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Method of luminescent analysis of the state of photosynthetic apparatus of plants

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Abstract. Photosynthetic apparatus and its reaction to changes in external conditions carry information about the state of the plant. Thus, under different lighting conditions, as well as with changes in nutrition and microclimate conditions, the amount and composition of chlorophyll in plant cells changes, which will lead to changes in the optical parameters of plant chlorophyll. You can track the state of the plant by changing the parameters of chlorophyll fluorescence. To monitor the state of the photosynthetic apparatus, the method of analysis of induction curves of variable fluorescence is used. The purpose of this article is to design a research facility for testing the technique of fluorescent analysis and further accumulation of statistical data on the dependence of fluorescence characteristics on lighting parameters.

1. Introduction

At the moment, greenhouse complexes and its lighting systems are developing very actively. In the process of photosynthesis, which is used by plants to convert electromagnetic radiation (light), plants produce chemical energy necessary for their growth. The decisive factor for successful cultivation is lighting, using of adaptive LED irradiation systems is the efficiency increasing solution. Knowing what the plant needs throughout the growing season, you can develop an adaptive lighting system that will change the luminous flux and spectral composition, selecting the necessary parameters depending on the phase of growth. You can see it by using the technique of analyzing the state of the photosynthetic apparatus in the plant. By analyzing the fluorescence intensity of the plant, it is possible to monitor the reaction of the photosynthetic apparatus to certain changes in the irradiation modes, which will allow you to choose the necessary parameters of the irradiation mode.

During photosynthesis, not all of the light energy consumed by the plant is converted into chemical energy. Part of the energy is lost in the form of heat and the other in the form of fluorescence. Fluorescence is emitted mostly by chlorophyll *a*, it is a secondary emission of light energy absorbed by the chlorophyll molecule [1, 2].

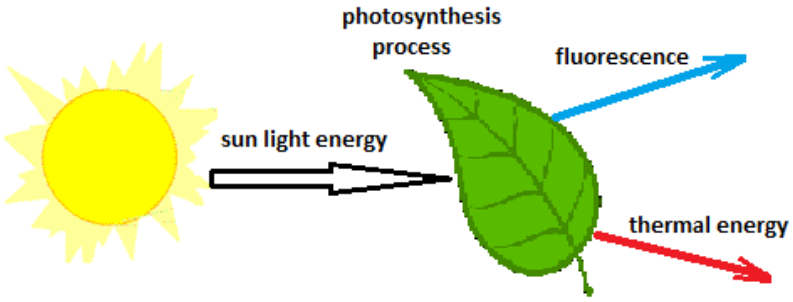

Figure 1. Process of energy redistribution in chlorophyll

Chlorophyll fluorescence *a* is a measure of photons unused during photosynthesis. In conditions of normal and effective work fluorescence remains on enough low level, and any violation of photosynthesis and lowering its efficiency to fast increasing fluorescence level. [1, 2]. Thus, by analyzing the quantum yield of fluorescence it is possible to judge the effectiveness of photosynthesis of the plant.

Plants adapted to the dark, irradiated by photosynthetically active radiation, emit fluorescence with a time-varying intensity, i.e. with a certain dynamic, called the induction curve of variable fluorescence [1].

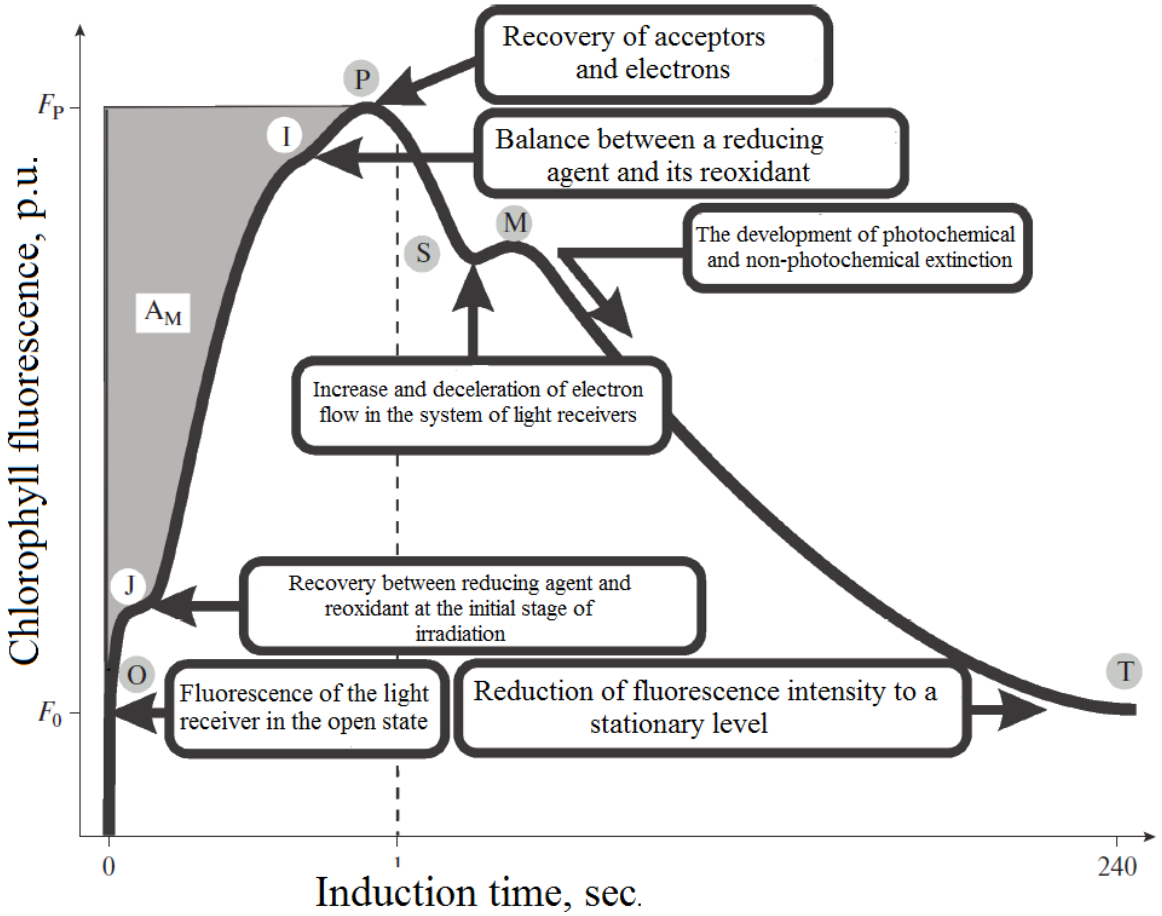

Figure 2. Typical induction fluorescence curve with an explanation of the processes of change in the fluorescence degree [1].

This curve describes the processes, which lead to changes in the level of fluorescence under different irradiation modes [1]. This technique is called JIP test (JIP – reference points of the induction curve) [1].

2. Materials and methods

This paper presents two methods for measuring chlorophyll fluorescence, namely a direct method for measuring the level of fluorescence and using amplitude modulation.

In the direct method, the pre-adapted to the dark plant is illuminated for 20-30 minutes by a constant LED light source, which has the function of changing the intensity and spectral composition of irradiation. The sample is illuminated by continuous light with an excitation wavelength in the range from 420 to 670 nm, the spectrofluorometer registers fluorescence light at wavelengths equal to 680-760 nm.

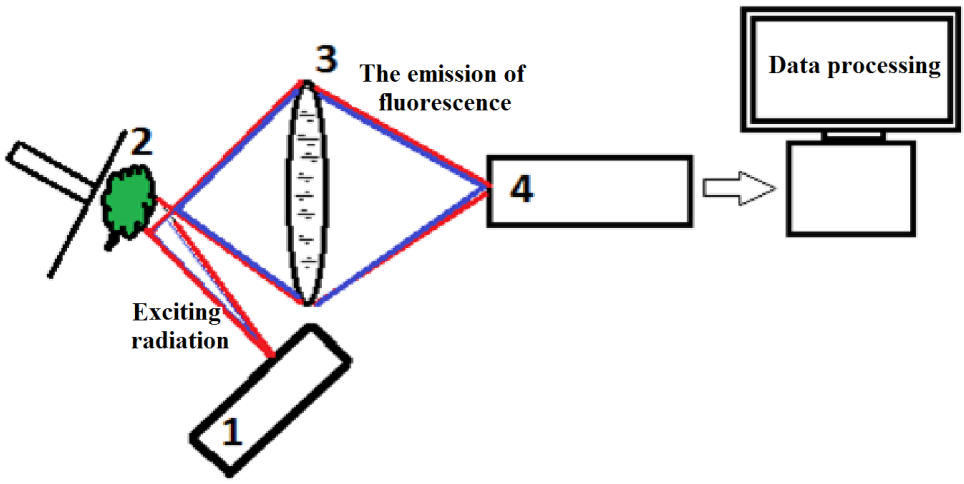

Figure 3. Installation scheme. 1) Light source; 2) Test sample; 3) Focus lens; 4) Spectrofluorimeter.

The installation has the following components:

1) Light sourse - it is intended for irradiation of the sample with light with the necessary characteristics such as: irradiation PPFD (Photosynthetic Photon Flux Density), spectral composition of light, time of illumination. The lighting system provides illumination in several irradiation modes, which are presented in Table 1:

Table 1. Dependence of irradiation intensity of red, white and blue LEDs on current.

Current, A	Voltage, W	Irradiance, W/m^2 (red)	Irradiance, W/m^2 (blue)	Irradiance, W/m^2 (green)	PPFD mol $m^{-2}s^{-1}$ (red)	PPFD mol $m^{-2}s^{-1}$ (blue)	PPFD mol $m^{-2}s^{-1}$ (green)
0.1	9.23	3.94	2.56	1.19	21.4	9.8	5.5
0.2	9.652	7.92	5	2.4	43	19.1	11.1
0.3	10.036	11.8	7.39	3.56	64.1	28.2	16.5
0.4	10.392	15.5	9.24	4.48	84.1	35.3	20.8
0.5	10.739	19.2	11.5	5.61	104.8	44.1	25.9
0.6	11.062	22.5	13.1	6.39	121.4	49.8	29.5

0.7	11.292	26	14.5	7.11	141.9	55.3	33

2) focusing lens with a focal length of 200mm-collects the rays coming from the test sample in the focal plane the monochromator slit is installed in the focal plane.
3) Monochromator MDR 204-designed for measurements of radiation spectra and kinetic measurements.

As a result of the measurements carried out on this installation according to the luminescence analysis technique, the fluorescence intensity was measured at different levels of irradiation.

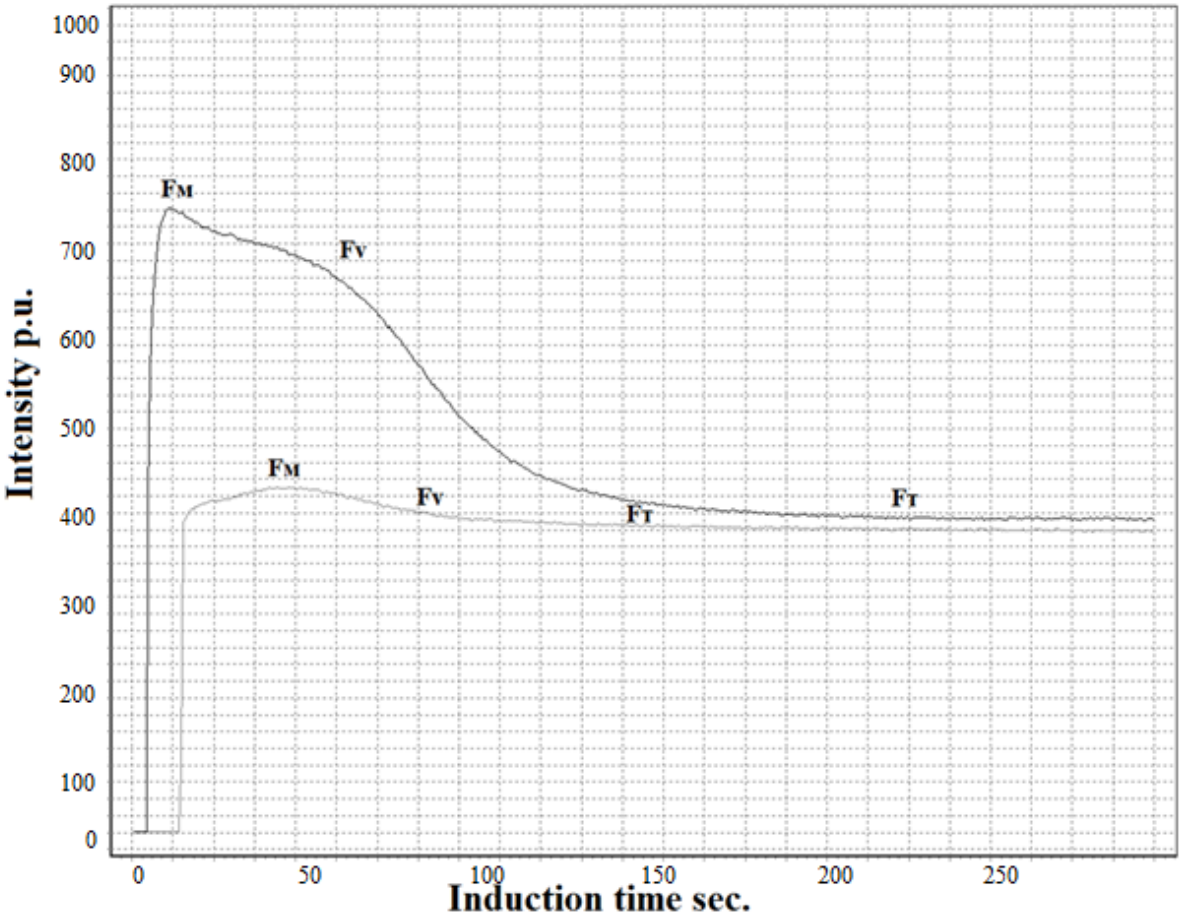

Figure 4. Induction curve.

The chart shows the change in fluorescence intensity over time. At two different levels of learning, characteristic points of the induction curve are obtained, thus the following parameters are determined:

F_M – maximum fluorescence is determined after the adaptation of the plant to the dark with the help of saturating radiation and shows the effectiveness of the initial processes of photosynthesis.

F_V – variable fluorescence is the difference between maximum and initial fluorescence, with the help of this indicator, the efficiency of photosynthesis is determined.

F_T – the steady - state fluorescence level shows the rate of adaptation of the plant to light, which also reflects the efficiency of photosynthesis.

The use of amplitude modulation in the second method expands the possibility of measuring the level of fluorescence, this method gives the opportunity to use of a saturating beam, which causes the processes of extinguishing luminescence, while using a source emitting modulated light, and the sensor in the spectrofluorometer registers only the variable component of radiation [1, 2]. Thus, it is possible to carry out measurements with application of saturating pulses and to investigate how chlorophyll reacts to them.

As part of the work the installation which has which has in its composition LED PPFD light source with adjustable wavelength in the range of 420 to 670 nm was designed. This source was created by saturating pulses with an intensity 1000 - 2000 mol $m^{-2}s^{-1}$ and a spectrofluorometer with amplitude modulation.

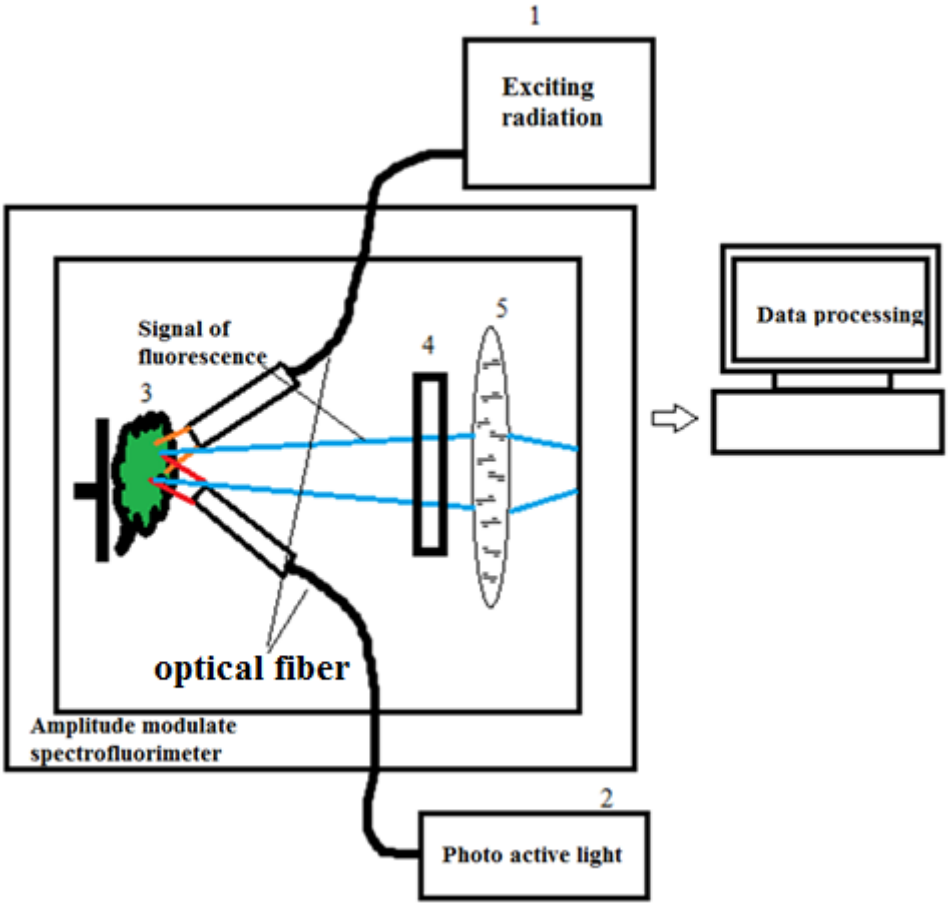

Figure 5. Installation scheme. 1) Exciting radiation source; 2) Photo active light source; 3) Test sample; 4) Light filter of monochromator; 5) Focusing lens.

The chart of induction curve using saturating light.

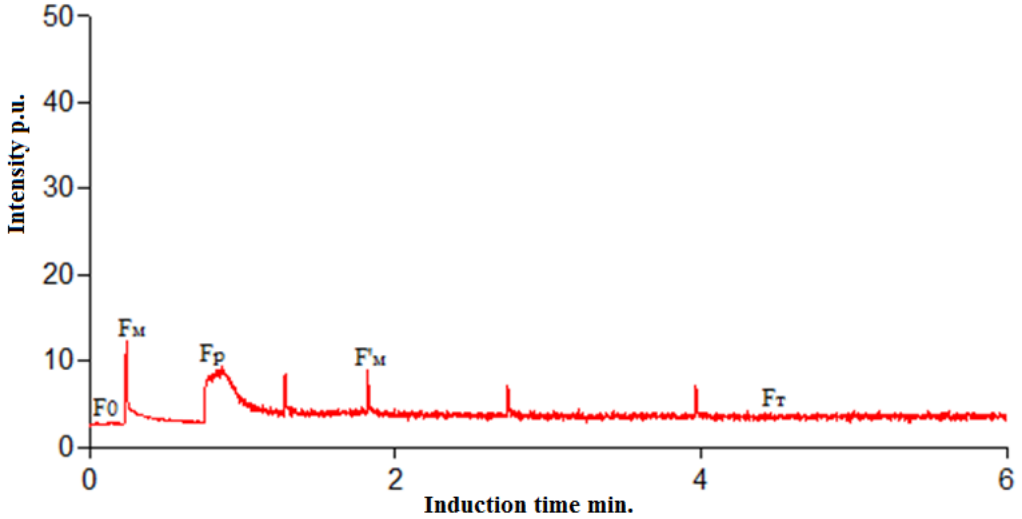

Figure 6. Induction curve with amplitude modulation light.

Max level and start level of luminescence which were obtained from saturating and photoactive radiation (F0, Fм, Fp), stationary fluorescence ($F_т$) [2]. According to this schedule, one can assess the state of the plant under study. Because the level $F'_м$ below the maximum of chlorophyll fluorescence $F_м$ since part of the fluorescence energy is absorbed as a result of extinguishing processes of petrochemical nature, such as heat. This suggests that the plant does not experience stress associated with lack of water or sol balance. $F_м$ – the maximum fluorescence is much higher than the initial fluorescence F_0, which indicates the effective photosynthesis of the plant. As a rule, this parameter is ten times higher than the start fluorescence, as it is seen in the chart.

3. Conclusion

Analysis of chlorophyll fluorescence gives a wide range of possibilities in the selection of the necessary lighting parameters. Such methods allow us to track the reaction of chlorophyll to changes in external conditions, which will contribute in the future to the creation of adaptive irradiation facilities and as a consequence will increase the efficiency of greenhouse complexes.

As part of the work, two research facilities were designed, which were adapted to the existing fluorescence analysis technique. These settings will help to accumulate statistical data on the reaction of chlorophyll to external lighting and allows you to link changes in the intensity of fluorescence with lighting parameters.

Acknowledgement

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