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Fluorescence spectra as an indicator of the influence of environmental conditions on fruit trees

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Abstract

Molecular fluorescence spectra determine possible structural changes of these molecules and provide data (by comparing them with absorption ones) on the efficiency of solar energy utilization. Fluorescence emission spectra provide information on the functionality of the photosynthetic apparatus by means of two bands red F690 and beyond red F735. The objective of the study is to highlight possible changes in the organization and operation of the photosynthetic apparatus, induced by different radiations, in the leaves, for a variety of pear (Santa Maria), based on the characteristics of the two bands of emitted fluorescence. The Santa Maria variety was studied in the period of June.

Keywords: Fluorescence spectra; Chl a; Chl b; F690; F735

1. Introduction

Plants make their own food by capturing sunlight through the pigment chlorophyll. Oxygen is released during the conversion of carbon dioxide into carbohydrates, or plant food. Photosynthesis is a clean, complete and harmless process. The photosynthetic process depends on a complex of protein molecules, which are observed in a highly organized membrane. Photosynthesis takes place in chloroplasts (in the thylakoid membrane). Photosynthesis is the most important biological process on earth.The energy of the solar rays falling on the unit of the earth's surface in the unit of time (insolation) depends on the angle of incidence and the intensity of the solar rays [3]. In higher plants, in addition to chlorophyll a, there are also chlorophyll b and carotenoids. Carotenoids are red, orange or yellow pigments.

Chlorophylls and other molecules are organized in thylakoid membranes in assemblies called photo systems. Each photo system contains several hundred molecules, including chlorophyll a, chlorophyll b, and carotenoids. This community of pigment molecules acts as a solar energy collecting panel according to the energy migration mechanisms in it. Sun-type chloroplasts of plants exposed to light have smaller sizes of pigment antennae compared to shade-type chloroplasts. Sun-type chloroplasts have many more reactive centers and photosynthetic transport chains per chlorophyll unit and far fewer light-harvesting pigment-protein complexes as well as fewer thylakoids and are smaller in size than shade-type plants or leaves grown in the shade [4].The highest rate of CO2 fixation has the leaves in the sun compared to the leaves of the shade that appear during the vegetation period from May to September.

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2. Material and methods

2.1. 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: Santa Maria (pear) part of a group of Pyrus Communis L pear species and the rose family. The results of the changes in the variety under study are related to the area that is lacking water and to the period of June, which is considered as the optimal period for the development of the photosynthetic apparatus.

2.2. Pigment determination

Leaf pigments were extracted with 100% acetone in the 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 [1], [2]. The represented values are the mean of six determinations from six leaves.

2.3. Fluorescence spectra

After turning on the device, the samples (leaves) are placed in the chamber of cuvettes, where they rotate in such a way that the samples are in the position of the direction of the light rays. To perform the measurements, one of the options can be selected: Fluorescence of a leaf - to perform the measurements to obtain a fluorescence spectrum. During this scan the excitation monochromator is fixed at the wavelength 470 nm (blue light) and 632 nm (red light), while the emission monochromator is shifted from the wavelength 660 nm-800 nm in 2nm steps. The resulting spectrum is referred to as the leaf fluorescence spectrum. In the measurement, the selected signal amplifier is "Gain" x 300 and slits 2 nm. The shape of the fluorescence emission spectrum of chlorophylls and the relative height of the red fluorescence band F690 and the infrared band of fluorescence F740, approximately at 730-740 nm, depends on the chlorophyll content of the leaves on the one hand and on the other hand the wavelength of the excitation radiation, which is even more important.

3. Results and discussion

3.1. Photosynthetic pigments

The Santa Maria variety (pear) has the highest chlorophyll concentration values in the south position compared to the other two positions (Tab. 1).

Table 1 Presentation of the values of the concentration of chlorophylls Chl (a+b), the ratio Chl a/b and (a+b)/(x+c) in three positions of the leaves for the Santa Maria. Mean values of 6 determinations per leaf-type

Leaf-type	**Chl (a+b)(mg dm^{-2})**	**Chl a/b**	**(a+b)/(x+c)**
Santa Maria - June			
Sun	8.80 ± 0.05	2.85	4.89
Blue-shade	6.38 ± 0.03	2.43	5.48
Half-shade/shade	4.20 ± 0.03	2.15	5.50

The values of the ratio of Chl a/b from the sun position in the shade are decreasing, while the ratio of (a+b)/(x+c) is increasing (Tab.1).

3.2. Fluorescence spectra

3.3. It is observed that the ratio of F690/F735 increases from the sun position in the shade (Tab. 2)

The shape of the fluorescence emission spectrum of chlorophylls and the relative height of the red fluorescence band F690 and the infrared fluorescence band F740, approximately at 730-740 nm, depends on the content of leaf

chlorophylls on the one hand and on the other hand the wavelength of the excitation radiation, which is even more important.

The F690/F740 ratio excited by blue light presents higher values than when green or red light serves as the exciting light [5], [6].

Table 2 Presentation of fluorescence ratio, F690/F735 in three positions for variety Santa Maria, June period

Leaf-type	**F690/F735 (λ_{ex}=632nm)**	**F690/F735 (λ_{ex}=470nm)**
Santa Maria - June		
Sun	0.568	1.145
Blue-shade	0.579	1.260
Half-shade/shade	0.604	1.358

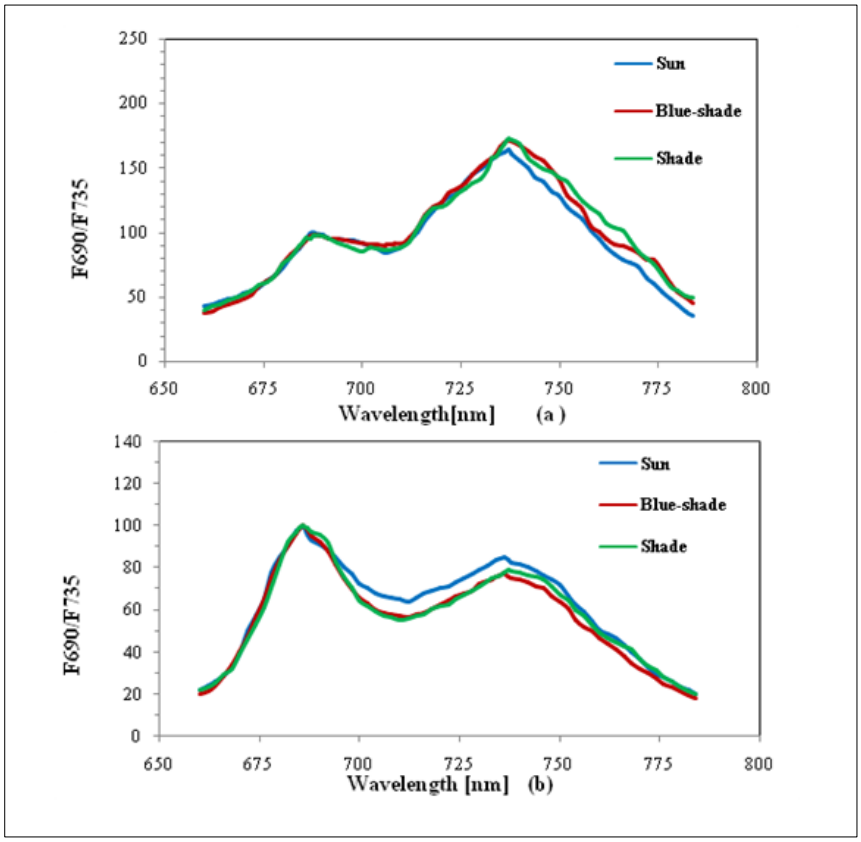

Figure 1 Presentation of fluorescence spectra for Santa Maria (pear): (a) λ_{ex}=632nm, (b) λ_{ex}=470nm

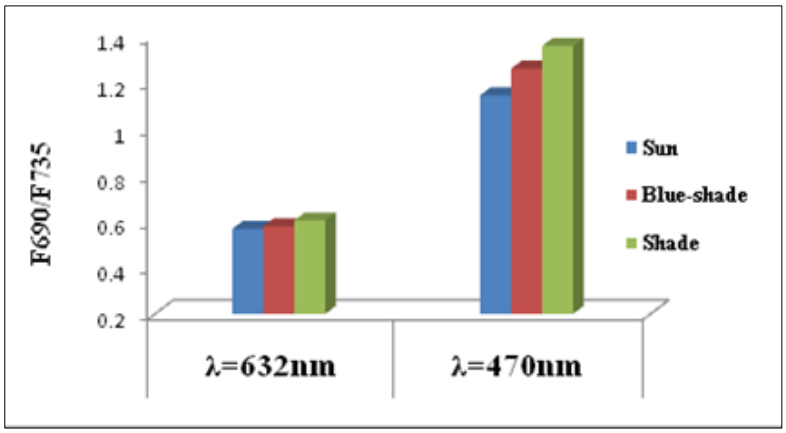

Figure 2 Report of F690/F740 for variety Santa Maria, June period

Table 3 Thickness of varieties, Santa Maria (pear), June period

Leaf-type	Thickness
Santa Maria - June	
Sun	0.342 ± 0.013
Blue-shade	0.295 ± 0.013
Half-shade/shade	0.267 ± 0.010

The variety Santa Maria (pear) in the study presents the highest thickness values for the leaves in the sun position. The leaves in this position are under the direct influence of solar radiation.

4. Conclusions

The concentration of pigments Chl (a+b) presents higher values in the south position compared to the other two positions in all periods and in the two areas. The values of the ratio Chl a/b from the south position - shade increases, while the ratio a+b/ x+c decreases from the south-shadow.

Shade leaves absorb less sunlight and reflect more. Low absorption of sunlight leads to lower chlorophyll concentration.

Fluorescence of chlorophylls excited by blue light is mainly emitted by the chloroplasts of the upper outer layers of palisade parenchyma cells of leaves. For this reason, the fluorescence emission spectrum of chlorophyll excited by blue light changes very little due to the reabsorption of the F690 band, compared to exciting green or red light that penetrates deeper into the leaf mesophyll cells.

Compliance with ethical standards

Disclosure of conflict of interest

Disclosure of conflict of interest - No conflict of interest to be disclosed.

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