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SMART PORTABLE FLUOROMETER FOR EXPRESS-DIAGNOSTICS OF PHOTOSYNTHESIS: PRINCIPLES OF OPERATION AND RESULTS OF EXPERIMENTAL RESEARCHES

Volodymyr Romanov, Volodimyr Sherer, Igor Galelyuka, Yevgeniya Sarakhan, Oleksandra Skrypnyk

Abstract: In the Institute of Cybernetics of National Academy of Sciences of Ukraine the smart portable fluorometer for express-diagnostics of photosynthesis was designed. The device allows easy to estimate the level of influence of natural environment and pollutions to alive plants. The device is based on real time processing of the curve of chlorophyll fluorescent induction. The principles of operation and results of experimental researches of device are described in the article.

Keywords: Kautsky effect, chlorophyll, chlorophyll fluorescence induction, fluorescence, fluorometer, portable device, vine plant.

ACM Classification Keywords: J.3 Life and Medical Sciences - Biology and Genetics

Introduction

Development of information technologies and microelectronic circuits allows filling of the world market with portable computer devices such as handheld PCs, laptops, media players, medical devices (tonometers, glucometers, cardiographs), navigation devices and so on. The achievements of Ukrainian scientists who work in the field of biosensors combined with modern capabilities of information technologies provided development of devices for express-diagnostics of plant state, evaluation of environmental parameters, exposure of infective diseases etc.

In the context of the program of Presidium of NAS of Ukraine "Development in the field of sensor systems and technology" in Glushkov's Institute of cybernetics of NAS of Ukraine the portable computer device was developed for express-diagnostics of stress factors which influence on the plant's state. The portable device measures chlorophyll fluorescence induction (CFI) without plant destruction. Using the curve of CFI (alike the cardiogram) allows diagnosing influence of one or other influential factor on the plant's state.

Features of biological objects' luminescence

As a result of external influence, different objects, including biological ones, can generate plenty of radiation that is independent of these objects temperature.

All the types of radiation that were caused by some external sources of energy are called luminescence. Duration of luminescence after external influence stopping exceeds period of light fluctuations. Luminescence is conditioned by fluctuations of relatively small number of atoms or molecules of substance that become excited under energy source activity. Radiation is a result of transformation of atoms' or molecules' states into fundamental (unexcited) or less excited (they have less energy) states.

This is well adjusted with quantum theory, according to what every stationary orbit conforms to definite value of atom's energy (Bore's postulate). Being placed on stationary orbits an electron doesn't radiate and doesn't absorb electromagnetic waves. According to the second Bore's postulate radiation and absorption can happen only when atom changes its state from one stationary state to another:

$$h\varpi_{mn} = h\nu_{mn} = E_n - E_m \,, \tag{1}$$

where ϖ_{mn} or ν_{mn} – photon's frequency, E_m , E_n – energy values of the states *m* and *n,* h – Planck's constant, *m* and *n* – the numbers of energy states. At the same time electron switches from one stationary orbit to another.

Luminescence is defined by the structure of substance energy spectrum, the average time of staying in excited states and rules of selection, which allow absorption or radiation of light of defined frequency. Short-timed luminescence is also called fluorescence. Luminescence which appears during lighting of substance (phosphor) with visible or ultraviolet light is called photoluminescence. Usually process of luminescence satisfies Stocks' rule that claims that wave length λ' of radiated light is greater than wave λ of excited light. According to the quantum theory this means that photon's energy $h\varpi(h\nu)$ is used partially for non-optical processes:

$$h\varpi = h\varpi' + E, \varpi > \varpi' \,, \tag{2}$$

where ϖ' – luminescence's frequency, E – energy waste on another process.

Luminescence is characterized by energy output which equals to ratio of luminescence energy to energy that was absorbed by substance under stationary conditions.

Energy efficiency of photoluminescence increases proportionally to wave length λ of absorbed light up to the definite maximum value at $\lambda = \lambda_{\max}$ and then rapidly decreases to zero at $\lambda > \lambda_{\max}$ (Vavilov's rule). A sharp decrease of energy at $\lambda > \lambda_{\max}$ is explained by the fact that at these wave lengths λ the energy of absorbed photons is not enough for the process of phosphor atoms and molecules transfer to the excited states.

Ratio of luminescence photons number to absorbed photons with fixed energy is called quantum yield of photoluminescence. According to Vavilov's rule, which is under Stocks' rule, quantum yield of photoluminescence doesn't depend on wave length of excited light and rapidly decreases for anti-Stocks radiation.

Intensity of luminescence *I* depends on behavior of elementary processes that causes this radiation. In case of spontaneous luminescence, when radiation starts after light absorption during which atoms or molecules are transmitted to the excited level that is placed higher than the level at which radiation takes place and then these atoms (molecules) are transmitted to the luminescence level, intensity is subordinate to exponential rule

$$I = I_0 \exp(-t/\tau) \,, \tag{3}$$

where I – lighting intensity at the moment *t*, I_0 – lighting intensity in a moment of excited radiation stopping, $\tau \approx 10^{-9} - 10^{-8}\tilde{n}$ – an average duration of excited state of phosphor atoms or molecules. Luminescence of compound molecules and phosphorescence (after lighting) of organic substance are subordinate to the low (3).

Under influence of light there can be happened photochemical transformation of substance (including photosynthesis), which is called photochemical reactions. In a process of such reactions light absorption takes place. Energy is spent on compound molecules and polyatomic ions decomposition to component parts and creation of compound molecules of primary ones. An example of photochemical reactions is decomposition carbon dioxide under influence of light

$$2CO_2 + 2h\varpi \rightarrow 2CO + O_2 \tag{4}$$

Carbon dioxide decomposition takes place in green parts of plants under sun light influence, as photochemical process, which is a part of photosynthesis.

Principles of Operation

One of the most important properties of the molecule of chlorophyll which is the basic pigment of plant cell is ability to fluoresce. For the first time this phenomenon was researched by Kautsky [Kautsky, 1931], [Kautsky, 1937]. Dependence of chlorophyll fluorescence induction on time passed after start of lightning of plant's leaves is known as an induction curve or a chlorophyll fluorescence induction curve (Fig. 1). The form of this curve is rather sensible to changes in the photosynthetic apparatus of plants during adaptation to different environmental conditions. This fact is a basic for extensive usage of Kautsky effect in photosynthesis research. The advantages of the method of CFI are the following: high self-descriptiveness, expressiveness, noninvasiveness and high sensibility.

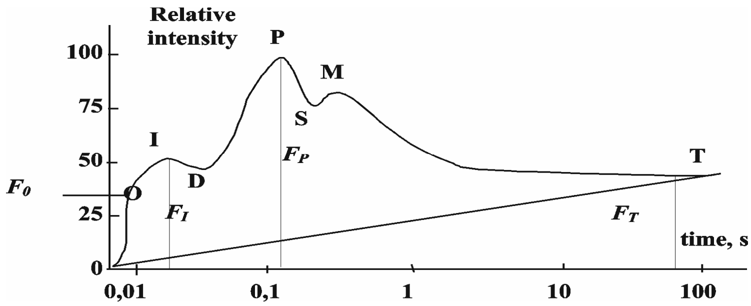

Figure 1. Chlorophyll fluorescence induction curve

The examples of changing form of this curve under influence external factors are listed in [Romanov, 2007]. The organization, scheme, basic components and advantages of the portable computer fluorometer "Floratset" are discussed in [Fedack, 2005] in detail.

Results of Experimental Research

The experimental researches of the "Floratest" were conducted in National Scientific Center "V.E. Tairov's Institute of viticulture and winemaking" of Academy of Agrarian Sciences of Ukraine. The conditions and results of the experimental researches are listed below.

Mature leaves of vine were used in the researches. Under changes of soil watering conditions there were observed sharp changes in behavior of induction transitions of chlorophyll fluorescence which were accompanied by quite essential changes of leaf tissue spectral characteristics.

Determination of fluorescence spectral characteristics was done by placing the device's sensor on the leaf's surface without integrity disturbance directly in a pot or in a field. It allowed to research on plastid and vacuolar pigments in their natural state and in that way approaching to understanding of the biophysical and physiology-biochemical processes which take place in the live leaf, and determination of important sides of photosynthetic activity.

Fluorescence intensity of the sample was determined in relative units.

It is significant that under natural conditions in the middle latitudes the drought is accompanied simultaneously by high temperatures of air, and that intensifies bad influence of ground water lack on agricultural plants.

Even in the first variant of experiment (drought) there appeared considerable changes of the behavior of fluorescence induction comparing to the control samples. Changes show in weakening of penetrability of the chloroplasts' membrane structures. That results in substantial increase of time characteristics of fluorescence induction slow decrease. At the same time noticeable variety differences become apparent. Sharp decrease of its value is typical for profound functional injuries of photosynthetic structures and cells of particular variety entirely.

Accordingly in this stage of drought influence significant variety differences in exsiccate factor resistance of both photosynthetic structures and lamina's parenchymal cells entirely became apparent.

More deep changes of destructive nature may be observed in case of high temperatures (+40 °C), which influence on leaves complementary to drought. In this case for all the varieties being studied significant and almost irreversible functional changes of plastid structures are noted. These functional changes show in sharp decrease of CFI intensity.

Disastrous changes of life activity of vine leaf cells which take place during these processes show in oppression of biosynthetic processes, intensive decomposition of cytoplasmic structures and intensification of oxide catabolism of plant cell's content. The consequence of these processes is decrease of CFI intensity as a result of its oxidizing transformation.

Diagrams of measuring of chlorophyll fluorescence intensity for vine plants under drought conditions and normal conditions accordingly are displayed on figures 2, 3.

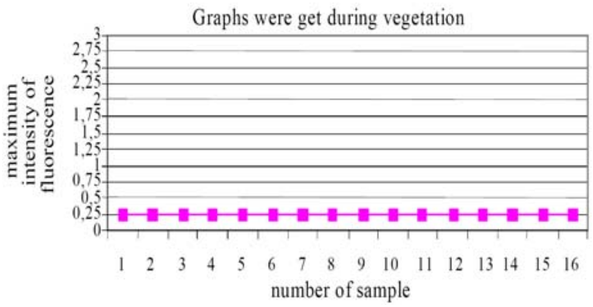

Figure 2. Maximum of CFI intensity of vine plant under drought influence (28-30% insufficient water capacity)

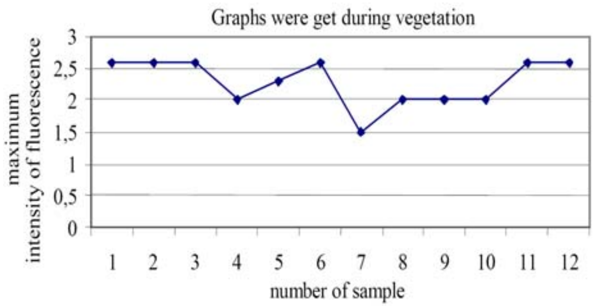

Figure 3. Maximum of CFI intensity of vine plant, control samples (68-70% insufficient water capacity)

Thus, a water deficit (WD) shows up on the Kautsky curve (figure 1) as difference of fluorescence ($F_p - F_0$) decrease. The most credible reason of this is oppression of oxygen emission which is related with slowing down of electrons transfer. Assuming that F_0 almost does not change for the test and control plants, in a maximum point the chlorophyll fluorescence intensity value can define the level of water deficit.

On the figures 4, 5 there are shown the diagrams of measuring of chlorophyll fluorescence intensity for two sort of vine plants (PxP 101-14 and Kober 5BB) during 5 months. The vine plants were under drought influence and normal conditions.

Examples of the practical usage of fluorometer "Floratest" in the National Scientific Center "V.E. Tairov's Institute of viticulture and winemaking" and the graph of CFI on the device's display are shown on figure 6.

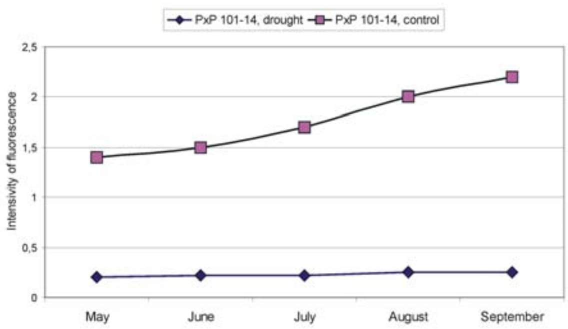

Figure 4. CFI intensity of vine plant (sort PxP 101-14) under drought and normal conditions

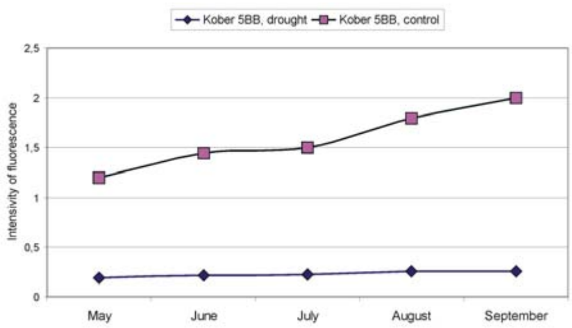

Figure 5. CFI intensity of vine plant (Kober 5BB) under drought and normal conditions

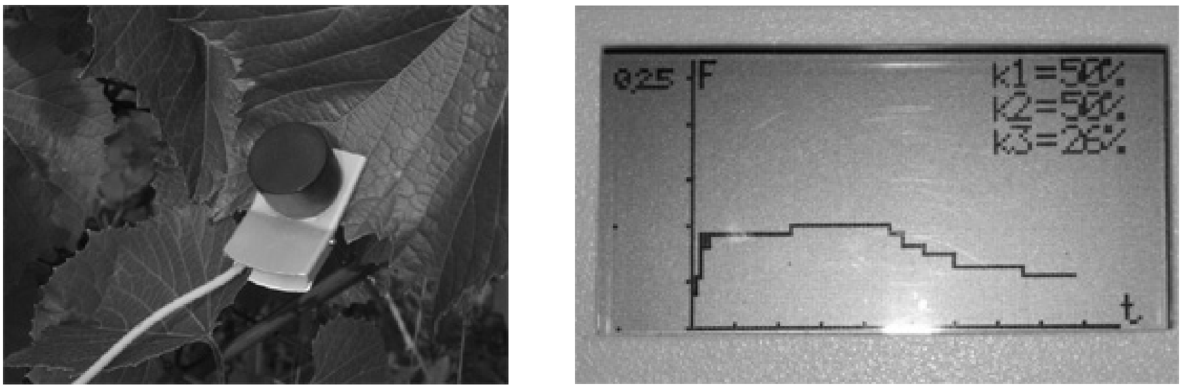

Figure 6. The sensor of the "Floratest" on the vine leaf and the image of CFI on the device's display

Experimental researches of fluorometer "Floratest" in National Scientific Center "V.E. Tairov's Institute of viticulture and winemaking" allow:

- determination of vine plants' state under the stress factor influence accordingly to the parameters of Kautsky curve;
- development of the recommendations on the fluorometer's software update and bringing output information on the device display to the recommendations, which accompany the Kautsky curve;
- development of recommendations on creation of the set of removable sensors, using which both detection of stress factors and express-diagnostics of plant disease can be performed.

Conclusions

- on the basis of modern information technologies and achievements in field of biosensorics original noninvasive portable fluorometer for express-diagnostics of plant state under stress conditions was developed;
- during the fluorometer designing and fast software and hardware tools adaptation to the conditions of exploitation the methods of virtual design created in the Institute of Cybernetics of NAS of Ukraine as a part of virtual laboratory for computer-aided design were used extensively;
- during experimental researches in National Scientific Center "V.E.Tairov's Institute of viticulture and winemaking" of Academy of Agrarian Sciences of Ukraine there were developed methodical tools which allow evaluating the state of vine plants under drought conditions and conditions of insufficient water capacity in express-mode.

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