

Review

Raman Spectroscopy for Plant Disease Detection in Next-Generation Agriculture

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Abstract: The present review focuses on recent reports on the contribution of the Raman method in the development of digital agriculture, according to the premise of maximizing crops with a minimal impact of agriculture on the environment. The Raman method is an optically based spectrum technique that allows for the species-independent study of plant physiology as well as the real-time determination of key compounds in a non-destructive manner. The review focuses on scientific reports related to the possibility of using the Raman spectrometer to monitor the physiological state of plants and, in particular, to effectively diagnose biotic and abiotic stresses. This review primarily aims to draw attention to and raise awareness of the potential of Raman spectroscopy as a digital tool capable of bridging the gap between scientists' detailed knowledge of plants grown under laboratory conditions and farmers' work. The Raman spectrometer allows plant breeders to take appropriate measures in a well-defined area, which will reduce the territory occupied by biotic and abiotic stresses, thus increasing yields and improving their quality. Raman technology applied to modern agriculture can positively affect the accuracy and speed of crop quality assessments, contributing to food safety, productivity and economic profitability. Further research and analysis on cooperation between farmers and scientists is indispensable to increase the viability and availability of Raman spectrometers for as many farmers and investors as possible.

Keywords: biotic and abiotic stresses in plants; digital agriculture; food production; innovations in agriculture; Raman spectroscopy



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1. Introduction

1.1. The Main Objective of Agricultural Transformation

Modern agriculture faces many challenges; it is struggling with climate and environmental crises. The key issues affecting modern agriculture are climate change, a lack of access to fertile soils and shrinking groundwater resources. Simultaneous rapid population growth is causing an increase in the global food demand and the need to increase agricultural efficiency [1]. Prediction models suggest that the global population will reach 9.8 billion people by 2050. It is estimated that to feed such a population, about 70% more food than is currently available would need to be produced [2]. An alternative solution to this problem is to increase agricultural efficiency [3,4], including protecting and improving existing crops [5]. In view of this, it is essential to search for and implement strategies to increase crop yields without increasing the surface area of agricultural land [6,7]. Developments in technology have made it possible to solve these problems by implementing innovative methods in farming [4]. The transformation of agriculture using innovative technologies is called digital agriculture [3,6–8]. The goal of digital agriculture is to increase agricultural efficiency through the development of modern technological solutions that will maximize yields while affecting natural ecosystems as little as possible [6,7]. Key to this issue is the transfer of knowledge from laboratory tests to agricultural crops in fields and under greenhouse conditions [9]. In order for digital agriculture to deliver the expected

results for farmers, appropriate detection methods are needed to provide information about the health statuses and current needs of plants in crop fields [3]. The same methods are essential in laboratory plant cultivation when developing new and more productive plant species. The laboratory techniques currently used to study new plant crosses are labor-intensive and time-intensive [3].

1.2. Advanced Tools in Digital Agriculture

Digital cultivation demands advanced methods for growing and selecting crops [10], the detection and identification of biotic and abiotic plant stresses and data collection of the health conditions and growth stages of plants in agricultural fields. These techniques are in high demand among plant growers [11]. Unmanned aerial vehicles (UAVs) enable plant phenotyping and disease monitoring with the spatial resolution of a single plant; additionally, chemoresistance sensors based on metal oxide semiconductors (MOSs) are a promising technology for detecting plant-produced ethylene in plants [8,12].

In the related literature, there is more and more evidence showing the effectiveness of Raman spectroscopy (RS) in identifying the physiological condition of plants, crop quality, plant species or varieties, in assessing the maturity of plants, in pre-symptomatic diagnostics or in detecting abiotic and biotic stresses in plants [2–4,11]. Raman spectroscopy allows phenotyping and in-crop digital plant selection. Fast access to plant health information enables its use in the discovery and detection of bacterial infections, secondary diseases, insect invasions, fungal infections and other pathogens and the diseases they transfer in greenhouses and crop fields [2,13,14]. The resulting information is used for highly precise and location-specific chemical interventions that could prevent the expansion of biotic stresses and prevent the death of up to 30% of crops. Crop losses due to abiotic stresses are much more significant and can amount to up to 70% of crops worldwide [14,15]. The quick detection of abiotic stresses, i.e., physiological drought and nutrient deficiencies, promotes the immediate and precise delivery of nutrients. Fertilizing a strictly defined area of the field limits the introduction of contaminants contained in fertilizers into crops and soil [15,16]. Figure 1 below illustrates the use of an innovative tool such as a Raman spectrometer for both laboratory and field plant research.

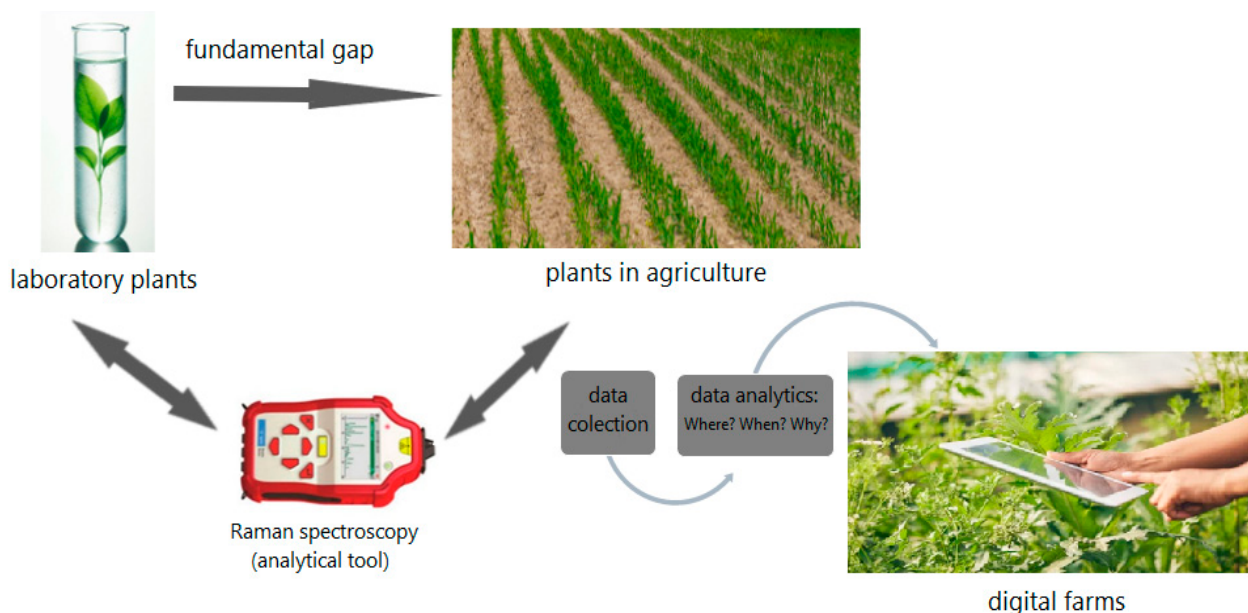


Figure 1. Application of Raman spectroscopy in digital agriculture.

1.3. Assumptions and Objectives of the Review

Recently published results indicate that Raman spectroscopy is the right tool to diagnose biotic and abiotic stresses [17–20]. Researchers have demonstrated, in a number

of studies, that Raman spectroscopy is a perfect method for the monitoring of field and greenhouse crops because of its high selectivity and specificity [4]. RS is a spectroscopic, laser method in which measurements are carried out without the need for the chemical labeling of the test material. The breeder does not incur any additional costs related to the purchase of reagents for analysis. In addition, carrying out plant analysis to investigate the growth of pathogens or the type of stress is immediate—it takes one second [21].

Agriculture and crop cultivation are relatively new areas for the use and development of research involving Raman spectroscopy [2]. This article presents the Raman spectrometer as a digital agricultural tool that can become a bridge between the extensive knowledge of plants grown by scientists in laboratories and of crops of agricultural importance in fields or greenhouses. The review's authors focus in particular on the use of RS to assess the physiological state of plants and effectively diagnose biotic and abiotic stresses. The review outlines the potential for using the Raman spectrometer with other research technologies to improve crops and collects information on how to increase the utility and availability of this method in agriculture. This review is primarily aimed at drawing attention to and raising awareness of the potential of Raman spectroscopy in agriculture among farmers and plant breeders. Further cooperative research and analysis between farmers and scientists is indispensable to increase the viability and availability of Raman spectrometers for as many farmers and investors as possible.

2. Principle and Instrumentation of Raman Spectroscopy

2.1. Principle of Raman Method

Raman spectroscopy was first discovered in 1928 by Sir Chandrasekhar Venkat Raman. It was observed that when light excites a particle, most of the light is scattered elastically—this is Rayleigh scattering, where $E_{\text{incident}} = E_{\text{scattered}}$, but also a small fraction of photons are scattered inelastically—this corresponds to Raman scattering, where $E_{\text{incident}} \neq E_{\text{scattered}}$. When $E_{\text{scattered}} < E_{\text{incident}}$, it is called a Stokes shift, and when $E_{\text{scattered}} > E_{\text{incident}}$, it is an anti-Stokes shift. Each peak on the Raman spectrum corresponds to a vibrational mode of the bonds of the molecule under study. Raman scattering can occur in the near ultraviolet, visible or near infrared [21,22]. The diagram in Figure 2 shows schematically the Raman and Rayleigh scattering process.

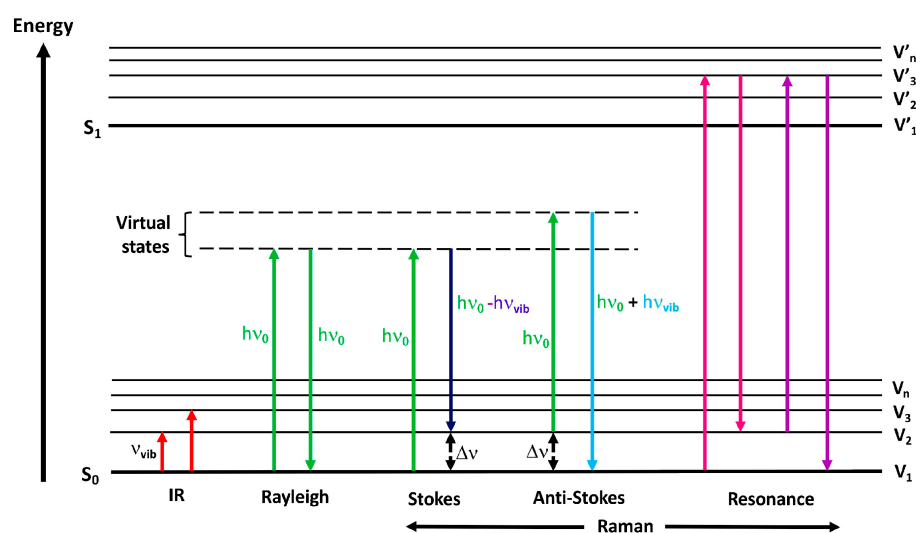


Figure 2. Jablonski energy diagram (transitions occurring during infrared absorption, Rayleigh scattering, Raman Stokes scattering, anti-Stokes scattering and resonant Raman scattering). Adapted from [1].

The Raman method is a vibrational spectroscopy technique used to study the structure, the dynamics of change and the functions of biomolecules. RS provides non-invasive views of tissue structure and insights into its molecular structure [22–25]. The Raman method

uses the so-called “fingerprint”—the characteristic spectrum pattern that each organic compound possesses, as well as those possessed by its functional groups, by which they can be identified. Meanwhile, peak heights can be used to calculate the concentration of an analyte in a sample [1,26]. The Raman spectrum can be used as a fingerprinting tool for various compounds [27], and the obtained analyte spectrum can be treated as a qualitative analysis for unknown samples or a mixture of components [28]. The principle of Raman spectrometry is to measure the frequency shift of inelastic scattered light while a photon of incident light strikes a molecule and a scattered photon is produced [29–35]. The biggest advantage of Raman spectroscopy in the study of biological materials is that we can obtain spectra containing a large amount of information from intact tissues without destroying their structure [36,37]. This is very useful during the chemical analysis of biological material, despite its high complexity. Due to the fact that Raman spectroscopy is sensitive to even negligible structural changes, this technique is used for comparative studies [38]. The Raman method for studying tissues gives a lot of information about the vibrational structure of their component proteins, glycosaminoglycans (GAGs), lipids and DNA. The benefit of Raman spectroscopy in tissue studies is the very low intensity of water bands, which, in other known spectroscopic techniques, makes it difficult to analyze biological materials [36]. By providing valuable information from complex biological materials, Raman spectroscopy is an extremely precise tool for studying various plant materials [39–45]; its mechanism enables the measurement and analysis of the composition and molecular structures of cell walls [46–48]. For many years, measurements using Raman spectroscopy were limited, due to the very low efficiency of normal Raman scattering and the high cost of instrumentation. In addition, Raman spectrometers were not adapted for on-site analysis of the test sample [19]. Recent technological advances in instrumentation have made Raman microscopy a remarkable analytical tool in biological and plant research [49].

2.2. Instrumentation of Raman Spectroscopy

A standard Raman instrument must have a light source and a spectrometer. The light source is usually one or more lasers, which are the source of the photon flux. The resulting photon flux is directed through a beam splitter and focused through a lens on the material under test. The scattered light is collected using the same optics and directed to a spectrometer, which is usually placed at a 90° angle from the incident illumination and may include an array of filters or a monochromator. Before the photons enter the spectrometer, elastically scattered photons are cut off using low-pass filters, while inelastically scattered photons (Raman photons) are scattered on the grids of the spectrometer according to their energies and are then recorded using a CCD (charge coupled device). An example of a Raman instrument configuration can be seen in Figure 3.

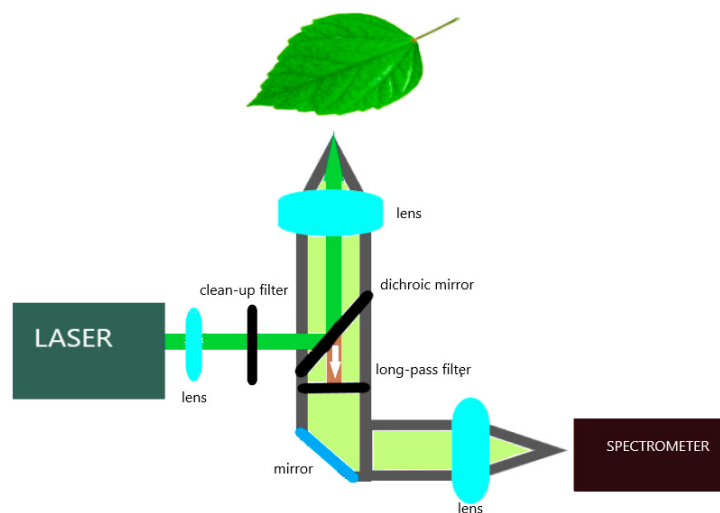


Figure 3. Schematic presentation of an example of a portable Raman spectrometer.

The Raman spectrometer was classified as laboratory equipment only for a long time, but portable spectrometers are now available. Portable Raman spectrometers provide quick and accurate access to plant responses to stress factors during field cultivation for both farmers and plant researchers [50]. These spectrometers are usually built with lasers that excite in the green ($\lambda = 532$ nm), red ($\lambda = 785$ or 830 nm) or infrared ($\lambda = 1064$ nm) parts of the electromagnetic spectrum [18,19,51–53]. Numerous studies have reported that the use of a laser in the blue and green parts of the electromagnetic spectrum shows carotenoid bands well [52]. In the case of a laser-emitting beam with a wavelength greater than 561 nm and less than 700 nm, extremely strong chlorophyll fluorescence occurs, so these lasers are unsuitable for the structural analysis of living tissues. Chlorophyll fluorescence decreases exponentially at wavelengths above 700 nm, so excitation wavelengths of 785–830 nm provide sufficient signal spectra for plants' leaf noise [50]. The most frequently occurring vibrational bands in the scientific literature and their assignment for spectra collected from a plant are presented in Table 1.

Table 1. Assignment of bands in the Raman spectra of cell wall polysaccharides based on the literature [2]. Copyright *Front. Plant Sci.* 2021.

Band (cm^{-1})	Vibration Mode	Assignment
480	C-C-O and C-C-C deformations; related to glycosidic ring skeletal deformations $\delta(\text{C-C-C}) + \tau(\text{C-O})$ scissoring of C-C-C and out-of-plane bending of C-O	Carbohydrates
520	$\nu(\text{C-O-C})$ glycosidic	Cellulose
747	$\gamma(\text{C-O-H})$ of COOH	Pectin
849–853	$(\text{C}_6\text{-C}_5\text{-O}_5\text{-C}_1\text{-O}_1)$	Pectin
917	$\nu(\text{C-O-C})$ in plane, symmetric	Cellulose and phenylpropanoids
964–969	$\delta(\text{CH}_2)$	Aliphatics
1000–1005	In-plane CH_3 rocking of polyene aromatic ring of phenylalanine	Carotenoids and protein
1048	$\nu(\text{C-O}) + \nu(\text{C-C}) + \delta(\text{C-O-H})$	Cellulose and phenylpropanoids
1080	$\nu(\text{C-O}) + \nu(\text{C-C}) + \delta(\text{C-O-H})$	Carbohydrates
1115–1119	Sym $\nu(\text{C-O-C})$; C-O-H bending	Cellulose
1155	C-C stretching; $\nu(\text{C-O-C})$, $\nu(\text{C-C})$ in glycosidic linkages, asymmetric ring breathing	Carotenoids and carbohydrates
1185	$\nu(\text{C-O-H})$ next to aromatic ring + $\delta(\text{CH})$	Carotenoids
1218	$\delta(\text{C-C-H})$	Carotenoids and xylan
1265	Guaiacyl ring breathing, C-O stretching (aromatic); -C=C-	Phenylpropanoids and unsaturated fatty acids
1286	$\delta(\text{C-C-H})$	Aliphatics
1301	$\delta(\text{C-C-H}) + \delta(\text{O-C-H}) + \delta(\text{C-O-H})$	Carbohydrates
1327	δCH_2 bending	Aliphatics, cellulose and phenylpropanoids
1339	$\nu(\text{C-O})$; $\delta(\text{C-O-H})$	Carbohydrates
1387	δCH_2 bending	Aliphatics
1443–1446	$\delta(\text{CH}_2) + \delta(\text{CH}_3)$	Aliphatics
1515–1535	-C=C- (in plane)	Carotenoids
1606–1632	$\nu(\text{C-C})$ aromatic ring + $\delta(\text{CH})$	Phenylpropanoids
1654–1660	-C=C- , C=O stretching, amide I	Unsaturated fatty acids
1682	COOH	Carboxylic acids
1748	C=O stretching	Esters, aldehydes, carboxylic acids and ketones

The Raman technique enables the detection and quantitative determination of key compounds in plant tissues without destructive effects on the sample. In addition, Raman spectroscopy shows little or no response to water. Thus, this technique can become a key tool in monitoring the condition of plants, and in assessing yield quality, pathology and crop maturation [1,54–58]. Although the use of Raman spectroscopy in farming and food safety analysis has been increasing recently, its capabilities are still not fully utilized [59]. So far, the applicability of Raman spectroscopy has not been fully exploited in agriculture and in the food analysis sector. Portable Raman spectrometers and the speed and non-invasiveness of the presented method make it possible to perform spectral recording and analysis at plant growth and harvesting sites, as well as at crop storages [21,60–62].

3. Influence of External Factors on Plant and Development

During agricultural cultivation, plants are exposed to various environmental stresses that limit their growth and adversely affect their cellular functions [20,63,64]. When environmental conditions are not disturbed, plants allocate their energy reserves for growth and, thus, for food production without any interference. Unfortunately, biotic and abiotic stresses often occur during plant growth. Biotic stresses arise as a result of the impact of other living organisms on plants, while abiotic stresses are caused by environmental changes. This is when plant growth is halted and energy is spent on fighting with stress [5].

3.1. Biotic Stress

Biotic stress is the result of pathogens such as bacteria, viruses and fungi. These organisms have a limiting effect on the maturation of crops, lowering their productivity; consequently, they can destroy entire agricultural ecosystems [65]. They cause huge losses in crops around the world. In the cases of the most common crop species (soybean, potato, wheat, corn and rice), approximately 20–30% of the global harvest is lost each year to plant pathogens and pests [66,67], and the greatest losses occur in areas where there are problems with food safety [66]. Pest and pathogen control is necessary to improve crops without increasing the agricultural land surface area [5,68]. Using targeted and location-specific chemical treatments may help to prevent the expansion of biotic pressure and save up to 30% of crop yields [14,15,68,69].

3.2. Abiotic Stress

Significant crop losses can be induced by different abiotic stresses, like nutrient deficiencies, salinity and drought [70]. Abiotic stresses are much more significant in crop yields and it is believed that they are linked to 50–70% of all crop losses worldwide [71]. The salinization of soil is a worldwide problem, particularly in many developing countries. High osmotic pressure during excessive salinity in the soil inhibits the plant absorption of water and minerals, which dramatically reduces crops and consequently, productivity, in high salinity regions. A nitrogen (N) shortage results in disrupted chlorophyll biosynthesis, which leads to low plant health and leaf chlorosis [72]. Nitrogen limitation contributes to premature leaf senescence, reduced crops and chlorophyll biomass. On the other hand, if available nitrogen is in excess of the plant's nutritional needs, the excess is removed through runoff and infiltration into groundwater, which results in the pollution of aquatic ecosystems. Consequently, optimizing nitrogen shortages has become a topic of particular importance for plants' conditions and for its environmental and economic impact [73,74]. Potassium (K) and phosphorus (P) deficiencies also lead to slower crop growth and the browning of leaf tips. The early detection and diagnosis of nutrient deficiencies can be used to apply fertilizers in a site- and dose-appropriate manner, alleviating losses associated with these deficiencies [75]. Rapidly preventing the spread of abiotic stresses is the key to increasing crop yields. In combating plant stress, the cultivation of plant varieties with increased stress resistance and high-yielding varieties is very important [5].

3.3. New Perspectives in Solving Problems in Agriculture

The growing importance of agriculture in meeting the world's food demand requires expanding current knowledge concerning plant growth and adaptation beyond the laboratory in order to make agricultural practices effective. In recent decades, significant progress has been made in understanding plant growth, signaling pathways and their adaptive responses to environmental stresses in the *Arabidopsis thaliana* model plant [76–78]. It is now possible to comprehensively manipulate *A. thaliana* genes to study plant development and physiology [79,80]. Despite this, modern knowledge on plant behavior in the field is more limited. The main reason for this is the difficulty of transferring genetic engineering to the study of agriculturally important plants and the lack of appropriate tools for studying the condition of plants in the field [81]. Genomic resources are available for model plants, i.e., rice (*Oryza sativa*), corn (*Zea mays*) and tomato (*Solanum lycopersicum*) [82–84], but they cover only a few families and do not adequately reflect crop biodiversity [85]. In addition, studying the physiology of these plants under varying field conditions remains a long-term challenge [86]. A key challenge for plant biology is bridging the gap between detailed knowledge of model plants and knowledge of physiological processes in plants under field conditions [9].

To solve problems in agriculture, modern spectroscopic technologies are needed to detect and identify biotic and abiotic stresses, as well as plant types. The use of spectroscopic methods by scientists can provide valuable information about the material being studied, enabling them to observe mutual interactions in spectral and chemical changes. Spectroscopic methods are usually fast, but they do not allow the examination of entire cultivated areas at the speed of imaging methods. Most spectroscopy techniques are non-invasive and non-destructive, so they can be used in the field [5]. The immediate results of health analyses of plants grown in fields would allow the detection and identification of the causes of plant diseases in these fields. These data would need to be applied to a specific action in a given area, which would reduce the territory occupied by biotic [66,67] and abiotic stresses [9]. The engineering knowledge gained can influence the development of next-generation agricultural technologies and solve the problems of energy-intensive use of water and fertilizers [87,88]. The optimal use of knowledge and available tools for crop management goes beyond agriculture and positively impacts environmental protection and consumers' health.

Scientists are currently using Raman spectroscopy to monitor biotic and abiotic stresses. Both biotic and abiotic stresses always trigger metabolic reactions in plants. The Raman spectrometer makes it possible, regardless of species, to study plant physiology in real time in a non-destructive manner. As an optical tool, Raman spectroscopy can be regarded as an enhanced version of imaging techniques that have previously been often used in field phenotyping. Nevertheless, the Raman method allows for much greater chemical specificity [9].

4. Raman Method in Monitoring Biotic and Abiotic Stresses of Plants

A growing number of research points to the use of portable Raman spectroscopy to analyze the health of plants and determine the type of stress (biotic or abiotic) [20,89–92]. An example of the use of a handheld Raman spectrometer for crop analysis is illustrated in Figure 4.

These data should be used for specific application of plant protection products, i.e., fertilizers and the irrigation of a precisely defined agricultural area, instead of supplying them for the entire crop. This will result in the quick inhibition of the growth and reproduction of pathogens and will reduce the costs of resource consumption [13,20,63,89,91,93–97]. Raman spectroscopy enables non-destructive biochemical analysis, allowing the study of many molecular species. This method is characterized by high chemical specificity [9]. The scientific literature reports that spectroscopic studies can help to explain the molecular machinery of plant responses to various biotic and abiotic pressures.



Figure 4. Scheme of using portable Raman spectroscopy to assess the health of crop plants.

4.1. Monitoring of Biotic Stresses

The literature describes techniques for using Raman spectroscopy methods to detect and identify plant diseases [17,18,20,51,54,89,91,92,95,96]. Scientific publications report Raman diagnostics confirming fungal infections in sorghum, wheat and corn [18,98]. Researchers also confirmed the potential of applying Raman spectroscopy to identify virus infections of wheat and rose and for the detection of bacteria-causing *Huanglongbing* (HLB, or citrus greening) found on citrus trees [20,92]. This diagnostic treatment is based on the identification of pathogen-induced modifications in the structure and chemical composition of plant molecules. Each pathogen species causes unique changes in the plant [92]. Raman spectroscopy has been used to test corn rot disease induced by *Colletotrichum graminicola* [98], rose rosette disease infection [91] and to test wheat and sorghum grains which are infected by ergot, black tip or mold [17]. Recently, RS has been used in conjunction with deep learning networks to detect wheat grains infected with *Fusarium head blight* (FHB) [99]. Thus, RS has a species-level sensitivity for pathogen diagnosis [63]. Sanchez et al. (2020) conducted a successful experiment using a portable Raman spectrometer for the detection of two haplotypes of *Liberibacter* bacteria in tomatoes. *Candidatus Liberibacter solanacearum* (*Lso*) is a Gram-nonreactive, phloem-limited bacterium that attacks crops all over the world. Using the Raman method, the researchers determined structural changes in carotenoids, xylan, cellulose and pectin caused by the bacterium. They showed that *Lso* disease infection can be successfully detected by the non-invasive spectroscopic analysis of tomato leaves as early as three weeks after infection, before airborne symptoms appear. Combined with chemometric analyses, Raman spectroscopy enabled the diagnosis, with an accuracy of 80%, of *Liberibacter* disease induced by any two different haplotypes [95]. Lin et al. (2020) successfully used Raman spectroscopy to diagnose banana wilt (FWB), which is known as Panama disease and is induced by the soil-borne fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Researchers have developed Raman spectroscopic fingerprints for *Foc* (including mycelium and conidia) and for samples of infected bananas with different levels of symptoms, to be able to distinguish *Foc*-infected bananas from healthy bananas [100]. Mandrile and his team investigated the potential of the Raman method in the identification of tomato [96] and grapevine [101] viruses, among others. Mandrile et al. evaluated the utility of RS in combination with chemometric analysis to control two different and particularly dangerous tomato infections caused by viral pathogens: tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato spotted wilt virus (TSWV). Plants were inoculated under laboratory conditions and observed for 28 days. During this time, measurements of the plants were taken with RS. RS was able to distinguish apparently inoculated (healthy) samples from those infected with the virus, achieving an accuracy of >70% as early as 14 days after inoculation for TYLCSV and >85% only after 8 days for TSWV, demonstrating its usefulness for the early detection of viral

infections. Differences in Raman spectra were observed between the two viruses studied. This study provided specific and detailed information about the infectious virus [96]. Mandrile et al. (2022) also conducted an experiment using RS to detect viruses that attack grapevines: grapevine fanleaf virus (GFLV) and grapevine stem pitting-associated virus (GRSPaV) in *Vitis vinifera* cv. *Chardonnay*. The experiment showed that, when using the Raman method, it is possible to successfully identify infected plants long before phenotypic symptoms appear. The accuracy of the RS method for GFLV was 100% and for GRSPaV was 80%. Researchers have demonstrated that it is possible to analyze the carotenoid content of grapevine leaves using Raman spectrometry. For leaves infected by GFLV, a decrease in carotenoid concentration was noted. The results obtained by the research team indicate that RS is a state-of-the-art technique for real-time monitoring of pathogens in grapevines and can be used to monitor metabolic changes resulting from plant diseases and stresses [101].

4.2. Monitoring of Abiotic Stresses

The literature on the subject increasingly reports the possibility of using Raman spectroscopy to measure plant responses to stress *in vivo*. The first work using Raman spectroscopy to identify stress responses reported measuring metabolites such as carotenoids and anthocyanins as tracers of abiotic stress in plants [93]. Altanger et al. (2017) used the Raman spectroscopy technique for early (in 48 h) *in vivo* detection of plant reactions to stress. The researchers subjected *Coleus* (*Plectranthus scutellarioides*) plants individually to four typical types of abiotic stress: drought, cold exposure, high soil salinity and light saturation. During the experiment, scientists studied plant induction after *in vivo* stress, and changes in the concentration levels of reactive oxygen uptake pigments were observed using Raman microscopy systems, among other techniques. Raman spectroscopy enabled scientists to simultaneously study different pigments in plants. The Raman confocal microscope system equipped with a 532 nm laser was used for microscopic measurements. Plants' leaves were placed directly on the sample holder, without physical separation from the plant. Hence, it is considered non-destructive *in vivo* detection. Twenty Raman spectra were collected from four leaves of each plant. The experiment made it possible to discover a negative correlation in the levels of anthocyanin and carotenoid concentrations, which indicates a finely tuned response of plants to stress. Altanger et al. (2017) demonstrated early detection of plant responses to stress using *in vivo* Raman spectroscopy methods that improved the sensitivity and ability to simultaneously examine individual stress indicators' pigment molecules [93].

Roy and Prasad (2022) used Raman spectroscopy to non-invasively monitor the growth of soybeans—a plant of major economic importance for organic fuel, feed and food. The researchers focused on compositional changes at several time points in plant development, as the plant moves from the vegetative phase to the reproductive phase, when exposed to nitrogen and nutritional pressure, in order to find some indicators of plant health. As a result of the experiment, it was noted that the longitudinal growth of cells was significantly reduced under complete nutritional stress. At the same time, early lignification of the cell wall was observed, which regulated growth and allowed the productive transport of limited resources. Healthier plants exhibited the activation of early defense strategies through tetraterpenes, a higher protein production capacity of glutamic acid and delayed lignification until the 12th week. Plants subjected to nitrogen stress also showed growth inhibition, although they had a similar defense mechanism and subsequent lignification as control plants. The reported differences in the concentrations of structural polymers, stress-signaling molecules and structural growth inhibitors at different time points and under varying stresses demonstrated the utility of Raman spectroscopy for the precise monitoring of plant conditions [87].

Gupta et al. (2020) used a portable Raman leaf-clip sensor for the early diagnosis of nitrogen deficiency in the *Arabidopsis thaliana* model plant and for a wide range of vegetable plants (Choy Sum and Pak Choi). The researchers reported that *in vivo* measurements, obtained using a portable Raman leaf-clip sensor under full-light growth

conditions, were consistent with results obtained under laboratory conditions with use of a benchtop Raman spectrometer. For the experiment, Gupta and al. chose the *Arabidopsis thaliana* model plant, in which metabolic pathways are well-studied, and two leafy vegetable plants from the Brassicaceae family: Pak Choi (*Brassica rapa chinensis*) and Choy Sum (*Brassica rapa* var. *parachinensis*). Three-week-old plants were grown in sufficient (N+) or nitrogen-poor (N−) hydroponic media. A Raman leaf-cutting sensor was used for the early detection of nitrogen levels in plants. The spectra were analyzed at 830 nm. Through the use of spectroscopic analysis, the team of researchers noted a reduction in nitrate content in plants grown on nitrogen-deficient media compared to plants under nitrogen-sufficient conditions, despite no differences in the phenotypic appearance of the plants and no change in chlorophyll content. The region of Raman spectra between 1030 and 1080 cm^{-1} indicated that the 1045 cm^{-1} peak with sufficient (+N) and deficient (−N) conditions were clearly distinguishable [50].

Sanchez et al. (2020), in their article, described the application of a portable Raman spectrometer for the high-precision, pre-symptomatic diagnosis of nitrogen, phosphorus and potassium shortages in rice (*Oryza sativa*). The researchers used the Presidio variety, a high-yielding rice with good grain quality. Plant seedlings in each stress group (nitrogen deficit (ND), phosphorus deficit (PD), potassium deficit (KD), salt stress and high salt stress) were placed in appropriate stressor solutions of equal pH (pH 5.0). Spectrum acquisition and measurements were performed on the 2nd, 4th, 6th, 8th, 11th and 13th days after the introduction of stress. Raman spectra were gathered using a portable spectrometer with an 830 nm laser source. The experiment proved the effectiveness of RS in the marker-free, non-invasive and non-destructive detection and identification of phosphorus, potassium and nitrogen deficiency, as well as excessive salinity, in rice plants. Spectra assembled from leaves of control plants showed vibrational bands attributable to pectin, cellulose, xylan, carotenoids, phenylpropanoids, proteins and aliphatic vibrations. The spectra assembled from ND, PD and KD test samples showed lower intensities of vibrational bands derived from pectin, cellulose, xylan, aliphatic vibrations and carotenoids, relative to vibrational bands in the spectra of control plants. The results obtained may prove that deficiencies in the elements N, P and K can be associated with reductions in pectin, cellulose, xylan and carotenoids in the plants studied. These data have shown that, during early development, these stresses can be detected with a high degree of precision and identified within as little as 1 s of spectrum recording [63].

The scientific literature reports that spectroscopic studies can help to explain the molecular mechanisms of plant defense against to different biotic and abiotic stresses.

5. Plant Diagnostics Using the SERS Method—Surface-Enhanced Raman Scattering

When diagnostics require additional sensitivity, a good alternative to conventional Raman spectroscopy is SERS—surface-enhanced Raman scattering [2]. SERS is a phenomenon discovered in 1974 by Fleischman and his team [102]. He noted that the Raman signal of pyridine molecules adsorbed on the surface of coarse-grained silver electrodes can be significantly enhanced. This is mainly related to two different amplification mechanisms: electromagnetic amplification [103] and chemical amplification [104]. Currently, miniature Raman spectrometers are being developed, which have become a breakthrough in SERS imaging from laboratory to mobile applications. These advantages make the SERS technique an ideal method for diagnosing plant pathogens [105]. Figure 5 shows an example of the use of the surface-enhanced Raman scattering (SERS) method to detect mercury (Hg^{2+}) ions, in a diagram.

Substantive literature has reported on positive attempts to use SERS to detect fungal toxins present at low concentrations in grain. Lee et al. used SERS to quantify the metabolite of a toxin, produced by *Aspergillus favus*, in corn, at a concentration range of 0–1206 $\mu\text{g}/\text{kg}$ [106]. Farber and Kourouski used SERS spectroscopy to analyze spectra collected from healthy roses and roses infected with rose rosette disease. The researchers succeeded in presenting a simple form of rose rosette disease diagnosis that, in its simplicity,

does not require other factors, e.g., the geographical location of the plants, plant varieties or abiotic stresses to which the plants may be exposed. However, the authors point out that the consideration of these factors is required to develop Raman spectroscopy into a reliable tool for analysis and detection [2]. Zhang et al. successfully used a portable Raman spectrometer with SERS for multiple detection of mycotoxins in corn. The researchers presented a SERS-based assay for the detection of multiple mycotoxins achieving an analytically relevant detection limit (~ 1 ng/mL). This sensor consists of a magnetic core and a mycotoxin-absorbing polydopamine shell, with SERS-active Au nanoparticles on the outer surface. The assay can concentrate multiple mycotoxins, which are identified through multiclass partite least squares analysis based on their SERS spectra [107]. Zeffino et al. developed a SERS sensor to detect anthocyanidins, which are produced by plants in response to abiotic stress factors. The researchers analyzed the pH dependence of anthocyanins. SERS spectra of six anthocyanin derivatives (cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin) showed significant differences, despite minor differences in molecular structure. SERS proved to be a good method for the rapid detection of anthocyanidin derivatives [108]. In addition, innovative SERS techniques are a very good tool for the real-time monitoring and detection of pesticides in plants [3].

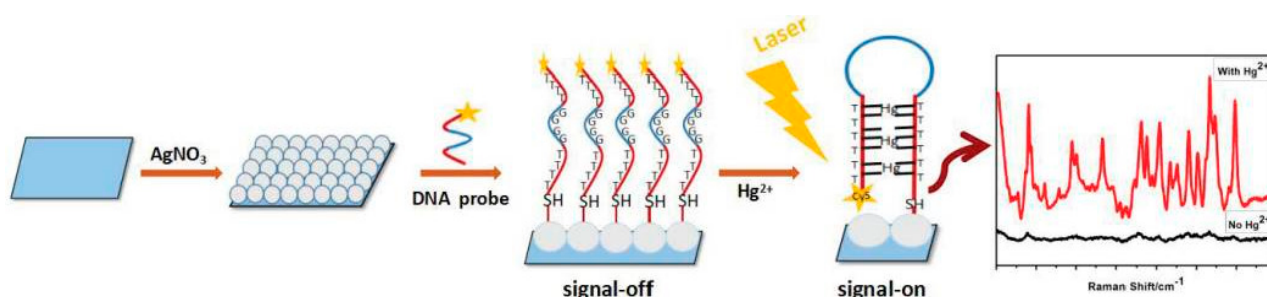


Figure 5. Mechanism of surface-enhanced Raman scattering sensor (SERS) using silver colloids. Adapted from [1].

6. Analysis of Plants with a Raman Instrument

The following table (Table 2) collects examples of the application of the Raman method in various types of plant research.

Table 2. Raman spectrometer in plant research.

Method	Purpose of Analysis	Plant	Laser	Conclusion	Ref.
Raman spectrometer	The detection of grapevine fanleaf virus (GFLV) and grapevine stem pitting-associated virus (GRSPaV).	Grapevine: <i>Vitis vinifera</i> cv. Chardonnay	785 nm	Raman spectroscopy applications in grapevine: metabolic analysis of plants infected by two different viruses.	[101]
Hand-held Raman spectrometer	The detection of two haplotypes of Liberibacter bacteria, <i>Candidatus Liberibacter solanacearum</i> (Lso).	Tomatoes	831 nm	Lso disease infection can be successfully detected by the non-invasive spectroscopic analysis of tomato leaves as early as three weeks after infection, before airborne symptoms appear.	[95]
Hand-held Raman spectrometer	The detection of <i>Colletotrichum graminicola</i> .	Maize or corn (<i>Zea mays</i>)	830 nm	Detection and identification of plant pathogens on maize kernels.	[98]
Hand-held Raman spectrometer	The detection of fungal pathogens <i>Aspergillus flavus</i> , <i>A. niger</i> , <i>Fusarium</i> spp. or <i>Diplodia</i> spp.	Maize or corn (<i>Zea mays</i>)	1064 nm	Detection and identification of plant pathogens on maize kernels.	[17]

Table 2. Cont.

Method	Purpose of Analysis	Plant	Laser	Conclusion	Ref.
Hand-held Raman spectrometer	The detection of rose rosette disease (RRD).	Garden shrub rose (<i>Rosa</i> sp.)		Raman spectroscopy can be used as an early detection tool for rose rosette infection.	[91]
Hand-held Raman spectrometer	The detection of bacteria causing <i>Huanglongbing</i> (HLB, or citrus greening).	Orange trees and grapefruit trees	830 nm	RS can be used for Huanglongbing (HLB) diagnostics on both orange and grapefruit trees.	[92]
Hand-held Raman spectrometer	The detection of the carotenoid content in the peel.	Citrus fruit	532 nm, 785 nm	The analyses found a strong correlation between the carotenoid content of the peel and the intensity of the Raman signal.	[37]
Hand-held Raman spectrometer	The identification of different genotypes.	Peanut leaves	830 nm	Farber and colleagues showed that, by acquiring Raman spectroscopy spectra of peanut leaves, it is possible to identify different peanut varieties and genotypes.	[2]
Confocal Raman microscopy	The determination of changes in different stages of growth and in different varieties.	Wheat grain (<i>Triticum aestivum</i>)		By examining wheat grain, the evolution of the protein content and structure during the growth of different cultivars of wheat grain can be followed.	[109]
Confocal Raman microscopy	Maturation stage assessment.	Tomatoes (<i>Solanum lycopersicum</i> cv <i>Cerise</i>)	532 nm	The researchers investigated the effects of biochemical parameters on cell wall microstructure and changes in polysaccharide distribution during the process of physiological development of tomato fruit.	[28]
Confocal Raman microscopy	The determination of changes in different stages of growth and during storage.	Apple	532 nm	RS was used to assess changes in the distribution of polysaccharides in the cell walls of the apple flesh of ripening fruit and during storage after harvest.	[110]
Spatially offset Raman spectroscopy (SORS) technique	The lycopene content, the main carotenoid content during fruit ripening.	Tomatoes	785 nm	The researchers analyzed lycopene content in tomato fruit samples representing different stages of ripeness (i.e., green, breaker, turning, pink, light red and red).	[111]
Confocal Raman microscopy	The mechanisms underlying fruit lignification.	Cultivars of loquat fruits (<i>Eriobotrya japonica</i> Lindl. cv. 'Luoyangqing', LYQ and Baisha, BS)	405 nm	The findings of the study showed that Raman spectroscopy can effectively be used to assess fruit lignification, in order to determine fruit maturity.	[112]
Raman spectrometer	Maturation stage assessment.	Olive fruit		The study demonstrated the ability of RS to track oil accumulation in olive fruit. The researchers showed that there are increases in carotenoid and phenolic contents during olive growth and decreases in these values during the ripening stage, which can effectively be monitored using RS.	[113]
Raman spectrometer	The identification of maize varieties.	Maize or corn (<i>Zea mays</i>)	831 nm	Krimmer and colleagues showed that RS can be used to identify six different maize varieties based on their unique spectra.	[52]

7. Future Prospects

The potential uses of Raman spectroscopy in agriculture were overlooked for a long time. This was a consequence of the low efficiency of normal Raman scattering and the high price of the equipment, which only allowed laboratory analyses [1,28]. The development of technology has led to the improvement of the Raman spectrometer, a reduction in its size and, finally, the construction of portable Raman spectrometers. The introduction of new and improved Raman spectroscopy techniques by scientists has increased its importance in discovering and defining tissues and the processes occurring in them [1]. The results of studies presented by researchers in recent years have shown the great potential of this technique in the following areas: the detection and identification of plant diseases [17,18,20,51,54,89,91,92,95,96]; the diagnosis of abiotic stresses [51,58,63,93]; the identification of plant species and varieties and the explanation of their phenotypes [13,53,90,114–116]; and the examination of the nutritional value of plants and seeds [13,53]. The undoubted advantages of Raman spectroscopy are its non-invasiveness, being able to obtain a real-time result and its non-destructive nature. Another advantage of Raman spectrometry, which is of great economic importance, is that samples for analysis do not require complex processing; thus, the cost of performing analysis is limited to the purchase of the apparatus. In digital agriculture, the mobility of the equipment is very important, i.e., the relatively inexpensive portable Raman spectrometers that are now available. Portable Raman spectrometers enable the method to be used in a crop field, greenhouse, grain elevator, unmanned aerial vehicle or harvester. However, the presented method also has its limitations. One of the biggest constraints is still the high cost of the apparatus. The price of portable Raman spectrometers is similar to that of portable Infrared (IR) Spectrometers and real-time PCR (polymerase chain reaction). Despite this, it will be impossible for most average farmers to purchase such an expensive equipment. Commercially available Raman spectrometers cost a minimum of USD 12,000 [117]. The solution to such a problem is to implement the method of Raman spectroscopy as an offering in agriculture that a farmer can commission to examine a crop field. It is expected that, with the development of digital agriculture, there will be an increase in the importance of the Raman method in agriculture. Currently available portable spectrometers, although suitable for use outside the laboratory, require direct contact between the apparatus and the sample under study during analysis. Despite the adaptation of portable Raman spectrometers for field work, only a few researchers have used Raman spectrometry for plant analysis directly in the field [20,51,54]. In the near future, it is very important to continue working on adapting and reducing the size of portable Raman devices and lowering their cost. A huge challenge for Raman spectroscopy is the diagnosis of more than one infection occurring on a plant at the same time. Equally challenging is the simultaneous diagnosis of biotic and abiotic stresses on the same plant. Researchers report that Raman spectrometry can be successfully used for the quick screening of plants to detect several diseases occurring simultaneously on a plant [3].

8. Conclusions

The Raman spectroscopy technique can be enhanced by coupling it with established imaging techniques [118,119] and molecular techniques [120–122]. Unmanned aerial vehicles (UAVs) with instrumentation, i.e., thermal and RGB (red, green, blue) color system sensors, may prove to be indispensable to analyze large areas of fields, which can guide Raman spectrometers to locations where changes have occurred [118,123]. UAV-based methods may result in limited resources for diagnosing biotic and abiotic stresses in crop fields. RS can work well as a “quick screening test”, which can be applied to a larger number of diseases present on a plant [3]. The Raman spectroscopy method presented in this article can help compensate for economic losses caused by biotic and abiotic stresses by detecting them early in the crop. To facilitate the widespread adoption of this technology in agriculture, its economic potential and reliability must be verified to assure farmers and entrepreneurs that it will remain affordable and more effective than current solutions. Research analyses

of the viability of portable Raman spectroscopy for use in precision agriculture have shown significant economic benefits in relation to the costs incurred. Advanced analytical tools such as RS not only increase efficiency from a cost point of view—i.e., they influence, for example, a reduction in fertilizer application, the limitation of sprays to local application and the adjustment of water quantity dosage—but they can also bring benefits on the revenue side, in the form of higher product quality. They may be used to help determine the optimum harvest time to achieve a certain flavor profile of a crop and may contribute to achieving a product for which consumers will be willing to pay a higher price. The biggest challenge for researchers is translating the raw data collected into meaningful information, which can enable growers to make cost-effective decisions [9]. Sustained collaboration with the public, growers and plant cultivation programs will be the way to speed up the acceptance and introduction of advanced Raman methods into daily farming practices. It is critical that more tests be conducted under field conditions with critical evaluation of their reliability and economic potential for sustainable implementation of these technologies in precision agriculture.

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