

COMPARATIVE ANALYSIS OF HYPERSPECTRAL VEGETATION INDICES FOR REMOTE ESTIMATION OF LEAF CHLOROPHYLL CONTENT AND PLANT STATUS*

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Abstract. Leaf chlorophyll (Chl) content, at the leaf and canopy level, is an important biochemical parameter because of its crucial role in photosynthesis and in plant functioning. Furthermore, it provides an indication of the plant nutritional state and stress. Due to the reliable, rapid, and non-destructive advantages, hyperspectral remote sensing plays a significant role in monitoring and assessing the plant biophysical variables. In this study, a set of Chl-related vegetation indices (VIs) derived from the leaf reflectance data of young pepper plants infected by Cucumber Mosaic Virus (CMV) were tested for estimating the changes in the Chl content and plant status. Hyperspectral reflectance data were collected by means of a portable fiber-optics spectrometer in the spectral range of 350-1100 nm. The effect of two growth regulators, MEIA (beta-monomethyl ester of itaconic acid) and BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester), on the Chl content and respectively on the development of the viral infection was investigated too. Four categories VIs: normalized difference (ND) VIs; simple ratio (SR) VIs; single-band reflectance or simple difference (SD) VIs, and some other forms of VIs, were tested using statistical analyses (ANOVA and Tukey-Kramer's tests) to explore their potentials in the Chl content estimation. To enhance the sensitivity of the VIs, modified VIs were tested in some other combinations of narrow bands. The statistical analyses showed that the Modified Red Edge Simple Ratio (MRESR) index, Vogelmann Red Edge index (VREI1), and Pigment index (PI) were most sensitive to the Chl content changes. The Normalized Difference VI (NDVI) and Triangular Vegetation Index (TVI) turned out to be insensitive to Chl variations. The rest of the VIs were responsible for Chl variations but with less sensitivity.

Key words: Biophysical variables, chlorophyll, hyperspectral reflectance, stress, vegetation indices, viral infection

1. INTRODUCTION

Leaf Chl content is a good indicator of photosynthesis activity, stress and nutritional state of plants. It is very important for assessment of crop health and prediction of crop yield [1]. The photosynthetic apparatus is a sensitive indicator of plant state due to the rapid changes in Chl content induced by the variations of the environmental conditions. Chl content can also be used to indirectly estimate soil nitrogen content or to define optimum fertilization. Therefore, much attention has recently been paid to the development of practical methods for an accurate Chl estimation using remote sensing (RS) techniques.

The RS technique based on leaf reflectance measurements in the visible (VIS) and near-infrared (NIR) spectral ranges has been shown to be useful for estimating plant chemistry and physiology from an individual plant to a canopy. Compared to conventional analytical methods, it is a rapid, reliable and non-destructive method, requiring less sample preparation,

with less or no chemical reagents [2]. This method is highly adaptable to automated and *in situ* measurements and has the potential to analyze various plant properties simultaneously.

The advances in RS technologies (optical sensors with high spectral and spatial resolutions) allow receiving accurate information for most important biophysical variables. Many algorithms have been developed for a remote estimation of biophysical characteristics of vegetation, such as combinations of spectral bands, derivatives of reflectance spectra, neural networks, inversion of radiative transfer models, and several statistical approaches [3, 4]. Quite simple and effective algorithms for quantitative and qualitative evaluations of vegetation biophysical variables are based on the mathematical combinations of VIS and NIR reflectance bands, in the form of spectral vegetation indices (VIs). Such algorithms improve the sensitivity to the investigated vegetation parameters by minimizing the influence of extraneous factors affecting leaf and canopy reflectance and reduce the abundance of data sets [5].

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Developments in hyperspectral RS have provided new VIs or indicators of plant biochemical and biophysical properties. Most of the studies involving the novel spectral VIs have been conducted at the leaf or canopy level and often are focused on mitigating unwanted reflectance effects, while increasing the indices' sensitivity to the investigated parameters [6, 7].

VIs introduced for the assessment of Chl content are initially developed at the leaf level because an assessment of the sensitivity of such a method at the leaf level is regarded as the first step for further research at the canopy scale and for the remote estimation of Chl content from satellite observation. Chl-related VIs are widely proposed for assessing various characteristics of vegetation as well as yielding prognosis [8]. Some ecological stresses can also be detected [9, 10]. The investigations are focused on evaluating the reflectance in individual narrow bands, two or more band ratios and combinations, slopes or other formulations.

The total Chl content in leaves decreases in stress vegetation, changing the proportion of light-absorbing pigments and leading to a lower overall absorption due to lower Chl *a* and Chl *b* concentrations at the leaf level. The graphs in Fig. 1 show the absorption spectra for the main leaf pigments - Chl *a*, Chl *b*, and carotenoids. Each type of pigment can be identified by the specific pattern of wavelengths it absorbs from VIs light. Chl *a* and *b* absorb radiation at the red and blue bands. Carotenoids absorb in the short-wavelength blue region, and reflect the longer yellow, red, and orange wavelengths. Recent studies show that the total carotenoid to Chl ratio increases during vegetation stress or senescence, as the declines in Chl content exceed any corresponding declines in carotenoid content [9].

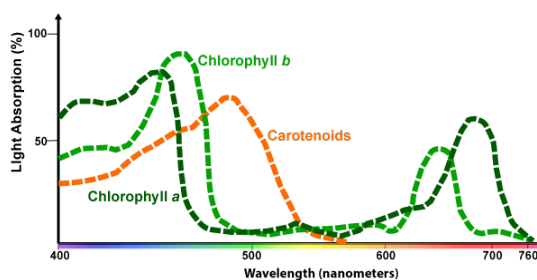


Figure 1. Absorption spectra for Chlorophyll a, Chlorophyll b, and Carotenoids

Significant differences in the reflectance spectra between healthy and stressed vegetation due to changes in pigment content have been detected in the reflectance *green peak* and along the *red edge*. Several studies have found that the wavelengths adjoining the Chl maximum absorption bands (700 nm) and the green (550 nm) bands are the most sensitive to changes in the wide range of Chl contents [11].

The objective of this study is to evaluate the sensitivity of a set of VIs for estimating the changes in the Chl content and physiological state of young pepper plants infected by Cucumber Mosaic Virus (CMV). The effect of two growth regulators: MEIA (beta-

monomethyl ester of itaconic acid) and BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester) on the Chl content and respectively on the development of a viral infection in the plants was investigated too.

2. MATERIALS AND METHODS

2.1. Plant material

Pepper plants cultivar Sivria, sensitive to CMV, were grown in a greenhouse under controlled conditions. Four groups of plants were used for the investigations. The first group included healthy (control) plants. At growth stage 4-6 expanded leaf the plants of the second group were inoculated with CMV. The plants of the other two groups were treated with growth regulators: MEIA (beta-monomethyl ester of itaconic acid) and BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester). On the next day, the plants were inoculated with CMV [12].

2.2. Leaf reflectance measurements

Spectrometric measurements of the reflected radiation from fresh detached leaves were carried out in laboratory conditions, one and two weeks after the inoculation. Hyperspectral reflectance data were collected by means of a portable fiber-optics spectrometer USB2000 (Ocean Optics) in the spectral range of 350-1100 nm at a spectral resolution of 1.5 nm. The spectrometer was placed 30 cm vertically above the leaf sample and the field of view was 6 cm in diameter. A halogen lamp was used as a light source in order to minimize a potential spectral variation between samples due to the illumination condition. Leaf reflectance spectra were recorded against barium sulphate plate as a standard, whereas black velvet was used as the background. The spectral reflectance characteristics (SRC) of the plants were determined as the ratio between the radiation reflected from the leaves and the one reflected from the standard.

2.3. Vegetation indices used in analyses

In this study, hyperspectral leaf reflectance data at different narrow bands in green, blue, red, and NIR spectral regions were used to evaluate a set of selected Chl-related VIs. The type, equations and sources of indices are given in Table 1. Only reflectance spectra in the VIs and NIR spectral range (450-850 nm) were considered in the spectral analyses because data in this part of electromagnetic spectrum are informative for leaf pigment variations.

Narrowband VIs were classified into four categories: normalized difference (ND) VIs, single-band (SB) or single-difference (SD) VIs, simple-ratio (SR) VIs, and other forms of indices. Two-band indices were evaluated using a custom developed computer programme to traverse several band combinations of the SD, SR and ND indices. These VIs were calculated using two wavebands (λ_1 and λ_2) to select the most effective two-band combinations for the assessment of Chl content, as shown in Eqs. (1)–(3):

$$SR(R_{\lambda_1}, R_{\lambda_2}) = \frac{R_{\lambda_1}}{R_{\lambda_2}}, \quad (1)$$

$$SD(R_{\lambda_1}, R_{\lambda_2}) = R_{\lambda_1} - R_{\lambda_2}, \quad (2)$$

$$ND(R_{\lambda_1}, R_{\lambda_2}) = \frac{|R_{\lambda_1} - R_{\lambda_2}|}{R_{\lambda_1} + R_{\lambda_2}}. \quad (3)$$

The Normalized Difference VI (NDVI) [13] is the most commonly used multi-spectral index. At the leaf level, the NDVI is correlated with leaf Chl content; however, at the canopy scale, the NDVI is much more affected by the changes in the leaf area index (LAI) [14]. Its calculation is based on the difference in reflectance at the red (660–680 nm) and NIR (750–850 nm) spectral bands. Table 1 contains the VIs selected based on the original NDVI equation, which are: modified NDVI (mNDVI), modified DATT index (MDATT), Photochemical Reflectance index (PRI), and Modified Red Edge Simple Ratio (MRESR) index.

Table 1. Chlorophyll vegetation indices used in this study

Type of VI	Equation	Source
ND		
NDVI	$(R_{845} - R_{665}) / (R_{845} + R_{665})$	[13]
mNDVI	$(R_{750} - R_{705}) / (R_{750} + R_{705})$	[15]
MDATT	$(R_{719} - R_{726}) / (R_{719} + R_{743})$	[8]
PRI	$(R_{531} - R_{570}) / (R_{531} + R_{570})$	[17]
MRESR	$(R_{750} - R_{450}) / (R_{705} - R_{450})$	[27]
SR		
NR1	R_{760} / R_{695}	[10]
NR2	R_{800} / R_{650}	[19]
ZM	R_{750} / R_{710}	[9]
G	R_{554} / R_{667}	[20]
BG	R_{677} / R_{554}	[21]
VREI1	R_{740} / R_{720}	[22]
SB		
or SD	$1 / R_{700}$	[23]
	$1 / R_{550} - 1 / R_{750}$	[23]
Chl_{green}	$R_{760} / R_{550} - 1$	[21]
Chl_{red edge}	$R_{760} / R_{714} - 1$	[21]
Others		
CARI	$(R_{700} - R_{670}) - 0.2(R_{700} + R_{550})$	[24]
MCARI	$[(R_{700} - R_{670}) - 0.2(R_{700} + R_{550})] R_{700} / R_{670}$	[25]
NGPI	$1 - (\lambda_r - \lambda_b)(R_r + R_b) / \left(2 \int_{\lambda_b}^{\lambda_r} R_\lambda d\lambda \right)$	in this study
TVI	$0.5[120(R_{750} - R_{550}) - 200(R_{670} - R_{550})]$	[26]

ND - normalized difference, SR - simple ratio, SB - single band, SD - simple difference, R - leaf reflectance

The modified NDVI with wavelengths of 705 nm and 750 nm [15] was developed to eliminate the effects of surface reflectance by incorporating the blue band. It is more strongly correlated with the total Chl content. The MDATT index is proposed as a new index for the remote estimation of Chl content in plants with varying leaf surface structures [8]. Based on the Datt principle [16], this VI was modified by introducing the third

band (λ_3) according to Eq. (4). It aimed to compensate for high leaf surface (specular) reflectance and scattering from the mesophyll, which tend to alter reflectance across the whole VIS and NIR spectrum.

$$MDATT(R_{\lambda_1}, R_{\lambda_2}, R_{\lambda_3}) = \frac{R_{\lambda_3} - R_{\lambda_1}}{R_{\lambda_3} - R_{\lambda_2}}. \quad (4)$$

PRI was originally developed [17] to estimate rapid changes in the epoxidation state of xanthophyll pigments, a major component of non-photochemical quenching. However, PRI has been increasingly used to detect photosynthetic activity at different scales from plant biochemistry to the canopy level. The MRESR index uses bands in the red edge and incorporates the correction of the leaf specular reflection. This VI is a robust measure of healthy green vegetation [18].

Simple ratio VIs: NR1, NR2 (Near infrared /Red), ZM (Zarco - Miller), G (Greenness), BG (Blue/Green) and VREI1 (Vogelmann Red Edge) were tested in several studies [10, 19], [9], [20–22], respectively, to evaluate leaf Chl content in different plant species and in different environmental conditions. VREI1 is sensitive to the combined effects of foliage Chl concentration, canopy leaf area, and water content. The VIs $1/R_{700}$ and $1/R_{550} - 1/R_{750}$ have recently received considerable attention and have been found to be well correlated with the leaf Chl content [23]. The green Chl and Red edge Chl indices (Chl_{green} and Chl_{red edge}) were proposed for a better prediction of Chl content [21].

The Chl Absorption Ratio Index (CARI) [24] measures the depth of Chl absorption at 670 nm relative to the green reflectance peak at 550 nm and the reflectance at 700 nm in order to reduce the effect of some non-photosynthetic materials. The modified CARI (MCARI) [25] is developed to further weaken the noise due to non-photosynthetic materials and is more sensitive to leaf Chl concentrations.

Due to the recent development of hyperspectral sensors, which acquire a quasi-continuous reflectance spectrum, new and more robust Chl detection VIs have been, and are being, developed. One example, proposed and tested in this study, is the Normalized Green Peak Index (NGPI), based on the changes in the SRC area in the green peak (500–670 nm). The triangular Vegetation Index (TVI) [26] is calculated as the area of a hypothetical triangle in the spectral space that connects the green peak, minimum Chl absorption, and the NIR shoulder.

2.4. Data analysis

In order to evaluate the potential of the selected VIs to discriminate the changes in leaf Chl content, statistical analyses were performed. A two-step procedure was adopted for the assessment of the presence and significance of the differences between VIs means of all pairs compared (the VIs of the control group with the VIs of every other group of the treated plants). First, to test whether differences exist between the VIs means, the null hypothesis $H_0: \mu_1 = \mu_2 = \mu_3 = \mu_4$ versus the alternative hypothesis $H_1: \mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4$, one-way analysis of variance (ANOVA) was used. Secondly, a multiple comparisons test using the Tukey-Kramer procedure was carried out in order to

determine which pairs of VIs means have statistically significant differences. This test was applied because of the small sizes of the datasets, as well as with an approximately equal size $n_i \cong n_j$, where n_i and n_j are the numbers of data in i and j datasets, respectively.

Every two sets of VIs were compared by calculating the difference between their means Δx and comparing it with the critical difference Δx_c

$$\Delta x_c = q_c \sqrt{\frac{MS_W}{2} \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}, \quad (5)$$

where MS_W is the mean square of error and q_c is the critical q -value taken from the Studentized Range q table [27] for the confidence level $\alpha=0.05$ and the degree of freedom $df = N - k$ (N - the total number of data in all datasets, k - the number of datasets). If the difference Δx_{ij} between the means of the i and j sets is higher than Δx_c ($\Delta x_{ij} > \Delta x_c$), it is accepted that the mean values of the pairs compared are statistically different at a given significance level.

3. RESULTS AND DISCUSSION

The SRCs of the four investigated groups of pepper plants, measured one and two weeks after the infection with CMV, were analyzed at the spectral range of 450-850 nm. The differences between the SRC of the control and treated plants were more expressed in the green (green peak, 500-670 nm) and NIR spectral ranges. The changes in leaf tissue caused an increase of the NIR reflectance. The decrease of Chl content, that is, Chl absorption, caused an increase of the reflectance in the green peak.

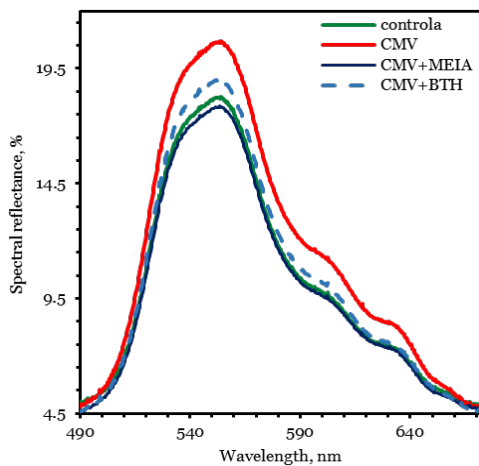


Figure 2. Averaged spectral reflectance characteristics of the control and treated pepper plants in the green peak measured one week after the inoculation with CMV

Averaged SRCs in the green peak, measured one week after the inoculation with CMV, are shown in Fig. 2. The differences between the SRC of the control plants and plants infected with CMV are most

significant. For the combined treatments with two growth regulators and CMV (MEIA+CMV) and (BTH+CMV), the differences are less pronounced. The SRC values in the case of the (MEIA+CMV) treatment are closer to the control SRC, thus indicating a stronger effect of MEIA for the suppression of the viral infection compared to BTH.

Descriptive Excel statistics was used to verify the normal distribution of the VIs datasets for each group of plants. Normality was investigated using the Pearson and Shapiro-Wilk procedure. In the majority of cases (small exceptions) the distribution was normal, with a high coefficient of determination R^2 . As an example, Fig. 3 shows the box-plots of the datasets of MRESR VI calculated for the control and treated plants for two measurements (one and two weeks after the infection).

The ANOVA results show that the mean values of the indices for the control and treated plants are equal only for the NDVI and TVI. For all other indices, the ANOVA results show that there is at least one pair of groups whose means are different.

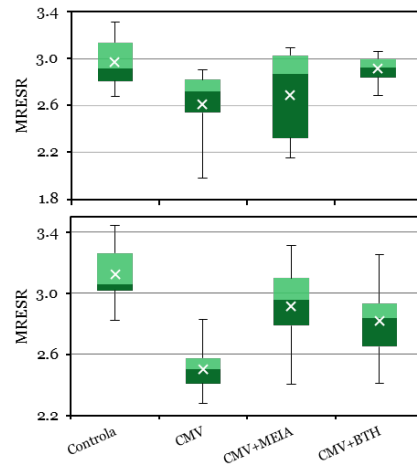


Figure 3. Box plots of the datasets of MRESR VI for the control and treated plants for the first (upper) and second (lower) measurements: median (the boundary between light and dark green boxes), mean value (white x), minimal and maximal values (lower and upper ends of the whisker, respectively), interquartile range containing 50% of values (both boxes)

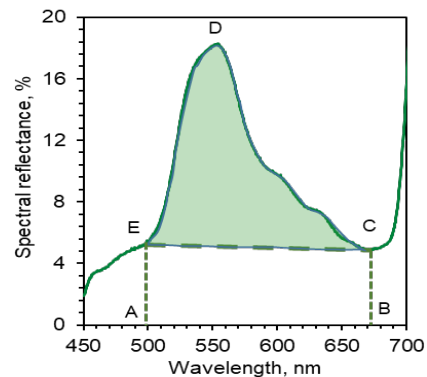


Figure 4. Normalized Green Peak Index (NGPI)

Therefore, the Tukey-Kramer comparison procedure was applied to the VIs means for the investigated plants. The confidence level was $\alpha=0.05$, the degree of freedom was $df=N-k=79-4=75$. The value of q_c taken from the Studentized Range q table is: $q_c = 3.7162$.

The results obtained for the five selected VIs (as the representatives of each category of indices) are given in Table 2. Δx_{ij} is the difference between the means of the i and j VI datasets, Δx_c is the critical difference, and p is the level of significance.

Table 2. Difference between VIs means for pairs compared, critical difference, and p-values at level $p=0.05$ for spectral data measured one week after the infection with CMV

Type of VI	mNDVI		mDATT		SR1		NGPI		MRESR	
Compared samples	Δx_{ij}	Δx_c	Δx_{ij}	Δx_c	Δx_{ij}	Δx_c	Δx_{ij}	Δx_c	Δx_{ij}	Δx_c
Control - CMV p	0.068 ***	0.024	0.007 *	0.006	1.434 ***	0.663	0.056 ***	0.027	0.621 ***	0.176
Control - CMV+MEIA p	0.008 ns	0.025	0.001 ns	0.007	0.143 ns	0.679	0.012 ns	0.028	0.208 **	0.181
Control - CMV+BTH p	0.018 ns	0.025	0.006 ns	0.006	0.195 ns	0.670	0.037 **	0.027	0.303 ***	0.179
CMV - CMV+MEIA p	0.060 ***	0.024	0.008 **	0.006	1.291 ***	0.663	0.044 ***	0.027	0.413 ***	0.176
CMV - CMV+BTH p	0.050 ***	0.024	0.002 ns	0.006	1.239 ***	0.654	0.019 ns*	0.027	0.318 ***	0.174
CMV+MEIA - CMV+BTH p	0.010 ns	0.025	0.007 *	0.006	0.053 ns	0.670	0.025 ns	0.027	0.095 ns	0.179

ns (no statistical significance) - $p>0.05$; * - $p<0.01$; ** - $p<0.005$; *** - $p<0.001$.

It is limited by the lowest reflectance in the green (point E) and red (point C) spectral ranges. This area was calculated for each of the four groups and normalized to the corresponding area ABCDE, Eq. 5:

$$1 - (\lambda_r - \lambda_g)(R_r + R_g) / \left(2 \int_{\lambda_s}^{\lambda_r} R_\lambda d\lambda \right) \quad (5)$$

The NGPI did not have the highest correlation with the changes in the leaf Chl content in this dataset. Similar results gave NR₁.

The averaged SRC of the control and treated pepper plants, measured two weeks after the inoculation, are shown in Fig. 5. The differences between the SRCs of the control and CMV infected plants remain most prominent.

The SRC values of the combined treatments (MEIA+CMV, BTH+CMV) are very close and lower than the control SRC which indicates an increase of the Chl content due to the stimulating action of the growth regulators.

The statistical analyses show that the number of statistically significant differences between the VIs means for most of the calculated VIs decreases by at least one. The result remains the same for the mNDVI, MDATT, ZM, and Chl_{red edge} indices. For the NDVI (greenness index), two differences are significant, which is indicated by a certain change in the colour (greenness) of the leaves. For NR₁, the number of significant differences increases with one. This was suggested in a previous work of the authors [10] on the

The results show that the NDVI and TVI are insensitive to changes in Chl content. Best results show the MRESR, VREI₁, and PI with statistically significant differences in investigated combinations 5, 4 and 4, respectively. The position of the mean values of the MRESR for all groups (white x) can be seen in Fig. 3. The rest of the VIs were responsible for Chl variations but with less sensitivity.

The NGPI, proposed in this study, is the area of the leaf reflectance spectrum in the green peak, Fig. 4, (CDE area).

detection of viral infection in potato plants and gave good results. For the NGPI, in the cases of Control-CMV+MEIA and Control-CMV+BTH, the differences were not significant.

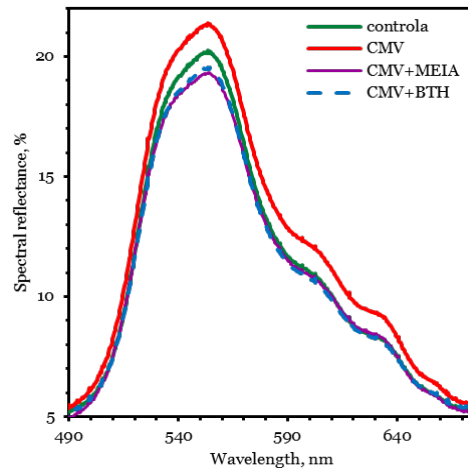


Figure 5. Averaged spectral reflectance characteristics of control and treated pepper plants in green peak two weeks after the inoculation with CMV

4. CONCLUSION

In this paper, nineteen hyperspectral vegetation indices from four categories were tested to explore their potentials in the Chl content estimation. A two-

step statistical analysis (ANOVA and Tukey-Kramer test) was adopted for the assessment of the presence and significance of the differences between the VI means. Three of the VIs (MRESR, VREI1, and PI) showed the best sensitivity. The rest of the VIs were responsible for Chl variations but with less sensitivity. One new VI (NGPI) was proposed and tested in this study based on the changes in the SRC area in the green peak (500–670 nm). The statistical analysis showed an adequate response of the NGPI to the quantity changes in the SRC due to Chl content variations. The proposed approach in this study, based on the analyses of the quantitative relationships between the changes in the Chl content and various narrow-band Vis, has a great practical application for a rapid and non-destructive assessment of Chl content and in particular for studying biophysical and physiological properties of plants under stress.

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