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Preliminary studies to evaluate the use of spectral data in monitoring of apple orchard parameters

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SUMMARY

The introduction/application of precision agricultural technologies has more important role in various fruit growing sectors among others apple growing. Remote sensing methods can detect electromagnetic waves where the green colour of the leaf is responsible for the chlorophyll content. The absorption of chlorophyll is in the wavelength range of 450–670 nm. Samples of apple tree leaves were taken on a weekly basis from the apple orchard at Horticultural Unit of Pallag on University of Debrecen in 2019 summer. Our studies were performed on 2 cultivars (Early Gold, Golden Reinders) and the samples were processed using 2 methodologies: a non-destructive spectral method and spectrophotometric method chlorophyll and carotenoid contents were calculated, which were created into some groups and compared with the spectral values. When the plant begins to lose strong green colour and turns yellow spectral measurements show that chlorophyll content decreases as the proportion of chlorophyll-carotenoid in the plant changes. In case of grouping into intervals, it can be observed that as the chlorophyll content increases the reflectance value decreases continuously due to the strong absorption. Based on the results, close relationship between the pigments can be detected.

Keywords: *apple, pigment content, stress, spectral analysis*

INTRODUCTION

The emergence of hyper- and multispectral technology has increasing role in the fruit production sectors among precision agricultural technologies (Burai et al., 2009; Milics et al., 2008; Riczu et al., 2014). Electromagnetic waves (400–7500 nm) can be detected with remote sensing methods. The reflective properties of the plant leaf sample can be examined in the visible wavelength range (400–700 nm). Plant chloroplasts absorbed maximum light at the wavelengths of 450 nm, 680 nm, and 700 nm, correspondingly, while the parenchyma tissue's structure and the lignin content of the cell wall account for the significant near-infrared reflectance. The reflectance properties of the leaves are determined by the sum of the surface area of the cell walls and not by the size of the space between the cells. The evolution of the reflection values depends on a lot of factors, e.g. in addition to the pigment composition, the quality of the leaf surface or the health status of the plant (Nagy, 2015). Various pigments such as anthocyanin, xanthophyll, carotenoid, and chlorophyll characterize leaf colour and these are also important for the conversion of and the energy of light (Winkel-Shirley, 2002; Nagy et al., 2016). Although photosynthetic pigments absorb light, the leaves in the red band have such a low reflectance value. Chlorophyll shows strong absorption in the 450–670 nm wavelength range. By measuring the chlorophyll contents of plants, we can analysis of the proposed about their physiological condition (Nagy et al., 2014) is the cause of the leaf's green color, which is easily destroyed in the lack of water, at high temperatures, and under strong light. In in addition to the primary damage induced by oxidative stress, which impacts lipids, proteins, nucleic acids, and chlorophyll degradation, plants under drought stress also experience secondary damage. In the case of extreme drought, the quantity of both types of chlorophyll shows a decreasing trend (Sircelj et al., 2007), so the chlorophyll values can be used to study the response of a plant to environmental stresses (Ghobadi et al., 2013; Neto et al., 2017). In addition to various stresses the plant aging can also be detected by the transformation or disappearance of pigment amounts (Gregersen et al., 2014; Kuska et al., 2018). The chlorophyll content is closely related to the nitrogen content of the plant; thus it is closely related to photosynthesis. Usha and Singh (2013) demonstrated that the reflectance sensitivity to stress-induced chlorophyll content is high in the 690–700 nm range and that, if stress is strong enough even to inhibit chlorophyll formation, increased reflection can first be detected at characteristic absorption wavelengths. For determining plant water stress, the 760–790 nm range is suitable (Jung, 2005; Nemeskéri, 2011), but the 730 and 960 nm wavelengths are associated with water absorption bands (Yu et al., 2014). The reflection of healthy vegetation increases exponentially at 700 nm moving towards the infrared range and the 40–50% of the received energy is reflected by healthy vegetation in the range of 700–1300 nm, which is primarily due to the internal structural properties of the foliage. Although these species appear to be very similar to the human eye, the measured reflection provides an opportunity to separate them into separate groups. In order to calculate spectral indices, which needs correct spectral measurement data, the values of the measured attributes can also be provided (Nagy et al., 2014). The aim of our research was to measure plant stress using a non-destructive spectrometric method with AvaSpec 2048 spectrometer based on the evaluation of the pigment content of apple foliage, supplementing the spectrophotometric measurement methodology with a

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Secomam Anthelie Light II UV-VIS spectrophotometer.

MATERIALS AND METHODS

Sample area and sample collection

The samples required for the research were taken from the intensive apple orchard of the Horticultural Unit of Pallag on University of Debrecen. In our studies, we observed two varieties: Golden Reinders and Early Gold, there are 100 individuals in the given sampling row for both varieties. Sampling was performed twice a week, between 9 and 10 am., from 7 July 2019 to 29 August 2019. 30 samples of each species were collected at each measurement period (total is 390). Based on Nemeskéri et al. (2009), leaf samples were taken from the middle of one of the branches at a height of 1.2 m to provide the homogeneity of the sample for determining the pigment content. Leaf samples were taken to a lab, transported in a fridge at 4 °C and processed using destructive and non-destructive spectrophotometry within 6 hours. For collecting spectral data on leaf samples, the AvaSpec 2048 spectrometer, which has a 0.6 nm precision range, was used. The AvaSpec 2048 consists of an optical fiber with an 8-mm diameter that connects a spectrometer to an AvaLight-HAL halogen light source. Laboratory measurement is usually disturbed by the ever-changing light conditions of the environment, which is why a special closed laboratory cabinet is used to isolate the sample for accurate measurement. The measurement is started by calibrating the spectrometer to a white and dark reference. The leaf sample placed on black cardboard is placed under the illumination of the spectrometer after calibration. Then placing the sample in the laboratory cabinet, the reflectance curve is obtained. The measurement was repeated three times. The measured reflectance values were exported to Excel and based on the results of the chlorophyll and carotenoid values measured with the spectrophotometer. The reflectance values and the relative standard deviation values were grouped into nearly identical intervals.

Process of measuring the pigment content of apples with a spectrophotometer

The collected leaf samples were analysed with a Secomam Anthelie Light II UV-VIS spectrophotometer to determine chlorophyll and carotenoid content. 0.10–0.20 g of the tested sample was weighted which was destroyed in a mortar by quartz sand and then 10 ml of 80% acetone were added to the suspension. The resulting liquid suspension was pelleted in a Hettich ROTOFIX 32A centrifuge for 3 minutes at 3000 rpm. Liquid suspensions taken from the pellet were pipetted into cuvettes and measured in a spectrophotometer at 3 wavelengths at 470 nm, 644 nm, and 663 nm. Data for chlorophyll values were calculated by Droppa et al. (2003) (1):

$$\text{Chlorophyll (a + b) fresh weight } (\mu g\ g^{-1}) = (20.2 * A_{644nm} + 8.02 * A_{663nm}) * V/w \quad (1)$$

The values of the carotenoid were calculated by Lichtenthaler et al. (1983) (2):

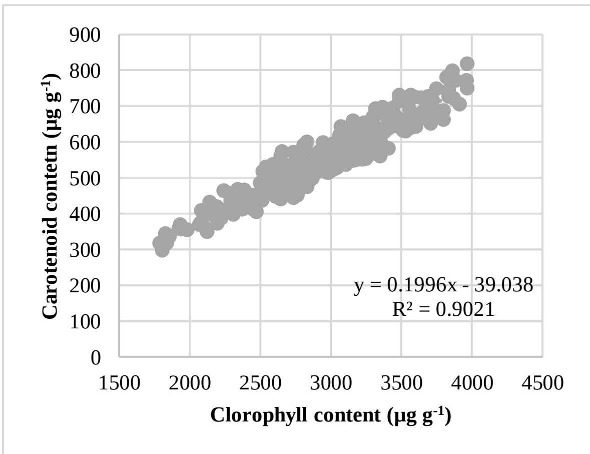
$$\text{Carotenoid fresh weight } (\mu g\ g^{-1}) = (1000*A_{470nm}–3.27\ (12.21*A_{663nm}–2.81*A_{644nm})–104\ *(20.13A_{644nm}–5.03A_{663nm}))/229 \quad (2)$$

where
V = volume of tissue extract (ml)
w = fresh mass of tissue (g)
A = absorbance

RESULTS AND DISCUSSION

The pigments responsible for photosynthesis are chlorophylls and carotenoids built into the thylakoid membrane. The two major groups of carotenoids are carotenes and xanthophylls, which also contain oxygen. Chlorophylls and carotenoids are found bound to the integral proteins of thylakoids within the plant (Ördög and Molnár, 2011). The relationship between the measured chlorophyll and carotenoid values was supported by linear regression, based on which we also supported the close correlation between the pigment contents in our research (R^2 = 0.9021) (*Figure 1*). In this way the changes in the pigments within the plant take place in parallel, they will interact. The chlorophyll and carotenoid ratio can be used to determine the ripening and stress processes of apples (Solovchenko et al., 2005).

Figure 1: **Investigation of the relationship between chlorophyll and carotenoid**



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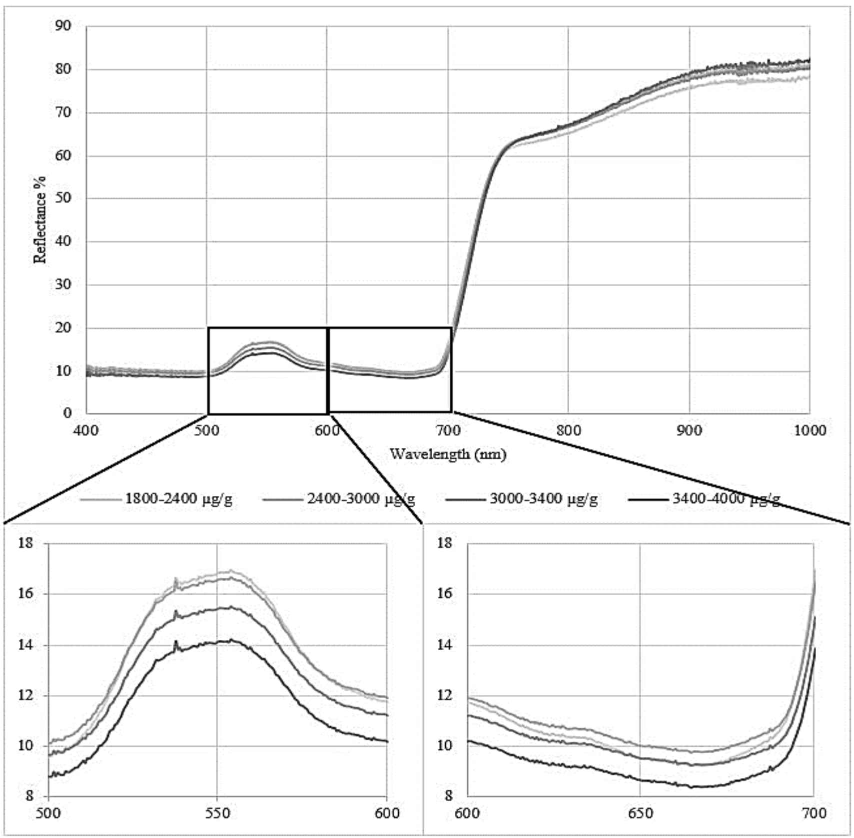
The lowest chlorophyll content value was 1805.83 µg $g^{-1}$and the highest was 3963.794 µg g^{-1}, the values between the two were divided into 4 intervals, 1800–2400 µg g^{-1}, 2400–3000 µg g^{-1}, 3000–3400 µg $g^{-1}$and 3400–4000 µg g^{-1} in the case of apple trees (*Figure 2*). We observed that the spectral values between the chlorophyll content groups changed. In the case of vegetation, the reflectance spectrum in the visible range of 400–700 nm is dominated by the light absorption of leaf pigments. For photosynthesis, chlorophyll pigments absorb blue light at 400–500 nm and red light at 600–700 nm. Blue wavelengths between 400 and 500 nm are substantially absorbed by the yellow or orange-red pigment (Huete, 2004). The absorption site of chlorophyll at 670 nm shows low reflectance due to the high chlorophyll value, thus showing strong absorption (Nagy et al., 2014). Because of the stress on various leaf pigments, the loaded vegetation produces a distinctive spectral signature. During the high chlorophyll values, we measured reflectance values between 8–9%, which shows an increasing reflectance in proportion to the decrease in chlorophyll values. Reflectance close to 10% were observed at low chlorophyll values of 1800–2400 µg g^{-1}. The maximum of carotenoid reflectance can be measured in the wavelength range of 520–580 nm, which gave a low reflectance value of around 14% in the case of high chlorophyll content. It can be observed that with decreasing chlorophyll content, the reflectance value will increase proportionally for carotenoid content. The carotenoid reached a reflectance value of 17% at low chlorophyll interval values. So, the plant stress can be detected in the wavelength range of 500–700 nm with high reflectance values. Depending on the leaf's structural properties, a large amount of energy is transmitted and reflected, resulting in a high near-infrared plateau (Huete, 2004). In our study, the reflectance is high at 65% nm in the NIR range, with a further increase. Since cellulose and leaf pigments are visible in the near infrared spectrum (700–1300 nm) and there is minimal absorption by the leaf, the reflectance in the 800–1000 nm wavelength range is insignificant. Based on the structural properties of the leaf, most of the energy is transmitted and reflected, creating a high near-infrared (NIR) plateau. The red edge, which is used to detect plant stress that is more closely tied to pigments, is a strong rise in reflectance between the red and NIR bands (Huete, 2004) *(Figure 2)*. Vegetation indices are derived mainly from reflectance data in the red and NIR bands. They are numerical measurements that measure biomass or vigour based on the spectral characteristics of vegetation (Roman and Ursu, 2016).

Figure 2: **Apple leaf reflectance values based on chlorophyll intervals**



The reflectance variance values calculated for the chlorophyll content were compared by proportioning the given reflectance means to investigate the further spectral properties of the apple leaves. The intervals including the spectral data for all plants from the standard deviation of the reflectance data with low

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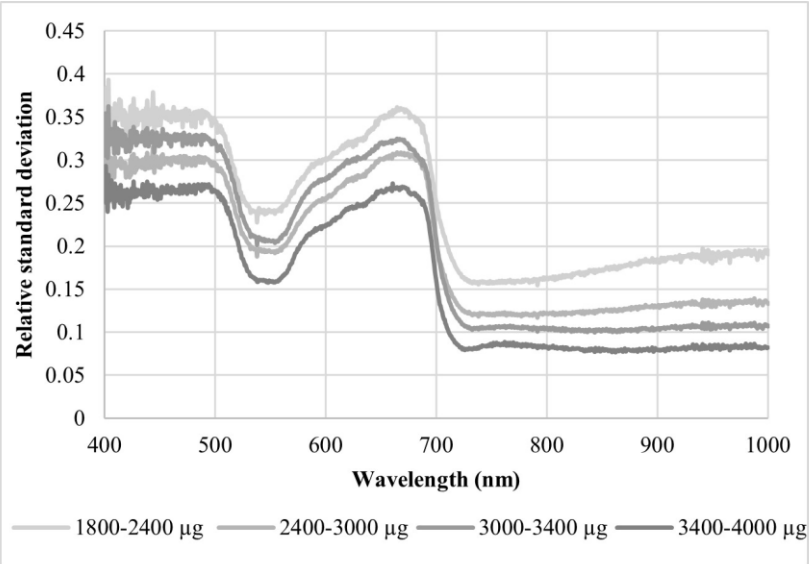
chlorophyll content are covered by the analysed groups. High standard deviation (up to 520 nm ± 30 nm) was observed in the low chlorophyll groups. The standard deviation of reflectance decreased in parallel with the chlorophyll content. Therefore, this range may be suitable for establishing plant maturity studies. The standard deviation peak is prominent due to the absorption characteristics of chlorophyll measured in the given wavelength range; it is sensitive at low chlorophyll content at 670 nm. It can be observed that the standard deviation of reflectance values calculated at 550 nm, 670 nm and 700 nm wavelength ranges are pigment sensitive. Due to an increase in absorption that occurs concurrently with the carotenoid content, this sensitivity decreases. Thus, this spectral feature vanishes as the carotenoid content rises. This is confirmed by Nagy et al. (2016) and Zur et al. (2000), who investigated changes in the pigment content of tree leaves and arrived to similar conclusions. The reflectance values measured in the NIR range showed low standard deviation, therefore they cannot be used to measure the change in pigment content (*Figure 3*).

Figure 3: **Relative standard deviation of reflectance %**



CONCLUSIONS

During our studies we observed the development of the pigment content of the foliage by spectrophotometric and spectral methods. Non-destructive determination of chlorophyll content is recommended in the carotenoid-sensitive wavelength range of 520–580 nm and reflectance values measured at 650–700 nm. We observed that the values belonging to the high chlorophyll group gave a low reflectance which increased in proportion to the decrease in chlorophyll. Low reflectance were measured in case of 8–9% of the groups with high chlorophyll content which increased continuously with the decrease of the chlorophyll content due to the close correlation between them. A similar result was observed for the relative standard deviations with a low standard deviation for the high chlorophyll group. In the future, it is recommended to supplement the spectrophotometric method with spectral measurements, primarily to reduce the number of samples, so that we can use a faster, cheaper data-generating method to achieve our results. It is also recommended to use Partial Least Squares Regression (PLSR), and Principal Component Analysis (PCA) for statistical analysis of the results to identify the wavelengths with the highest variances, from which chlorophyll prediction indices can be determined. In addition, it can provide a basis for the development of a field methodology as the given test parameters can be measured in the field in a non-destructive way.

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