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ESTIMATION OF THE PHYSIOLOGICAL STATE OF MAIZE PLANTS GROWN UNDER STRESS CONDITIONS USING HIGH SPATIAL RESOLUTION SPECTROMETER – CONDITIONS FOR SPECTRAL CLASS FORMATION

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Summary. The spectral reflectance, respectively the spectral reflectance coefficients (SRC) of vegetation provide an express and significant information for the impact of abiotic factors (water stress, heavy metals, herbicide pollution etc.) on important bio-agricultural parameters of vegetation in different phenophases. The high spatial resolution (HSR) of spectrometric systems in current use (in laboratory conditions they operate with HSR of the order of several square mm) reveals possibilities for examining individual leaves as well as the fine structure of the leaves. To obtain a reliable average SRC of the leaves making use of the SRC of a set of leaf areas (SRCLA) with dimensions defined by the HSR, the minimal number n_l of SRCLA is to be determined so that the average SRCLA would be an estimate of SRC at a given confidence probability, i.e. it would belong to the spectral class defined by the whole leaf.

In this study n_l was determined for fresh and dry leaves (grown to phenophase 4–5th leaf) of maize, cv. Knezha, hybrid No 655. For this purpose known statistical methods and own examinations were utilized. SRC were obtained by means of the spectrometric system "Spectrum 256" developed by scientists of STIL – Bulgarian Academy of Sciences (BAS).

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***Key words*:** spectral reflectance chracteristics, vegetation, spectral class, maize

***Abbreviations*:** HRC – high spectral resolution; RWC – relative water content; SRC – spectral reflectance coefficients; SRCLA – spectral reflectance coefficients of a set of leaf areas

Introduction

Remote sensing methods applied in the investigation of the Earth's surface, especially spectral reflectance characteristics and SRC, allow us to obtain an express and authentic information about the influence of abiotic agents on the agrobiological indices of agricultural plants in different stages of their development. Recently investigations have been carried out focusing on the use of the spectral reflectance characteristics of agricultural plants under stress conditions – herbicide and heavy metal pollutions, water stress etc. This investigations are still in their laboratory stage and adequate relationships between changes in SRC and the stress factors mentioned above have to be found (Carter, 1994). Due to the specific absorption of solar energy by plant pigments, SRC in the visible and near infrared ranges of the electromagnetic spectrum are particularly important. The possibility of using SRC as indicator of plant water status (Bowman, 1989) has been proven. In normal state, green plants may absorb more than the half of the solar radiation in the visible range, while in the infrared the largest part of it is reflected (Fig. 1). Under stress conditions – drought, treatment with herbecides etc., the chlorophyll content in plants changes, the reflected

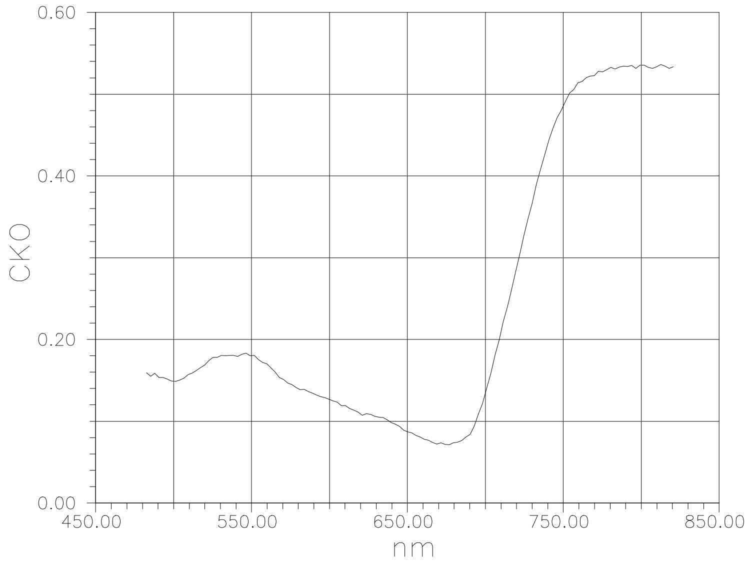

Fig. 1. SRC of maize leave

solar radiation is redistributed along the wavelengths and statistically significant differences in the function of SRC appear (Carter, 1994; Penuelas, 1994).

Modern multichannel spectrometric systems for remote sensing of the earth surface are characterized by HSR (Vane et al., 1993). It is of the order of several square mm under laboratory conditions and allows a study of the objects surface.

The determination of a minimal number n_l of pixels (areas, equal to or larger than the area, defined by HSR of the spectrometric system) in the objects under examination (e.g. leaves) is the aim of the present investigation. It's spectral reflectance characteristics determine adequately the average of all spectral reflectance characteristics of the object considered as a spectral class and are of great importance in agrobiological research. The determination of the limiting (minimal) number n_l of pixels, meeting the requirements for a spectral class formation of the studied object at an increase in the number of measurements, was completed by means of known statistical methods and own developments as well.

Materials and Methods

Spectral data were obtained using the trace multichannel spectrometric system "Spectrum 256", developed by scientists from STIL – BAS (Mishev, 1989) obtained through the formula:

$$SRC = SR_{\lambda}/SR_{scr\lambda} \,,$$

where: SR_{λ} is the spectral reflectance in the λ wavelength and $SR_{scr\lambda}$ stands for the spectral reflectance in the same wavelength of reference (white) screen (Lambertian Surface, i.e. flat surface from which the incident radiation is uniformly reflected in all directions of the hemisphere).

The system measures the radiance from natural objects in 256 or 128 spectral channels in the visible and near infrared regions of the electromagnetic spectrum (450–830 nm), with a half-bandwidth of each channel 1.3 and 2.6 nm, respectively. Under laboratory conditions, the HSR of the "Spectrum 256" was 1.8×0.8 mm at a distance of 3 m and coincided with the pixels of measurement.

Experiments were carried out with leaves of maize plants of the cultivar Knezha, hybrid No 655 grown in pots up to phase 4th–5th leaf. Spectra were recorded from pixels belonging to an area with dimensions 25×100 mm of the leaves. The pixels were located along a spiral (40 pixels on average) which ran from the edge to the centre of the investigated area. Three samples every one of them with volume of 3 leaves were examined: sample 1 of fresh leaves with average RWC by Turner 0.88; sample 2 of fresh leaves with RWC by Turner 0.9 and sample 3 of dried up leaves with average RWC = 0.5. Data sets were obtained with "Spectrum 256" operating in the 128 spectral channels mode. Two most informative channels in the working spectral range – 27th channel (the region of maximum reflection of green pigments, 548 nm) and 124th channel (in which SRC are significantly affected by the water content in leaf structure, 800 nm) were taken into consideration.

Taking n as the number of the set of pixels over which the SRC taken from a given channel are averaged in order to obtain the SRC plateau, the minimal number n_l of pixels for which spectral reflectance characteristics have to be recorded to obtain a representative sample from a spectral class, is determined by the requirement for a spectral class presence, i.e. by the presence of unimodal distribution of the signs, describing the class. In our case, those are the obtained SRC. The minimal number of pixels meeting the condition that SRC distribution parameters satisfy the requirements for stability according to a previously chosen criterion at an increase of n_l is further considered as limiting number n_l. It is usually accepted, that SRC distribution of agricultural plants is close to the normal one. In such case, the arithmetical mean and the covariation matrix of SRC contain exhaustive information about the distribution of SRC by channels, when n_l tends to infinity. In a first approximation, conditions for stability of the SRC averages by channels should be obtained. In conformity with Yanev et al. (1978), the criterion for stability of SRC averages of x in certain channel determines such a value n_l, that the values of x are within the confidence interval of $\bar{x}_{n_{\max}}$ for $n > n_l$:

$$\bar{x}_{n_{\max}} - \Delta < \bar{x}_{n_{\max}} < \bar{x}_{n_{\max}} + \Delta \text{ , at } n = n_{\max}, \quad (1)$$

where 2Δ is the size of the confidence interval and $n_{\max}$ is the number of pixels examined for a certain leaf.

With normal SRC distribution in the individual channels, Δ is determined in accordance with the mathematical statistics, from:

$$\Delta = \mathrm{t.S_x} \text{ ,} \quad (2)$$

where S_x is the standard deviation of SRC sample, $S_x = \sigma/\sqrt{n}$, and the coefficient t defines the confidence probability of Δ, e.g. at $t = 2$ it is about 95%. The graphic expression of this criterion shows the formation of a “plateau” by x depending on n, which plateau is inscribed into the corridor defined by $\bar{x}_{n_{\max}}$ confidence interval (Fig. 2).

An estimation of the of $n_{\max}$ values in the beginning of the experiments was performed by known statistical methods (Yessen, 1985). Expression (2) was also used for solving the inverse problem: determination of the minimal number of experiments (in this case SRC measurements), which provides the given *a priori* value of Δ at a confidence probability defined by t. For this purpose estimates of t, Δ and S_n were obtained using small samples.

Results and Discussion

SRC distributions for 27th and 124th channel of the pixels examined ($n = 36$) for each of the fresh and drying up leaves were studied. SRC histograms were satisfactory fitted

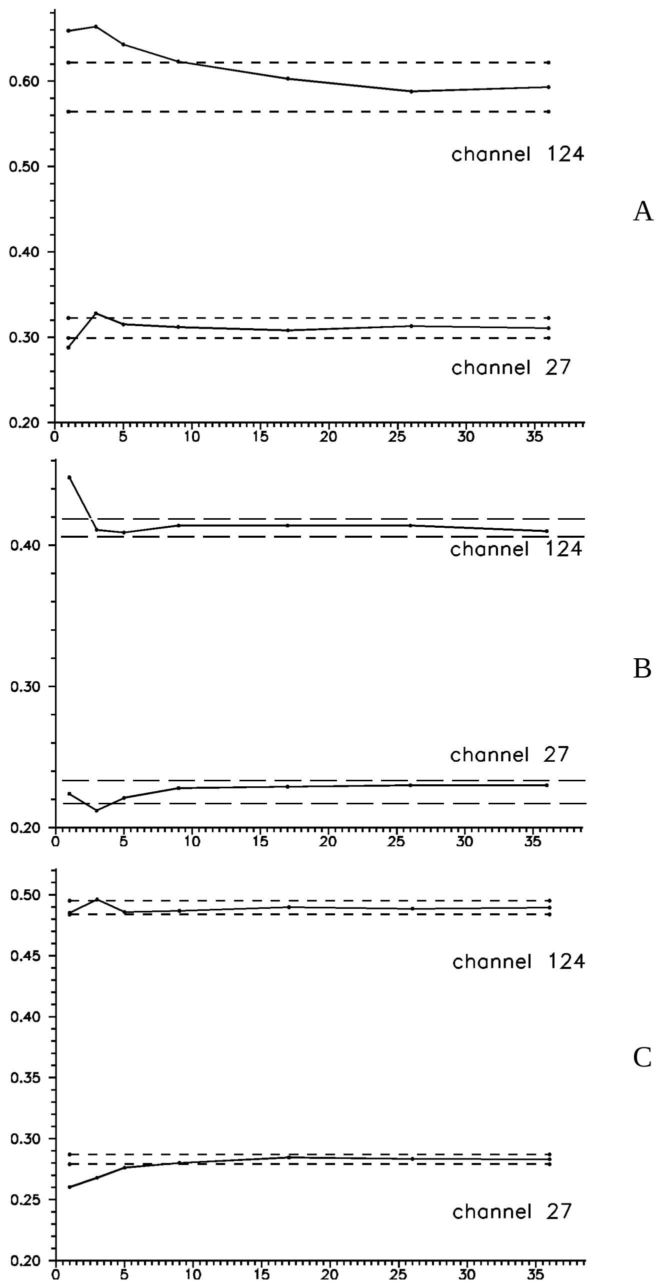

Fig. 2. Run of averaged over *n* pixels SRC in 27th and 124th channels: A) fresh leaf from sample 1; B) fresh leaf from sample 2; C) dried up leaf from sample 3. The confidence corridor (dashed lines) and the entrance of the averaged SRC in it (solid line) are shown

by normal distributions. This result leads to the consideration that SRC of the investigated pixels of one and the same leaf belonged to only one spectral class. The goodness-of-fit was estimated by the X^2 criterion at a confidence level $p > 0.05$ (sample 1: for 27th channel $p > 0.325$, for 124th channel $p > 0.4$; sample 2: for 27th channel $p > 0.28$, for 124th channel $p > 0.35$; sample 3: for 27th channel $p > 0.73$ and for 124th channel $p > 0.58$). SRC histogram of a leaf from sample 2 for 27th channel is shown as an example in Fig. 3.

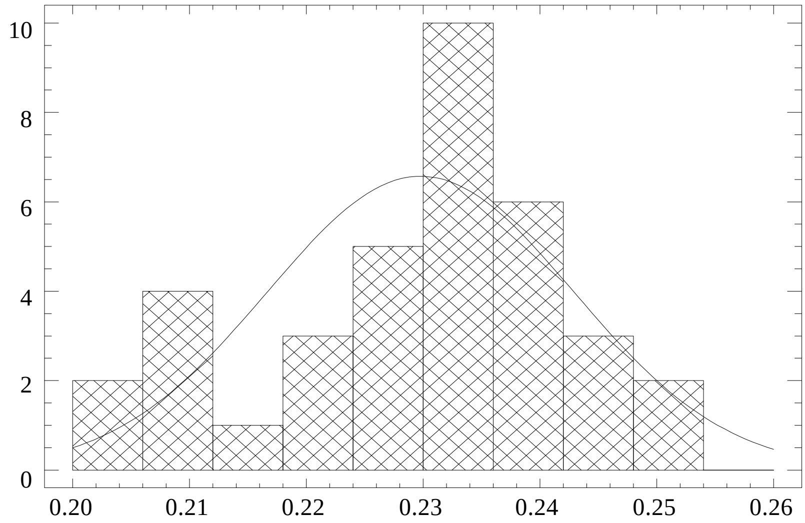

Fig. 3. SRC frequency histogram of fresh leaf from sample 2

The SRC belonging to one and the same class for each one of the samples 1, 2 and 3 was also confirmed by cluster-analysis. Over 90% of SRC of the leaves examined for 27th and 124th channel were gathered in one cluster. The cluster-analysis was completed by the algorithm "Euclidean distance" included in STATGRAPHICS 5.0 package (see also Tou and Gonzales, 1978). The result of SRC cluster-analysis for a leaf from sample 1 at $n = 36$ in channels 27 and 124 is shown as an example in Fig. 4.

The SRC (x) averages and their standard errors (S_x), calculated with different numbers of pixels ($n = 136$) for leaves from samples 1, 2 and 3 for both channels, are shown in Tables 1, 2 and 3. Subsets with n = 1, 3, 5, 9, 17, 26 and 36 ($n = n_{max}$) pixels, distributed approximately uniformly along the spiral of the investigated pixels, were chosen. k=0.05 is commonly considered as an acceptable error for the SRC averages, obtained through samples of finite volume. In this investigation, Δ=0.04 was assumed and estimates for standard deviation $S = S_{x_n}$ at $n = 5$ were used. Hence, applying expression (2) we obtained the following approximate values about n_{max} for leaves 1, 2 and 3: 32, 10, 7 (rounded to the larger integer). We accepted n_{max}= 36 for all leaves.

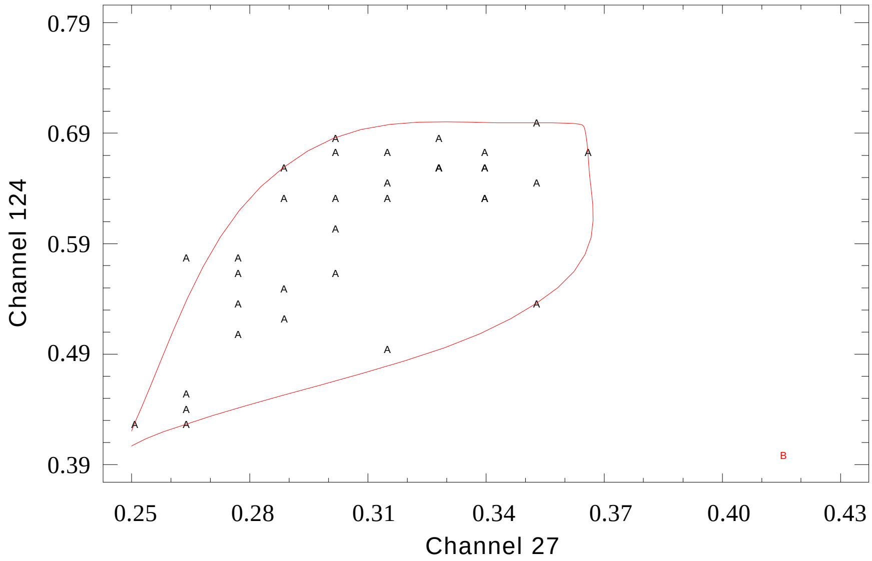

Fig. 4. SRC clusters of fresh leaf from sample 1. A - cluster 1. B - cluster 2

Table 1. Sample averages and standard errors of the spectral reflectance coefficients of leaf 1

n	Channel 27		Channel 124	
	$\bar{x}$	S_x	$\bar{x}$	S_x
1	0.288		0.659	
3	0.328	0.0223	0.664	0.0080
5	0.315	0.0157	0.643	0.0379
9	0.312	0.0092	0.623	0.0379
17	0.308	0.0123	0.603	0.020
26	0.313	0.0077	0.588	0.0179
36	0.3107	0.0059	0.593	0.0144

The results obtained for samples 1, 2 and 3 showed that n_l values in 27th and 124th channel were in the interval 5–15 for all the leaves examined, i.e. $n_l = 15$ provided the fulfillment of conditions (1) and (2) at t = 2 (the inclusion in x "plateau") – Fig. 2. Similarly, the approximate value $n_{max} = 36$ was large enough for estimation of n_l of the examined leaves.

The techniques proposed allows to study the influence of different factors, defining certain agrobiological class (variety of agricultural plants, plant development phase, growing and stress conditions, etc.) on n_l.

Table 2 . Sample averages and standard errors of the spectral reflectance coefficients of leaf 2

n	Channel 27		Channel 124	
	$\bar{x}$	S_x	$\bar{x}$	S_x
1	0.224		0.448	
3	0.212	0.0043	0.411	0.0203
5	0.221	0.0059	0.409	0.0117
9	0.228	0.0048	0.414	0.0076
17	0.229	0.0032	0.414	0.0049
26	0.23	0.0026	0.414	0.0037
36	0.23	0.0022	0.410	0.0043

Table 3. Sample averages and standard errors of the spectral reflectance coefficients of leaf 3

n	Channel 27		Channel 124	
	$\bar{x}$	S_x	$\bar{x}$	S_x
1	0.2603		0.4852	
3	0.2680	0.00505	0.4962	0.00662
5	0.2762	0.00622	0.4857	0.00793
9	0.2800	0.00454	0.4868	0.00498
17	0.2846	0.00346	0.4898	0.00418
26	0.2834	0.00253	0.4886	0.00334
36	0.2831	0.00204	0.4895	0.00281

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