An image analysis method for the evaluation of green pigment reduction in wheat leaves under the influence of heavy metals

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Abstract - **In this paper we present the method of leaf greenness visual assessment based on the analysis of scanned images. It involves the comparison of hue distribution of a reference leaf group with corresponding distribution of single tested leaf. The proposed method was utilised in biological experiment examining the influence of heavy metals (nickel and selenium) on wheat seedlings.**

Keywords – **wheat seedlings, heavy metals, chlorophyll, *HSV* colour space, hue histogram, greenness assessment.**

I. Introduction

A significant content of chlorophylls in photosynthetic organisms is responsible for their green colour. In higher plants mainly chlorophylls *a* and *b* occur, which deal 85% of total amount of photosynthetic pigments. Chlorophyll content in leaves provides valuable information about plant condition, because it can change with plant physiological status depending on growth stages and environmental conditions.

There are several physical and chemical methods for chlorophyll content determination. The most of them are destructive for blades and time-consuming, because need a procedure of pigment extraction. One of non-destructive method in this group is leaf reflectance and transmittance measurement in 700–1000 nm wavelength [1]. But in that case we lose also useful information about chlorophyll distribution in leaves, which is sometimes as important as quantitative amount. Because of the drawbacks of chlorophyll measurement methods mentioned above there is a need to develop a fast and approximate method for the comparative assessment of green pigment changes in leaves.

In this paper the authors proposed solution of the problem of pigments assessment in young wheat leaves cultivated with the presence of two heavy metals: nickel and selenium, which can affect the oxidative activity and thus change chlorophyll content [6]. In many plant species photosynthetic pigment decreases with increase nickel concentration due to oxidative stress [2] and significantly increases with selenium treatments. We expect a relationship between chlorophyll concentration and the degree of leaf greenness, but in the case of this particular study and results of biological experiment only visual inspection of leaves and chlorophyll distribution was taken into account.

II. Material and biological experiment

The materials for studies were young wheat plants. The aim of the experiment was to investigate the negative effect of nickel on oxidative activity at the early growth stage and to determine the neutralizing effect of additional selenium fertilization. One group of seven days old wheat seedlings was subjected to different concentrations of nickel sulfate ($NiSO_4$) every alternate day. In the same time other two groups were treated with different concentrations of sodium selenite pentahydrate ($Na_2O_3Se{\bullet}5H_2O$) and with both compounds. All groups were compared with control group not treated with heavy metals.

III. The measurement system

The image measurement system for the detection of leaf blades and their substantially green pixels includes (Fig. 1):

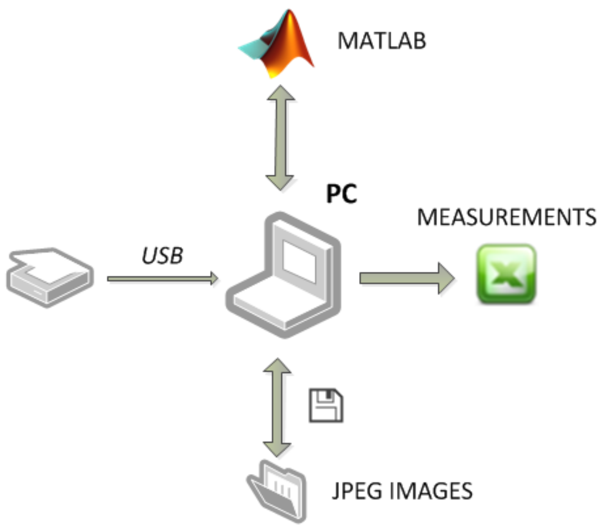


Fig.1 The block diagram of leaf's histochemical reaction measurement system

- a standard flatbed scanner with 300 *dpi* resolution and 24 *bit/pixel* colour depth equipped with appropriate software package controlling the scanning process,
- a laptop with at least dual core processor, minimum 4 *GB* RAM and operating system *Windows* 7 32 or 64 *bit*,

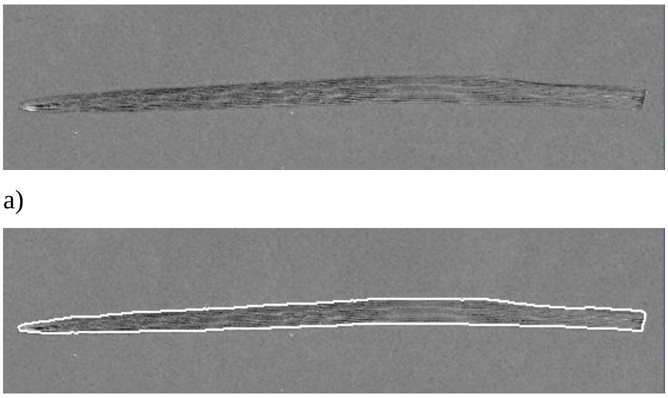
- MATLAB 2008 environment and *Visual Studio Express 2008* C++ compiler plus M language application executing the proposed image segmentation and evaluation method,
- *Excel* application to preserve the measurement results: green pigment ratios, computed areas of regarded as green pixels and the whole leaf blade.

The scanned leaves of wheat have been exposed on a highly saturated and uniformly coloured blue background. After scanning the images have been saved as disk files in JPEG format before reading them into MATLAB environment.

The developed MATLAB application provides further processing and analysis of leaf images with the help of appropriate M functions and C++ functions stored in MEX files. The application uses several library functions from *Image Processing Toolbox* [5].

IV. Green assessment ratio evaluation

The evaluation of any leaf features must be preceded by the leaf blade extraction in the scanned images.



a) b)

Fig.2. The image of an example wheat leaf a) before segmentation, b) after segmentation with visible boundaries of detected leaf mask contained in the image I_M. Original image background is blue; leaf blade is green with yellow-green regions (brighter places).

The images are initially divided into partial images including single leaf objects (Fig. 2a, b). The idea of leaf blade extraction consists in thresholding the difference of image colour components I_R and I_B of the image I_{RGB} from *RGB* colour space as shown in Equ. (1).

$$I_M = \neg T(I_B - I_G), \tag{1}$$

where I_B, I_G – blue and red leaf image components, I_M – binary image of leaf blade mask, '¬' – binary image negation symbol, $T(\cdot)$ – Otsu thresholding operation [4]. Because of using colour component difference global image thresholding is fully acceptable even in the presence of foreground object shadows which are in fact minimal for naturally flat leaves. To assess green pigmentation of the tested leaves the original image I_{RGB} has been converted into *HSV* colour space as follows in Equ. (2) [3],[5].

$$\{I_H, I_S, I_V\} = rgb2hsv\left(I_{RGB}\right), \tag{2}$$

where *rgb2hsv*(·) means a function available in MATLAB's *Image Processing Toolbox*.

In the successive steps of the proposed algorithm only the hue image component I_H has been taken into account. The flowchart of complete algorithm is presented in Fig. 3.

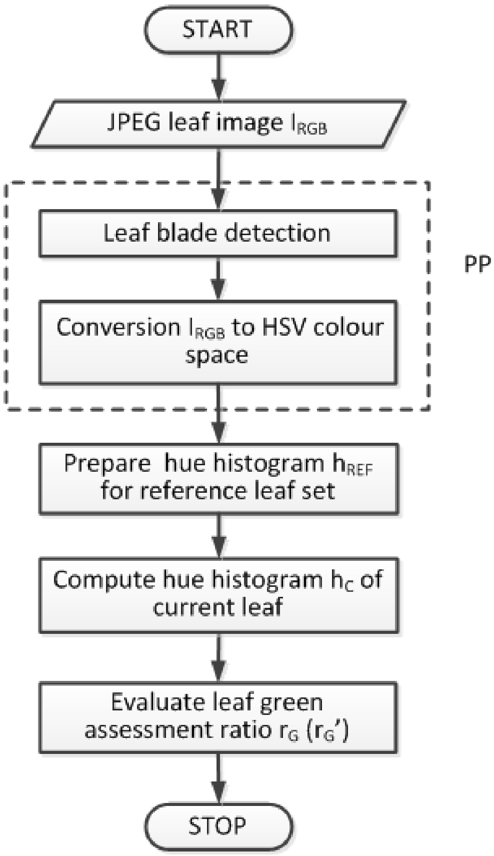


Fig. 3. Flow diagram of the algorithm for the proposed leaf green assessment method, PP – image preprocessing module

In the paper two approaches to the evaluation of green assessment ratios have been proposed. Both of them apply a control group of leaves, which carries information of a reference green colour. For this group a normalised colour hue histogram h_{REF} has been computed according to Equ. (3), which serves as a reference of pigment distribution.

$$\begin{aligned} h_{REF}(H) &= \frac{1}{N_R}\sum_{i=1}^{N_R}\frac{1}{\left|M_i\right|}\sum_{p\in M_i}\left(I_H^{(i)}(p) = H\right), \\ M_i &= \left\{p : I_M^{(i)}(p) > 0,\ I_S^{(i)}(p) > S_{MIN}\right\}, \end{aligned} \tag{3}$$

where: p – the symbol of an image pixel, M_i – the set of leaf blade mask pixels in an image $I_M^{(i)}$ with saturation value above S_{MIN}, $I_H^{(i)}(p)$ – the value of hue for a pixel p in an image $I_H^{(i)}$, $I_M^{(i)}(p)$ - the mask value for a pixel p in a mask image $I_M^{(i)}$, N_R – the cardinality of reference image set. It is well known from the definition of *HSV* model components that the determination of colour hue is highly uncertain for very low saturation values. Therefore all hue histograms are calculated for image pixels p with the saturation value $I_S(p)$ above some minimum value $S_{MIN.}$

Method 1 applies the mean value of the histogram h_{REF}, to determine the reference hue of a green colour H_G computed according to Equ. (4).

$$H_G = mean\left(h_{REF}\right). \qquad (4)$$

Symmetric hue band $B_G = [H_G - W_B/2, H_G + W_B/2]$ of width W_B is then introduced around H_G as a representation of green hues. It is assumed that every "truly" green pixel p of a leaf coming from the reference leaves or outside of this group must have its hue value $H(p)$ placed inside of the green hue band i.e.:

$$H(p) \in \begin{cases} \{G\} & if\ H(p) \in B_G \\ \{G\}' & otherwise \end{cases}, \qquad (5)$$

where $\{G\}$ means the set of "green" hues. Therefore green assessment ratio can be defined like in Equ. (6):

$$r_G = \frac{\sum_{H=H_G-W_B/2}^{H_G+W_B/2} h_C(H)}{\sum_{H=H_G-W_B/2}^{H_G+W_B/2} h_{REF}(H)}, \qquad (6)$$

where $h_C(H)$, $h_{REF}(H)$ – hue histograms of current leaf and reference group of leaves respectively. The bandwidth W_B used for computation of r_G should be relatively narrow, e.g. about 20° in the full hue range [0, 360°] because the appropriate histogram counts are averaged inside of W_B as stated in Equ. (6). This idea of r_G ratio calculation has been depicted in Fig. 4. It is assumed that the average of h_{REF} sum located in the denominator of Equ. (6) is always greater than a given minimal value h_{MIN}.

The green assessment ratio r_G' in method 2 defines mean conformity between the hue histogram of a reference leaves group and the hue histogram of a currently tested leaf. The hue range taken into account is typically full (W_B=360°) or at least widely spread around H_G in comparison with the method 1. The proposed similarity measure between a current leaf and the reference group hues has been given in Equ. (7). It includes the proportions f_H of minimum to maximum counts for every bin H of the normalised hue histograms h_{REF} and h_C. Additionally the ratios f_H can be provided with different weights w_H incorporating different importance of bin count dissimilarities closer to the reference hue H_G (Equ. (8)). Currently all w_H are assumed equal to $1/N_B$, where N_B means the number of bins pairs with maximum count in each pair greater than a certain value h_{MIN}.

$$r_G' = \sum_{H \in B_G} w_H f_H, \qquad (7)$$

where

$$\sum_{H \in B_G} w_H = 1, \quad f_H = \frac{\min\left(h_C(H), h_{REF}(H)\right)}{\max\left(h_C(H), h_{REF}(H)\right)}. \qquad (8)$$

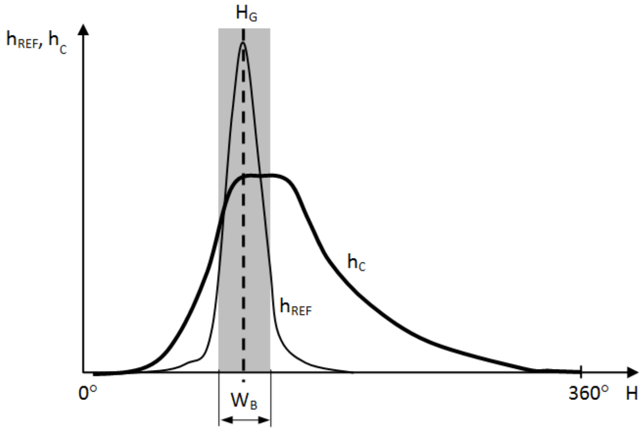


Fig. 4. The hue histograms: h_C - of a tested leaf, h_{REF} – of a referenced group of leaves. H_G – the hue of green.

All considered hue histograms have typically 256 bins. The second method regards each difference of hue histogram profiles as a measure of dissimilarity to the reference green population.

The proposed algorithm has several parameters and options, most of them can be set in the application main window (Fig. 5). Among them are:

- the number of histogram bins (levels of hue) - taken as 256,
- green bandwidth $W_B \in [0, 360°]$ – typically 20°,
- lower limit of pixel saturation $S_{MIN} \in [0, 1]$ – typically 0.05,
- h_{MIN} minimum normalised histogram value in a bin – assumed as 0.0001.

The application window enables to load JPEG leaf image file ("*Open...*" button), to start the measurement ("*Start*"), to display the hue histogram h_C of currently tested leaf (Fig. 6). The additional button "*Comp histo*" initialises simultaneous visualisation of the current leaf histogram h_C and the reference histogram h_{REF}. Every single result of the similarity green ratio r_G (r_G') is displayed as a list entitled "*Measurement result*". It includes the area of currently tested leaf and the virtual area of green pixels from the band B_G named as "*green area*". The evaluated ratio r_G can also be understood as the quotient of these two data.

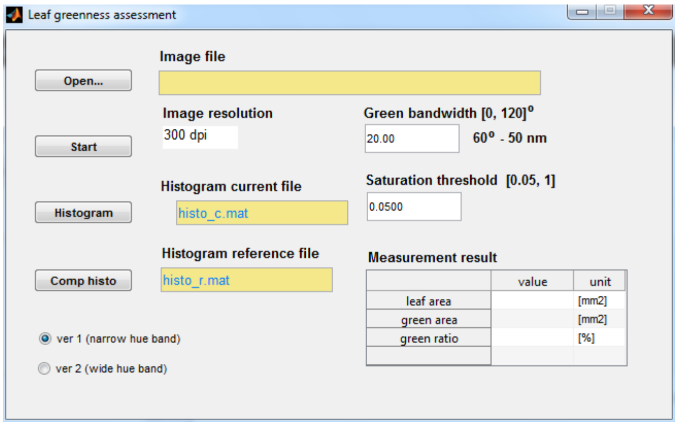
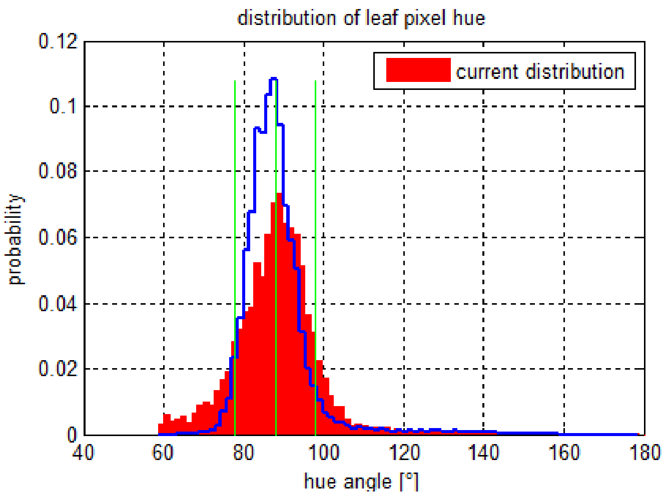


Fig. 5. Main window of the application; [*ver1*, *ver2*] – selects evaluation of r_G or r_G' shown as "*green ratio*" in the list "*Measurement results*", "*Green bandwidth*" – the bandwidth W_B for narrow band version.

a)



b)

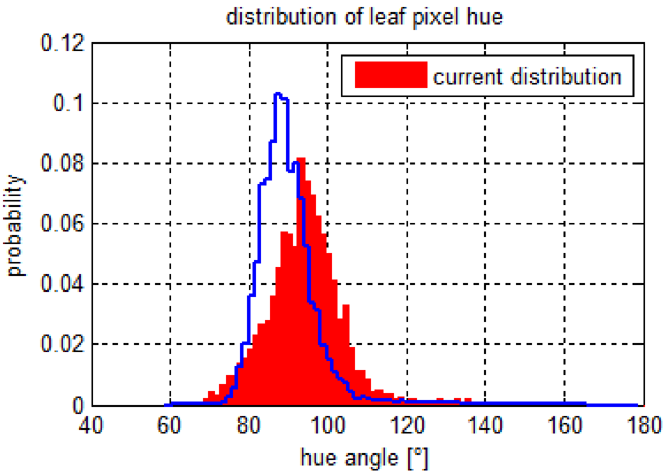


Fig. 6. Example histograms of leaf pixel hue distribution; stairstep plot – reference set distribution, bar plot – current leaf; a) plot for nickel treated (70 mg/l) leaf and narrow hue band used; vertical lines – predefined narrow band b_G for the purpose of r_G calculation, b) plot for selenium treated (89 mg/l) leaf and wide hue band used.

TABLE 1

RESULTS OF GREEN RATIO ASSESSMENT IN WHEAT SEEDLINGS USING THE NARROW (1^{TH} METHOD) AND WIDE (2^{ND} METHOD) HUE BAND

Experimental groups	Solution concentr.	r_G (1^{th} method)	$r_{G'}$ (2^{nd} method)
selenium treated	102mg/l	0.84	0.57
	92mg/l	0.82	0.59
	89mg/l	0.72	0.44
	85mg/l	0.99	0.82
	77mg/l	0.97	0.63
mean		**0.87**	**0.61**
nickel treated	78mg/l	0.92	0.49
	70mg/l	0.83	0.48
	66mg/l	0.88	0.54
	55mg/l	0.88	0.56
	52mg/l	0.86	0.48
mean		**0.87**	**0.51**
nickel and selenium treated	84mg/l	0.92	0.62
	82mg/l	0.87	0.59
	74mg/l	0.83	0.58
	71mg/l	1.00	0.74
	58mg/l	0.84	0.52
mean		**0.89**	**0.61**

V. EXPERIMENTAL RESULTS & CONCLUSIONS

The algorithm for greenness assessment has been applied to the four groups of leaves including one reference group and three other groups treated with different combination of heavy metals. The assumptions of the experiment have been explained in section II. The results are listed in table 1. Both the reference group of leaves and other groups have been represented by a set of 5 images each containing single leaf object. Due to the activity of nickel or selenium within the period of wheat cultivation chlorosis (spots or scratches with yellow-green or washy green colour) appeared in the leaf blades. The algorithm confirms observed colour changes by the reduction averaged green assessment ratios in plants treated with nickel down to the value of r_G ≈0.87 (ver 1) and r_G ≈0.51 (ver 2). The treatment of wheat seedlings with the combination of both nickel and selenium at the same time lowers the r_G ratio to above 0.89 and 0.61 for each method respectively and therefore seems to have less impact on the loss of chlorophyll. The expected correlation between computed r_G ratios and associated heavy metal concentrations is unfortunately unnoticed. The reason for this may be to small range of these concentrations. The results obtained by the proposed method should be compared with the measurements of chlorophyll content provided by biochemical methods applied to the same plant groups.

The processed images including single leaf blades are scanned at the 300 dpi resolution. Their typical sizes lay in the range from 800×400 pixels to 1500×500 pixels. The execution time of the algorithm for such an image in *MATLAB 2008* environment varies from 10 *s* to 20 *s* and therefore the analysis of several series of leaf images stored in disk files in one session is fully realistic.

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