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REMOTE SENSING OF SPECTRAL RESPONSES OF PLANTS TO ADVERSE ENVIRONMENTAL CONDITIONS

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Abstract: *This research outlines the ways for detecting and recognizing plant stress caused by adverse environmental conditions by combining hyperspectral reflectance data between 450 and 850 nm and chlorophyll fluorescence data between 600 and 900 nm. Young tobacco (Nicotiana tabacum) plants, cv. Nevrokop 1146, infected with Tomato mosaic virus (ToMV) were investigated. The spectral reflectance of healthy and infected plants was taken by a portable fibre-optics spectrometer. The fluorescence data were collected with the same spectrometer using UV-blue excitation. Statistical analysis, derivative and spectral normalization procedures were used to account for differences in spectral properties of the plants. The red edge position in the reflectance spectra of infected leaves was determined to be shifted to the shorter wavelengths, which is a reliable indicator for presence of a stress in plants. These results are in agreement with serological analyses carried out using DAS-ELISA test for the viral infection assessment.*

ДИСТАНЦИОННИ ИЗСЛЕДВАНИЯ НА СПЕКТРАЛНИТЕ ОТГОВОРИ НА РАСТЕНИЯТА НА НЕБЛАГОПРИЯТНИТЕ УСЛОВИЯ НА ОКОЛНАТА СРЕДА

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Ключови думи: *отразена радиация, флуоресценция на хлорофила, вирусна инфекция, вирус на доматената мозайка (ВДМ), Nicotiana tabacum L.*

Резюме: *Това изследване очертава начините за откриване и разпознаване на стрес в растенията, причинен от неблагоприятни условия на околната среда чрез комбиниране на хиперспектрални данни за отразена радиация между 450 и 850 nm и флуоресценция на хлорофила между 600 и 900 nm. Изследвани са млади тютюневи (Nicotiana tabacum) растения, сорт Неврокоп 1146, заразени с вируса на доматената мозайка (ToMV). Спектралната отражателна способност на здравите и заразените растения е регистрирана с преносим спектрометър с гъвкав световод. Флуоресцентните данни са получени със същия спектрометър като е използван източник за възбуждане в UV и синята област на спектъра. Статистически анализ, първа производна и спектрална нормираща процедура са използвани за отчитане на разликите в спектралните свойства на растенията. Установено е, че позицията на червения ръб на спектрите на отразената радиация на заразените листа е отместена към по-късите дължини на вълната, което е надежден показател за наличие на стрес в растенията. Тези резултати са в съответствие със серологичния анализ, проведен чрез теста DAS-ELISA за оценка на вирусната инфекция.*

Introduction

Recent researches in remote sensing have demonstrated the advances and merits of hyperspectral data in the environmental monitoring and sustainable agriculture. A large variety of applications includes classifying vegetation species and type, quantification of agricultural crops, detecting crop stress and disease, identifying plants affected by contaminants, demonstrating sensitivity to plant nitrogen content, characterizing wetlands, mapping invasive species, biophysical and biochemical modeling, etc. The need for significant improvements in quantifying, modeling, and mapping of plant chemical, physical, and water properties is more critical than ever before in order to improve our understanding of the Earth and to achieve sustainable development [1].

Nowadays remote sensing techniques allow presymptomatic monitoring of changes in the physiological state of plants nondestructively. In this respect, spectral reflectance and chlorophyll fluorescence have proved their potential by detecting stress-related changes in the pattern of light emission from plant leaves. These techniques can be applied on scales ranging from on ground to airborne remote sensing. The effective use of remote sensor systems in resource management, agriculture and environmental monitoring requires an understanding of the nature and limitations of the high-resolution remote sensing data and of various strategies for processing and interpreting it [2]. In developing the necessary knowledge base, the ground-based measurements are the expedient source of information.

Vegetation analysis using remotely sensed data requires knowledge of the structure and function of vegetation and its reflectance properties. Spectral behavior of vegetation depends on the nature of the vegetation itself, its interactions with solar radiation and other climate factors, and the availability of chemical nutrients and water within the host medium (usually soil) [3]. The spectral properties of the leaves are usually directly related with the surface characteristics, leaf structure, water and chlorophyll content [4]. The pigments are critical to the function and health of vegetation although the relative concentrations of these pigments in vegetation can vary significantly. Vegetation with a high concentration of chlorophyll is generally very healthy, as chlorophyll is linked to photosynthetic rates. On the other hand, carotenoid and anthocyanin pigments often appear in higher concentrations in vegetation that is less healthy, typically due to stress or the onset of senescence (dormant or dying vegetation that appears red, yellow, or brown).

In the visible (VIS) spectral range, the main signal comes from the absorption of incident radiation by the leaf pigments chlorophyll, carotenoids, and anthocyanins. In the near-infrared (NIR) range, the primary contribution comes from the absorption of water. The reflectance in the shortwave infrared range is partially determined by water, but the reflectance also receives significant contributions from the reflectance of nitrogen and various forms of carbon [5].

A small percent of ultraviolet and VIS light absorbed by plant's pigments is re-emitted at longer wavelengths as fluorescence in blue, green, red and far-red bands. As this process is in competition with photosynthesis, the efficiency of the photochemistry of the plant, i.e. its physiological status, can be probed by means of chlorophyll fluorescence signal. It allows distinguishing normal from stressed condition in intact plant material [6].

Vegetation stress is the result of complex physiological processes. Stress symptoms show up as photosynthesis decline (strain phase). With the persistence of the stress (i.e. pollution, water deficiency, diseases), stress induced mechanisms become dominant and give rise to acute or chronic injury (damage phase), depending on the stress tolerance threshold [7]. The plant response to stress implies biochemical and morphological changes during this phase that is therefore irreversible. Monitoring vegetation stress in time and space is necessary to improve the sustainable use of environmental resources. The possibility to detect early plant response to stress before the damage occurs, during last decade has driven most research in vegetation stress detection [8].

This article aims to highlight the efficiency of application of the techniques of hyperspectral reflectance and chlorophyll fluorescence for detecting changes in the physiological state of plants arising from adverse environmental conditions.

Plant material

Inoculation of the plants with ToMV

Nicotiana tabacum plants, cv. Nevrokop 1146, grown in a greenhouse under controlled conditions (22-25°C, humidity 75-85%, photoperiod of 16/8 h, light intensity 3000–4000 lux) were investigated. At growth stage of 4-6 expanded leaf the tobacco plants studied were inoculated with Tomato mosaic virus (ToMV). The plants were inoculated with one gram infected leaves homogenized in 1 ml 4°C 0.1M potassium-sodium buffer, pH 8.0, with 0.2% Na_2SO_3 and 0.2% Ascorbic acid. Before virus inoculation the plants were darkened and dusted with carborundum 400-600 mesh [9]. Inoculations were performed by gently rubbing the leaves with this homogenate. After 3-5 minutes the plants were washed with water. Spectral measurements of the leaf reflectance and fluorescence have been carried out on randomly harvested leaves from young healthy and infected tobacco plants on the 7th day after inoculation. A few of the investigated leaves with different symptoms of the effect of the ToMV are shown in Fig. 1.

Fig. 1. Two pairs of investigated tobacco leaves: a) healthy and b) infected with ToMV

Methods

Spectral reflectance was collected in the VIS and NIR spectral ranges (450-850 nm) at a spectral resolution of 1.5 nm in 1170 spectral wavebands using a portable fiber-optics spectrometer USB2000 (Ocean Optics, USA). For leaf reflectance measurements a halogen light source was used. The spectral reflectance characteristics (SRC) were obtained as a ratio of the intensity of leaf reflected light to the light reflected from a diffuse reflectance standard for each wavelength in VIS and NIR ranges.

Chlorophyll fluorescence measurements were carried out by the same spectrometer in the spectral range 600-900 nm in 915 spectral wavebands. As a source of actinic light a LED diode emitting monochromatic light in the blue spectral range with light output maximum at 470 nm was used. The abaxial side of the leaves is irradiated with actinic light and the exited fluorescence is measured from the adbaxial leaf surface.

Serological method DAS-ELISA (Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay) was applied according to the method of Clark and Adams [10] for determination of virus concentration. The analysis was made on the 7th day after inoculation using a commercial kit (LOEWE Biochemica GmbH, Sauerlach, Germany) with polyclonal IgG, specific for ToMV.

All samples were crushed in extraction buffer containing 1% PVP (polyvinyl pyrrolidone) at a ratio 1:10. Plates were incubated 16 hours at 4° C. After washing three times were added alkaline phosphatase conjugate for ToMV and plates remained four hours at 37° C. We used as a substrate para-nitrophenyl phosphate (Sigma) in diethanolamine buffer (pH 9.8) at a ratio 1mg/1ml. The reaction takes place in daylight and at room temperature. The reaction was stopped with 3N NaOH. The reaction has been measured with Multimode Detector (DTX 880) at a wavelength of 405 nm.

Leaf reflectance, fluorescence, and sample collection for serological analysis have been completed at approximately the same time and on the same subset of leaves for each plant.

Data analysis

Statistical analysis was conducted using the STATISTICA software (Version 6.1, StatSoft Inc. Tulsa, Oklahoma, USA, 2002). The Student's t-criterion was applied for determination of the statistically significance of differences ($p<0.05$) between the means of sets of the values of the reflectance and chlorophyll fluorescence of control and infected plants. The reflectance analysis has been performed in spectral ranges: green (520-580 nm), red (640-680 nm), red edge (680-720 nm) and NIR (720-770 nm) at ten wavelengths (λ_1 = 475.22 nm, λ_2 = 489.37 nm, λ_3 = 524.29 nm, λ_4 = 539.65 nm, λ_5 = 552.82 nm, λ_6 = 667.33 nm, λ_7 = 703.56 nm, λ_8 = 719.31 nm, λ_9 = 724.31 nm, and λ_{10} = 758.39 nm) chosen to be positioned uniformly over the investigated ranges [7]. The fluorescence spectra have been analyzed in five characteristic spectral bands: the middle of the forefront, first maximum, middle between first and second maximum, second maximum, and the middle of the rear slope.

Derivative analysis of spectral reflectance was used primarily to locate the position and height of the inflection point of the red edge. The first derivative has been calculated using a first-difference transformation of the reflectance spectrum according to Dawson and Curran, 1998 [11]. In this case, the red-edge peak in the derivative spectra was composed of a peak maximum usually between 680 and 740 nm.

Results

The averaged spectral reflectance characteristics over 25 measurements of the healthy and infected tobacco leaves are shown in Fig. 2. It is seen that the values of SRC of infected with ToMV leaves are higher in the green, red and red edge position (REP) ranges and are lower in NIR. These changes in the reflectance values call for changes in physiological state of the plants.

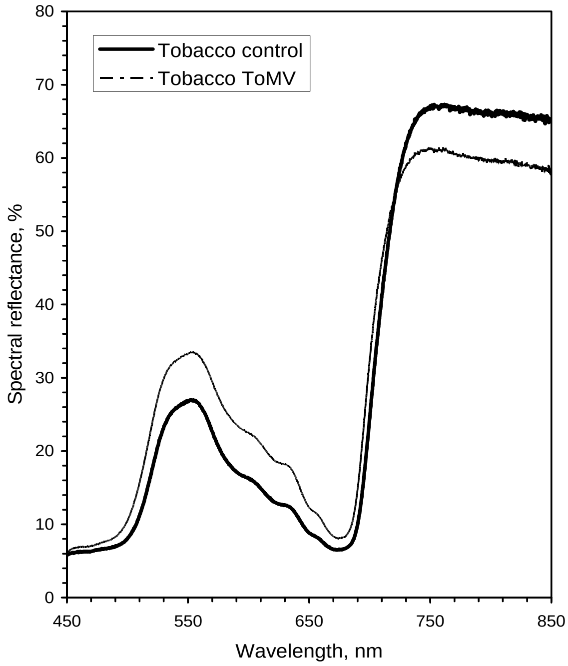

Fig. 2. Averaged spectral reflectance characteristics of control (thick line) and infected with ToMV tobacco leaves (thin line)

Tomato mosaic virus (ToMV) is one of the highly infectious viruses that are very easily spread from plant to plant by contact. It can survive for long periods in crop debris and on infected equipment. Although this virus affects field crops, it is more often a problem in greenhouse crops where plants are generally grown at a higher density and handled more frequently. ToMV infects a wide range of hosts, including crop plants, weeds and ornamentals.

Table 1. p-values of the Student's t-criterion in the case of tobacco leaves infected with ToMV

Pairs compared	Control mean	p	ToMV mean
λ_1 / λ_{1c}	6,539	0.040	7,345
λ_2 / λ_{2c}	6,941	0.003	8,176
λ_3 / λ_{3c}	20,157	0.001	26,627
λ_4 / λ_{4c}	28,684	0.000	32,189
λ_5 / λ_{5c}	26,907	0.001	33,512
λ_6 / λ_{6c}	6,739	0.001	8,764
λ_7 / λ_{7c}	30,717	0.000	38,00
λ_8 / λ_{8c}	53,044	0.411	53,805
λ_9 / λ_{9c}	57,983	0.203	56,691
$\lambda_{10} / \lambda_{10c}$	66,935	0.001	61,102

The symptoms caused by ToMV can vary considerably with the strain of virus, time of infection, variety, temperature, light intensity and other growing conditions. Foliar symptoms include mosaic, mottling, leaf distortion (Fig.1) and sometimes leaf death and defoliation.

The changes between SRC of control and infected tobacco leaves were assessed by statistical analysis using the Student t-criterion. The statistical significance (p-level) and means of the sets of data at ten investigated wavelengths are given on Table 1. The SRC of infected with ToMV leaves differed statistically significant ($p<0.05$) against the SRC of control leaves in eight of investigated wavelengths with the exception of two within the NIR region.

The first derivative analysis of the SRC of control and infected leaves was applied to assess the position of the inflection points in the red edge region. The derivatives of the averaged SRC of two groups in the 680-780 nm range, where the maximums are located, are shown in Fig. 3. The maximum of the derivative of healthy (control) leaves is located at 699.52 nm while for the group of leaves infected only with ToMV the maximums occur at 695.14 nm.

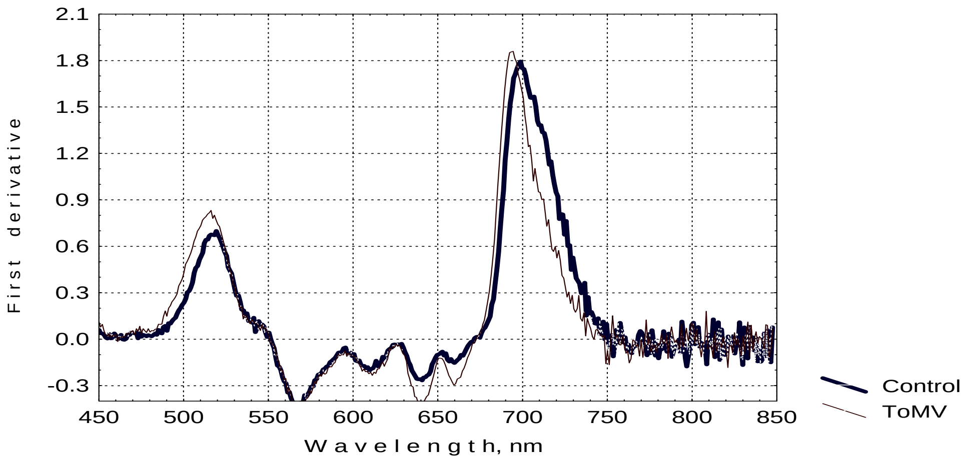

Fig. 3. Maximum of the first derivative on SRC of control and infected with ToMV tobacco leaves

The average fluorescence spectra over 20 controls and 20 infected with ToMV leaves are displayed in Fig. 4. All spectra are normalized to their second spectrum maximum which in this case coincide with the wavelength λ = 738 nm. Changes in the spectra of infected plants against the controls were predominantly observed in the arising forefront. The curve of the average leaf fluorescence spectra of inoculated plants differed against the control curve significantly within the spectral range 630-740 nm which is a sign of alterations that have occurred in the physiological state of the plants. These results are in agreement with the findings concerning the changes in the leaf spectral reflectance of the same tobacco plants, treated with ToMV.

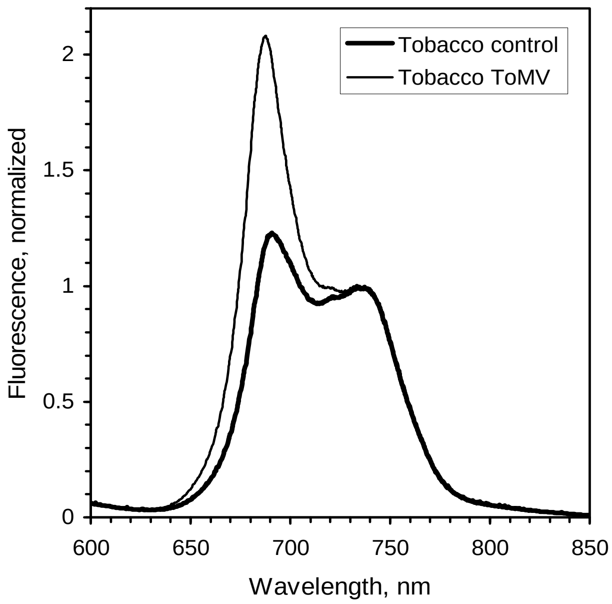

Fig. 4. Averaged normalized fluorescence spectra of control (thick line) and infected with ToMV tobacco leaves (thin line)

The serological analysis by DAS-ELISA (OD 405 nm) yielded a positive result for presence of an extensive viral infection of the leaf sample of cv. Nevrokop 1146 on the 7th day after inoculation with ToMV. The extinction value of 2.45 is approximately twelve times higher than the negative control value (K-) 0.127. OD is the optical density (absorbance).

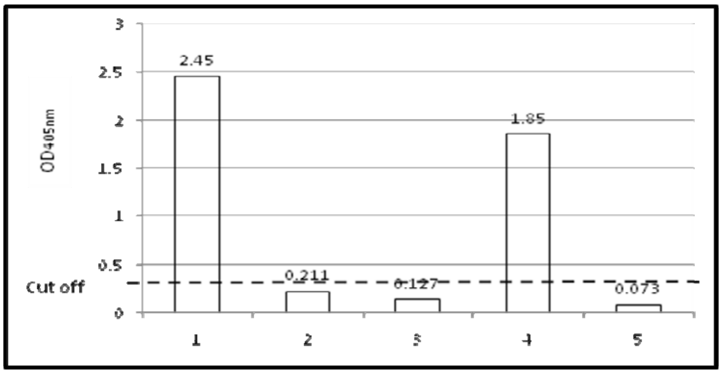

Fig. 5. DAS-ELISA of leaf samples from tobacco plants, cv. Nevrokop 1146, infected with ToMV.

Legend: 1 – leaf sample taken on the 7th day after virus inoculation (2.45), 2- healthy plant (0.211), 3 – negative control (0.127), 4 – positive control (1.85), 4 – buffer control (0.073)

Conclusions

This article emphasized the efficiency and sensitivity of the remote sensing techniques, hyperspectral reflectance and chlorophyll fluorescence, for monitoring of the natural resources and preservation of the Earth's ecosystems. Our results demonstrate the great potential of these methods for assessing changes in various biophysical and biochemical properties of plants in response to the adverse environmental conditions, as well as for making timely management decisions for the rational use and preservation of the vegetable ecosystems.

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