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ORIGINAL ARTICLE

Detection of significant wavelengths for identifying and classifying *Fusarium oxysporum* during the incubation period and water stress in *Solanum lycopersicum* plants using reflectance spectroscopy

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Abstract

Spectroscopy has become one of the most used non-invasive methods to detect plant diseases before symptoms are visible. In this study it was possible to characterize the spectral variation in leaves of *Solanum lycopersicum* L. infected with *Fusarium oxysporum* during the incubation period. It was also possible to identify the relevant specific wavelengths in the range of 380–1000 nm that can be used as spectral signatures for the detection and discrimination of vascular wilt in *S. lycopersicum*. It was observed that inoculated tomato plants increased their reflectance in the visible range (Vis) and decreased slowly in the near infrared range (NIR) measured during incubation, showing marked differences with plants subjected to water stress in the Vis/NIR. Additionally, three ranges were found in the spectrum related to infection by *F. oxysporum* (510–520 nm, 650–670 nm, 700–750 nm). Linear discriminant models on spectral reflectance data were able to differentiate between tomato varieties inoculated with *F. oxysporum* from healthy ones with accuracies higher than 70% 9 days after inoculation. The results showed the potential of reflectance spectroscopy to discriminate plants inoculated with *F. oxysporum* from healthy ones as well as those subjected to water stress in the incubation period of the disease.

Keywords: band selection, hyperspectral reflectance, plant diseases, relevant specific wavelength, vascular wilt

Introduction

Remote sensing and nearly sensing methods, like multi or hyperspectral sensors, have been widely applied in agriculture, livestock, industries, and even some sectors of pharmaceutical and human medicine (Huang *et al.* 2007; Hatfield *et al.* 2008; Berzaghi and Riovanto 2010; Teixeira *et al.* 2013). Additionally, remote sensing technology provides an alternative method that is unbiased and automatic for visual evaluation of plant diseases (Mahlein *et al.* 2012), even in the early stages of evolution when the symptoms are not visible (Khaled *et al.* 2017).

After symptom expression of a plant disease, the disease can be verified through detection techniques. Detection techniques of plant diseases that are currently available can be divided into four groups: 1 – serological methods: flow cytometry, Enzyme Linked Immunosorbent Assay (ELISA) and immunofluorescence, 2 – "Purely molecular" methods: Fluorescent *In Situ* Hybridization (FISH), Polymerase Chain Reaction (PCR) and DNA Arrays, 3 – disease detection based on biomarkers: profiles of metabolites in the gas phase and profiles of plant metabolites,

4 – disease detection based on plant properties and stress, which includes: imaging techniques (hyperspectral and fluorescence images) and spectroscopic techniques (Vis/NIR), infrared (NIR), fluorescence and multispectral bands (Sankaran *et al.* 2010). In recent decades, research in this last group of technology has led to the development of methods based on spectroscopy for the detection of diseases and stress in plants. These methods are faster, non-destructive, sensitive and selective for detection of disease during its incubation phase.

These techniques are based on measuring the amount of radiation reflected on a surface in function of the wavelengths to produce unique reflectance spectra for each material. These spectra can be used as a "fingerprint" (spectral signature), since they sense healthy and diseased plants in different states of evolution, even in cases when the symptoms of the disease are not yet visible (Zhang *et al*. 2003; Huang *et al*. 2004; Larsolle and Muhammed 2007; Mahlein *et al*. 2010; Marín-Ortiz *et al*. 2018; Marín-Ortiz *et al*. 2019). Characteristics of spectral radiation, reflected, transmitted or absorbed by the leaves, can provide a deep understanding of the histological, physiological and biochemical responses to growth conditions and adaptations of plants to the environment. However, as a result of increased interest in remote sensing, leaf reflectance has been studied more than absorbance and transmittance as stress responses in plants (Carter 1994; Gregory *et al*. 2001).

The efficiency of measuring spectral reflectance for detection of diseases is based on the identification of the most significant spectral wavelength, which is highly correlated with a specific disease (Song *et al*. 2011; Mahlein *et al.* 2013), since it is found in only some regions of the electromagnetic spectrum of interest. Jacquemoud and Ustin (2001) divided the spectral range from 400 nm to 25,000 nm into three large bands. The first is the Vis range (~380–750 nm), in which photosynthetic pigments have a greater impact on spectral signature, characterized by low reflectance values and a maximum peak located ~550 nm. The second one includes the near infrared plateau (7,500–1,100 nm), a high reflectance spot due to multiple dispersion within the leaf in relation to the fraction of air spaces within the tissue (internal structure) and/or its water content. Finally, the third one includes near infrared (1,100–2,500 nm), which is a low reflectance zone, due to high absorption of water mainly intissues such as fresh leaves and dry matter.

Significant wavelengths have been identified as a base to develop a large number of Spectral Vegetation Indices (SVE) (Robert *et al*. 2011), as well as a method to detect and analyze changes in physiological and biochemical parameters in plants (Merzlyak *et al*. 2003a, 2003b; Gitelson *et al*. 2003; Gitelson *et al*. 2007). However, it is not possible to perform a quantitative definition or identification of a particular disease based on common SVEs because the method lacks specificity for diseases (Mahlein *et al.* 2013). Therefore, it is necessary to determine the Relevant Specific Wavelengths (RSW) for the construction of Spectral Disease Indices (SDI) that can be used to simplify the detection of diseases by spectral sensors, since each disease uniquely influences the spectral signature in a characteristic way.

In this research were conducted experiments under semi-controlled conditions to identify important spectral wavelengths for the early detection of *F. oxysporum* in tolerant and susceptible plants of *S. lycopersicum*. This organism model is widely accepted for the study of pathogenicity in plants (Baayen *et al*. 2000). First, were carried out a characterization of spectral variation in leaves of *S. lycopersicum* infected with *F. oxysporum* during the incubation period of the disease and subjected to mild water stress. Then were identified RSW on 380–1,000 nm, that could be used for detection of spectral signatures in *S. lycopersicum* plants infected with *F. oxysporum* before the expression of visible symptoms. Finally were tested or discriminated infected *S. lycopersicum* plants with *F. oxysporum* from healthy plants as a test of the RSW identified in the previous step.

Materials and Methods

Biological material

The plants used in this study were maintained under semi-controlled greenhouse conditions, located at the National University of Colombia Medellín (Antioquia, Colombia). Average temperatures ranged between 18 and 24°C, relative humidity between 60 and 70% and there was a photoperiod of 12 h during the experiments. In this study, the Ponderosa tomato variety, which is susceptible to all races of *F. oxysporum* (Reis and Boiteu 2007), and the Santa Cruz variety, which is resistant to races 1 and 2 were used. The seeds were planted in germination trays of 86 wells with sterile peat as a substrate and kept in the greenhouse for the duration of the respective experiments. The plants were irrigated daily, fertilized once a week with a nutritious solution and a protective action fungicide was applied every 7 days, starting when the plants were 7 days old, according to the management plan. After 4 weeks of germination, the inoculation procedure was carried out and individual plants were placed in 900 cm^3 plastic cups containing the same substrate as that used to plant the seeds. In this study, five

treatments were evaluated: 1 – tomato plants (var. Ponderosa) inoculated at 4 weeks after germinating with a pathogenic strain of *F. oxysporum* (Fo5), 2 – healthy plants (var. Ponderosa) submitted to hydric stress sustaining 60% of field capacity, 3 – healthy plants (var. Ponderosa) and substratum maintained with 100% field capacity as control treatment, 4 – plants of tomato (var. Santa Cruz) infected with Fo5, 5 – healthy plants (var. Santa Cruz) and substratum maintained at 100% field capacity. The plants subjected to these treatments were kept under almost the same conditions as the greenhouse during the rest of the experiment. Fertilization with nutritious substances was increased to twice every week. *F. oxysporum* Fo5 strain isolated from *Passiflora edulis* (passionfruit) was used because it is highly pathogenic on tomato plants (Marín-Ortiz *et al*. 2018; Marín-Ortiz *et al*. 2019). This strain has an incubation period of 24 days post infection (dpi) on tomato plants.

Inoculation

Four-week-old tomato plants were inoculated according to the modified procedure described by Ortiz and Hoyos-Carvajal (2016) with a suspension of spores of isolate Fo5. Ten milliliters of spore suspension of *F. oxysporum* were prepared in distilled water. The concentration of the spore suspension used was 1×10^6 spores · ml^{-1}. The tomato seedlings were removed gently from the nurseries and the roots were washed with tap water to remove the remains of peat. Then wounds (cuts) were made on the secondary roots of all the plants with a sterile scalpel and only the roots were immersed in 15 ml of the solution of distilled water and spores for 20 min. The same procedure was performed on the inoculated plants. The control plants were inoculated only with distilled water, and subjected to water stress. The seedlings submitted to the different treatments were transplanted to the vessels with sterile peat of 900 cm^3. To verify the efficiency of the inoculation (postulate three of Koch), a cross section from the neck of roots from five plants of each treatment was made (days 0, 12 and 24 dpi). The tissue was disinfected and diluted in distilled sterilized water 1 : 10 (w/v). The homogenized solution (100 ml) was placed on Potato Dextrose Agar (PDA) + malachite green oxalate supplemented with 200 ppm chloramphenicol. During the first 4 days after sowing, observations were made under a microscope and the *F. oxysporum* colonies that grew in the medium were counted according to the following formula:

$$CFU/g = CFU/(V_p + D_f)$$

where: CFU/g – the number of colonies per one gram; CFU – the number of viable fungal cells; V_p – plant volume; D_f – dilution factor.

Spectroscopy

For the acquisition of Vis/NIR reflectance spectra, a HR2000 portable spectroscope (Ocean Optics, USA) with a tungsten halogen light source HL-2000-HP (wavelength range of 360–2,400 nm), a diffuse reflectance standard model WS-1 (reflectivity >98% in the range of 250–1,500 nm) and a 600 μm premium grade reflectance probe QR600-7-VIS-125F were used. The measurements were made with the optical fiber attached to the adaxial surface of the leaf, in which five spectra were taken for each leaf. Different parameters required for spectroscope calibration, such as integration time, average readings per measurement and interval time were determined at the beginning of the experiments.

Pathogenicity test

Destructive samplings were executed to confirm plant infection in the following way: a cut of 1 cm was made from the neck of the stem of each plant and each segment was cut into five equal parts, approximately 2 mm long each. Then, the segments were disinfected in 70% alcohol, 2% sodium hypochlorite and water. Finally, the five cuts of the stem of each plant were placed in Petri dishes with PDA medium + 300 ppm of Gentamicin. Seven days after sowing the five stem segments, each Petri dish and stem segment were observed for the presence and growth of the pathogen.

Data analysis

The results obtained in this work are presented as a function of the treatment realized in two tomato varieties infected with a strain of *F. oxysporum* and their respective controls through the incubation period of the disease (before the appearance of visible symptoms). A comparison of the effects caused by the treatments was carried out on leaves in the same stage of growth and development. Five reflectance spectra of the adaxial face of the second leaf of each tomato plant were measured in the 380–1,000 nm spectral range with a spectral resolution of ~0.5 nm, using an "Ocean Optics HR2000 spectroscope".

Initially, a spectra selection was made to remove noise, either by being deformed and/or with reading error. The spectra that showed very different patterns were confirmed with analysis of "outliers" identified in a Principal Component Analysis (PCA) without prior data treatment. After the elimination of the spectra with noise and previous treatments, several types of transformations were applied to reduce the impact of the difference in illumination, variety of the plant or specific effects of the sensor. After several analyses, the normal standard variate transformation (SNV) was

chosen as one of the best pre-treatments that allow a good grouping of the plants through the treatments. After performing the pre-treatment of raw data, an analysis of the variance was made from the absolute differences between the reflectance means of the plants of *S. lycopersicum* (two varieties) subjected to biotic stress (infected with *F. oxysporum*) and abiotic (water stress) with healthy plants, and standard deviations of reflectance. Subsequently, a binary classification of healthy leaves and diseased plants was made to test the detection and the later classification of disease by RSW. To reduce the information of the measured spectrum and obtain these RSW to separate diseased and healthy plants, the RELIEF algorithm was used (Robnik-Šikonja and Kononenko 2003). Finally, the RSW identified in the previous step were used to perform Linear Discriminant Analysis (ADL) in order to characterize or separate tomato plants subjected to biotic stress (infected with Fo5) and abiotic (hydric stress) during the incubation period of disease. All statistical analyses were performed using Software R. The main libraries and functions used are summarized in Table 1.

Results

Variation of the spectral signatures

Changes in treatments were analyzed as the absolute differences between statistical average reflectance of *S. lycopersicum,* and subjected to biotic stress, less the reflectance on healthy plants (Fig. 1). Difference in reflectance increased with *F. oxysporum* colonization on two tomato varieties evaluated (380–750 nm) 22–24 dpi. On these days, the susceptible variety displayed drastic changes at same time as visible alterations or symptoms occurred, while the tolerant plants revealed slight differences in spectra, and there were no visible symptoms of disease. In the infrared (750–1,000 nm) showed a sustained increase in the difference of the tolerant variety with their respective healthy controls until 21 dpi, decreasing markedly 24 dpi. In the susceptible variety there was an increase 12 dpi, and then, fell. Taking into account the standard deviations, it can be affirmed that only the mean varied, but there was no disparity in the dispersion of the data in each treatment. The highest differences on reflectance were on limit of red (750 nm), usually after the first week of infection. Besides, plants with water stress displayed different patterns, with a lack of reflectance from the first week on visible and NIR, 750–1,000 nm being the region with a high difference of reflectance, during which the assessment standard deviation was kept constant.

Relevant Specific Wavelengths (RSW) for detection and classification of plants subjected to two types of stress

Since vascular wilt is a systemic disease in tomato plants, a binary classification of healthy leaves and diseased plants was made to test the detection and subsequent classification of the disease by RSW. To reduce the information of the measured spectrum and obtain these RSW the RELIEF algorithm was used to separate diseased and healthy plants (Fig. 2).

On day 0 (plants without infection and without water stress) weights for wavelengths were constant and close to "0" for the two types of stress evaluated in the measured range, without highlighting any relevant RSW (Fig. 2A and F). The specific wavelengths relevant to infection by *F. oxysporum* (biotic stress) were 510–520 nm, 650–660 nm and 750 nm, clarifying that the ranges of 510–520 nm, 650–660 nm were relevant from 3 dpi (Fig. 2B), while the wavelength of 750 nm began to be important approximately 9–12 dpi (Fig. 2C and D). No RSW was observed in plants subjected to biotic stress in the infrared range measured (750–1,000 nm).

Table 1. Description of the main analyses used with the Software R

Analysis	Library name	Function name	Description
Pre-processing	mdatools, prospectr	prep.snv, gapDer	apply the transformations: standard normal variate (SNV) and derivatives of different orders to the rows of the data matrix
Detection and elimination of extreme points	mvoutlier, rrcov	pcout, PcaHubert	fast algorithm to identify multivariate outliers in high dimensionality data, using the Filzmoser algorithms (Filzmoser *et al*. 2007), and ROBPCA (Hubert *et al*. 2005)
Data visualization	mdatools, ggplot2 graphics	mdaplot, ggplot, plot	functions used for data visualization (scatter plots, bars, histograms)
RSW Selection	dprep	relief	this function implements the RELIEF feature selection algorithm (Kira and Rendel 1992)
Discrimination of treatments	MASS	lda, predict	functions used to calculate linear discriminant analyses and matrices of confusion

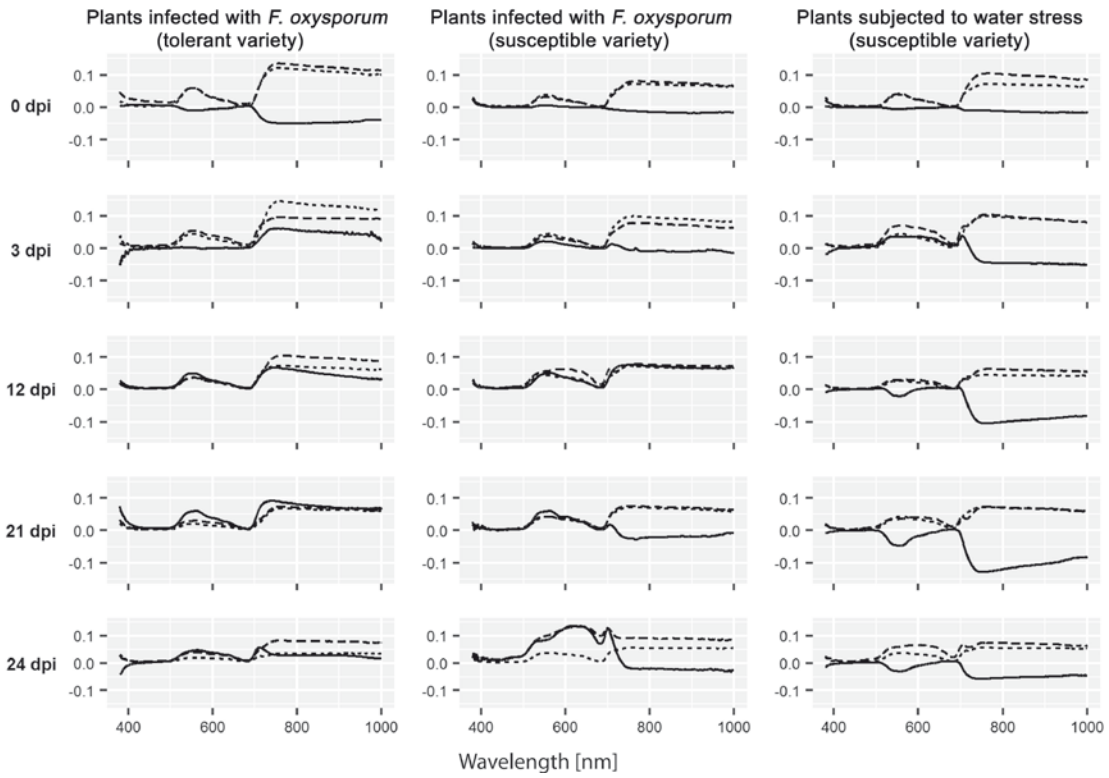

Fig. 1. Differences between the average reflectance (———) and standard deviation of tomato leaves subjected to both types of stress compared to healthy controls: infected with *F. oxysporum* and subjected to water stress (- - - - - - - -) standard deviation on control plants, (– – – – –) standard deviation on treatment); dpi – days post infection

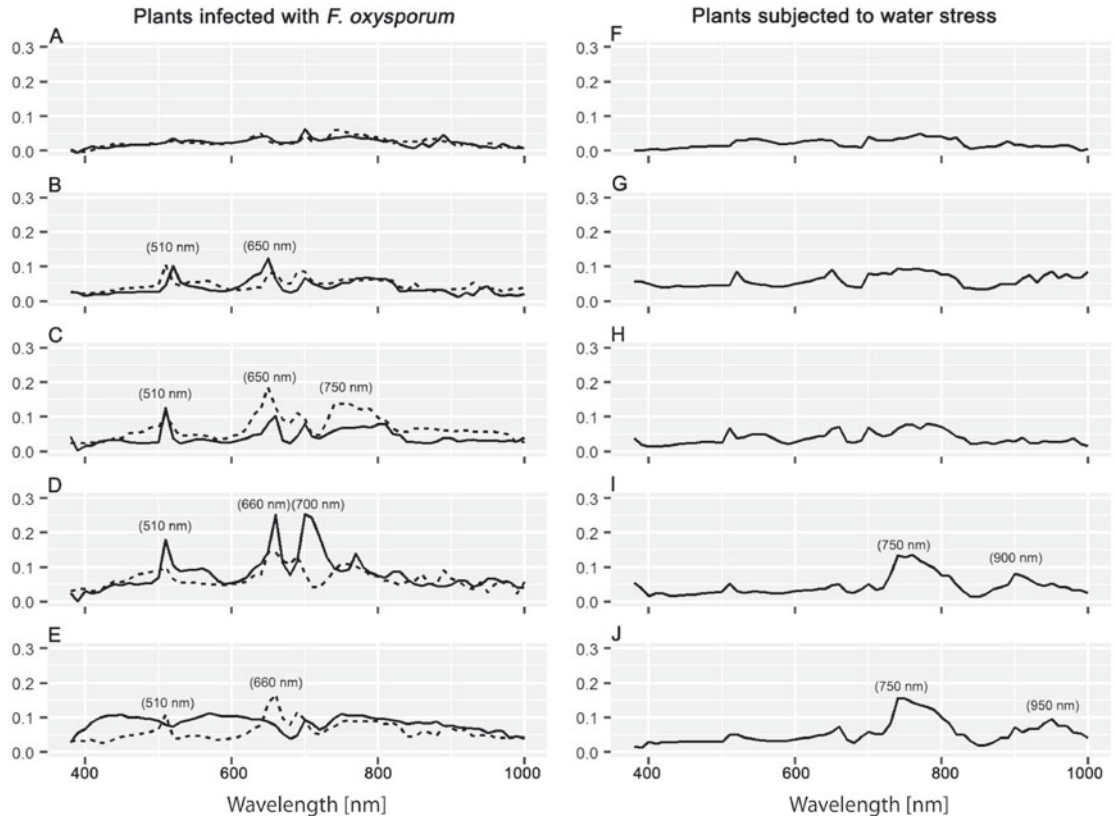

Fig. 2. Relevant specific wavelengths for the two tomato varieties during the incubation period of *Fusarium oxysporum* infection (A–E): 0 dpi (A), 3 dpi (B), 12 dpi (C), 21 dpi (D) and 24 dpi (E); tolerant variety (- - - - - - - - - -) and susceptible variety (———); the R for the susceptible variety of tomato subjected to water stress are illustrated in F–J

Table 2. Relevant specific wavelengths selected for the detection and classification of plants of both tomato varieties (tolerant and susceptible) subjected to two types of stress, biotic (*Fusarium oxysporum* – Fo5, during the incubation period) and water stress (ws); dpi – days post infection

dpi	Biotic stress (Fo5)		Abiotic stress (ws)
	tolerant	susceptible	susceptible
0	–	–	–
3	510	520, 650	–
6	510, 550, 710	520, 650	–
9	650, 760	520, 650	–
12	510, 650, 750, 880	510, 660	–
15	650, 740, 890	510, 650	–
18	–	–	750, 900
21	510, 650, 770	510, 660, 710	750, 950

The scores of these RSW increased with the period of incubation of the disease in the plant, but decreased markedly in the susceptible variety to 24 dpi (their weights tended to be constant), when the symptoms were observed on them, whereas the RSW in the tolerant plants continued with the same pattern observed during the previous days (Fig. 2E). The RSW for plants subjected to water stress were 750 nm and the range 900–950 nm, was observed after 18 dpi (Fig. 2I and J).

The RSW with scores greater than 0.1 that were selected after analysis with the RELIEF algorithm are summarized in Table 2.

Classification of tomato plants infected with *Fusarium oxysporum*

Figure 3 summarizes classification percentages of plants compared to their healthy controls during the incubation period of disease, this is a result of the confusion tables in Linear Discriminant Analysis (LDA) using the RSW selected with the RELIEF algorithm. The tolerant variety of tomato infected with *F. oxysporum* exhibited a progressive increase in the classification percentage compared to its controls since infection, reaching the highest value (92.76%) 12 dpi and maintained little variation until 24 dpi (Fig. 3, series A). Otherwise, susceptible tomato plants showed intermediate values of correct classification, between 68 and 72% from 3 to 15 dpi, respectively, abruptly increasing on day 18 from coincident at the same time as early visual symptioms with early visual symptoms (Fig. 3, series B). Susceptible plants subjected to water stress showed a pattern similar to the one described above, maintaining constant classification percentages between 71.00 and 76.00%, but increasing rapidly on day 15 and reaching 93.00% 21 dpi (Fig. 3, series C).

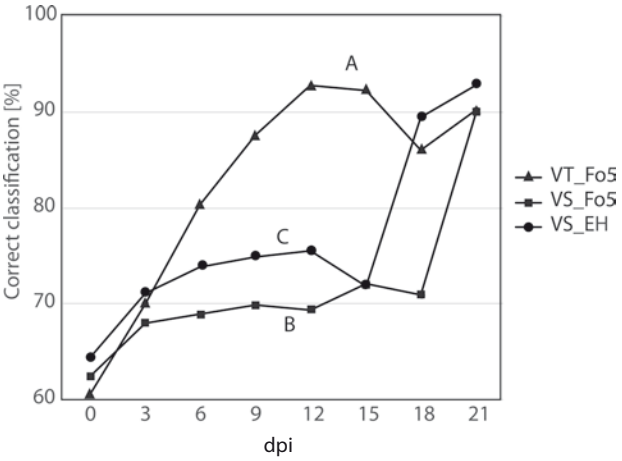

Fig. 3. Temporal variation on classification percentage for plants subjected to biotic stress (Fo5) and abiotic stress (ws). A – tolerant variety infected with *Fusarium oxysporum* (VT_Fo5); B – susceptible variety infected with *F. oxysporum* (VS_Fo5); C – susceptible variety subjected to water stress (VS_EH); dpi – days post infection

Histograms for each group in the first discriminant dimension were made to visualize post-infection during 24 dpi, when the separation of the groups of plants can be observed compared to their respective controls (Fig. 4). Tolerant tomato plants infected with *F. oxysporum* are clearly discriminated from healthy plants from 9 to 12 dpi (Fig. 4A). Also, the plants of the susceptible variety achieved an acceptable classification after 12 dpi (Fig. 4B), although it was appreciably lower than the previous treatment. Regarding plants subjected to water stress, it should be noted that the water deficiency to which they were subjected was slight (field capacity at 60%), so a classification percentage >85% was observed for 18 and 21 dpi (Fig. 4C).

Discussion

Spectral reflectance analyses are useful for detecting different types of biotic and abiotic plant stress due to changes in the light absorption incident in the Vis/NIR range of the electromagnetic spectrum (Sankaran *et al.* 2010; Khaled *et al.* 2017). Additionally, the relationships between the physiological, histological and biochemical changes generated in the plant by different pathogens affect the spectral characteristics and can be detected before the expression of symptoms in the Vis/NIR regions. Currently there are few studies dedicated to the *S. lycopersicum-F. oxysporum* pathosystem using the reflectance spectroscopy technique focused on the detection of the disease (Salman *et al.* 2012; Abu-Khalaf 2015). However, these did not investigate in depth early detection during the incubation period or the search for LOER that are related to the disease.

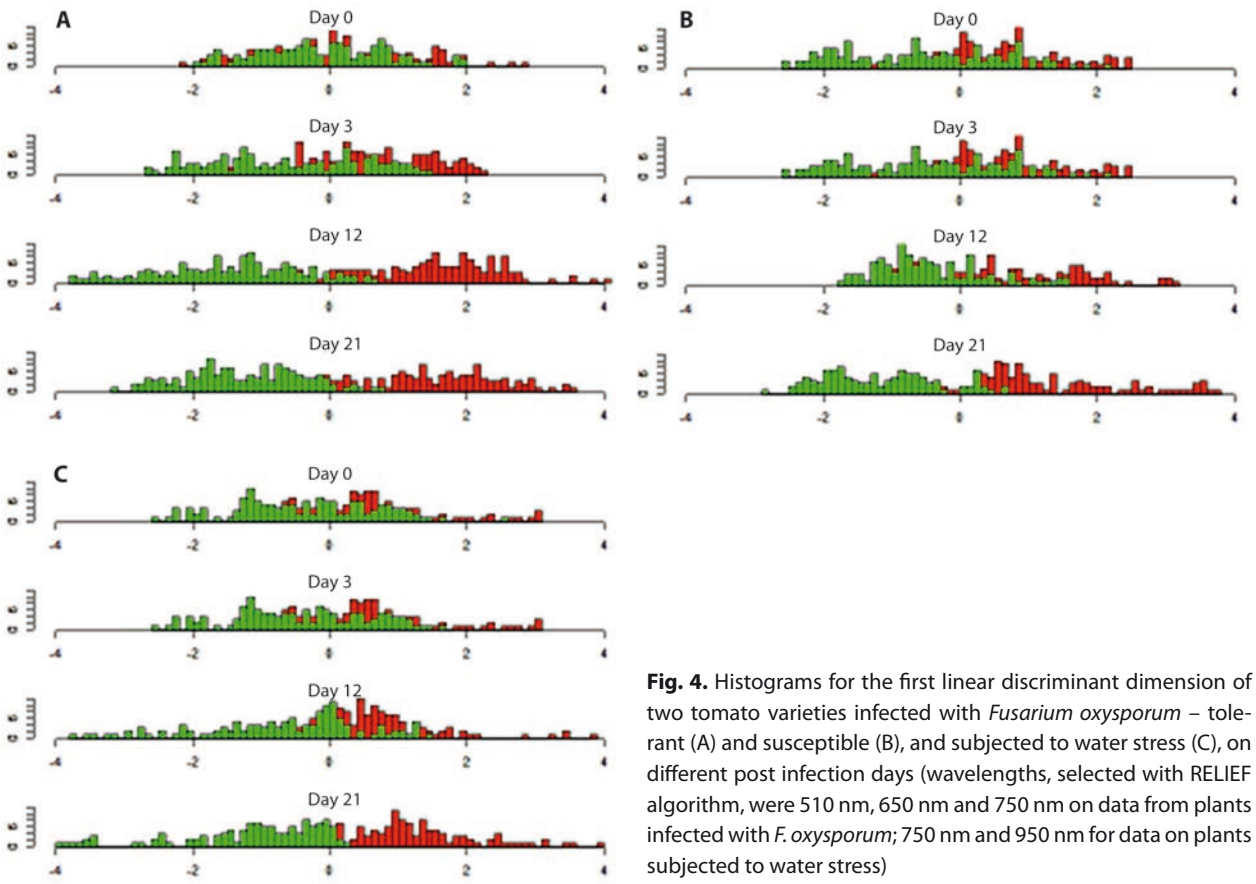

Fig. 4. Histograms for the first linear discriminant dimension of two tomato varieties infected with *Fusarium oxysporum* – tolerant (A) and susceptible (B), and subjected to water stress (C), on different post infection days (wavelengths, selected with RELIEF algorithm, were 510 nm, 650 nm and 750 nm on data from plants infected with *F. oxysporum*; 750 nm and 950 nm for data on plants subjected to water stress)

The data variance set analyzed from the absolute differences between the reflectance means of *S. lycopersicum* plants subjected to stress by *F. oxysporum* (biotic) and water stress (abiotic) with healthy plants agrees with the theoretical basis proposed by Zhang *et al*. (2003). This is a basis for the use of spectroscopy in the discrimination of different forms of stress in plants, which suggests that the wave magnitude will typically vary in different lengths, increasing the reflectance in the Vis range and decreasing in the NIR (750–1,100 nm) on plants infected with pathogens.

During the incubation period of the disease the difference of reflectance values in tomato varieties evaluated (tolerant and susceptible) fluctuated between 380 and 750 nm compared to the positive controls ($Dif = \lambda_{Fo5} - \lambda_{CON}$), indicating higher values in the reflectance of infected plants in this spectral range. This increase in reflectance in the Vis range (decrease in absorbance) suggests that the content of the different photosynthetic pigments is lower in leaves of infected plants rather than the healthy ones (Carter and Knapp 2001). This fact is related to physiological responses against stress produced by reported pathogens (Berger *et al*. 2007). On the other hand, the small differences between plants infected with Fo5 and their controls, in the range of 750–1,000 nm, may indicate a minor disturbance in the hydric state of the leaves infected (Genc *et al*. 2013; Jin *et al*. 2017). These results contrast with the strong decrease in reflectance in plants subjected to water stress in the same range, from the start of data collection (day 3 of the beginning of the stimulus) to the death of the leaf (day 24), suggesting a strong relationship between water reduction in leaf tissues and wavelengths in this spectral range. Additionally, plants with an increasing level of water deficiency showed a decrease in the reflectance, which has also been observed in plants with a decrease in the relative water content of the leaf (Zhang *et al*. 2012; Genc *et al*. 2013).

This study provides evidence regarding the finding of specific wavelengths more relevant to vascular wilt in tomato plants caused by *F. oxysporum* that can help to improve the detection and discrimination of asymptomatic infected plants and water stress from hyperspectral data. These RSW are characterized by having high sensitivity and specificity in the pathosystem studied and can be used in the future to make definition indices that incorporate two or three bands of the spectrum in the Vis/NIR range. In general, the method most commonly used to detect RSW for the development of disease indices is by correlation with biophysical or biochemical traits (Hatfield *et al*. 2008). However, the use of the RELIEF feature selection algorithm offers many advantages, since it works with non-linear classes and efficiently separates the classes by performing an iterative process. In this process, each iteration

of a "X" instance of the data set is chosen randomly and the weight of each characteristic becomes updated according to the distance from "X" to the nearest instance of the same class ("NearHit"), and in turn to the nearest instance of a different class ("NearMiss"). Finally, all the data are added in a class (Kira and Rendel 1992; Kononenko *et al.* 1997).

The detection of a specific relevant wavelength range around 510–520 nm for tomato vascular wilt caused by *F. oxysporum* coincides with the maximum absorbance peak of the carotenoids (Zur *et al.* 2000; Merzlyak *et al.* 2003b). In plants, carotenoids fulfill different functions, mainly as light-gathering molecules and photoprotection (Demmig-Adams *et al.* 1996). Additionally, recent studies show that carotenoids play a key role in the adaptation of plants to mild stress and other unfavorable factors (Strzalka *et al.* 2003). The majority of specific wavelengths relevant to the pathosystem *S. lycopersicum-F. oxysporum* was found in the range of 650–750 nm, which can be related with an importance of the reflectance near 700 nm as a fundamental characteristic of green vegetation produced by a balance between biochemical and biophysical characteristics of plants (Gitelson and Merzlyak 1996, 1997). It has been observed that the displacement towards the blue of the red edge of the reflectance curve frequently accompanies the stress generated by pathogens in plants, whereby it could be used in the early detection of diseases, since an increase in reflectance around 700 nm can be a first indicator to detect cultures infected by pathogens. However, this relevant wavelength (700 nm) is not specific to a disease, since in plants there can be an overlap with important nearby wavelengths, like 680 nm, which is related to the chlorophyll content. With respect to water stress, there are decreases in reflectance in the Vis/NIR, which becomes more evident after the 15th day, considering that the plants were subject to 60% field capacity, which can be considered as a slight stress in the state of development of the plants. These RSW were present in the near-infrared region in which the main information is generated regarding the water absorption of the leaf (760–1,100 nm), with peaks in the ranges 750–760 nm and 900–960 nm. Water absorption characteristics, as a result of absorption by O-H bonds, can be found at approximately 760 nm, 970 nm, 1,200 nm, 1,450 nm and 1,950 nm (Li 2006).

It is important to note the lower magnitude on the reflectance spectra obtained in the plants of the tolerant variety. These results in the region suggest greater photosynthesis and subsequent synthesis of different types of polysaccharides compared to susceptible plants. *In vitro* studies from the 1980s and 1990s showed that tolerant phenotypes infected with *F. oxysporum* generated high contents of polysaccharides and callose, and induction of peroxidase, phytoalexins synthesis and inhibition of pathogens in dual crops (Storti *et al.* 1989). In contrast, moderately tolerant phenotypes had lower polysaccharide content and showed no hypersensitivity reaction when treated with the pathogen. The authors proposed that the presence of high levels of polysaccharides in incompatible interactions generally should be considered as evidence of direct inhibition of the fungus by these compounds and with their recognition by the plant, limit their defensive factors.

Results support the hypothesis that the differences in spectral responses during the incubation period of the disease of evaluated varieties (susceptible and tolerant) are due to physiological changes generated in the plant–pathogen recognition process and the generation of polysaccharides important to inhibiting the pathogen. These changes at different times of the incubation period may cause differences at the time that make it possible to discriminate each variety with percentages of classification greater than 80%: 9–12 dpi (tolerant) and 18–21 dpi (susceptible), under these particular test conditions. The colonization of susceptible and tolerant plants is systemic and similar in terms of the amount of inoculum used for both. In tolerant plants, pathogen recognition occurs quickly and therefore important compounds are synthesized to suppress growth and spread of the pathogen. In contrast, susceptible plants have delayed responses. The plants respond to the invading pathogen with physical barriers, producing depositions in the cell walls, blockages of the xylem vessels, and by chemical defense, synthesizing antimicrobial substances (Fradin and Thomma 2006; Cregeen *et al.* 2015). The different physical and chemical responses to the pathogen by the susceptible and tolerant varieties generate spectral changes that can be detected in real time with spectroscopic techniques and different types of multivariate analysis.

The ability to identify healthy tomato plants and those infected with *F. oxysporum*, or subjected to water stress with RSW seems to have been demonstrated in this work. There were varying percentages of success in the classification by increasing time after infection of the plant. Values between 85–93% were reached in the varieties evaluated (although at different dpi). Previous studies on the detection and classification of plant diseases using Vis/NIR spectroscopy and different multivariate analysis techniques (including the Linear Discriminant Analysis, Partial Least Squares and Regression by Main Components) have shown percentages in the classification accuracy greater than 80% in a wide variety of pathosystems, such as Wheat-Yellow Rust (90.0%), Cotton-*Verticillium* (82.4%), GLAVV–Vid--virus (81.0%), Tomato-*F. oxysporum* (85.0–100%), Tomato-*R. solani* (85.0%), Palm oil-*Ganoderma boninense* Pat. (92%), Sugar beet-*Uromyces betae* (80.3%), Sugar beet-*Cercospora* (85%), Citron-*Candidatus Liberibacter americanus* (Lam) (80–90%), among others (Mahlein *et al.* 2013; Abu-Khalaf 2015;

Alfadhi *et al*. 2017; Marín-Ortiz *et al*. 2018; Marín-Ortiz *et al*. 2019).

Even though of an appreciable amount of research has focused on the detection and classification of plant diseases using reflectance spectroscopy in the Vis/NIR as well as on the use of multivariate techniques for the analysis of high dimensionality data matrices, more detailed research is needed in the search for WSR. These subsequent specific indexes and analysis of data could be used for detection and early discrimination of systemic diseases in plants.

Conclusions

Plants of *S. lycopersicum* infected with *F. oxysporum* presented a clear spectral response compared to their respective controls, increasing their reflectance in the Vis and decreasing slowly in the NIRs measured (750–1,000 nm) during the incubation period of the disease. The tomato varieties evaluated (tolerant and susceptible) presented the same pattern of response in the spectra in the Vis/NIR range evaluated, but with a delay of the tolerant variety, mainly in regard to the decrease of reflectance on the measured infrared region. Traditionally vascular wilt has been related to the death of the plant due to hydric stress, caused by the plugging of vascular bundles, which prevents the flow of water in the plant and causes its death in advanced stages of the disease. These results showed marked differences in the plants subjected to water stress in the Vis/NIR, which suggests that there are different physiological and structural response mechanisms to the two types of stress during the incubation period in which the symptoms are not visible.

The RSWs related to infection by *F. oxysporum* were found in the Vis range (mainly the ranges 510–520 nm, 650–670 nm and 700–750 nm), which suggest physiological changes in the plants in response to the pathogen. Otherwise, the RSW related to water stress which were found (750 nm, 900–960 nm) were in the near infrared range measured, in which the main information is generated regarding water absorption of the leaf (760–1,100 nm), suggesting high specificity and sensitivity to detect and discriminate *F. oxysporum* infection from hydric stress in tomato plants in the asymptomatic stage of the disease. However, it is important to highlight the importance of performing comparative studies with specific indices developed from RSW for different diseases and other indexes proposed in current literature, in order to evaluate the specificity and sensitivity of the wavelengths found in each type of infection.

The detection of the disease in tomato plants with correct classification greater than 70% was 12 dpi on the two varieties evaluated; although the tolerant variety presented higher correct classification from 9–12 dpi, but without symptoms visible after 21 dpi, as presented by the susceptible variety. Linear discriminant models on spectral reflectance data were able to classify plants infected with *F. oxysporum* from healthy ones with high precision (85–93%), due to minor changes in the reflectance of diseased leaves at this stage. This study showed that the discrimination of systemic diseases in early infection stages is possible, but remains a challenge. Therefore, future research is required to provide additional information about factors that affect the spectral response in plants, such as differences between plant varieties, responses to various environmental conditions and nutritional considerations.

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