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EXPRESS INSTRUMENTAL STABILITY ASSESSMENT OF ORDINARY BEANS TO ABIOTIC STRESS FACTORS

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The results of studying the influence of abiotic stress factors on the state of the photosynthetic apparatus of bean by the conventional methods based on the biosensors allowed the rapid diagnostics are presented. It was characterized a number of such indexes: IFH, the intensity of photosynthesis adaptation and viability of plants to abiotic stress factors.

Keywords: *biosensor, IFH, indexes adaptation, sustainability indexes, common bean, abiotic stress factors.*

Determining the impact of environmental factors on plant requires the use of express and informative methods that would allow to conduct tests in the laboratory and in the field with minimal violation of the integrity of the objects. These methods include induction method for detecting chlorophyll fluorescence (IFH), based on modern principles and biosensors that can be widely used in modern studies of photosynthetic processes [7, 13]. This method reflects the changes of the photosynthetic apparatus, which occur at the earliest stages of external influence on the plant. Interest in the study of slow induction of chlorophyll fluorescence (PIFH) is caused by the fact that this phenomenon is largely manifested regulatory processes that ensure optimal functioning of the totality of photosynthetic reactions. Study PIFH plants is very promising in terms of the development of rapid methods for assessment of plants in the environment that changes [10, 14]. Because the instrumental approach of this method is somewhat new, then work it is compared with the traditional, that is, the determination of the intensity of photosynthesis way of air flow.

Beans - a valuable legumes, the total area of cultivation which is the world's 25.6 million. Including Ukraine 20 thousand. In fruits beans contain proteins, carbohydrates, nitrogenous compounds, flavonoids and organic acids, vitamins, pyridoxine, thiamine , pantothenic and ascorbic acid. But recently this useful vegetable growing culture was problematic because different stress factors badly influencing its development and performance [1, 3, 4].

The aim of research was to study the characteristics of IFH, establishing sustainability indices and adaptation to stress Common Bean (Phaseolus vulgaris L.) under different conditions of growth, namely, acidified, salted and dry soils.

Materials and methods

The starting material for the study was selected beans of course, since this plant has a wide leaf blade, which simplifies the experiments, and the high rate of growth. Plants planted in acidic, alkaline soils and dehydrated that served stressors [6, 5].

For information about the impact of factors on the photosynthetic apparatus of plants used biosensor Floratest (manufactured Institute of Cybernetics. NAS of Ukraine) and portable fluorometers (Department of Physics made NUBiP Ukraine), based on the definition of IFH, and classic method for determining the intensity photosynthesis. To control the humidity of soil acidity and pH-use device 300 [9].

Measurements using the device Floratest carried out as follows: an initial fixed the procedure; then performed dark adaptation controlled plane sheet and then determine the level fluorestsents for 3 min and 160 s, respectively. Sheet plate for measurement was taken from identical layers in each series of experiments. For curves IFH and analysis software tool used Microsoft Office Excel 2007 [8].

IFH or induction lines - shows the intensity of fluorescence of time after the lighting. By this device can be fixed Floratest kinetics as fast and slow changes in the signal and interpret typical plot induction curves. In the induction kinetics of chlorophyll fluorescence transitions are reflected processes as light and dark phase of photosynthesis. To assess the state of the photosynthetic apparatus using a range of options, including the main ones being:

1. (Fmax-Fo) / Fmax = Fv / Fmax - depends on the efficiency of photochemical reactions of photosynthetic system 2 (PS2) where: (Fv = Fmax-Fo - variable fluorescence);

2. (Fpl-Fo) / Fv - if the current light intensity sufficient to reach a state of maximum i QA system at the time of reaching the level of Fmax.

3. t1 / 2 - time which corresponds to half the achievement variable fluorescence;

4. (Fmax-Fst) / Fst - value quenching of fluorescence, which affect both photochemical.

The shape of the curve IFH sensitive to changes of the photosynthetic apparatus as a result of unfavorable factors or physiologically active substances such as herbicides. Measurements do not require time-consuming and reagents can be executed without damaging the native structure of the object. Due to these advantages of fluorescence induction method has been widely used in studies of the photosynthetic apparatus [2, 7].

To determine the indexes adapt to the stress applied by fluorescence spectroscopy based portable fluorometers Department of Physics NULES of Ukraine. Before the measurement sample was kept in the clip 4 minutes in the dark, and then registering IFH once both wavelengths (690 and 740nm) over the next 4 minutes. Fluorescent indexes were recorded at liquid-crystal screen display [3]. As fluorestsent parameters used: viability index (Rfd), which was measured at two wavelengths: Rfd (690) and Rfd (740) and the index of adaptation to stress (Ar). Sustainability indices I investigations by the formula:

where fmax - maximum and fst - stationary fluorescence, fd = fmax - fst reflects a decrease in fluorescence. Adaptation to stress index calculated by the following equation:

$Ap = 1 - [Rfd(740) + 1] \div [Rfd\ (690) + 1]$ [4, 12, 13].

Determining the rate of photosynthesis by classic performed as follows: photosynthetic leaf placed on a flat transparent chamber; made barite (in a volume of 100 ml at a concentration of 9 g /l) in a flask connected with the absorber tube; first rubber tube were attached to a cylinder fixed to a tripod, the other end is lowered into the flask and then fixed time (t) and missed the water with a 10-liter capacity; were taken in a flask 10 ml of barite, which did not pass through the air, adding 1-2 drops of phenolphthalein indicator to slightly pink color; titration of oxalic acid was carried out to determine the disappearance of color and its quantity (B), with This 1 ml of oxalic acid equal to 0.2 mg CO2); calculated photosynthetic leaf surface area (S). and finally, experiments several times, but without the leaf chamber and measured the amount of oxalic acid used for titration (C).

Calculated rate of photosynthesis by the formula [11]:

$$\text{Iф} = [(A - C) - (A - B)] \times 0{,}2 \times 100 \times 60 \div S \times t,$$

Where: IGF-rate of photosynthesis, $CO_2 \times dm^2$ mg / h; S - area of leaf cm^2; t - time experiment, h.

Results and discussion

Research of plant-based IFH.

Direct express evaluation methods of photosynthesis does not exist, but analysis of the competitive process of photosynthesis, which is fluorescence, makes possible rapid diagnosis of the photosynthetic apparatus of plants. Application of assessment IFH plant enables to receive objective information on the operation of express photosynthetic apparatus of the investigated plants during changes in the conditions of its life cycle.

When changing growing conditions Common Bean observed changes in the nature of transitions IFH, accompanied by a fairly significant changes of the spectral characteristics of leaf tissue of plants (Fig. 1).

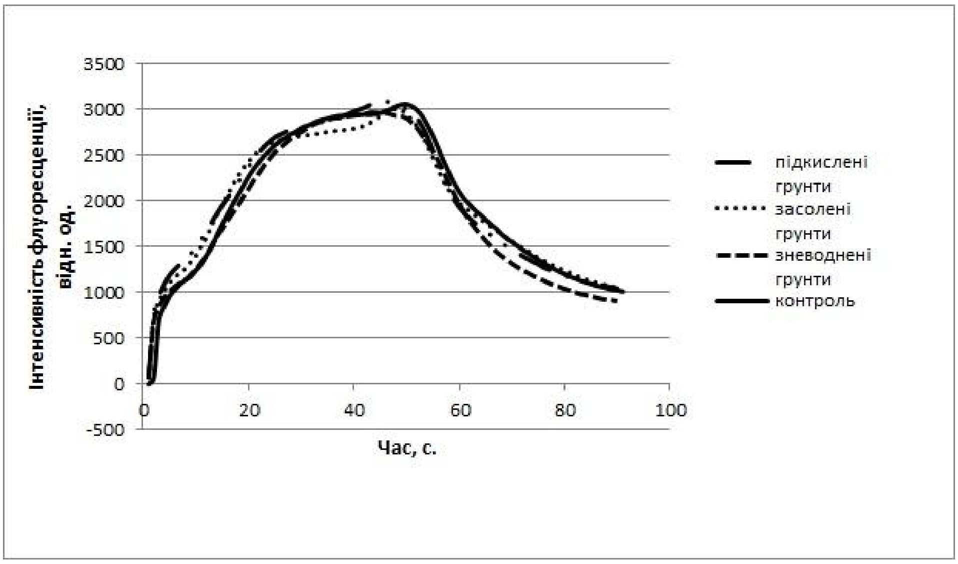

Fig.1. Lines IFH Common Bean in phase 2-leaf for the actions of abiotic stress factors

By measuring the IFH under the influence of stressors in phase two leaves, one could argue that the plant operates poore acidic environment, because the background level of fluorescence F0 = 772 acidic, therefore, this stress factor causes the greatest loss of excitation energy during migration in pigmented matrix and reduces the content of chlorophyll molecules. Background fluorescence level plants that grow in saline soils and dehydrated too high: F0 = 736 and alkaline dry F0 = 725 compared to control acid F0 = 700. The efficiency of photochemical reactions of PS2, Fv / Fmax plants that grew under the influence of stressors, lower as compared to the control (Table. 1).

The value of fluorescence quenching, which affect both photochemical and processes (Fmax-Fst) / Fst plants that grew on acidified and saline soils, lower than in controls and plants growing on soil dehydrated.

Tab. 1. Effect of acidified, salted and dehydrated soil parameters of chlorophyll

Soil	Parameters									
	F_o	F_{pl}	F_{max}	F_{st}	F_v	K_i	dF_{pl}	dF_{pl}/F_v	F_v/F_{max}	$(F_{max}-F_{st})/F_{st}$
control	700 ±62	980± 61	3048± 70	1008± 63	2348	0,7703	280	0,1193	0,7703	2,0238
acidified	772 ±55	1152± 63	3000± 68	1040± 61	2328	0,7509	380	0,1632	0,7509	1,9808
alkaline	736 ±53	1020± 64	2992± 64	1052± 62	2256	0,7540	284	0,1259	0,7540	1,8441
dehydrated	725 ±51	1024± 61	2992± 64	904± 61	2300	0,7687	332	0,1443	0,7687	2,3097

fluorescence induction Common Bean in phase two leaves.

Analyzing the curves shown in Fig. 2, we got the data included in the table. 2, are convinced that in the flowering stage beans by conventional indicators increased.

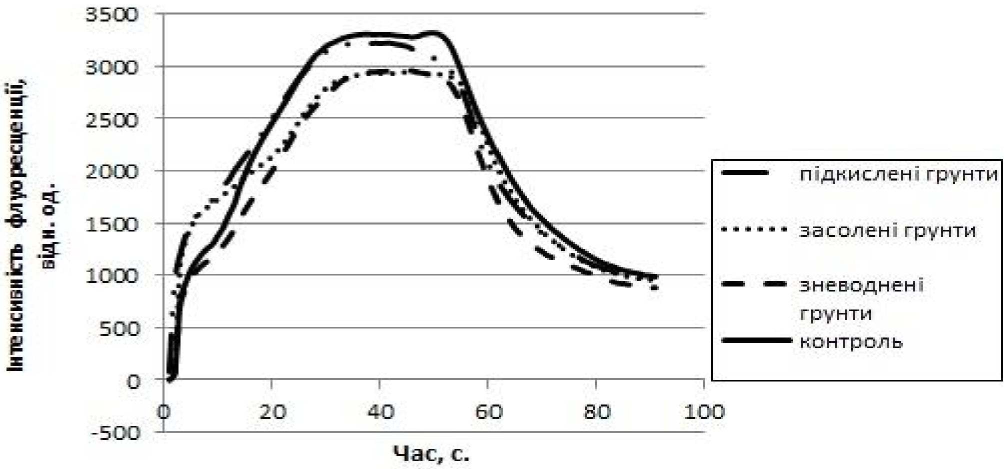

Fig. 2. Line IFH Common Bean in the flowering stage for the actions of abiotic stress factors

Poor plant operates on alkaline alkaline F0 = 1104. So, this stress factor leading to major loss of excitation energy during its migration in pigmented matrix and reduces the content of chlorophyll molecules.

Tab. 2. Effect of acidified saline and desiccated soil parameters of chlorophyll fluorescence induction Common Bean in the flowering stage.

Soil	Parameters									
	F_0	F_{pl}	F_{max}	F_{st}	F_v	K_i	dF_{pl}	dF_{pl}/F_v	F_v/F_{max}	$(F_{max}-F_{st})/F_{st}$
control	720 ±53	960 ±61	3316 ±69	988 ±56	2568	0,7744	212	0,0826	0,7744	2,3563
acidified	944 ±55	1468 ±57	3228 ±67	960 ±52	2284	0,7076	524	0,2294	0,7076	2,3625
alkaline	1104 ±56	1486 ±57	2960 ±65	960 ±52	1856	0,6270	382	0,2058	0,6270	2,0833
dehydrated	722 ±52	988 ±56	2974 ±65	882 ±57	2252	0,7572	266	0,1181	0,7372	2,37198

Background levels of plants growing on acidified and dehydrated soil too high: F0 = 944 acid and dehydrated F0 = 722 compared with control - F0 control = 720. The intensity of the photochemical reactions of PS2, Fv / Fmax plants under the influence of stressors lower as compared with the control.

By measuring the IFH under the influence of stressors in fruiting phase (Fig. 3, Tab. 3) background level of fluorescence in all plants has not changed, which can be attributed to the gradual adaptation of stress.

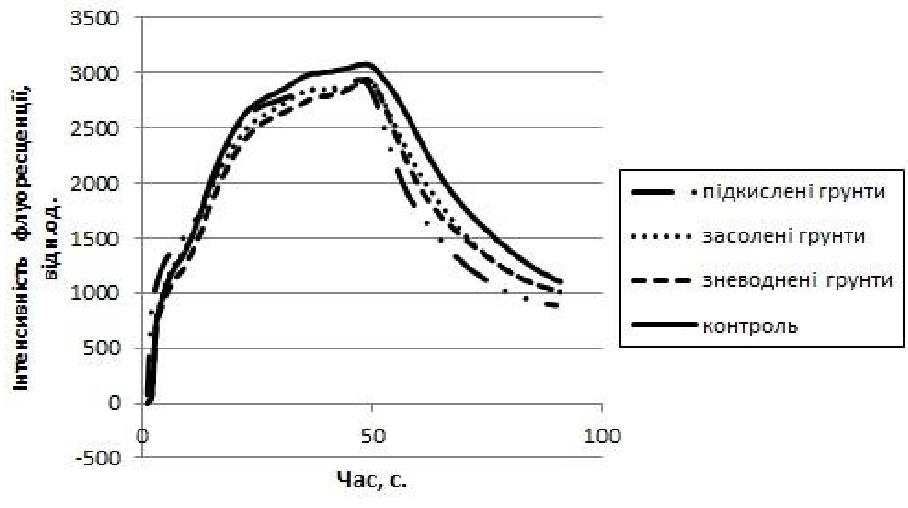

Fig. 3. Curves IFH Common Bean in fruiting phase under the influence of abiotic stress factors

The intensity of the photochemical reactions of PS2, Fv / Fmax plants under the influence of stress factors remained lower compared with the control.

Table 3. Effect of acidified saline and desiccated soil parameters of chlorophyll fluorescence induction Common Bean in the phase of fruiting.

Soil	Parameters									
	F_o	F_{pl}	F_{max}	F_{st}	F_v	K_i	dF_{pl}	dF_{pl}/F_v	F_v/F_{max}	$(F_{max}-F_{st})/F_{st}$
control	705 ±53	1148 ±65	3084 ±70	1104 ±64	2348	0,7613	412	0,1755	0,7613	1,7935
acidified	705 ±53	1324 ±63	2936 ±61	888 ±55	2076	0,7070	464	0,2235	0,7071	2,3063
alkaline	752 ±63	1184 ±66	2932 ±67	1008 ±64	2180	0,7435	432	0,1982	0,7435	1,9087
dehydrated	707 ±56	1108 ±64	2932 ±67	1012 ±63	2228	0,7599	404	0,1813	0,7599	1,8972

The maximum level of chlorophyll fluorescence highest in the control group, but these other groups like that again confirms the ability of plants to adapt to stressors.

The measurement results indexes and viability index adaptation depending fluorescent indexes Common Bean from the effects of stress factors in Table. 4.

Tab. 4. Dependence of sustainability indexes (Rfd (690) and Rfd (740)) from abiotic stress factors

Parameters	Control	Acidified	Alkaline	Dehydrated
Rfd(690)	1,077±0,09	0,466±0,04	0,992±0,05	1,550±0,11
Rfd (740)	1.618±0,20	0,508±0,03	1,133±0,11	2,136±0,23
Rfd (740)/ Rfd(690)	1,502	1,090	1,142	1,378

They indicate that fluorescent indices sensitive to abiotic stress factors.

Influenced pidkyslenosti, salinity and dehydration in the ratio of fluorescence indices bean plants are significantly lower than in control, as they are exposed to stress. Reduced viability indices involving serious violations of the photosynthetic apparatus. Effect of water stress on photosynthetic activity of plants and fluorescent indexes can be explained by excess water loss through transpiration compared with its supply through the root system and stem that leads to destruction of chloroplasts and the violation of their functions. Acidity is not less stress factor for plants. A significant change in pH in either direction is harmful and sometimes devastating impact on the plant. Less harmful to plants bias soil pH to the alkaline side. This is because the cells of the root plants emit CO2, and sometimes organic acids that neutralize excess alkalinity. The dramatic shift reaction in the soil acid side has undesirable effects of several factors: direct damaging effect on the surface layers of protoplasm;

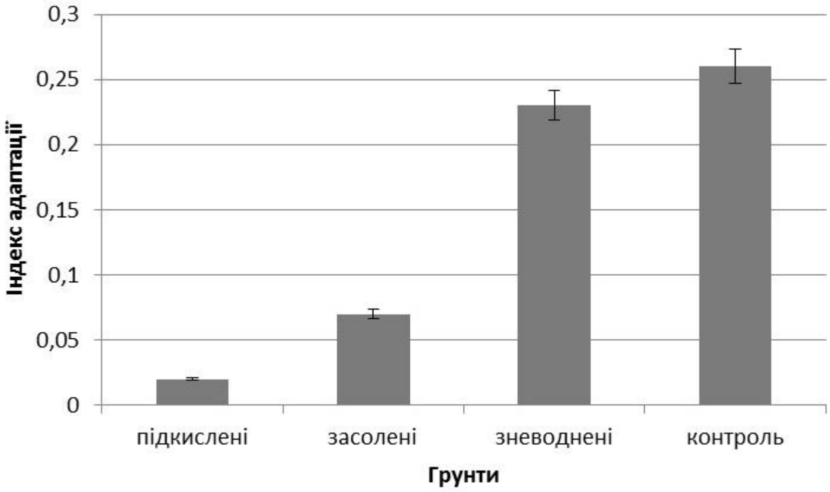

Figure 4. Dependence codes adaptation of various stress conditions.

The results presented in Fig. 4 indicate that the level of adaptation to stress in leaves of beans that grew in acidified saline, excessively drained soils were respectively 0.02, 0.07 and 0.23 relative units. At the same time, in control experiments, the figure was 0.26 value. The results indicate that fluorescent indices sensitive to stressors such as acidification, salinization, dehydration. These conditions lead to a reduction indexes Rfd (690) and Rfd (740), in addition to dehydration.

The results of experimental determination of the intensity of photosynthesis by classic by the number of assimilated carbon dioxide (CO2) photosynthetic leaf Common Bean per unit time are shown in Table. 5.

Tab. 5. Determination of the rate of photosynthesis beans hazometrychnym conventional method.

Soil	The intensity of photosynthesis, mg CO_2×dm^2/h (Iph)
Control	21,07±3,49
Acidified	9,13±0,98
Alkaline	16,25±1,61
dehydrated	14,32±1,32

Table. 5 confirm that the rate of photosynthesis under the influence of stressors begins to subside, and poore on photosynthetic activity of plants acidified soil acts as the intensity of photosynthesis decreased significantly to 56.7% compared with the control.

Conclusions

1. A study of the photosynthetic apparatus of plants beans for the actions of a number of environmental factors with three types of instrumentation devices. The most effective for simplicity/ The express and informative application of the results proved biosensor Floratest.

2. Based on the parameters of the IFH, found that in phase two leaves of beans compared with the phases of flowering and fruiting, nayzhubnishe the plant has an acidic environment, because the background level of fluorescence F01k = 772 is significantly different from the control, this causes the most stress factor excitation energy loss during the migration of pigment in the matrix and reduces the content of chlorophyll molecules. The intensity of the photochemical reactions of PS2, Fv / Fmax fluorescence quenching and the value of such plants significantly lower than in controls.

3. Revealed that soil salinity gradually inhibits photosynthetic apparatus of plants. The level of inhibition IFH observed in the flowering stage and has the highest rates F01l = 1104, higher than under the influence of acidified soils in phase two leaves. The maximum fluorescence in these conditions also significantly reduced. Thus, the rate Fmax plants in saline soils is 2960, and it is stored in the control at the level of 3316.

4. Stressors - dehydration, compared to acidification and salinity less detrimental effect on the plant. In this case, the highest level of background fluorescence was observed in phase two leaves (F01k = 725), the efficiency of photochemical reactions (PS2, Fv / Fmax) and the magnitude of fluorescence quenching in these plants close to the control.

5. It is established that the level of adaptation to stress in leaves of beans under the influence of acidification, salinization, drought, was 0.02, 0.07 and 0.23 relative units, essentially conceding control (0.26). The results indicate that the fluorescent indices sensitive to stressors such as acidification, salinization, dehydration. These conditions lead to a significant reduction index Rfd (690) and Rfd (740).

6. The results obtained during diagnostic classic method confirm rates determined using a portable biosensor fluorymetrs and Floratest - significantly decreased rate of photosynthesis under the influence of stressors compared to the control sample. Under the influence of salinity it was 16.25 × mhSO2 dm2 / h pidkyslenosti - 9,13 × mhSO2 dm2 / h dehydration - 14.32 mhSO2 × dm2 / h and in control - 21,07mhSO2 × dm2 / h.

References

1. Дудчак Т. В. Стан і перспективи виробництва в Україні зерна квасолі / Т. В. Дудчак // Збірник наукових праць. – Кам’янець-Подільський – 2007. – № 15. – С.92-96.

2. Інструментальне вивчення фотосинтетичного апарату за допомогою індукції флуоресценції хлорофілу [О. В. Брайон, Д. Ю. Корнєєв, О. О. Снєгур, О. І. Китаєв] Методичні вказівки для студентів біологічного факультету – К.: Видавничо-поліграфічний центр «Київський університет», 2000.– 15 с.

3. Клиша А. І. Взаємозв’язок ознак продуктивності та їхній вплив на урожайність квасолі / А. І. Клиша, І. В. Хорошун // Вісник Полтавської державної аграрної академії. – 2009. – № 2. – С. 41-44.

4. Клиша А. І. Мінливість господарсько-цінних ознак у квасолі і добір урожайних форм / А. І. Клиша, І. В. Хорошун // Бюлетень Інституту зернового господарства. – 2009. – № 36. – С. 159-162.

5. Коломієць О. Д. Неспецифічні реакції рослинних клітин на стресові фактори / О.Д. Коломієць // Фізіологія рослин в Україні на межі тисячоліть. – 2001. – Т2. – с.41-47.

6. Колупаєв Ю. Є. Стресові реакції рослин (молекулярно-клітинний рівень) / Є. С. Колупаєв. – Харків: Хар. держ. агр. ун-т. , 2001. – 173 с.

7. Корнеев Д. Ю. Информационные возможности метода индукции флуорысценции хлорофила / Д. Ю. Корнеев. – К.: «Альтерпрес», 2002. – 188с.

8. Корсунский В. М. Инструкция к прибору "Флоратест ФТ-1" / В. М. Корсунский – К.: Научно-производственная фирма "Рост", 1997. – 11 с.

9. Методичні вказівки до виконання лабораторних робіт з дисципліни „Ґрунтознавство" для студентів спеціальності „Екологія та охорона навколишнього середовища" / За ред. Стороженко Д. О. – Полтава: Полтавський нац. технічний ун-т, 2005. – С. 15-24.

10. Посудін Ю. І. Флуоресцентний аналіз гороху посівного *Pisum sativum* впродовж розвитку та під впливом зовнішніх факторів / Ю. І. Посудін, О. В. Богдашева // Наукові доповіді НУБіП України. – 2010. – №5(21).

11. Физиология и биохимия сельскохозяйственных растений [Н. Н. Третьяков, Е. И. Кошкин, Н. М. Макрушин и др.] ; Под ред. Н.Н. Третьякова. – М.: Колос, 2000. – 640 с.

12. Posudin Y. I. Application of portable fluorometer for estimation of plant tolerance to abiotic factors / Y. I. Posudin, O. O. Godlevska, I. A. Zaloilo, Ya.V. Kozhem'yako // Agrophysics – 2010. – V. 24, No 4. – P.363-368.

13. Romanov V. O. Biosensors for express-diagnostics of photosynthesis and acute viral infections / V. O. Romanov, M. F. Starodub, I. B. Galelyuka // Proceedings: Measurements Control Computers in Economy and Environment Protection – 2010. – 3. – P. 3-7.

14. Smart portable fluorometer for express-diagnostics of photosynthesis: principles of operation and results of experimental researches [V. Romanov, V. Sherer, I. Galelyuka, at all] Information research and applications (I.TECH 2007): Firth International Conference, 26-30 June 2007, Varna, Bolgaria : Proceedings. – VI., 2007. – Vol. 2. – P. 399-403.

ІНСТРУМЕНТАЛЬНА ЕКСПРЕСНА ОЦІНКА СТАНУ СТІЙКОСТІ КВАСОЛІ ЗВИЧАЙНОЇ ПРОТИ АБІОТИЧНИХ СТРЕСОВИХ ЧИННИКІВ

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Наведено результати вивчення впливу абіотичних стресових чинників на стан фотосинтетичного апарату квасолі звичайної методами біосенсорики, що забезпечують експрес-діагностику. Визначено показники ІФХ, інтенсивність фотосинтезу, індекси адаптації рослин до абіотичних стресових чинників та індекси життєздатності.

Ключові слова: *біосенсор, ІФХ, індекси адаптації, індекси життєздатності, квасоля звичайна, абіотичні стресові чинники.*

ИНСТРУМЕНТАЛЬНАЯ ЭКСПРЕССНАЯ ОЦЕНКА СОСТОЯНИЯ УСТОЙЧИВОСТИ ФАСОЛИ ОБЫКНОВЕННОЙ К АБИОТИЧЕСКИМ СТРЕССОВЫМ ФАКТОРАМ

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В статье представлены результаты изучения влияния абиотических стрессовых факторов на состояние фотосинтетического аппарата фасоли обыкновенной методами биосенсорики, обеспечивающие экспресс-диагностику. Определены показатели ИФХ и интенсивности фотосинтеза, а также индексы адаптации и жизнеспособности растений под влиянием абиотических стрессовых факторов.

Ключевые слова: *биосенсор, ИФХ, индексы адаптации, индексы жизнеспособности, фасоль обыкновенная, абиотические стрессовые факторы.*

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