Spectral Data for Plant Chlorophyll Assessment

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An increasing role in plant phytodiagnostics becomes to play different spectrometric techniques used as a part of remote sensing applications. The radiation behavior of land covers and the spectral response to changing conditions lies at the root of these studies. The visible and near infrared (400 - 900 nm) measurements have proved abilities in vegetation monitoring. The reason is that this wavelength range reveals significant sensitivity to plant biophysical properties. The information is carried by the specific vegetation spectral characteristics which depend on such plant parameters as chlorophyll content, biomass amount, leaf area, etc. These parameters are associated with plant development and stress factors being closely related to vegetation physiological state. In our study, multispectral data of reflected, transmitted and emitted irradiance have been used to show the possibility for plant chlorophyll assessment. Different methods such as vegetation indices, red edge analysis and fluorescence spectra have been applied and compared.

Key words: – multispectral data, chlorophyll estimation, vegetation indices, red edge, fluorescence

INTRODUCTION

Recent developments in environmental studies are greatly connected with worldwide ecological problems related to anthropogenic impacts on the biosphere and first of all on vegetation. Advanced monitoring and alerting techniques, on-time information extraction, modeling and forecasting methods are a preposition for successful data application and decision support in environmental studies.

The spreading acceptance of the concept of precision agriculture running [1] generated much interest in the early detection of plant growth stress. The implementation of modern remote sensing technologies is one of the basic assumptions of this concept, special attention being paid to vegetation monitoring in relation to stress detection. That is why the assessment from spectral data of crop state [2,3] and growing conditions has been and still is at the focus of numerous investigations and experimental studies [4,5].

Important here are early warning signs of plant inhibition which should be directly connected to fundamental physiological processes. Such a process is the photosynthesis and the connection has been found in vegetation fluorescence [6,7]. The optical signatures of leaves are mostly defined by the composition of photosynthetic pigments and their stress-induced changes, and as such they are indicative of plant short-term or long-term stress. Though being studied for decades, light induced fluorescence has not lost its attractiveness. In recent years, the screening of plant fluorescence signatures is developing as a specific tool which could be applied to detect the functioning and health status of plants [8-10]. Compared to reflectance, induced fluorescence might be a more accurate indicator of plant state and be able to detect stress impacts at earlier growth stages.

In our study, multispectral data of reflected, transmitted and emitted by plant leaves irradiance have been used to show the possibility for plant chlorophyll estimation. Different methods such as vegetation indices, red edge analysis and fluorescence emission have been applied and compared. A comparison has been made between the stress bioindicator (reduced concentration of chlorophyll pigments) and a variety of spectral stress indicators (vegetation indices, red edge position, chlorophyll fluorescence). Good correspondence has been found between both physiological and spectral indicators of plant stress.

MATERIALS AND METHODS

The investigations were conducted on various agricultural species (winter wheat, spring barley, peas, alfalfa) grown under different conditions (soil type, fertilization and heavy metal pollution). The study comprised laboratory, green-house and field experiments. The soil acidity, nutrient deficiency and heavy metal contamination (Cd, Ni,) were stress factors that affected the development and caused variations of crop state. Their impact was evaluated through plant spectral features (reflectance, absorption, transmittance, fluorescence) which were examined for their ability to serve as sustainable stress indicators during plant growth.

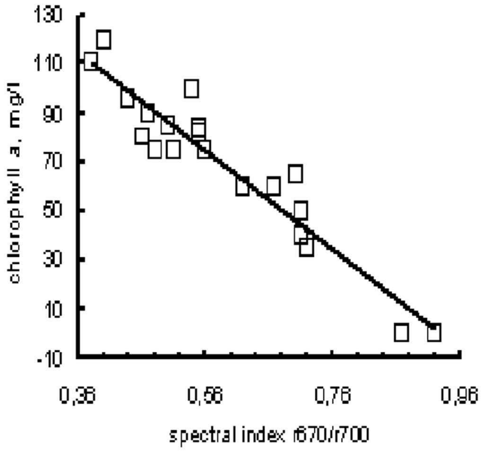

Fig. 1. Linear fit between spring barley chlorophyll and a ratio vegetation index

Spectral and biometrical data were gathered at different stages of plant ontogenesis. The ground-based reflectance measurements were carried out at canopy level with a multichannel radiometer operating from the nadir position in the visible and near infrared wavelength range 400-820 nm at a 10 nm step. The transmittance and fluorescence measurements were performed over detached plant leaves with a laboratory spectrometric system [11]. On the same day as the spectrometric measurements were made, plant chlorophyll a and b concentrations were determined spectrophotometrically by grounding 100 mg fresh weight of leaf material in 10 ml of 80% acetone and calculated by Arnon (1949).

Spectral and chlorophyll data. were statistically analyzed to determine the significance of the spectral variations due to changes in leaf pigments, and to derive empirical relationships. Regression analysis was run on plant spectral response and chlorophyll content Different approaches were used for chlorophyll estimation from multispectral data including reflectance features transformations (vegetation indices), determination of the absorption ”red edge” position, transmittance and fluorescent spectra characteristics.

RESULT AND DISCUSSION

A common technique for multispectral data processing is the use of spectral transformations called vegetation indices (VI). They represent various combinations [12-14] of the measured reflectance factors $r(\lambda)$ at two or more wavelengths λ usually in the form of different ratios $r(\lambda_i)/r(\lambda_j)$, $[r(\lambda_i)-r(\lambda_j)]/r(\lambda_i)$, $r(\lambda_i)/[r(\lambda_i)+r(\lambda_j)+r(\lambda_k)]$, weighted sums $ar(\lambda_i)+br(\lambda_j)+br(\lambda_k)$ and normalized differences $[r(\lambda_i)-r(\lambda_j)]/[r(\lambda_i)+r(\lambda_j)]$. The wavelengths are selected corresponding to vegetation high reflectance and absorption bands in the green (550 nm), red (670 nm) and near infrared (800-900 nm) range or are located within the R-NIR interval (700-780 nm) of steep reflectance increase. In our study we have examined various spectral indices for their correlation with plant chlorophyll. Some examples are given in Table 1. Figure 1 illustrates the high correlation (R^2=0.89) between spring barley chlorophyll and vegetation ratio index $r_{(\lambda=670)}/r_{(\lambda=700)}$.

Table 1 Linear correlation coefficients between spring barley VI and chlorophyll (a+b) at different phenological phases

	NIR/R	*G*NIR/R*	*(NIR-G)/(NIR+G)*	*NIR/G*
Chl(a+b)	0.92	0.84	0.92	0.93
Chl(a+b)	0.93	0.87	0.92	0.93
Chl(a+b)	0.94	0.88	0.93	0.96

The tristimulus values *XYZ* derived from spectral reflectance curves are colorimetric features of the vegetation canopy that have been examined as plant spectral indicators related to chlorophyll concentration. Figures 2a and 2b present the relationship between the tristimulus values sum X+Y+Z and chlorophyll (a+b) concentration in spring barley (with R^2=0.84-0.85) cultivated on two soil types – grey and black. The different behaviour of the fitted models is explained by the different (opposite) way in which the light and dark soils effect the vegetation canopy reflectance in the visible spectral range.

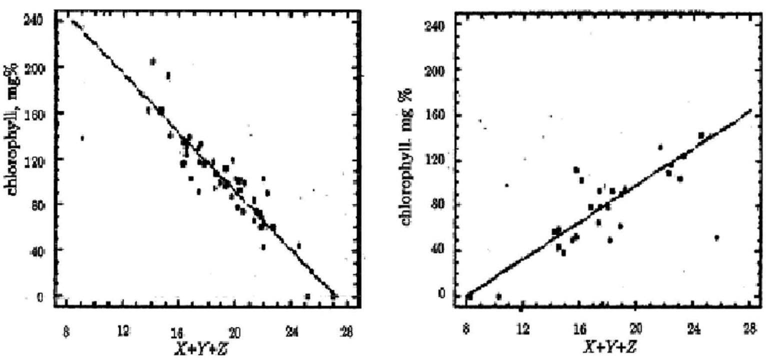

Fig. 2. Regression of tristimulus values sum on chlorophyll (a+b) concentration in spring barley on grey (a) and black (b) soil

The chlorophyll content is associated with plant growth and maturing. In the active vegetative stages of development the chlorophyll depression is an indicator of plant stress. The senescence effects change plant reflectance characteristics especially around the red band of the chlorophyll absorption. The decreased absorption along with its shift to shorter wavelengths is exploited for the assessment of chlorophyll content and stress detection [15-17]. One of the methods is the determination of the "red edge" position (wavelength) by derivative analysis.

In Figure 3 the obtained dependence of the “red edge” wavelength on the chlorophyll (a+b) concentration in alfalfa is presented where the chlorophyll variations are due to Cd contamination of the soil. The sensitivity of this spectral indicator to chlorophyll (R^2=0.87) and the availability of high spectral resolution data have the potential for reliable detection and quantification of chlorophyll deprivation.

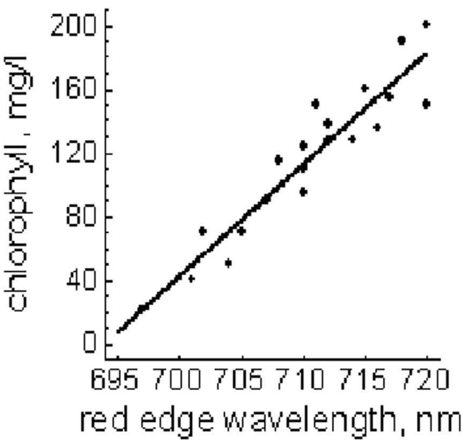

Fig. 3. Correlation between the “red edge” wavelength and the chlorophyll concentration in alfalfa

Compared to reflectance, chlorophyll fluorescence might be a more accurate indicator of plant state and be able to detect stress impacts at earlier growth stages [9,10]. The relationship between induces fluorescence emission spectrum and barley leaves chlorophyll concentration was examined. Fluorescence changes appeared to be high-sensitive to chlorophyll decrease at a very early stage of plant development before visual color or morphological signs had been observed.

Fluorescence excitation wavelength was 470 nm. Each measurement record consisted of 10 consecutive spectra, automatically averaged. The fluorescence was obtained from the upper side of leaf samples after 3 min predarkening. Visible light illumination excites chlorophyll fluorescence of high intensity in the red and far red spectral band (675-740 nm) with peaks at about 685-690 nm and 735-740 nm [8]. Typical fluorescence spectra obtained in our experiments from barley leaf samples are shown in Figure 4.

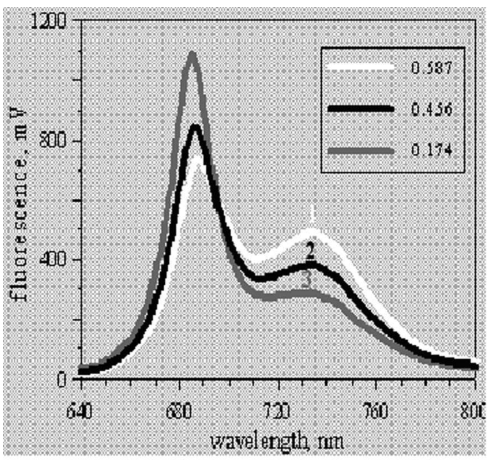

Fig. 4. Red and far-red fluorescence of barley leaves with different chlorophyll concentration in [mg/g]

Changes of the fluorescence intensities were observed as well at the minimum emission values at about 710 nm. Plants with higher degree of stress (lower chlorophyll) exhibited increased red fluorescence and decreased far-red intensity along with decreased F710 minimum. The wavelength of the red fluorescence F690 peak manifested shifts towards longer wavelengths with increasing chlorophyll content while the position of the far-red maximum remained consistent. Small wavelength shifts were observed also in the F710 emission minimum but with the opposite to λF690 behavior, i.e. to shorter wavelengths with increasing chlorophyll content. All these changes are due to varying chlorophyll and as such chlorophyll changes can be readily traceable through fluorescence measurements. Vigorous vegetation (more chlorophyll) manifested low fluorescence emission in the red spectral band (F690) and lower in the far-red band (F740). Fluorescence response in terms of F690/F740 ratio was exponentially related to leaf chlorophyll. The established empirical relationship between fluorescence F690/F740 ratio and barley chlorophyll concentration (R^2=0.88 at $p<0.001$) is presented in Figure 5. It shows the increasing ratio of the red to far-red fluorescence F690/F740 with leaf chlorophyll decrease. Besides the high correlation, the adequacy of the fitted model was tested and confirmed by an independent data set from repeated experiments.

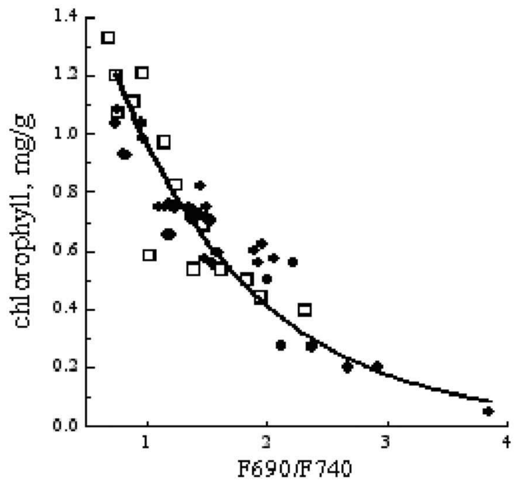

Fig. 5. Regression between chlorophyll concentration of barley leaves and fluorescence red to far-red intensity ratio (●); independent data from repeated experiments (□)

Multispectral data of peas leaves transmittance in the 500 – 800 nm wavelength range is presented in Figure 6a. The shape of the spectral curve is indicative of the heavy metal-induces stress quantified through plant chlorophyll inhibition.

Figure 6b shows the statistical dependence between the ND (λ=670 nm) and the pigment ratio (chlorophyll (a+b)/carotenoids). Here ND is the normalized difference between the spectrograms of the control and stressed samples. Indicators of the model adequacy and accuracy are the high correlation (R^2=0.95) and the narrow confidence limits.

CONCLUCIONS

Good correspondence is found between both physiological (chlorophyll) and spectral indicators of plant biostatus. The high correlation allows quantitative dependences to be established and used for plant stress diagnostics.

The obtained results indicate that chlorophyll causes statistically significant variations of plant spectral signatures. These differences have been used to established empirical dependences between plant chlorophyll and different spectral indicators. High correlation and good correspondence has been found between measured (actual) values and spectral (model) estimates of plant chlorophyll using a variety of spectral features (vegetation indices, red edge, fluorescent emission). This allows, for instance, the use of such like relationships for plant stress diagnostics in terms of chlorophyll deprivation. Moreover, some spectral features (fluorescence parameters) are early detectors of plant stress revealing physiological abnormality before visual changes have been observed.

The performed study does not only illustrate the informational potential of spectral data providing for crop monitoring and detection of stress situations but attaches to it a quantitative dimension which allows the evaluation of stress affects. The state assessment of natural vegetation resources and agricultural crops is of equal importance for biodiversity preservation, farmland management and timely response to alert situations.

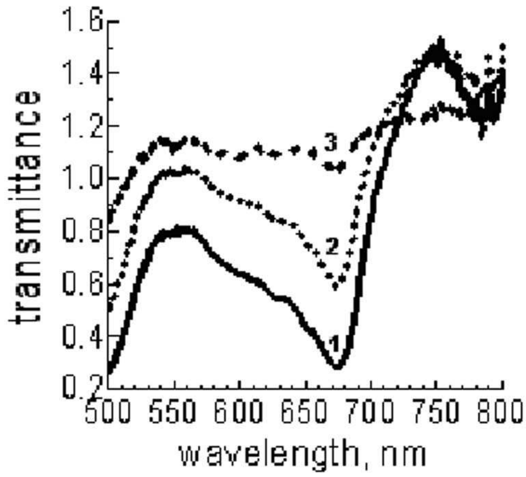

Fig. 6a. Transmittance spectra of pea leaves with different chlorophyll concentration

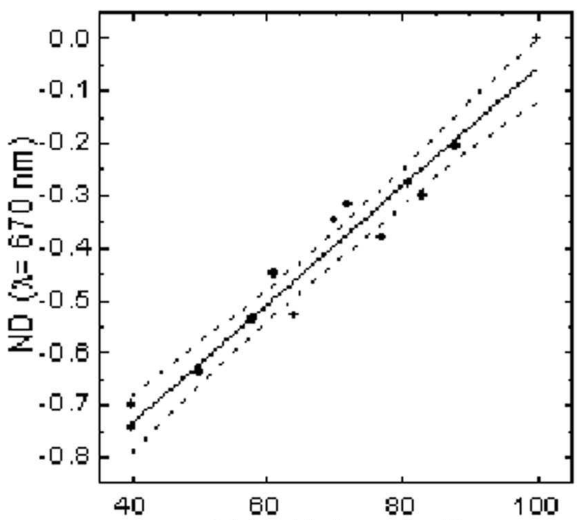

Fig. 6b. Regression between ND(λ=670 nm) and pigment ratio in percentage to control sample

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