Quantification of pigments in tomato leaves using reflectance spectroscopy

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Abstract - Reflectance spectroscopy is a non-invasive technique, which can be performed in a real-time mode for studying pigment composition and content in plants *in vivo*. In this work reflectance spectra were measured in laboratory for the leaves of three tomato cultivars. Good correlation with analytically measured pigment concentration was achieved for (1) chlorophyll *a* concentration and reverse reflectance values at wavelength 600 nm for the direct leaf side, (2) anthocyanins concentration and reverse reflectance values at wavelength 550 nm for the reverse leaf side. Reflectance spectroscopy has an advantage over any analytical techniques in possibility to measure the pigment distribution between leaf sides.

Keywords: reflectance spectroscopy, plant pigments, chlorophyll, tomato, leaf

1. INTRODUCTION

In the higher plants are present a large group of pigments as well as chlorophyll and carotenoids. These pigments are involved in energy capture during photosynthesis and they are responsible for variations of color from dark-green to yellow. Other pigments involved in leaf and fruit coloration are flavonoids and in particular, anthocyanins give yellow, red and blue coloration. These pigments are also very important for plant physiology and they can be used as indicators of plant reaction to different kinds of stress. (Chalker-Scott 1999; Markstädter, et al 2001).

Pigments absorbing light in the visible spectral range, and which absorption bands do not interfere, in principle can be measured quantitatively from their reflectance spectra. Reflectance spectroscopy technique gives information on pigment composition, and content in addition to pigment degradation during plant tissue growth, stress condition, maturation and senescence. The internal structure of epidermis layer (the volume of intercellular air spaces in the mesophyll layer of leaves) can be also evaluated using reflectance values in the near-infrared (NIR) region (700-1300 nm). The technique is non-invasive and express, can be performed in a real-time mode.

However for plant pigment quantification using reflectance spectra the technique has serious limitations. First, the algorithm of data processing must be adjusted to the type of the sample (leaves from a certain plant, certain fruit, etc) using the empiric model of light reflectance based on light absorbance, scattering and transmittance in the plant tissues. For leaf samples the inhomogeneous distribution of the pigment between both leaf sides must be taken into account. Interference of absorption bands for different pigments and their dependence on local environment (pH, temperature) complicates the problem of quantitative pigment measurements.

Since plants strongly absorb the UV light it is not possible to measure concentration of colourless compounds like simple phenolics, proteins and many flavonoids by means of reflectance spectroscopy.

Chlorophyll absorbs the majority of incident red and blue radiation, resulting in little red or blue reflectance by green vegetation. The blue absorbance peak of chlorophyll overlaps with the absorbance of carotenoids, so blue reflectance is not generally used to estimate chlorophyll concentration (Sims and Gamon, 2002). Maximum red absorbance occurs between 660 and 680 nm (Curran, 1989), but relatively low chlorophyll concentrations can saturate this absorption region (Sims and Gamon, 2002). Therefore, chlorophyll concentration is usually predicted from reflectance in the 550 nm or 700 nm ranges, because these regions require higher chlorophyll concentrations to saturate.

Leaf chlorophyll concentration has also been well-correlated with reflectance in the green and red spectral regions. Leaf reflectance factors in the 550 nm and 660 nm range show high correlation with leaf nitrogen concentration (Fernández et al., 1994) and chlorophyll concentration (Adams et al., 1999). The shape of the visible reflectance spectra of leaves changes between the maximum reflectance near 550 nm and the minimum near 660 nm as they become chlorophyll-deficient, and changes in this shape can also be used to identify chlorosis in some instances (Adams et al., 1999; Carter and Spiering, 2002).

The tomato is a good model plant for these studies. Different tomato mutants for over accumulation anthocyanins are available. Besides among vegetables tomato is one of the most representatives of Mediterranean diet and it is important for its richness in health-related food components (La Vecchia 1997; Leonardi et al 2000; De Stefani et al 2000).

The objective of this work was to study the correspondence of reverse reflectance values at different wavelengths to concentration of main pigments in tomato leaf. The findings could provide an empiric model of light reflectance by the leaf surface, and could be used for non-invasive rapid measurements of pigment concentration.

2. MATERIALS AND METHODS

2.1 Tomato plants growing and leaves sampling

Tomato (*Lycopersicum esculentum*) plants of wild type, anthocyanin-rich and rin (ripening inhibitor) mutants, were grown in the green-house for 60 days at the same conditions.

The leaves of different age were analyzed for reflectance measurements. In order to quantify pigments the same leaves were used for pigments extraction (three reps of 1 cm^2 each leaf) frozen (-30°C) till analytical measurements.

2.2 Reflectance measurements

The reflectance measurements were performed in the spectral range from 385 nm to 1100 nm with the Perkin Elmer Lambda 25 spectrometer equipped with the integrating sphere and the certified Spectralon Reflectance Standard. The leaflets of each leaves were placed behind the integrating sphere with a matt black paper as a background. Reflectance spectra were measured for both sides of each collected leaflet.

2.3 Pigments extraction

Concentrations of chlorophyll (Chl) *a* and *b*, Carotenoids, Anthocyanins were measured on the leaf samples according to Mancinelli modified (1984) and Lichtenthaler, (1987). Extraction was performed by methanol 90% acidified with HCl 1N (1:1:6 v/v). 1 cm^2 area were put in solvent and kept at 4°C overnight (24 hours). 5 ml of solution were put in 1-cm quartz cuvettes and measured on the spectrophotometer Perkin Elmer Lambda 25 with the standard configuration.

Each leaflets were named $T_{Nnumber}$.(T=tomato; 1-9 represents each leaflets from younger to older leaf of wild type tomato; 11-18 for anthocyanins mutant and 21-29 rin mutant).

3. RESULTS

3.1 Tomato leaf pigments concentrations

The results of concentration, expressed in mg/cm^2, of main plant pigments on the leaflets analysed are shown in Fig. 1-3. Each concentration value represents the average of three replicates.

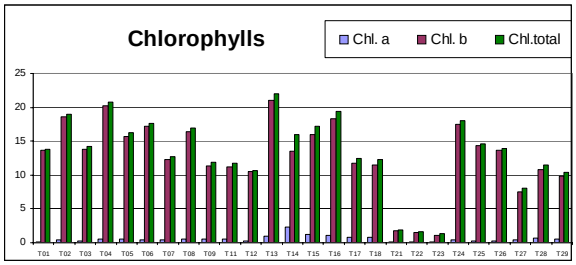


Figure 1. Distribution of chlorophylls (mg/cm^2) in different tomato leaflets.

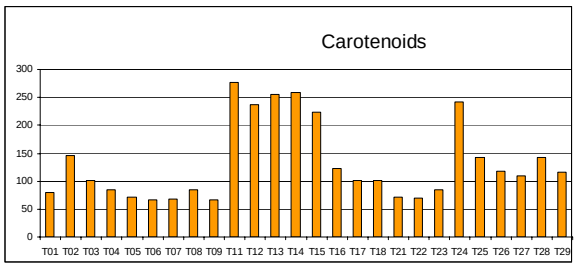


Figure 2. Distribution of carotenoids (mg/cm^2) in different tomato leaflets.

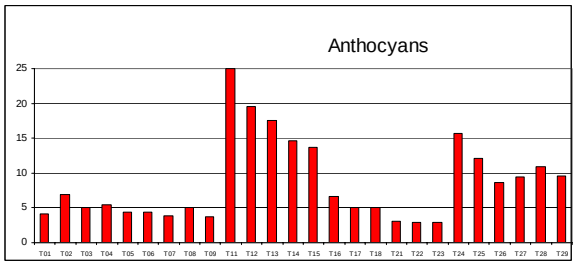
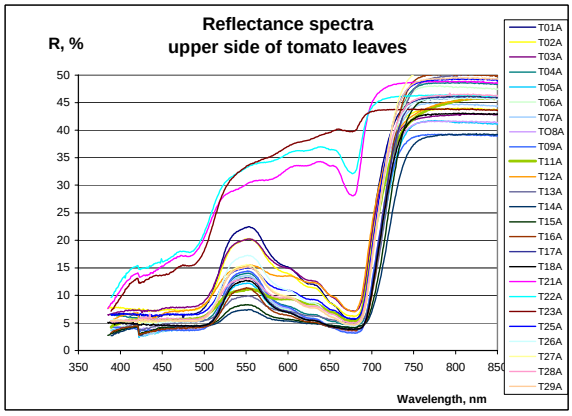


Figure 3. Distribution of anthocyanins (mg/cm^2) pigments in different tomato leaflets

Differences in concentration pigments were found compared with leaf age and the tomato cultivar. As expected higher anthocyanins pigments concentration were found in anthocyanin mutant (T11-T18 leaflets) compared with wild type (T1-T9 leaflets) and rin (T21-T28 leaflets) (fig 3). Differences in concentration are shown in relation of different age. Juvenile leaflets (T11-T13) shown higher concentration compared with mature leaflets (T16-T18) because of different physiological stage.

3.2 Reflectance spectra of tomato leaves

Original reflectance spectra are presented in Fig. 4 for each leaf side separately. One can notice the difference in reflectance for the upper (direct) and reverse sides of leaves: wavelength-independent scattering level is higher for reverse side.



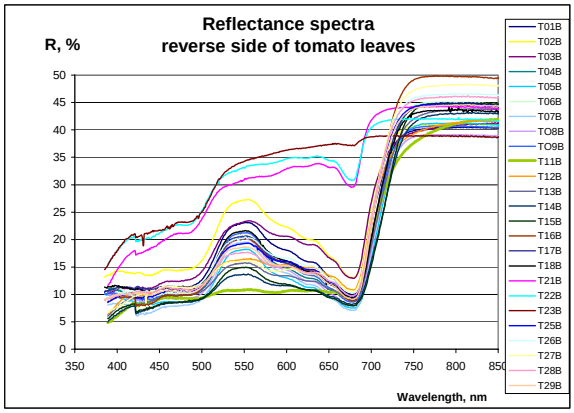


Figure 4. Original non-processed reflectance spectra for both sides of tomato leaflets.

Reflectance spectra after correction and normalisation are shown in Fig. 5. Normalised reflectance at given wavelength is calculated as

$$\text{Normalised Refl}(\lambda) = (R((\lambda)-R(UV))/R(NIR),$$

where R(UV) and R(NIR) were taken at 385 and 800 nm correspondingly.

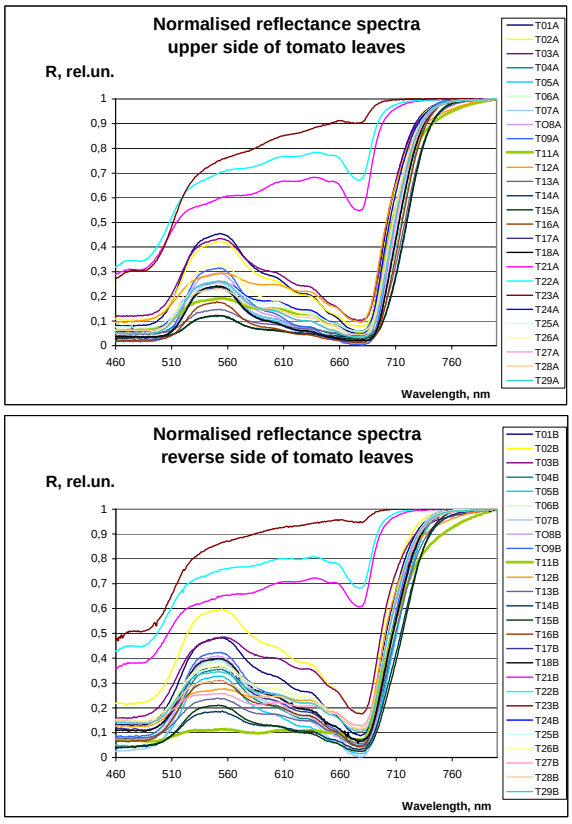


Figure 5. Corrected and normalised reflectance spectra for both sides of tomato leaflets.

Correction procedure for reflectance spectra eliminates the background caused by direct reflectance from the leaf surface, and normalises all spectra to the reflectance value in the near IR region, which is important for leaves of different thickness.

3.3 Correlation of reflectance indices and pigment concentration

After correction and normalisation of reflectance spectra were made, the data at different wavelengths within visible range were extracted, appropriate reflectance indices were calculated as reverse values of normalised reflectance. Reflectance indices and pigment concentration were averaged over three leaflets of each measured leaf and compared with analytically measured concentrations.

The best correlation with analytically measured pigment concentration was achieved for the following pigments and reflectance indices:

Chlorophyll *a* concentration and reflectance indices derived at wavelengths within Chl absorption band, but not close to its absorption peak maximum. This correlation is better for the upper side of the leaves. The dependence of reverse reflectance values at all tried wavelength was parabolic versus Chl concentration, and the highest r^2=0.94 was observed for the index = 1/Norm.Refl600, where corrected and normalised reflectance values are taken at 600 nm (Fig.6).

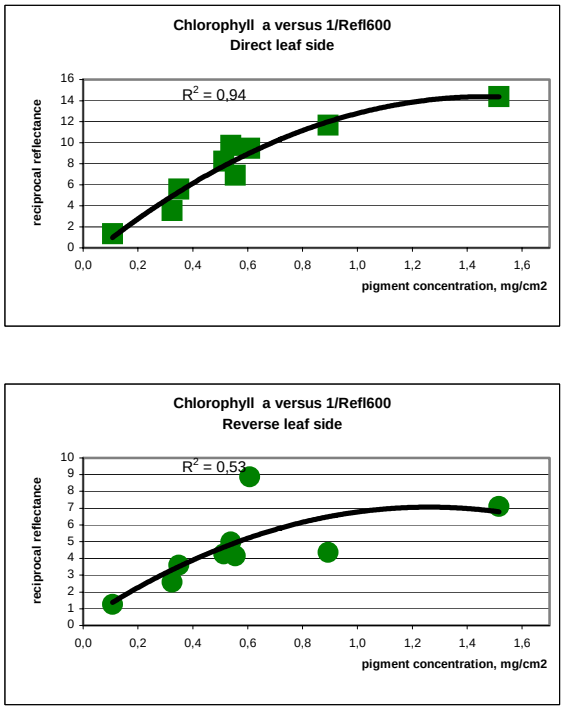
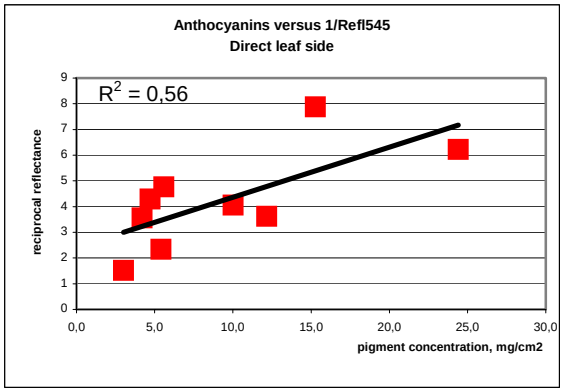


Figure 6. Comparison of correlation of reflectance indices and chlorophyll *a* concentration for both sides of the leaf.

Anthocyanin concentration and reverse normalised reflectance values at wavelengths within the absorption band 520...550 nm (see Fig.7 for reflectance taken at 545 nm). This correlation is high only for the reverse side of the leaves. Dependence of reverse reflectance values at 545 nm and anthocyanins concentration is linear.



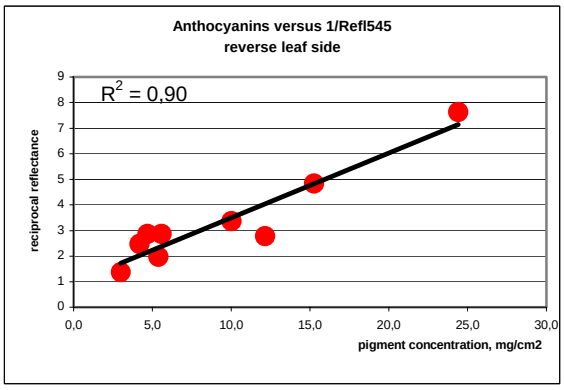


Figure 7. Comparison of correlation of reflectance indices and anthocyanins concentration for both sides of the leaf.

For other pigments (Chlorophyll b, total chlorophylls, carotenoids) was not found good correlation ($r^2 < 0.6$) with pigment concentration within all visible range for the same set of tomato leaves from three cultivar.

4. CONCLUSIONS

In this work reflectance indices derived as reverse reflectance values at different wavelengths for the leaves of different age of three tomato cultivars were compared with analytically measured concentrations of chlorophylls, carotenoids and anthocyanins in leaf tissue.

Good correlation with analytically measured pigment concentration was achieved for (1) chlorophyll *a* concentration and reverse reflectance values at wavelength 600 nm for the direct (upper) leaf side, $r^2 = 0.94$, non-linear (parabolic) dependence; (2) anthocyanins concentration and reverse reflectance values at wavelength 550 nm for the reverse leaf side, $r^2 = 0.90$, linear dependence.

Reflectance spectroscopy has an advantage over any analytical techniques in possibility to measure the pigment distribution between leaf sides.

5. REFERENCES

Chalker-Scott, L., "Environmental Significance of Anthocyanins in Plant Stress Responses", Photochem. Photo-biol., 1999, vol. 70, pp. 1–9.

Curran, P.J. 1989. Remote sensing of foliar chemistry. *Remote Sens. Environ.* 30:271-78.

Dawson, T.P., and P.J. Curran. 1998. A new technique for interpolating the reflectance red edge position. Int. J. Remote Sens. 19:2133-2139.

De Stefani E., Oreggia F., BoffettaP., Deneo-Pellegrini H., Rocco A., Mendilaharsu M., "Tomatoes, tomato-rich foods, lycopene and cancer of the upper aerodigestive tract: a case control in Uruguay" Oral oncology 2000 36:47-53

Fernández, S., D. Vidal, E. Simón, and L. Solé-Sugrañes. 1994. Radiometric characteristics of Triticum aestivum cv. Astral under water and nitrogen stress. Int. J. Remote Sens. 15:1867-1884

LaVecchia C., "Mediterranean epidemiological evidence on tomatoes and tomatoes and the prevention of digestive tract cancer" Proc. Soc. Exp. Biol. Med. 1997 218; 125-128

Leonardi C., Ambrosino P., Esposito F. and Fogliano V. "Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes" J. Agric. Food Chem. 2000, Vol 48 (10) 4723-4727

Lichtenthaler, H.K., Chlorophyll and Carotenoids: Pigments of Photosynthetic Biomembranes, Methods Enzymol., 1987, vol. 148, pp. 331–382.

Lichtenthaler, H.K., Gitelson, A.A., and Lang, M., Non-Destructive Determination of Chlorophyll Content of Leaves of a Green and an Aurea Mutant of Tobacco by Reflectance Measurements, J. Plant Physiol., 1996, vol. 148, pp. 483–493.

Mancinelli A.L. "Photoregulation of Anthocyanins synthesis" Plant Physiology 1984, 75: 447-453

Markstädter, C., Queck, I., Baumeister, J., Riederer, M., Schreiber, U., and Bilger, W., "Epidermal Transmittance of Leaves of Vicia faba for UV Radiation as Determined by Two Different Methods" Photosynth. Res., 2001, vol. 67, pp. 17–25.

Merzlyak, M.N., Gitelson, A.A., Chivkunova, O.B., and Rakitin, V.Yu. Non-Destructive Optical Detection of Leaf Senescence and Fruit Ripening, Physiol. Plant., 1999, vol. 106, pp. 135–141.

Sims, D.A., and J.A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ. 81:337-354.

Taiz, L., and E. Zeiger. 1998. Plant Physiology. 2nd ed. Sinauer Associates, Sunderland, MA.