COMPLEX OF OPTICAL BIOSENSORS FOR CONTROL OF TOTAL STATE OF VEGETABLES AND ESTIMATION OF THEIR LOADING BY VIRUSES

N. F. Starodub[1,2], R. V. Son'ko[1], V. O. Romanov[2], I. P. Galelyuka[2]
National University of Life and Environmental Sciences, 15 Herojev Oboroni Str., Kyiv, 03041 Ukraine, nikstarodub@yahoo.com
V.M. Glushkov Institute of Cybernetics of National Academy of Sciences, Kiev, 03680, Ukraine
VRomanov@i.ua

Summery.
It was developed the complex of the portable biosensors for the non-invasive measures of the chlorophyll fluorescence induction and express control of vegetable loading by some viruses. The first type of biosensor named "Floratest". It allows to obtaining information about the activity of the photosynthetic process in the tissues which reflects the total state of vegetables. The main advantages of this approach are in its very sensitivity and rapidity. For the control of the viral loading of some vegetables it was developed the portable part of the immune biosensor. In this case as a basis it was used so called laboratory on the crystals Spreeta TSPR1A170100, which produced by the Nomadics, Inc. Spreeta.

Reliability of the proposed methods of the express-diagnostics based on the estimation of the induction of the chlorophyll fluorescence in the vegetable flats confirms by the results of the physiology-biochemical analyses: vegetables which are less stable to drought had more high level of chlorophyll fluorescence. Generally it was obtained the conclusion that the determination of the last index by "Floratest" allows to us optimizing the technology of grapes and hot in real time. The results of the analysis of 21 samples with the help of the developed immune biosensor have a good correlation with that obtained by others above mentioned methods. There is necessary to pay attention that the results obtained by the PCR was chosen conditionally as absolute correct since the serological methods have some restrictions which are absent in gene engineering ones. Analysis data are transferred from device to the medical centre or the laboratory by means of radio channel. As radio-transmitter it is used the original unit, which is developed by the company "VD MAIS". Besides the obtained data also the information about the place of the analysis is transferred by means of radio-channel. For this goal it is used GPS-system, which is built in the radio-transmitter. Principle of the operation is based on the transferring all data through the radio-channel (by means of the GSM-technology) directly to the Internet, and then to the server of the medical organization.

In any case there are not any doubts that the both developed biosensors may be used at the practice, in particular, for the fulfilment of the screening observations. The together application of both sensors allows to determining not vegetable state only and to reveal the reason of such situation. Moreover, since the principle of work immune biosensor is based on the registration of the bioaffinic reactions it may be considered that this biosensor may help at the diagnostics of many diseases which is stipulated by viruses, bacteria and fungi's. And it will be perspective at the registration of molecular hybridization.
Key words: *Chlorophyl, fluorescence, vegetable, state, control*.

Introduction.

Plants as other living organisms are affected by numerous environmental factors. The control of vegetable state is necessary from number of sides including possibility to predict the amount of yield and to estimate changes of environment. Finding general relationship in the complex system of the plant also required selection of appropriate key objects [1, 2]. One of the main processes of plant vital activity is photosynthesis provides generation of carbohydrates, glucose and number of organic substances using solar energy, water, carbon dioxide and producing oxygen. From other side, the fluorescence of chlorophyll is still the only indicator that allows us to study flow photochemical reactions in living objects. They are connected with functioning so called photo system 2 which is the most sensitive to the influence of environmental factors. The regulator mechanisms of photosynthesis occur predominantly in a variety of inductive phenomena and, in particular, the phenomenon of so-called slow induction of fluorescence of photosynthesizing objects that together with a simple experimental technique makes the perspectives of its study.

For the first time the phenomenon of chlorophyll fluorescence was researched by Kautsky [3, 4]. Dependence of the 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 are the following: high self-descriptiveness, expressiveness, non-invasiveness and high sensibility.

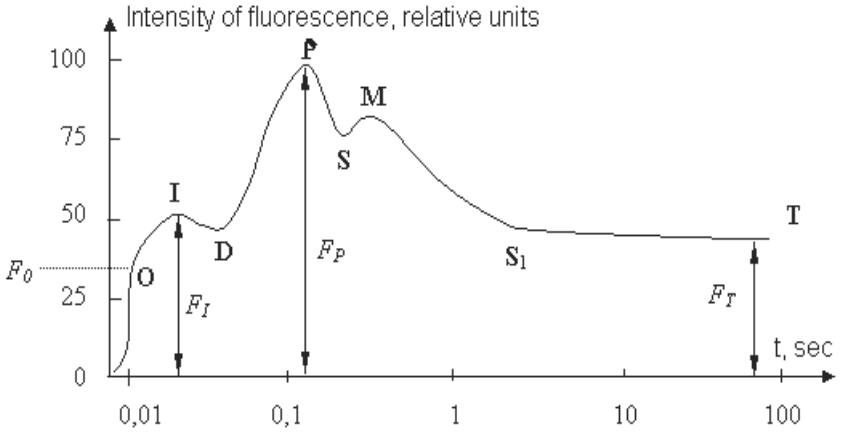

Fig. 1. Chlorophyll fluorescence induction curve. When: (O-I) – background level of fluorescence; (I-D) – rapid recovery Q_A in the complexes of the photosynthetic system 2 which take part in electron transport on the pool of plastoquinones; (D-P) – more slowly reduction of Q_A in the complexes of the photosynthetic system 2; (P-S) – activation of the feredoksin-$NADP^+$-reductase, accumulation of gradient of protons; (S-M) – slowing the outflow of electrons from electron-transport chain to feredoksin-$NADP^+$-reductase caused by decreasing $NADP^+$ pool in case of the delay in CO_2 fixation and reduction of gradient of protons due to activity of ATP-synthetase; (M-T) – start in active CO_2 fixation, T – stationary level.

To estimate the state of plant photosynthetic system, as a rule, the number some characteristic values are used: 1) F_0 – initial level of fluorescence after switching of illumination; 2) F_{max} – maximal value of fluorescence; 3) F_T – steady-state value of fluorescence after light adaptation; 4) $\zeta_{0,5}$ – time to reach half the maximum fluorescence; 5) $K_1 = F_{max}\text{-}F_0/F_{max}$ – indicator index of the effect of exogenous factors; 6) $K_2 = F_{max}\text{-}F_T/F_{max}$ – coefficient of fluorescence induction (indicator measure of the fluorescence quantum yield); 6) $K_3 = F_T/F_{max}$ – indicator measure of viral infection.

The main fields of application of the method of fluorescence registration are in the estimation and prediction of the influence of a number of factors on the vital functions of the chlorophyll contented organisms: 1) climatic conditions; 2) fertilizers; 3) substances contaminated environment; 4) viral infection; 5) chemical plant protection and 6) water regimes. Moreover, there is possible to select optimal processing methods for the commercial cultivation of crop plants, to provide monitoring and control of productive plants in real time and perform screening control of the environment in certain regions.

Today a lot of different instrumental analytical devices are proposed for the chlorophyll fluorescence registration (Table 1). They differ by the sensitivity, dimensions, set of some characteristics, costs and most importantly the fact that usually they are pre-programmed and it can not be changed by the user in line with his desire [5, 6].

Table 1. Issued types of devices for the measurement of chlorophyll fluorescence [5].

Item	Type of device	Instrument brand	Producer	Citation
1.	Portable Chlorophyll Fluorometer	MINI-PAM	Heinz Walz Gmb (Germany)	[7]
2.	Modulated Chlorophyll Fluorometer	OS5-FL	Opti-Sciences (USA)	[8]
3.	Chlorophyll Fluorometer	OS30	Opti-Sciences (USA)	[9]
4.	Modulated Fluorometer	OS1FL	Opti-Sciences (USA)	[10]
5.	Algae Fluorometer	AFM	Opti-Sciences (USA)	[11]
6.	Fiber-Optic Fluorometer	GFP-Meter	Opti-Sciences (USA)	[12]
7.	Chlorophyll Fluorometer	UV-A-PAM	Gademann Instruments Gmb	[5]

The collaborators of V.M. Glushkov Institute of Cybernetics of Ukrainian National Academy of Sciences have proposed variant of similar device which was named as “Floratest” [6]. The overall view of device, form of the registered results and principal block scheme are shown in Fig. 2, 3 and 4, respectively.

The efficiency of this device work was examined by us at the investigations of number of the chlorophyll fluorescence parameters in leaves of maize, some garden trees, grapes and wheat in different conditions. Second part of this article is the presentation of the efficiency of work of the portable immune biosensor based on the surface plasmon resonance for the control of loading of vegetables by viruses. Both this devices are as unique complex intended for control of vegetable state with the final purpose to predict grope and for estimation of direction of environment changing at the climate warming [13].

The characteristics of the developed device for the measurement of chlorophyll fluorescence in the comparison with existed types are presented in Table 2. This device is as open system and has the remote and replaceable sensor parts as well as original methodological software's that allows quickly its orientation on concrete application.

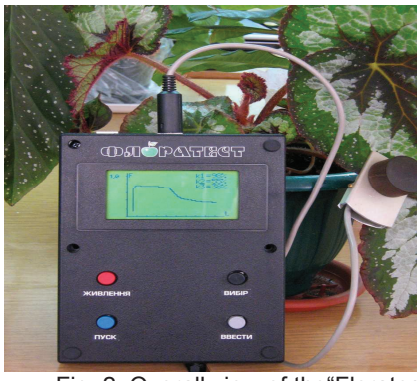

Fig. 2. Overall view of the"Floratest".

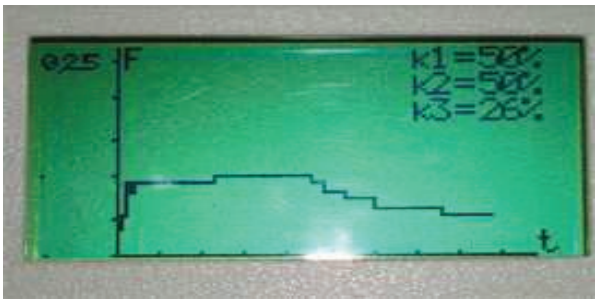

Fig.3. Overall view of the result presentation desk of the "Floratest".

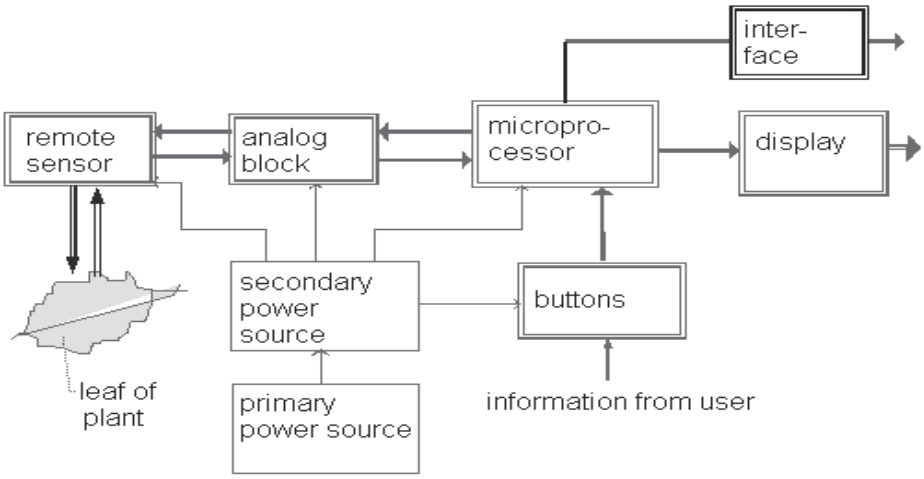

Fig. 4. The principal block scheme of "Floratest".

Table 2. Comparison of parameters of the developed device with the existed analogues.

Type of device	Floratest	OS-30	CL-01	HANDY-PEA	PAM-2 100	PPM-100
Producer	V.M. Glushkov Institute of Cybernetics of UAS	OPTI-Sciences	Hansatech Instruments	Hansatech Instruments	Heinz Walz GmbH	EARS
Specification	Portable	Portable	Portable	Portable	Portable	Portable
λ of measurement, nm	670	660	620, 940	660	655	637
Time of measurement, sec	10…180	2…255	0,5…60	0,1…300	0,1…300	0,1…300
Type of interface	RS-232/USB	RS-232	RS-232	RS-232	RS-232	RS-232
Changing of program and methodical supplements	Possible	–	–	–	–	–
Cost, Euro	~300	~2495	~1275	~1350	~1500	~1395

Experimental.

Principle of measurements of chlorophyll fluorescence by "Floratest". Leaf of plant was situated between plates of the remote optical module of sensor (Fig. 5). Part of leaf in these conditions is isolated from light and goes dark adaptation during 3-5 min. The remote sensor illuminates portion of the surface of the leaf blade with a diameter of 5 mm by blue light. At the effect of this light in the chlorophyll of the illuminated spot the red fluorescence appears. Through a red filter fluorescent signal arrives to the photo detector which transforms its in electrical one. At last, the electrical signal proportional to the intensity of fluorescence is enhanced to some value and the signal proportional to the intensity of fluorescence comes for the processing in the processor module fluorometer.

It was measured the next indexes: F_v – value of electron transport activity which characterizes light stage of photosynthesis; F_v/F_o – index of photochemical activity of photo system 2; $(F_{pl} - F_o)/F_v$ – index of content of Q_b – not renewing complexes of photo system 2 which taken part in linear electron transport and which lead to decreasing initial products of photosynthesis; F_{max}/F_{st} – indexes depended on efficiency of reaction of CO_2 mobilization; F_v/F_{max} – values depended on quantity of active photo system 2 complexes in sample; $(F_{pl} - F_o)/F_{max}$ – value characterized quality of reaction centres of photo system 2 and $(F_{max} - F_{pl})/ F_{max}$ – index characterized efficiency of work of electron transfer lain.

Fig. 5. Overall view of optical part of sensor fixed on the leaf.

Control of viral loading of plants. For this purpose it was developed the portable part of the immune biosensor with GPS system which gives possibility to immediately transfer results in the special laboratory. In this case as basis it was used so called laboratory on the crystals Spreeta TSPR1A170100, which produced by the Nomadics, Inc. Spreeta [14]. This portable biosensor was used at the revealing of the Carla viruses in the hop (*Humulus lupulus L.*). In this case the sap from the tissues of the wild hop was obtained and to concentrate viroides the polyethylene glycol (6.0 kDa) was used in the presence of 0.3 M NaCl. There is necessary to underline that the results obtained with the help of the portable immune biosensor were confirmed by the ELISA-method and the polymerase chain reaction (PCR). Both last methods were fulfilled according to the standard procedure.

Results and discussion

The results of measurements of number of indexes of chlorophyll fluorescence of plants after their putting into the darkness and return to the previous light conditions trough some times are given in Table 2.

Table 2. Some indexes of chlorophyll fluorescence of plant in the dark and illuminate conditions.

Measured indexes	Control	Darkness (1 hour)	Returning to light
Fv	0,391	0,635	0,566
Fv/Fo	0,909	2,166	1,611
(Fpl-Fo)/Fv	0,300	0,461	0,397
Fm/Fst	2,154	2,568	2,000
K_i (K_2)	0,536	0,611	0,500
Fv/Fm	0,476	0,684	0,617

At the analysis of the obtained results it may be considered that the photosynthetic process are active in the experimental plants but the increasing of $(F_{pl} - F_o)/F_v$ index indicates about accumulation of not-renewing complexes (Q_b) and F_v/F_m index testify about destruction of complexes of photo synthetic system 2. In plants which were returned to light the indexes of fluorescence were recovered.

In next experiments we analyzed changing of the intensity of chlorophyll fluorescence in two sorts of the hop (wild and industrial grade – Humulus Lupulus L.) which were in the condition of low (28–30%) and high humidity (68–70%). These results are presented in Fig. 6. It can see that the intensity of the

chlorophyll fluorescence sharply decreased in case of low humidity and, in particular, it has place for hope of industrial grade in the comparison with the wild sort. At the high level of humidity the value of this index approximately is the same for both sorts. This fact is understandable when we will take to consideration that the wild sort may be more evolutionarily adapted to changes of humidity of growing environment.

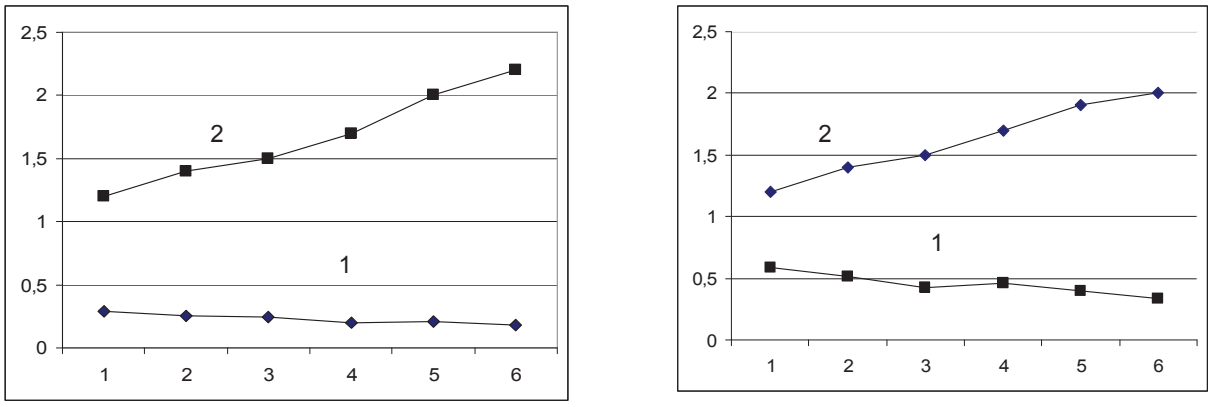

Fig. 6 The intensity of the chlorophyll fluorescence industrial grade hope (left) and wild type (right) during effect of different level of humidity: 1 – 28-30, 2 – 68–70%.

The efficiency of the "Floratest" application was examined at the control of the chlorophyll fluorescence indexes in the vegetables loaded by viruses. In particular it was done in case of infection of the hop (Humulus Lupulus L.) by the Carla virus. Simultaneous it was checked the efficiency of the developed portable immune biosensor based on the surface Plasmon resonance for the control of the virus content in the vegetable tissues.

The results of the analysis of 21 samples with the help of the developed immune biosensor have a good correlation with that obtained by others above mentioned methods (Table 3). There is necessary to pay attention that the results obtained by the PCR was chosen conditionally as absolute correct since the serological methods have some restrictions which are absent in gene engineering ones.

Certainly the obtaining of the positive result by the immune biosensor depends on the viroid content in the vegetable tissues and on the way of the preparation of the samples to be analyzed. This way should be improve as in the respect of the providing of the viroid concentration and the simplicity of the method of the sample preparation.

Table 3. Correlation of the results obtained by the developed immine biosensor, the ELISA-method and the PCR at the revealing of the Carla viruses in the sup of the hop (Humulus Lupulus L.).

Method of analysis	Quantity of the coincident results, %	
	Negative	Positive
PCR	100	100
ELISA-method Portable immune biosensor	91 87	80 72

In any case obtained results testify that the developed portable instrumental device may be really used for the express diagnostics of the different acute viral infections including the viral loading of some vegetables. It may be used for the preliminary screening of the plant area. Moreover there are any doubts in the possibility of this biosensor application for the determination of number of others biochemical quantities which have a big significance for the practice and which may form the immune complexes with the specific Ab.

Analysis data are transferred from device to the medical centre or the laboratory by means of radio channel. As radio-transmitter it is used the original unit, which is developed by the company "VD MAIS" [15-17]. Besides the obtained data also the information about the place of the analysis is transferred by means of radio-channel. For this goal it is used GPS-system, which is built in the radio-transmitter. Principle of the operation is based on the transferring all data through the radio-channel (by means of the GSM-technology) directly to the Internet, and then to the server of the medical organization.

Conclusion

In any case there are not any doubts that the both developed biosensors may be used at the practice, in particular, for the fulfilment of the screening observations. The together application of both sensors allows to determining not vegetable state only and to reveal the reason of such situation. Moreover, since the principle of work immune biosensor is based on the registration of the bioaffinic reactions it may be considered that this biosensor may help at the diagnostics of many diseases which is stipulated by viruses, bacteria and fungi's. And it will be perspective at the registration of molecular hybridization.

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