SPECTRAL IMAGING IN THE EVALUATION OF THE CHLOROPHYLL CONTENT IN WHEAT

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ABSTRACT. *The objective of our research was to evaluate the nutritional status expressed through the chlorophyll content in wheat crops, by using the analysis of the digital images taken in the visible spectrum. The study is based on observations of different colors expressed by plants in the visible spectrum, following a different nutritional status. These data about colors were associated and correlated with the chlorophyll content. The biologic material was represented by Alex variety of wheat (Triticum aestivum ssp. Vulgare). The nutritional stress which generated the variation in the chlorophyll content was induced by controlled nitrogen fertilization, in doses between 0 – 200 kg active substance* ha^{-1} *with four levels of PK 0 – 150 kg active substance* ha^{-1}*. Chlorophyll content of wheat plants was determined with a portable chlorophyll meter (Konica Minolta SPAD 502 Plus) which measures the absorbance of leaves in red and near infrared region. Determination of chlorophyll content was made in the same period of time to capture digital images. Capturing digital images was taken with a Nikon D80, at uniform parameters for all variants. The digital images, captured in the visible spectrum, were analyzed from the point of view of their color spectrum by nonlinear perceptual representation systems RGB, HSB and HSL. Chlorophyll content ranged from 40.26 ± 1.36 (SPAD units) to control variant* ($P_0K_0N_0$) *and 55.57 ± 0.55 (SPAD units) at* $P_{150}K_{150}N_{200}$ *variant. After the correlative analysis of the numerical values resulted on color channels (H,S,B and H,S,L) and chlorophyll, we obtained correlations with high significance level (very significantly negative correlation between the chlorophyll content and color channel* B_{HSB}, $r = -0.930$*). Multi-parameter analysis of the variables, accomplished through cluster analysis and data grouping based on Euclidean distances, places the experimental variants according to the fertilization level. Their disposition is also correlated with the chlorophyll content and HSB and HSL values resulted from measurements that were made and from the information stored in digital images.*

Key words: *imagistic spectral analysis, digital image, visible spectrum, RGB, HSB, HSL, chlorophyll, wheat*

INTRODUCTION

In recent years, the technological developments in electronics and informatics came into prominence among other new fields or through successful applications in processing of images captured by digital cameras.

Taking into consideration the structural and functional characteristics of bodies regarding absorption, reflectance and transmission in relation to the wavelength of the electromagnetic spectrum (Figure 1), imaging is practically an approach with very wide range, both on macroscale and on microscale.

Non-destructive techniques based on imaging have been ever more developed and promoted for the assessment of crops and ground cover regarding their floristic composition, biomass, phyto-sanitary status, reaction to stress and estimation of yield, CARTNER 1993, CECCATO et al. 2001, FISCHER et al. 2003, MERZLYAK et al. 2003, ZYGIELBAUM et al. 2009. At the same time, imaging is an method for investigation on a microscale, in genomics and proteomics in the analysis of cell activity, of tissues and organs, based on the capacity of biological molecular structures of having types of behavior that are quantifiable qualitatively and quantitatively in various electromagnetic spectra, especially in IR (NIR, FTIR) and UV, PRENDERGAST and MANN 1978, TSIEN 1998, PHILLIPS 2001.

Infrared investigation methods reveal structures with spectra in the general domain IR (750 nm – 1 mm) or on a limited segment within this domain (NIR, SWIR, MWIR, LWIR, FIR). Each spectrum generated is a mean of a larger number of scannings (20 or bigger) which ensures accuracy in sample analysis, MORAN et al. 1997, HACKMANN at al. 2001.

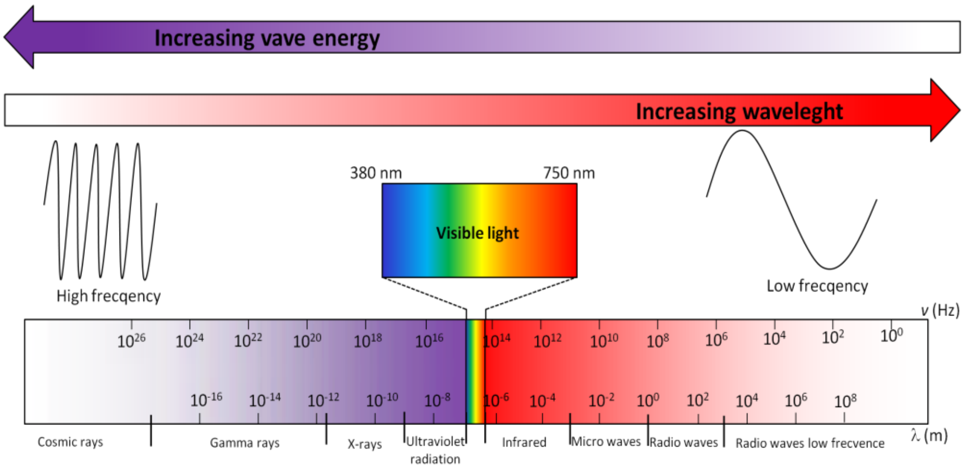


Figure 1. Electromagnetic spectrum - visible domain of capturing digital images

Near infrared chemical imagistic through technique NIR – CI is a method for assessing qualitatively and quantitatively various structural components of a sample, with importance in the qualitative analysis of pharmaceutical substances and products, RAVN et al. 2008, medical analysis and diagnosis, MEHAGNOUL-SCHIPPER et al. 2002, JINGDAN ZHANG et al. 2011.

In agriculture, NIR and FTIR investigation technique is used in determining quality yield for cereals, oil-bearing plants, fruit, vegetables, and the quality of fodder, animal products and other agricultural produce, WATSON 1977, BEZANT et al. 1997, WESLEY et al. 2001, MONICA HĂRMĂNESCU et al. 2010, 2012.

Recent years have brought the emergence of the technique which involves satellite capturing images in fluorescence, with a number of advantages regarding the accuracy of capturing details and of assessing the status of the ground cover. In 2011, NASA made public the first world maps made in fluorescence. ELIZABETH MIDDLETON 2011, member in the NASA research team states that: "Chlorophyll fluorescence offers a more direct window into the inner workings of the photosynthetic machinery of plants from space. With chlorophyll fluorescence, we should be able to tell immediately if plants are under environmental stress - before outward signs of browning or yellowing of leaves become visible", (23).

Investigation technologies based on hyperspectral images (hyperspectral imaging) offers high resolutions, scans and captures a much larger quantity of the spectrum of electromagnetic frequencies in comparison to the UV, IR methods or with common photography, KIM et al. 2001, SCHULTZ et al. 2001, FISCHER and KAKOULLI 2006. On each image (spectral cube), special cameras make a large number of specific images, each image having unique characteristic, which is invisible to the human eye. This is ideal for a large range of uses, such as night vision, assessment of the ground cover and of the phyto-sanitary status of crops. The only drawback is that hyperspectral sensors are not at all cheap (24).

The technique based on hyperspectral imaging is still very expensive, and therefore its availability for current operators is limited by the high prices of equipment or images. The calculation technique with high power of processing and the software for data processing and interpreting are other factors that limit the above-mentioned technology for current use at accessible prices.

The visible spectrum includes radiations with wavelengths between 380 – 750 nm and it refers to that portion of the electromagnetic spectrum which is directly accessible to the human eye, Picture 1. Nevertheless, analysis in the visible spectrum has some shortcomings, since the human eye only perceives a part of the electronic transitions expressed by plants, namely those with high wavelengths and small frequencies in the domain 8000 – 4000 Å – visible domain, (25, 26).

Alternative methods, faster, low-cost and with available equipment would be very useful for practice, even if their accuracy is lower than the dedicated technologies, but with high efficiency, immediate practical applicability and availability to a large number of operators.

In this sense, we embraced an imagistic approach based on the analysis of digital images captured in the visible spectrum by a commercial photo camera, in order to assess the chlorophyll content in a winter wheat crop in different states of nutritional stress induced by controlled fertilization.

MATERIAL AND METHOD

The objective of our research was to analyze the digital images captured in the visible spectrum in correlation with chlorophyll content, as an expression of the nutritional status.

These studies were based on observations of differences in color expressed by plants in the visible spectrum, as a result of different nutritional status. These data regarding color were associated and correlated with chlorophyll content.

The aims of our research was to fundament, check and parameterize the imagistic method based on digital images in the visible spectrum, for the purpose of promoting into current practice an accessible method for evaluating the chlorophyll content and nutritional status in wheat crops.

The vegetal biologic material was wheat, *Alex* variety (*Triticum aestivum ssp. Vulgare*).

Taking into consideration the medium fertility level of the soil in the experimental field, we differentiated nitrogen into doses between 0 and 200 kg ha^{-1} on four PK variants (0 to 150 kg ha^{-1}).

We assessed the chlorophyll content in correlation with the nutritional status with the help of a portable chlorophyll meter (SPAD 502 Plus Chlorophyll Meter).

The digital images were captured with a Nikon D80 photo camera, resolution 10.2 MP DX format. The images were captured in identical conditions for all variants. The digital images were analyzed from the point of view of their color spectrum, by RGB, HSB and HSL nonlinear perceptual systems, Vertan 1999.

Statistical data processing was performed with various mathematical tools, multivariate allometry, correlations, regressions, multiparameter analysis of the variables.

RESULTS AND DISCUSSIONS

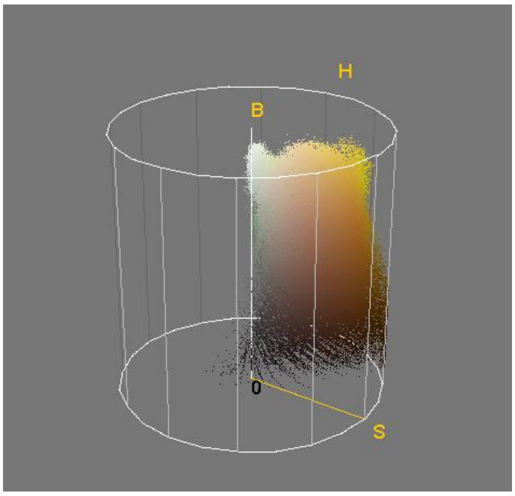
Results on the chlorophyll content and the values obtained by the decomposition of the data stored in the digital images on color channels, according to systems HSB and HSL are presents in Table 1.

The information on color, presented in Table 1, is very eloquently expressed in graphic form in the HSB and HSL analysis systems used. Images a, and b, in Figure 2 illustrate the color information expressed in HSB representation system, containing the images of two variants with extreme values for chlorophyll content; V1 (Chl. = 40.26 ± 1.36) and V11 (Chl. = 55.57 ± 0.55) as a result of nutritional stress.

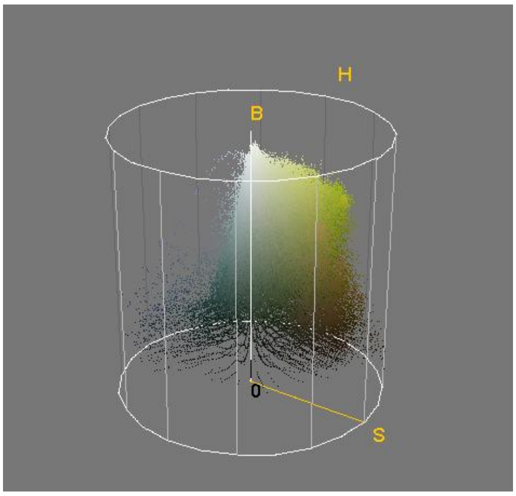
Table 1

Chlorophyll content and numerical data of HSB, HSL systems of decomposition of digital images

Parameters / Variants		Chlorophyll content (SPAD)	Numerical values on systems and color channels					
			HSB Color system			HSL color system		
			H	S	B	H	S	L
PK0N0	V1	40.26±1.36	75	61	58	75	44	40
PK0N100	V2	52.78±0.98	98	40	45	98	25	36
PK0N200	V3	51.38±1.02	100	39	45	100	25	36
PK50N50	V4	48.69±1.22	87	58	48	87	41	34
PK50N100	V5	50.64±0.68	98	42	45	98	26	36
PK50N200	V6	52.31±1.11	96	45	40	96	29	31
PK100N100	V7	51.47±1.02	91	48	47	91	32	36
PK100N150	V8	52.10±0.91	99	40	44	99	25	35
Pk100N200	V9	52.41±0.89	100	39	42	100	24	34
PK150N150	V10	54.04±1.25	93	48	44	93	31	34
PK150N200	V11	55.57±0.55	103	22	34	103	36	41



a, V1 – control variant, PK_0N_0



b, V11 – $PK_{150}N_{200}$

Figure 2. Graphic representation of the color information stored in digital images through HSB system; a - V1 (control variant, PK_0N_0); b - V11 ($PK_{150}N_{200}$)

In order to assess data confidence, we evaluated the variation amplitude of the parameters studied, for assuring the confidence threshold of 95%, Figure 3. The % of variation on PC1 is 91.18.

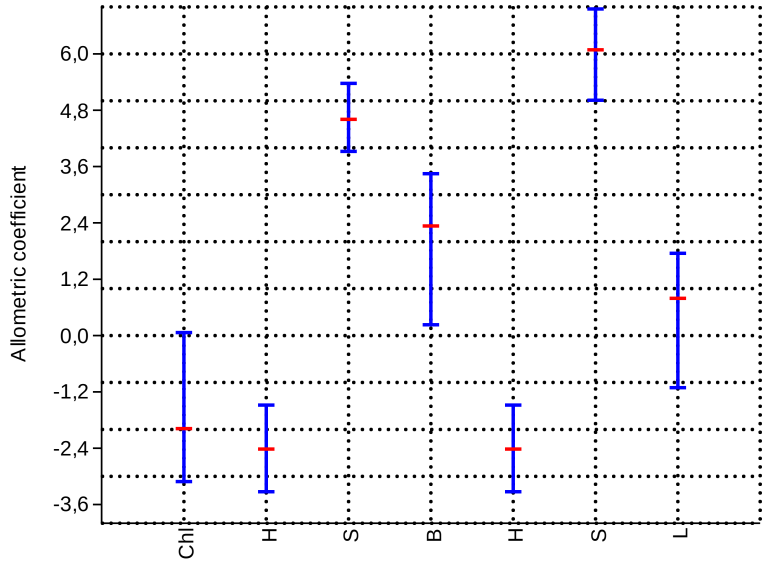


Figure 3. Multivariate allometry, 95% confidence

In order to assess the link and the degree of correlation between the chlorophyll content and the values of the color spectrum resulted from the analysis of the digital images, we performed a number of correlations, both individual correlations, based on pairs of index values, and multiparameter correlations.

The correlation analysis of the values resulted in the two representation systems (HSB and HSL) and the chlorophyll content gave very significantly negative correlation between the chlorophyll content and the color channel B_{HSB} (r = - 0.930). In addition, we registered significantly positive correlations between chlorophyll and color channel H_{HSB} (r = 0.886) and chlorophyll and color channel H_{HSL} (r = 0.886). We obtained significantly negative correlation between chlorophyll and color channel S_{NSL} (r = - 0.815). As for the relation between chlorophyll and the other color channels, we observed correlations of lower intensity: Chl / channel S_{HSB} (r = - 0.794) and Chl / channel L_{HSL} (r = - 0.718), Table 2.

The values of the correlation coefficients reflect in a high level of confidence the correlation between the chlorophyll content, as the direct expression of the nutritional deficit, and the numerical values obtained for color channels through the analysis of the digital images captured in the visible spectrum.

Multiparameter analysis of variables through function cluster analysis and data grouping based on Euclidean distances place the experimental values according to the fertilization level expressed correlated through the chlorophyll content and the HSB and HSL values resulted from the data stored in digital images, Figure 4.

From graphical representation 2 it becomes clear that there are three variant groupings. The control variant stands alone, it being the expression of the maximum level of nutritional stress and with the lowest chlorophyll content.

Table 2

Values of the correlations between the chlorophyll content and the numerical values for color channels in systems HSB, HSL

	Chlorophyll	H	S	B	H	S	L
Chlorophyll	1						
H	0.886	1					
S	-0.794	-0.960	1				
B	**-0.930**	-0.898	0.790	1			
H	0.886	1	-0.960	-0.898	1		
S	-0.815	-0.965	0.996	0.813	-0.965	1	
L	-0.718	-0.566	0.379	0.859	-0.566	0.408	1

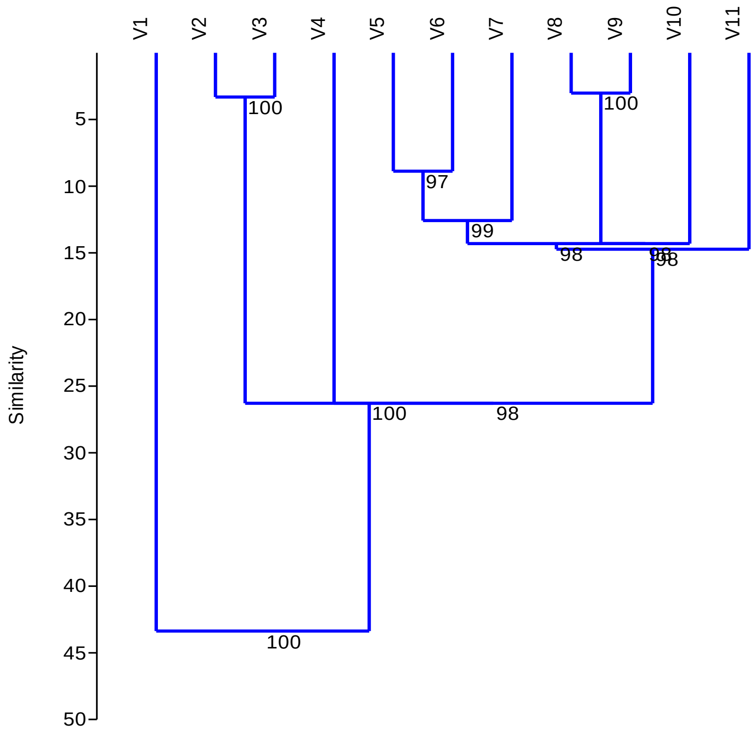


Figure 4. Multiparameter grouping of variables through cluster analysis, Euclidean distance and similarity

A group contains three variants V2, V3 subgrouped and variant V4 solitary, also as an expression of the high level of nutritional stress and low chlorophyll level. The third group contains 7 variants, which benefited from higher fertilization levels, within them variants V7, V10 and V11 being isolated and variants grouped in microgroups: V5 and V6, and V8 and V9 respectively.

The horizontal lines in the dendrogram represent the level of similarity between clusters. The cophenetic coefficient indicates to what extent the dendrogram reflects the structure of similarity of the original data. Its value is 0.879, which indicates high significance ($I_{Cph} \leq 1$). The cluster has a robust structure.

Taking into consideration the distribution of variants, they are differentiated based on the effect they generate, and the common traits that put them together into groups and subgroups are the level of active substance, the level of nutritional stress expressed by the chlorophyll content and the numerical data on HSL and HSB analysis systems.

CONCLUSIONS

The experimental results we obtained reflect the dependence between the variation in the fertilization system, the status of nutritional deficit in plants, the chlorophyll content and the system of expressing color data in HSB and HSL system.

Individual correlations between chlorophyll content and the values of color channels HSB and HSL support the link between the information stored in digital images and the chlorophyll content for the winter wheat crop.

Multiparameter analysis groups the experimental variants based on the affinity of the values of the variables analyzed, and this grouping coincides with the doses of nitrogen administered, assuring the correspondence: determining factor the dose of nutrients – variables determined (chlorophyll) and the color information in the digital image.

Therefore, this imagistic approach based on digital images accumulates information that helps in assessing the nutritional status of wheat crops, in correlation with the fertilization level.

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