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Hyperspectral remote sensing applications for early stress detection of young plants

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Remote sensing technologies have advanced significantly at last decades and have improved the capability to gather information about Earth's resources and environment. They have many applications in Earth observation, such as mapping and updating land-use and cover, deforestation, vegetation and water dynamics and quality, etc. In this study, the physical principles and some applications of two hyperspectral remote sensing techniques, reflectance and fluorescence, are briefly discussed with a view to achieve an early diagnosis of stress in young deciduous trees (Paulownia tomentosa) in response to adverse environmental conditions (abiotic stresses). Leaf reflectance and fluorescence data were collected in the visible and near infrared spectral ranges (350–1000 nm) using a portable fiber-optics spectrometer. Statistical analyses and spectral normalization procedures were used to account the changes in the spectral features of the trees in response of adverse conditions. Spectral analyses were performed at ten narrow bands in green, red, red edge and near-infrared spectral ranges. Fluorescence spectra were investigated at five characteristic wavelengths in a spectral region 600–850 nm. Spectral data analyses were compared with the results from the accompanying biochemical tests for the assessment of damage to the trees.

Key words: Hyperspectral remote sensing, reflectance, fluorescence, Earth observation, abiotic and biotic stresses

INTRODUCTION

Earth observation from space and ground through various remote sensing technologies and instruments has provided vantage means of monitoring land surface dynamics, natural resources management, and the overall state of the environment itself [1-4]. Remote sensing is basically a multi-disciplinary science including various disciplines such as optics, spectroscopy, photography, electronics and telecommunication, etc. Remote sensing is broadly defined as a technique of obtaining information about properties of an object without coming into physical contact with that object. A more specific definition of remote sensing relates to studying the environment from a distance [5]. Remote sensing involves the use of ground-, aircraft-, or satellite based sensors to gather information by measuring the electromagnetic radiation (EMR) that is reflected, transmitted and absorbed by the objects in various spectral regions, from gamma-rays to radio waves. After interaction of the EMR with the surface of terrestrial materials a number of changes are acquired in its magnitude, direction, wavelength, polarization and phase [6,7]. These changes are detected by the remote sensors and enable to obtain useful information about the objects.

Different types of sensors including aerial photographs, airborne multi-spectral scanners, satellite imagery, low and high spatial and spectral resolution and ground based spectrometers and fluorometers collect electromagnetic information. In last decades, the used sensors and techniques have improved significantly the capability to gather information about Earth's resources and environment [8]. Future sensors will continue the progress toward more comprehensive and more accurate measurements. Further improvements in the spatial, spectral, radiometric, and temporal characteristics of the measurements are expected.

One of the recent applications of hyperspectral remote sensing is monitoring and preservation of plant ecosystems. The distribution of vegetation, its properties and state, is of major importance for a wide range of applications, such as environmental management, natural hazards monitoring, agriculture and forestry, climate change studies, numerical weather forecast models [9–11]. The spectral features of the plants

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may be investigated by measuring reflected (spectral reflectance) and emitted radiation (chlorophyll fluorescence) directly from the plant surface and identifying unique spectral features that change in response to changes in environment [12]. Understanding the physical and biological responses of plants to environmental stresses, governing leaf reflectance, transmission, and absorption at the leaf level represents the first step to identifying unique spectral responses for extension to the canopy and regional scales [13]. Recent research has demonstrated the advances and merit of hyperspectral data in a range of applications including quantifying agricultural crops, modeling forest canopy biochemical properties, detecting crop stress and disease, mapping leaf chlorophyll content as it influences crop production, etc. [14–16].

With the progress of the hyperspectral remote sensing technology, more detailed data are potentially available. Therefore, extracting of meaningful relationships from the vast quantity of data is necessary. Currently, a variety of techniques have been implemented including a number of different vegetation indices, band absorption analysis, spectral mixture analysis, "red edge" position, statistical analysis, wavelet transform and neural networks [17–19]. Common problems in the area of hyperspectral analyses concerning data relevancy include optimal selections of wavelength, number of bands, and spatial and spectral resolution. Hyperspectral narrow-band spectral data are fast emerging as practical solutions in modeling and mapping vegetation. The goal is to develop some special algorithms and models for hyperspectral data processing, information extraction, classification and identification. In the field of vegetation study some successful progresses are already achieved by using derivative spectral analysis model for background noise elimination, radiative transfer models, "red edge" determination or biochemical parameter detection [20–22]. The need for significant improvements in quantifying, modeling, and mapping plant chemical, physical, and water properties is more critical than ever before to reduce uncertainties in our understanding of the Earth and to better sustain it.

In this study, the physical principles of two hyperspectral remote sensing techniques, reflectance and fluorescence, are briefly considered and their applicability with a view to achieve an early diagnosis of stress in young deciduous trees (Paulownia tomentosa) caused by adverse environmental conditions (enhanced content of hydrogen peroxide, treatment with herbicide 2,4-D and their combination) is discussed. The remote sensing findings were validated through accompanying biochemical tests broadly implemented in plant science practice.

REMOTE SENSING TECHNIQUES

Spectral reflectance

When electromagnetic radiation reaches an object three different interactions may occur. From the incident light on a surface, there may be a fraction which is transmitted, another which is absorbed, and a third fraction that is reflected. This portion of reflected radiation is the reason why we can actually see the objects. Moreover, the colour of the object results from the combination of wavelengths of the reflected portion of light from the object [23]. The proportions of reflected, absorbed, and transmitted fractions of the EMR vary for different terrestrial objects (rocks, minerals, soil, vegetation, water, etc.), depending on their material type and condition [24]. Thus, the objects can be differentiated in the remotely sensed images by the reflected EMR at varying wavelengths in ultraviolet (UV), visible (VIS, 400–700 nm), near infrared (NIR, 700–1200 nm), and short wave infrared (SWIR, 1200–2500 nm) spectral ranges, known as spectral signature. The property that is used to quantify the spectral signatures is called spectral reflectance $R(\lambda)$, a function of wavelength (λ), defined by the ratio:

$$R(\lambda) = [E_R(\lambda)/E_I(\lambda)] \times 100$$

where: $E_R(\lambda)$ – energy of wavelength λ reflected from the object, $E_I(\lambda)$ – energy of wavelength λ incident upon the object. $R(\lambda)$ is expressed as a percentage.

The plot between $R(\lambda)$ and λ, called spectral reflectance curve (SRC), of three different classes of natural objects (conifers, water and soil) are shown in Fig. 1 [25]. Soil, water and vegetation have clearly different patterns of reflectance and absorption over different wavelengths.

Green vegetation species all have unique spectral features which evolve with the plant life cycle. Spectral reflectance is influenced by leaf surface features, internal architecture and biochemical composition (pigments, water, nitrogen, etc.) [26,27]. The configuration of the spectral reflectance curves is important in the determination of the wavelength regions in which remote sensing data is acquired as the SRC give insight into the spectral characteristics

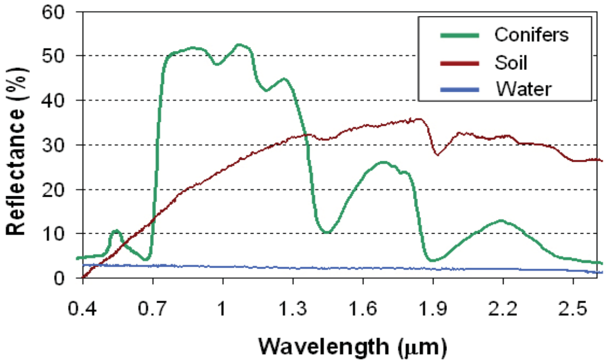

Fig. 1. Spectral reflectance curves of three different natural objects.

of an object [22]. Spectral reflectance of green vegetation has several characteristic features. The values of $R(\lambda)$ in the VIS portion of the EMR (0.4–0.68 μm) are low (Fig. 1) and are dictated by the pigments in plant leaves, mainly by strongly chlorophyll (Chl) absorption for photosynthesis. The other feature is the dramatic increase in the reflectance for healthy vegetation in the spectral region 0.68–0.72 μm (red edge position). In the NIR range (0.72–0.85 μm) the values of the reflectance are highest and the magnitude depends on leaf development and cell structure. Because this structure is highly variable between plant species, reflectance measurements in this range often permit us to discriminate between species, even if they look the same in VIS wavelengths [22]. In the SWIR range (0.95–2.5 μm) strong absorption bands around 1.45, 1.95 and 2.50 μm appear and the reflectance is mainly determined by the leaf tissue and water content.

If a plant is subject to some form of stress that interrupts its normal growth and productivity, it may decrease or cease chlorophyll production. The result is less chlorophyll absorption in the blue and red spectral bands (centered at about 0.45 μm and 0.65 μm, respectively) [28]. Often the red reflectance increases to the point that we see the plant turn yellow. Shifts in the red edge position can be a sensitive indicator of plant stress by degree of reduced absorption owing to falling levels of chlorophyll and a decrease in the NIR reflectance due to changes in plant cell structure [29].

Chlorophyll fluorescence

Chlorophyll fluorescence (ChlF) allows studying the different functional levels of photosynthesis indirectly (e.g. process at pigment level, primary light reaction, electron transport reaction, slow regulatory process) and can be used to study components of the photosynthetic apparatus and their reactions to changes in the environment [30,31]. Together with other spectroscopic and biochemical methods, recording of ChlF helps elucidate many important mechanisms of photosynthesis and nowadays ChlF is widely used as a nondestructive diagnostic tool in photosynthesis research [32]. Chl is the primary pigment of the plants that absorbs light energy from the sun for photosynthesis. Excess energy can be dissipated as heat or re-emitted as light at longer wavelength, i.e. chlorophyll fluorescence, Fig. 2. The increase in efficiency of one of these three processes (absorption, fluorescence and thermal emission) will result in a decrease in yield of the other two. As such, the relative intensities of ChlF are strongly related to the efficiency of photochemistry and heat dissipation [33] and may provide additional data to detect plant stress in an early stage [29]. Generally, fluorescence yield is highest when photochemistry and heat dissipation are lowest.

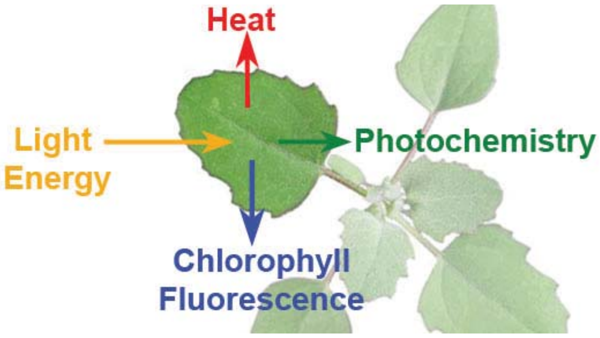

Fig. 2. Plant chlorophyll fluorescence technology.

Although the total amount of ChlF is very small (only 2 or 3% of total light absorbed), measurement is quite easy. The spectrum of fluorescence is different to that of absorbed light with the peek of fluorescence emission being at longer wavelength than that of absorption. At room temperature, Chl is emitting fluorescence in the red and NIR spectral ranges between 650–800 nm, in two broad bands with peaks at $\lambda_{\max 1}$ (684–695 nm) and $\lambda_{\max 2}$ (730–740 nm) [34].

It is well known that plant photosynthesis is a sensitive indicator of environmental perturbations such as excessive ozone, pollutants, cold or heat stress, salinity, deficiency of light and nutrients or water. Currently, vegetation stress is poorly described in numerical models. Changes in Chl function take place before changes in Chl content, before any physical signs of tissue or chlorophyll deterioration are manifested in the plant, and therefore alterations in the fluorescence signal occur before any visible signs are apparent [35,36]. Under conditions of stress, some plant mechanisms for disposing of excess energy do not work efficiently, thus causing changes in the competing reactions of photochemistry, heat loss and fluorescence.

PLANT MATERIAL

Leaf reflectance and chlorophyll fluorescence techniques were applied for investigation of the responses of paulownia trees to stress factors – applying the herbicide 2,4-D (2,4-Dichlorophenoxy acid) and H_2O_2 (hydrogen peroxide) and their combination. Paulownia is ideal tree species for afforestation and improvement and restoration of contaminated and poor soils. Paulownia grows in contaminated with heavy metals and harmful substances soils where other trees would not survive. Consuming these substances, it released them from the soil. Our investigations were conducted with three months old paulownia seedlings (fully developed 5th leaf) grown as soil cultures in a growth chamber under controlled conditions (12 h light/12 h dark photoperiod, photon flux density 90 μmol m^{-2} sec^{-1}, humidity 60–70%, and temperature $25 \pm 1^{\circ}$C). The trees were divided into four groups. The first group included healthy, untreated (control) trees. The trees from the second group were sprayed with 2.0 mM H_2O_2. After 24 h the third group trees were sprayed with 1 mM 2,4-D. The forth group trees were treated with both H_2O_2 and 2,4-D. Spectral measurements were conducted after three days on leaf samples without visual symptoms.

DATA ACQUISITION AND ANALYSESS

Spectral measurements

Hyperspectral reflectance data were collected in the VIS and NIR spectral ranges by using a portable fibre-optics spectrometer USB2000 (Ocean Optics) [37]. In the range investigated (450–850 nm) the main part of the reflected from leaves radiation is concentrated. Data were analyzed at 1170 spectral bands with a step of 0.3 nm and a spectral resolution of 1.5 nm. 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. As a light source a halogen lamp providing homogeneous illumination of measured leaf surfaces was used.

The spectral measurements of the chlorophyll fluorescence were carried out under laboratory conditions using the same portable fibre-optics spectrometer (USB2000). Data were investigated in the VIS and NIR spectral ranges (600–900 nm) at 910 spectral bands with a step of 0.3 nm where the main part of the emitted from the plants fluorescence radiation is concentrated. As a source of actinic light, a LED diode with light output maximum at 470 nm was used. The tested leaves were dark adapted before the measurements for ten minutes.

Data processing

The Student's t-test was applied for determination of the statistical significance of differences between the means of sets of the values of the reflectance spectra of healthy (control) and subjected to adverse environmental conditions paulownia trees. Hierarchical cluster analysis (tree graph) was performed on reflectance data to examine and visualize how the groups of data are merged. The clustering method uses the dissimilarities or distances between objects when forming the clusters.

The spectral reflectance analyses were performed in four most informative for investigated plants spectral ranges: green (520–580 nm, maximal reflection of green vegetation), red (640–680 nm, maximal chlorophyll absorption), red edge (680–720 nm, maximal slope of the reflectance spectra) and the NIR (720–770 nm). The statistical significance of the differences between SR of control and treated trees was examined in ten wavelengths ($\lambda_1 = 475.22$ nm, $\lambda_2 = 489.37$ nm, $\lambda_3 = 524.29$ nm, $\lambda_4 = 539.65$ nm, $\lambda_5 = 552.82$ nm, $\lambda_6 = 667.33$ nm, $\lambda_7 = 703.56$ nm, $\lambda_8 = 719.31$ nm, $\lambda_9 = 724.31$ nm, and $\lambda_{10} = 758.39$ nm) chosen to be disposed uniformly over these ranges. The fluorescence spectra were analyzed in five characteristic spectral bands, chosen at specific wavelengths: the middle of the forefront edge, first maximum, the middle between first and second maximum), second maximum, and the middle of the rear slope.

RESULTS AND DISCUSSION

The averaged SRC (over 24 measured leaves) of control and treated with H_2O_2 and 2,4-D and their combination trees are shown in Fig. 3. It is seen that

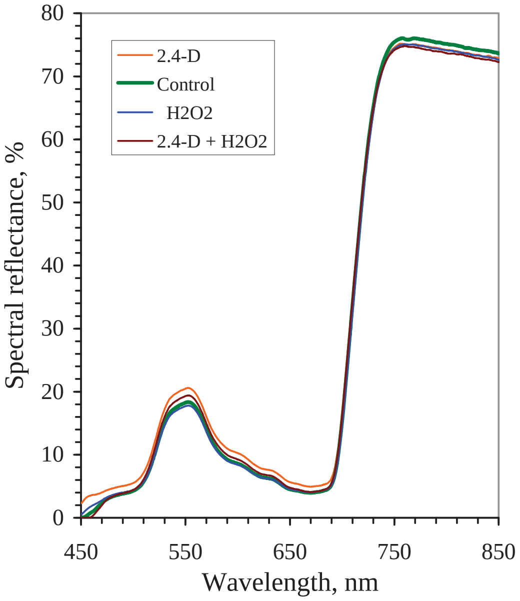

Fig. 3. Averaged spectral reflectance characteristics of paulownia trees treated with H_2O_2 and 2,4-D and their combination.

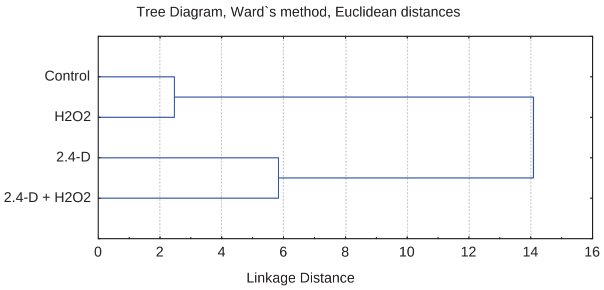

Fig. 4. A hierarchical tree diagram of data sets of paulownia trees under adverse environmental conditions.

the values of the SRC of the group treated with 2,4-D differed most significant against the control. SRC of the group treated with H_2O_2 is closed to the control. In the case of combined treatment SRC values approached to the control.

The Student's t-test was applied for determination of the statistically significance of differences between the means of sets of the values of the SRC of control and treated paulownia trees. The results are set in Table 1. In the case of treatment with H_2O_2 the differences are not statistically significant ($p > 0.05$) against the control SRC at eight of the ten investigated wavelengths which indicates that these curves are very closly lying each to other. For the treatment with herbicide 2,4-D the number of not statistically significant differences (ns) decreased to three in the wavelengths in NIR spectral range which means that this SRC differs signifficantly from the control SRC. For combined treatment not statistically significant difference was assessed at six wavelengths – SRC is more close to the control than in the case of herbicide treatment only.

Hydrogen peroxide in used concentration has a protective effect and almost no effect or beneficial effect on the treated plants. Therefore, differences of SRC were not statistically significant against the control. The herbicide 2,4-D applied in field concentration does not act quickly. Initially it stimulates growth. Plants grow rapidly and lose their power as the membranes are damaged. This is manifested in the increase of the values of SRC in VIS range and statistically significant differences. In combined treatment, H_2O_2 inhibits the action of the herbicide and changes against the control SRC decreased.

Hierarchical cluster analysis (tree graph) was applied to the SRC of four group of trees in the

Table 1. p-Values of the Student's t-criterion for pairs of the control and treated paulownia leaves

Pairs compared	Control	P<	2,4-D	P<	H_2O_2	P<	2,4-D + H_2O_2
λ_1/λ_{1c}	2.98	***	4.35	*	3.42	ns	2.79
λ_2/λ_{2c}	3.73	***	4.96	*	4.09	ns	3.76
λ_3/λ_{3c}	12.12	***	14.06	ns	12.03	***	12.93
λ_4/λ_{4c}	17.16	***	19.31	ns	17.03	***	18.12
λ_5/λ_{5c}	18.15	***	20.30	ns	17.98	***	19.14
λ_6/λ_{6c}	3.92	***	4.92	ns	4.16	ns	4.01
λ_7/λ_{7c}	21.20	**	22.85	ns	20.63	**	23.57
λ_8/λ_{8c}	50.06	ns	51.05	ns	50.01	ns	50.85
λ_9/λ_{9c}	57.77	ns	58.41	ns	57.93	ns	58.14
$\lambda_{10}/\lambda_{10c}$	75.20	ns	74.72	ns	75.86	ns	74.16

ns – no significance between obtained differences ($p > 0.05$); * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$

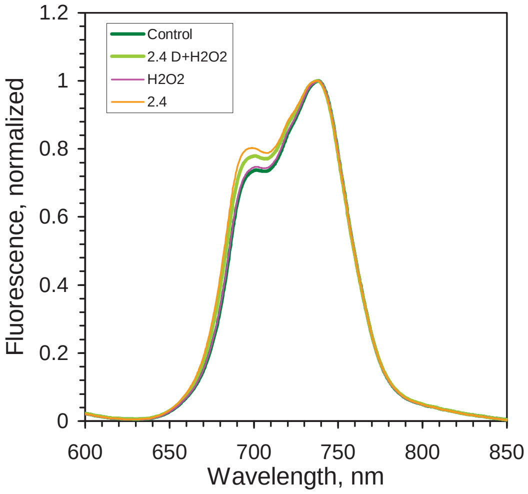

Fig. 5. Averaged fluorescence spectra of paulownia leaves treated with H_2O_2, 2,4-D and their combination.

spectral range 520–580 nm, Fig. 4. Two completely separate clusters were received. The first one includes the spectral data of control and H_2O_2 groups and the second – data of the rest two treatments.

The averaged fluorescence spectra of control and treated paulownia leaves are shown in Fig. 5. Most significant are differences between SRC of control and treated with herbicide 2,4-D. For combined treatments differences decreased because of regenerating action of hydrogen peroxide.

The Student's t-test was applied to fluorescence data in above mentioned five characteristic wavelengths. The significant differences were obtained in first three wavelengths, most significant ($p < 0.001$) in the case of H_2O_2 treatment.

The remote sensing findings were validated through complementary biochemical tests commonly applied in plant science practice in studies of abiotic and biotic plant stresses.

The biochemical parameters – stress markers such as phenols, proline, and thiol groups (-SH) were determined using a spectrophotometer Multiskan Spectrum (Thermo Electron Corporation). Phenols and proline are important protective components of the plant cells and they accumulate when cells are in stress conditions. Treatment with 2,4-D causes increased levels of free proline and reduced levels of free thiol groups and total phenols. When pre-treated with H_2O_2 plants the proline content is not increased, corresponding level of thiol groups and total phenols is higher, in comparison with plants treated with 2,4-D. On the basis of these investigations it could be concluded that the preliminary application of H_2O_2 reduces oxidative damage.

Table 2. Results from biochemical analysis – stress markers.

Investigated groups	**Proline mkmol/gFW**	**Phenols nmol/gFW**	**-SH nmol/gFW**
control	0.381	66.811	0.512
H_2O_2	0.330	34.085	0.406
2,4-D	0.426	36.524	0.306
H_2O_2+2,4-D	0.366	53.253	0.455

CONCLUSIONS

In this research hyperspectral reflectance and fluorescence data from leaves of paulownia trees were tested upon their sensitivity to two abiotic stresses. Hydrogen peroxide and herbicide 2,4-D were applied in low concentrations. Measurements were conducted on the fourth day after the treatment on leaf samples without visual symptoms. It was demonstrated that the spectral behavior of stressed and unstressed leaves was clearly different. The examination of differences of mean reflectance values of stressed and healthy leaves indicated that some regions in the spectrum were more sensitive than others. Furthermore those regions were situated around places in the spectrum where from the biophysical point of view we expected them to occur.

The strong correlation which was found between the results from the two remote sensing techniques and the biochemical analyses of plant stress markers indicates the importance of remote sensing hyperspectral data for conducting, early and without damage, rapid assessments of plant health condition. These data have great potential for assessing changes in various biophysical and biochemical properties of plants and ecosystems in response to the changes in the environment. This study exemplifies the benefits of integrating remote sensing and ecology and conducting of interdisciplinary investigations of vegetation.

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ПРИЛОЖЕНИЕ НА ХИПЕРСПЕКТРАЛНИТЕ ДИСТАНЦИОННИ ИЗСЛЕДВАНИЯ ЗА РАННО ОТКРИВАНЕ НА СТРЕС В МЛАДИ РАСТЕНИЯ

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(Резюме)

През последното десетилетие хиперспектралните данни от дистанционни изследвания на земната повърхност доказаха, че са много ефективни за идентифициране на растежни аномалии в културни растения вследствие на влиянието на естествени неблагоприятни фактори на околната среда, вирусни инфекции и др. Промените във физиологичното състояние на растенията, симптоми на стрес, се отразяват върху техните спектрални отговори и могат да се установяват чрез спектрални измервания във видимата и близката инфрачервена области на електромагнитния спектър. Целта на това изследване е да се постигне ранна диагностика на стрес в млади растителни видове чрез прилагане на два метода за дистанционни изследвания (отразена радиация и флуоресценция на хлорофила) и да се вземат навременни управленчески решения за предотвратяване или ограничаване на последствията. Отразената от изследваните растения радиация е регистрирана с портативен спектрометър с гъвкав световод в спектралния диапазон 450-850 nm със спектрална разделителна способност 1.5 nm. Данните за флуоресценция на хлорофила са получени със същия спектрометър в спектралния диапазон 600-900 nm, като е използван източник за възбуждане в синята област на спектъра. Статистически анализи, първа производна и спектрални нормиращи процедури са използвани за отчитане на разликите в спектралните свойства на растенията. В някои от случаите е установено, че позицията на червения ръб на спектрите на отразената радиация на увредените растения е отместена към по-късите дължини на вълната, което е надежден показател за наличие на стрес в растенията. Тези резултати са съпоставени с резултатите от проведени съпътстващи биохимични и серологични анализи за оценка на увреждането на растенията.