ACTIVITY OF THE PHOTOSYNTHETIC APPARATUS IN PLANTS UNDER THE INFLUENCE OF DIRECT SOLAR RADIATION

MATILDA MEMA[1], EMIL XHUVANI[2] , KEJDA KRISTO[2], FATBARDHA BABANI[2]

[1]Department of Physics, Faculty of Mathematical and Physical Engineering, Polytechnic, University of Tirana

[2]Department of Physics, Faculty of Natural Sciences, University of Tirana

e-mail: mema_matilda@yahoo.com

Abstract

Transmission and reflection spectroscopy are used as a non-destructive method for the assessment of chlorophyll in leaves. Reflectance is the best indicator of chlorophyll in the spectral region around 700nm and for this method the ratios R750/R700 are used. Reflection spectra demonstrating the optical properties of leaves provide information on reflectance in some specific wavelength. The measurements were carried out in three different types of leaves (sun, partial shade and shade) for Abbas (pear) variety in an area under water. The spectra allow determination of leaf color with x and y coordinates brightness, luminance as well as "dominant" wavelength. In the narrow band of wavelengths 531 nm and 570 nm, the diffuse reflectance values allow the calculation of the photochemical diffuse reflectance index (PRI).

Key words: *Reflection spectra, Chl a, Chl b, a+b/x+c, R550, R750/R550, PRI*

Përmbledhje

Spektroskopia e transmetimit dhe pasqyrimit përdoren për vlerësimin jo-destruktiv të klorofilës në gjethe. Reflektimi në zonën spektrale rreth 700nm është treguesi më i mirë i klorofilës dhe për këtë përdoret raportet R750/R700. Spektrat e pasqyrimit të shpërhapur karakterizojnë vetitë optike të gjetheve dhe japin informacion mbi pasqyrimin në gjatësi vale specifike. Matjet u kryen me tre lloje gjethesh (diell, gjysmë-hije dhe hije) për varietetin Abbas (dardha) në një zonë nën ujë. Spektrat lejojnë përcaktimin e ngjyrës së gjetheve me koordinatat x dhe y, si dhe të gjatësisë së valës *"dominante". Nga vlerat e matura të shkëlqimit, ndriçueshmërisë pasqyrimit të shpërhapur llogaritet indeksi fotokimik i pasqyrimit të shpërhapur (PRI) në bandën e ngushtë të gjatësive të valës 531 nm dhe 570 nm.*

Fjalë kyçe: *Spektra reflektimi, Kla, Klb, a+b/x+c, R550, R750/R550, PRI*

Introduction

Plants during their development, are adapted to the sunlight of the environment in which they have been exposed. This adaptation refers to growth and development of the entire plant, but also to leaves, cells and particularly to chloroplasts.

This includes the structure of the thylakoids as well as the relative amounts of the photosynthetic pigments such chlorophylls and carotenoids. The light adaptations involve both structural and functional differences (Boardman 1977;Bjorkman 1981; Lichtenthaler et al. 1981, Meier and Lichtenthaler 1981; Anderson et al. 1995) and also adaptations in pigment levels (s. review Lichtenthaler 2007;Urban et al. 2007a).

In most cases, this results in optimization of light interception and utilization for the functioning of photosynthesis. In this manner a leaf developed under full sunlight (sun leaf) has a higher photosynthetic activity when receiving high irradiance as compared to a leaf that developed in the shade (shade leaf) (Wild et al. 1975; Lichtenthaler and Babani 2000, 2004; Lichtenthaler et al. 2007). Sun leaves with their sun chloroplasts (low and narrow grana stacks) possess higher values for the ratio Chl a/b and lower values for the weight ratio total chlorophylls to total carotenoids, known as ratio (a + b)/(x + c), as compared to shade leaves with their shade chloroplasts (broad and high grana stacks) (s. review Lichtenthaler 2007).

The purpose of the paper is to evaluate the photosynthetic apparatus on fruit trees (pears) in Tirana region in presence of different environmental conditions such solar radiation, temperature and humidity to which they are exposed.

Material and methods

1. Plants

Measurements were made with leaves selected in three types of positions (sun - southern part of the crown, blue shade - northern part and semi-shade/shade - inside a tree crown) for the variety: Abbas (pear), part of a group of *Pyrus Communis* L pear species in the rose family. The study of the variety was done in an area under water in two periods, April and July.

2. Pigment determination

Leaf pigments were extracted with 100% acetone in one circular piece of 9mm in diameter cut from the leaves using a mortar. The pigment extracts were centrifuged for 5 min at 500 X g in glass tubes to obtain the fully transparent extract. The pigment contents, Chl a, Chl b and total carotenoids, were determined spectrophotometrically from acetone extract using the extinction coefficients and equations re-determined by Lichtenthaler (Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001). The represented values are the means of six determinations from six leaves.

3. Reflectance spectra

Leaf reflectance (R) was recorded from upper side of the leaf in a spectral range from 400nm to 800nm with a spectral resolution of 2nm with a spectrophotometer equipped with an integrating sphere attachment (Bushmann et al., 2012; Gitelson et al., 2003). Leaf reflectance spectra were recorded against barium sulphate as a white reference standard. Leaves were placed on black velvet used as a background which has a reflectance less than 0.5% over the spectral range of measurements. Reflectance (R) was represented as the ratio of the radiation intensities reflected by the leaf sample and the white standard respectively.

The leaf spectra were taken in the intercostal fields between the larger leaf veins. These spectra represent an integrated signal over several square centimeters. The measurement of spectral reflectance is a nondestructive and a rapid method (Gamon J.A, Serrano L, Surfus J.S, 1997).

4. Photochemical index (PRI)

The photochemical index of diffuse reflectance serves as a photosynthetic indicator of radiation utilization efficiency (Gamon, Serrano, Surfus, 1997). The photochemical reflectance index (PRI), calculated from the reflectance at 531 and 570 nm, is sensitive to the photochemical changes induced by the photoprotective xanthophyll cycle, acting upon light saturation of the chlorophyll antenna (Gamon, 1990; Gamon, Surfus, 1999). The values of the photochemical index of diffuse reflectance fluctuate in the range from -1 to 1. The PRI values are calculated using the reflectance values at 531nm and at 570nm as reference wavelength:

$$PRI = \frac{R531 - R570}{R531 + R570}$$

The photochemical index of diffuse reflectance (PRI) depends on photosynthetic (leaf) pigments, the amount of energy falling from the sun on the surface, the angle of the sun's rays falling on the leaf surface and the water content (Gamon & Berry, 2012).

5. Colorimetry

Evaluation of the visual impression of a leaf sample was assessed by the chromaticity coordinates in the CIE 1931 color space which allow defining quantitative links among wavelengths in the electromagnetic visible spectrum and physiological perceived colors in human color vision. In order to help to assess the visual impression of a sample, the reflectance spectra of the leaf samples were used to define the color as x and y chromaticity coordinates in the CIE 1931 color space, a colorimetric standard widely used in the textile and coating industries, (Malacara D, 2002). The coordinates x and y, which define a visual color in the CIE 1931 color space chromaticity diagram, were determined using the reflectance data and the color matching functions for daylight illumination (D65).

Furthermore, we determined the brightness (values between 0 = dark and 100 = completely bright), the dominant wavelength (the wavelength characteristic for the color of the sample determined by the intersection point with a curved outer boundary line, also called spectrum locus, of the line connecting the achromatic point, i.e., “white” with x = y = 0.33, and the detected color point), and the color saturation (percentage of distance of the color point between the achromatic point and the boundary line: 100% at the spectrum locus, 0% at the achromatic point).

Results

1. Photosynthetic pigments

The highest value of the chlorophyll content Chl (a+b) is presented in the variety Abbas (pear) on the period of April compared to the period of July. It is also observed that the content of chlorophylls Chl (a+b) decreases in variety from sun leaves to blue-shade and shade leaves (Tab. 1). The ratios of the photosynthetic pigments, Chl a/b and (a+b)/(x+c), reflecting the light adaptation of the photosynthetic apparatus (Lichtenthaler 2013) showed different values in the three leaf types.

The mean values of the ratio Chl a/b are higher in sun leaves as compared to blue-shade and shade leaves (Tab. 1). Sun leaves displayed lower values of the ratio (a+b)/(x+c) as compared to two other leaf types (Tab. 1). The period of April is considered as a period with suitable conditions for the development of the photosynthetic apparatus for the variety Abbas (pears).

Table 1. Content of Chl (a+b) and total carotenoids (x+c) per leaf area unit as well as the pigment ratios Chl a/b and chlorophylls (a+b) to carotenoids (a+b)/(x+c) between sun, blue-shade, shade/half-shade leaves of *Abbas* variety of pear trees. Mean values of 6 determinations per leaf-type.

Leaf-type	**Chl (a+b) (mg dm^{-2})**	**Chl a/b**	(a+b)/(x+c)
Abbas - April			
Sun	8.498 ±0.012	2.894	4.033
Blue-shade	5.589 ±0.021	2.458	4.353
Half-shade/shade	4.283 ±0.015	2.016	4.968
Abbas - July			
Sun	7.428 ±0.040	2.664	4.611
Blue-shade	6.807 ±0.050	2.384	4.813
Half-shade/shade	4.790 ±0.044	2.258	5.175

2. Reflection spectra

Reflectance spectra of the three types of leaves showed a higher reflectance in the green-to-orange range of the spectrum at wavelengths 500nm to 650nm and mainly in the near infrared from 680nm to 740nm on both pear varieties. In addition, reflectance spectra exhibited a low reflectance from 400nm to 500nm in blue part of visible spectra and near 680nm in red part of visible spectra (Fig. 1). The observed variations correspond to the absorption region of the in-vivo chlorophyll bands.

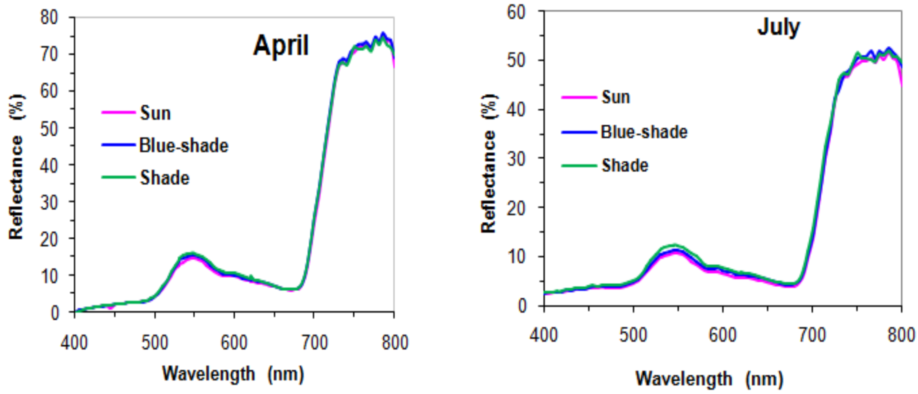

Figure 1. Reflectance spectra of the sun (south part), blue-shade (north part) and shade/half shade leaves of Abbas pear variety.

The reflectance spectra exhibit the highest values in the green-orange range of the spectrum of shade leaves compared to two other leaf types of both varieties. Also, could be observed a blue shift of the "red edge" (inflection point of the rise of signal at wavelengths between 680nm and 740nm towards shorter wavelengths to the shade leaves. Abbas (pears) in the two periods under study for three types of analysed leaves are related to the chlorophyll content being lower in shade leaves and higher in sun leaves (Tab. 1). The highest diffuse reflectance values R550 represents the Abbas in the period of April. It is observed that the highest values R550 of variety are presented in the shade position compared to the other two positions (Tab 2). The values of R700, R750 and R800, for variety Abbas are presented in the shaded position. The high values of reflection in the wavelengths 700nm, 750nm and 800nm are explained by the low absorption in the shadow position.

Table 2. Reflectance on sun, blue-shade, shade/half-shade leaves of *Abbas* variety of pear trees. Mean values of 6 determinations per leaf-type.

Leaf-type	R550	R700	R750	R800
Abbas- April				
Sun	14.7 ±0.31	24.3	70.5	66.5
Blue-shade	15.6 ±0.62	26.0	71.7	69.1
Half-shade/shade	16.0 ±0.51	26.0	72.0	69.6

Abbas- July				
Sun	10.5 ±0.72	13.6	49.1	49.8
Blue-shade	11.2 ±0.48	14.3	50.5	50.6
Half-shade/shade	12.2 ±0.09	15.7	51.5	50.8

The values of R550 are higher in the shade position for variety Abbas of pear trees in the periods of study, while the content of chlorophylls is lower in comparison to the other two positions (Tab. 2).

3. Photochemical index (PRI)

Highest value of the photochemical index (PRI) presented in the period of July for Abbas (pear), (Tab 3). PRI as a parameter is closely related to the action of solar radiation on the surface of the leaves.

Table 3. PRI values for Abbas (Pear) variety

Leaf-type	**PRI**
Abbas - April	
Sun	0.047
Blue-shade	0.038
Half-shade/shade	0.034
Abbas - July	
Sun	0.077
Blue-shade	0.068
Half-shade/shade	0.064

Abbas variety presents the highest values of the photochemical index (PRI) in the sun position, in the two periods under study (Fig.2).

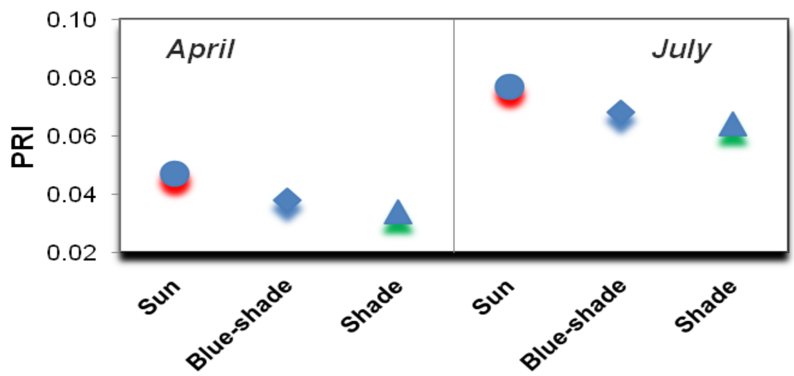

Figure 2. Photochemical index (PRI) values of the leaves of the sun, blue-shade and shade type leaves; of Abbas (pear) variety.

4. Colorimetric parameters

In the study, from the diffuse reflectance values, in the three positions, some chromatic parameters can be determined which are: "dominant" wavelength and Y-brightness based on the algorithm of the CIE 1931 system, for variety Abbas (pear), area under water.

The highest values of the "dominant" wavelength are presented for the leaves in the shade position, compared to the leaves in the other two positions, for the April-July period. In the two periods under study, the highest values are presented in April.

Table 4. Colorimetric determination according CIE 1931 for the leaf samples: sun, blue-shade, shade/half-shade leaves of *Abbas* variety trees, in periods April- July. Mean values of 6 determinations per leaf-type.

Leaf-type	**Brightness -Y**	**Dominant wavelength (nm)**
***Abbas* - April**		
Sun	10.5	563.2
Blue-shade	11.8	563.3
Half-shade/shade	13.7	563.5

***Abbas* - July**		
Sun	7.84	554.1
Blue-shade	8.42	555.8
Half-shade/shade	9.13	556.5

The values of: "dominant" wavelength, brightness-y are higher in the period of April, the period with optimal conditions for the development of the photosynthetic apparatus (Tab 4).

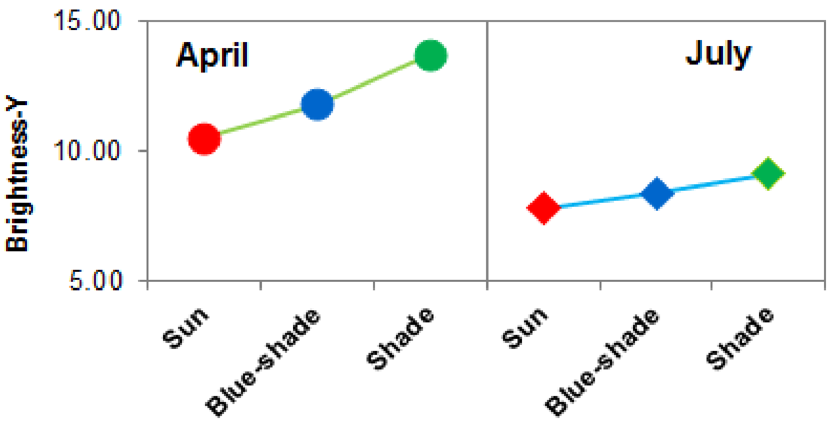

Figure 3. Brightness-Y values of the leaves of the sun, blue-shade and shade type leaves; of Abbas (pear) variety.

The "dominant" wavelength depends on the concentration of chlorophylls. The dependence between them is oblique, which means that the leaves in the shade position have a lower concentration of chlorophylls, but a higher "dominant" wavelength value. It is also noticed that the values of "dominant" wavelength present lower values in the shade position, compared to the other two positions. Brightness-Y results in higher values in the April period, the shadow position.

Conclusions

The reflectance spectra, as well as the values (R550, R750, R800) show the characteristics and differences between the analyzed leaves demonstrating structural changes in the photosynthetic apparatus as a result of adaptation to the environment.

The pigment content Chl (a+b) represents the highest values on the sun leaves (sun position) and the lowest values on half-shade/shade leaves (inside a tree crown). Whereas blue shade leaves (northern part) show values lower of Chl (a+b) than sun leaves but higher than shade leaves. The ratio Chl a/b decreases from the sun to blue shade and shade leaves while the ratio a+b/x+c increases representing higher values in shade leaves.

The areas under study (under water), represent values of the photochemical index (PRI) higher in the south position, compared to the other two positions. The variety, Abbas (pear), presents higher values of the "dominant" wavelength and brightness-Y in April in the shade position. The two parameters are related to the structure of the leaf itself.

All, calculated parameters such as: PRI, colorimetric parameters (brightness-Y, "dominant" wavelength) make it possible to distinguish between different periods of fruit trees and evaluate the impact of growth conditions on the photosynthetic apparatus

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