EFFECT OF NITROGEN FERTILIZATION ON THE COLOUR OF WHEAT LEAVES AS AN INDICATOR OF APPLICATION DEFICIENCY

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Abstract
The article is focused on comparing vegetation indices tested on plants grown in controlled environment. The indices were derived from scanned RGB images of leaves of spring wheat, variety Dafne. They were the Kawashima index (R-B)/(R+B), the excess green index (2G-(R+B))/(R+G+B) and hue. The images were processed in the ImageJ program and compared based on histograms of index values over the leaf areas. There were four variants with varying degree of fertilizer, herbicide and fungicide application to the plants, including a control variant with no application. Leaf samples were analysed for 11 weeks after the emergence of plants. The indices did not reliably distinguish between the variants, however greater differences were found between early and later stages of the plant development.

Key words: *Triticum aestivum; rgb channels; vegetation index.*

INTRODUCTION
The response of plants to environmental factors can express in the change of spectral reflectance of the foliage *(Ayala-Silva & Beyl, 2005)*. However, since getting a full reflectance spectrum requires costly instruments, a cheaper option is to use a hyper-spectral or multi-spectral camera to receive an image with a multitude of channels in various frequency bands ranging from ultraviolet to near-infrared light. *(Akhtman, Golubeva, Tutubalina, & Zimin, 2018)*. Most widely used approach is the employment of vegetation indices, numbers obtained by arithmetic operations usually over intensity values of red, green, blue and near-infrared bands *(Wang, Zhou, Zhu, & Guo, 2017)*. The most well-known such index is the normalized difference vegetation index (NDVI) used to evaluate the proportion of vegetation cover.

A still cheaper option is to use a regular RGB colour camera, which creates an image from three overlapping frequency bands that approximate human colour vision. RGB images can be used to distinguish the maturity status of plants *(Alharbi, Zhou, & Wang, 2018)*, to detect the presence of diseases *(Dhingra, Kumar, & Joshi, 2018)* and even to estimate the nitrogen status of plants *(Gupta, Ibaraki, & Trivedi, 2014)*.

For multiple crop plants, correlations have been found between indices calculated from RGB channels captured by a digital camera and leaf nitrogen content or chlorophyll content *(Baresel et al., 2017; do Amaral et al., 2018; Kawashima & Nakatani, 1998; Niu et al., 2019)*. Such relations have been studied using various methods ranging from simple regression to machine learning algorithms *(Barbedo, 2019)*, but predominantly they have been done on field data. There has been a lack of comparisons on plants grown in controlled environment.

Therefore, this research was focused primarily on determining the effect of different levels of pesticide application and nitrogen nutrition on the colour differences of the spring wheat leaves grown in controlled conditions.

MATERIALS AND METHODS
The research was carried out under laboratory conditions as a pot experiment. The experiment was carried out in a closed phytotron with adjustable air temperature and humidity under artificial light. The main reason was the need for precise application of fertilizers and plant care products, the possibility of adjusting the temperature and the ease of immediate measurements. In this way, unaccountable effects were eliminated. The cultivation area of each pot was 0.24 m^2.

The tested crop was spring wheat, Dafne variety. The experiment was performed at four different dosage levels, each in four replicates. The water input of each individual vessel was monitored. In later

growth stages, it was facilitated through bottom watering in combination with an automatic misting system.
Wheat was sown on March 21, the seed area density was 5.2 10^6 seeds per hectare, the sowing depth was 30 mm. Applied preparations were Roundup Klasik (pre-emergence application), nitrogen (basic fertilization and two-time urea application) and the fungicide ARTEA plus. The dosages were adjusted in multiples (0, 0.5, 1, and 2) of a base application rate. Thus, four variants of different application intensity were tested (see Tab. 1). Plants emerged March 28th.

Tab. 1 Types of application, dates and amounts

	Preemergent herbicide Roundup klasik	Basic fertilization	Preemergent herbicide Roundup klasik	Fertilization – 7% urea	Fertilization – 7% urea	Fungicide ARTEA plus
Variant	10.3. ($l.ha^{-1}$)	15.3. ($kgN.ha^{-1}$)	24.3. ($l.ha^{-1}$)	20.4. ($kgN.ha^{-1}$)	4.5. ($kgN.ha^{-1}$)	5.5. ($l.ha^{-1}$)
0	0	36.8	0	0	0	0
0.5	5		5	18.4	14.7	0.25
1	10		10	36.8	29.4	0.5
2	15		15	73.6	58.8	1

For each analysis 1 leaf was taken from each container. At the tillering stage, they were selected randomly. During the stem extension, heading and ripening stages, the leaves from the penultimate joint were sampled.
The colour of leaves was scanned immediately after removal with the CANON CanoScan 8800F scanner against a white background and saved in 24-bit bmp format. Images were analysed in the program ImageJ 1.52n. First the white background was removed using threshold function and replaced with black. The RGB channels were then split into greyscale images and subsequently arithmetic operations were performed on the split channels using the expression parsing utility of the program. The expressions were selected from literature based on good performance in sources. The expressions based on RGB values were the Kawashima index *(Kawashima & Nakatani, 1998)* (equation 1) and excess green index *(Woebbecke, Meyer, Von Bargen, & Mortensen, 1995)* (equation 2).

$$KI = (R - B)/(R + B) \quad (1)$$

$$ExGI = (2G - (R + B))/(R + G + B) \quad (2)$$

These operations resulted in 32-bit depth images from which histograms were acquired. The histograms were made from values in the range from -1 to 1 and sorted into 256 bins. The histogram counts were converted to relative frequencies and the distribution of expression values was summarized by average value and standard deviation.
As a third index, hue was selected. For this the images were converted into HSB (hue, saturation and brightness) colour model and only hue histogram was used. Hue values are reported in degrees in 360° scale.
All used indices are expected to be reasonably independent on the brightness of the image, significantly more so than the original RGB channels and they have been used to distinguish between plant canopies and soil, therefore being in practice useful also for the estimation of vegetation cover ratio. However, they still could change according to white balance of the image etc. Therefore, each image was taken with the same setting of the device. Figure 1 shows an example of histograms of RGB channels and indices made from the same image. It is evident, that the indices have narrower distributions, thus making them more suitable to distinguish between images.

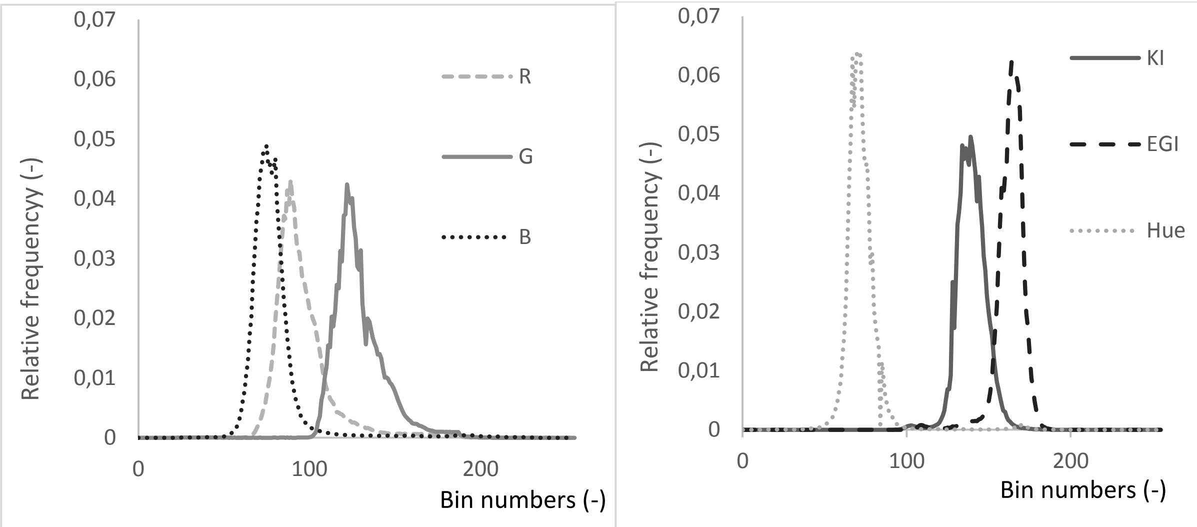

Fig. 1 Histograms of RGB channels and indices of an image, horizontal axes show histogram bin numbers.

RESULTS AND DISCUSSION

Table 1 presents the Kawashima index values. The KI values received were in the range from -0,032 to 0,091 and there was a decreasing tendency with succeeding sampling dates. In *(Kawashima & Nakatani, 1998)* chlorophyll content was correlated with the expression 0.952 – 1.76 KI, however the range of KI values was higher (ca. 0.1-0.5). However, there is a weak tendency towards lower values of KI in variants with more nitrogen input. This is more evident in the last three samplings. However, the differences between variants at the same date are always well within one standard deviation.

Tab. 2 Kawashima index (KI) average values and standard deviations

Variant	weeks after emergence							
	2	5	6	7	8	9	10	11
0	**0,091**	**0,084**	**0,044**	**0,061**	**0,016**	**0,013**	**0,000**	**0,015**
	±0,069	*±0,055*	*±0,054*	*±0,055*	*±0,048*	*±0,050*	*±0,045*	*±0,044*
0.5	**0,085**	**0,075**	**0,053**	**0,029**	**0,029**	**0,001**	**-0,017**	**-0,008**
	±0,071	*±0,049*	*±0,057*	*±0,064*	*±0,059*	*±0,053*	*±0,047*	*±0,049*
1	**0,067**	**0,072**	**0,051**	**0,051**	**0,011**	**-0,027**	**-0,027**	**-0,021**
	±0,065	*±0,056*	*±0,052*	*±0,055*	*±0,044*	*±0,046*	*±0,049*	*±0,046*
2	**0,087**	**0,069**	**0,056**	**0,036**	**0,029**	**-0,017**	**-0,032**	**-0,027**
	±0,069	*±0,059*	*±0,048*	*±0,054*	*±0,056*	*±0,045*	*±0,049*	*±0,048*

Table 3 shows the values of excess green index. EGI is generally useful to distinguish green plant parts from e.g. soil, however it has been also shown to correlate weakly with chlorophyll content in rice *(Saberioon et al., 2014)* but with varying correlation coefficients in different development stages. In present case it shows similar behaviour to KI since it also tends to decrease with the development of the plants, although with multiple exceptions. The values for variants are very close again except for the last three sampling dates when the variants with lower applications show higher EGI.

Tab. 3 Excess green index (EGI) average values and standard deviations

Variant	weeks after emergence							
	2	5	6	7	8	9	10	11
0	**0,275** *±0,069*	**0,271** *±0,061*	**0,247** *±0,056*	**0,243** *±0,050*	**0,235** *±0,049*	**0,219** *±0,053*	**0,197** *±0,053*	**0,219** *±0,048*
0.5	**0,257** *±0,078*	**0,276** *±0,053*	**0,241** *±0,058*	**0,235** *±0,054*	**0,244** *±0,055*	**0,203** *±0,055*	**0,175** *±0,054*	**0,199** *±0,050*
1	**0,267** *±0,079*	**0,275** *±0,063*	**0,256** *±0,049*	**0,250** *±0,055*	**0,224** *±0,045*	**0,182** *±0,054*	**0,156** *±0,058*	**0,178** *±0,047*
2	**0,268** *±0,083*	**0,267** *±0,060*	**0,255** *±0,046*	**0,253** *±0,052*	**0,233** *±0,058*	**0,188** *±0,046*	**0,154** *±0,059*	**0,172** *±0,045*

Table 4 shows hue values expressed in degrees and which can be easily expressed in a circular diagram for easy visual comparison. In studied data the hue values tend from warm green (hue 105) towards mid green and cool green (hue 120-135) with the development of the plants. The variants are very close until week 7. Again, the last three weeks show the largest differences between variants.

Tab. 4 Hue average values and standard deviations in degrees

Variant	weeks after emergence							
	2	5	6	7	8	9	10	11
0	**103** *±18*	**102** *±15*	**109** *±14*	**105** *±11*	**115** *±12*	**116** *±16*	**119** *±16*	**115** *±14*
0.5	**104** *±23*	**103** *±10*	**107** *±17*	**113** *±14*	**113** *±14*	**120** *±17*	**125** *±18*	**122** *±16*
1	**107** *±22*	**105** *±14*	**107** *±12*	**108** *±13*	**116** *±14*	**128** *±17*	**130** *±21*	**126** *±16*
2	**104** *±25*	**104** *±15*	**106** *±11*	**111** *±12*	**111** *±17*	**125** *±12*	**132** *±21*	**128** *±16*

In the presented data, varying degrees of fertilizer, herbicide and fungicide application do not show significant differences. Since the values could not be correlated with leaf nitrogen or chlorophyll contents, it cannot be determined whether they are useful for nitrogen measure on spring wheat. On the other hand, differences are more obvious between early and later growth stages. Therefore, they could still be used for comparing development and ripeness level in a field.

In practice, colour sensing methods can be sensitive to light conditions. For example, in sunny conditions the increased surface reflections will yield wider distribution of colour intensities. This makes reliable differentiation between index values more difficult. Also, it means that there is a potential for errors if conditions change during image acquisition over tested area. It is, therefore, reasonable to conduct both experiments in controlled environment and in field to ascertain the effectiveness of an image treatment algorithm.

CONCLUSIONS

Three indices derived from RGB images of spring wheat leaves from a colour scanner have been tested for their differences for 11 weeks after plant emergence in four degrees of fertilizer, herbicide and fungicide application doses. The tested indices were the Kawashima index, excess green index and hue. The indices performed generally similarly in that they were capable of distinguishing between stages, however differences between application variants were not significant, although they were not correlated with chlorophyll or nitrogen content.

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