abiotic stresses and biotic infections at the lab scale.

Keywords: Raman, spectroscopy, precision agriculture, chemometrics, plant pathogens

Introduction

Nowadays, while world population and food demand are rapidly growing worldwide, the yield and the quality of agricultural products are greatly affected by plant diseases, devastating entire crops, and causing severe economic losses. [1] For these reasons, expensive chemical treatments and pesticides and drugs are needed in modern agricultural and food production. Nevertheless, irrational or excessive usages of these chemicals cause severe concern for environmental and food safety reason. Thus, to limit the use of phytosanitary treatments, rapid detection of pathogen-infected plants is an important first step in plant disease management. Most disease symptoms usually manifest only at relatively later stages of infection or when it is already too late for a possible precision therapeutic intervention. [2] Therefore, early detection of infected plants would allow their rapid and selective removal, thus greatly reducing the opportunity for further disease spreading. This approach falls more generally into what is defined as "precision agriculture", a protocol of tailored treatments applied only when they are really needed, for a more sustainable farming. [3] Currently, the gold standard methods for analyte detection correlated to the presence of plant pathogens are chromatographic-based techniques. Although these methods are sensitive, accurate and reliable, the major limitations are the requirement for dedicated lab space, prolonged analyses, costly systems with expensive upkeep and the need for highly trained operators, as well as cumbersome analyte extraction and time-consuming sample preparation steps. [1] The use of Raman spectroscopy in agriculture is recently emerging for its relatively fast and non-destructive analysis that does not require sample preparation and offers selective and early detection of biochemical markers (*i.e.*, carotenoids, chlorophylls and anthocyanins) over multiple stress conditions. [4] By comparing the differences in the Raman spectra of a diseased sample vs. a healthy leaf, compositional analysis, rapid diagnosis of plant diseases, and biotic and abiotic stress response of plant tissue can be easily evaluated. [2][5] Most importantly, the recent development of portable and handheld Raman spectrometers allows for a quick and easy on-field monitoring, reducing the gap between sample collection and laboratory analysis. Finally, compared to already existing near-infrared monitoring techniques, the Raman spectrum is of much simpler interpretation. In the CN-Agritech project, the feasibility of the application of Raman spectroscopy in precision agriculture was evaluated, with the aim of analyzing biotic and abiotic stresses in green-leaf plants.

Results

In this work we have optimized an experimental protocol for the early detection of plant pathogens, starting from the study of different types of green-leaf plants (*i.e.*, tomato, rocket). Many physical variables which could affect the measurement were tested, including the working wavelength and the leaves to be chosen. Rocket plants have been sowed simulating a saline accumulation in the soil, in order to study the abiotic stress, while tomato plants were infected with a commercially interesting virus, to evaluate the outcomes of biotic stresses. In particular, rocket plants were grown in a greenhouse: a control group was left without treatment, one group was treated with 150 mL NaCl solution, and another group was treated with 300 mL NaCl solution. Similarly, three tomato plants were infected by *Tomato Spotted Wilt virus* (TSWV) and three plants were used as a control group. The Raman measurements have been recorded either with a Horiba iHR 320 microspectrometer coupled with a 532 nm laser line, or a Rigaku Progeny hand-held Raman spectrometer in contact mode, with excitation wavelength at 1064 nm. Strategies to remove the stray light in outdoor environment were studied. Since signal variations were small and biological variability very high, a chemometric statistical approach was required to analyze the data collected. In fact, machine-learning algorithms (*e.g.*, PCA, LDS, PLS-DA) are at the core for predictive classification methods. Concerning rocket leaves, tests on fresh and lyophilized leaves were performed. The use of either 532 nm or 1064 nm as excitation source was achieved for fresh leaves, while with lyophilized specimens, intense fluorescence signal impeded the use of visible light. The interest in measuring dried samples consisted in the possibility to freeze the leaf and to exchange samples between different laboratories to compare and correlate conventional analytical techniques with Raman spectroscopy. The investigated data range was from 700 a 1700 cm^{-1}, as in this region, the Raman spectrum of green-leaf plants essentially displays the vibrational patterns of bio-pigments and metabolites, *e.g.*, carotenoids, chlorophyll, and polyphenols (Fig. 1). The classification accuracy strongly depends on the data collection method and was as high as 97.5% for lyophilized samples. For tomato plants, the aim of the investigation was to detect any Raman signal that could be correlated with the infection, before the appearance of any symptoms on the leaf. For this, infected leaves and mock-infected ones have been analyzed after three, six and nine days after the virus injection. After nine days each leaf measured has been evaluated with the destructive Polymerase Chain Reaction (PCR) diagnosis test. After data processing with PLS-DA, we demonstrated that a classification of healthy *vs.* infected plant was achieved by means of the multivariate analysis and that the error rate diminishes as the time increases. It is very interesting to consider that the technique is somewhat independent of the plant type. It is therefore potentially usable for leaf crops other than those considered in this study.

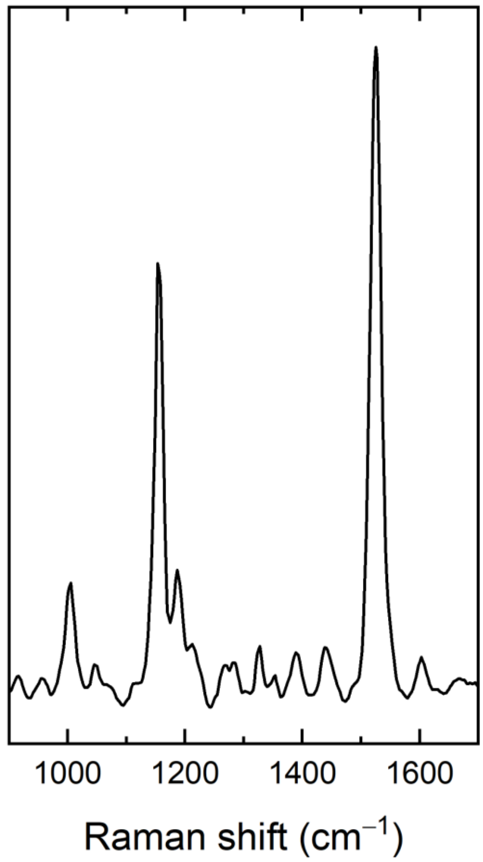

Fig. 1 *Raman spectrum of a fresh tomato leaf, recorded with the hand-held device (1064 nm).*

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