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Application of portable fluorometer for estimation of plant tolerance to abiotic factors

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A b s t r a c t. A portable, two-wavelength fluorometer based on recording chlorophyll fluorescence induction of agronomic plants is proposed. The effects of various fertilizers on fluorescence of soybean and rapeseed were studied. It was shown that the most effective fertilizer combinations were $N_{15}P_{15}K_{15}$ for soybean and $N_{75}P_{60}K_{75}$ for rapeseed. The effects of high-intensity solar radiation on chlorophyll fluorescence of bush bean can be explained by the process *qE*-quenching which depends on the presence of a proton gradient across the thylakoid membrane, and *qI*-quenching which occurs with excessive radiation; this type of quenching provokes photoinhibition. It is possible to suggest an effect of protein structural change in chlorophyll fluorescence quenching. Ultraviolet (especially UV-B) radiation predominantly damages DNA. The main molecular alteration in UV-B-irradiated DNA is the formation of dimer photoproducts – pyrimidine dimers of cyclobutane structure which are responsible for disrupting the genetic code and damaging the photosynthetic apparatus of bush bean. In detached leaves the water deficit develops faster and therefore it is accompanied with a decline of the fluorescence indices. The proposed portable fluorometer is characterised by compactness, an independent power supply, high sensitivity, and gives a non-destructive estimate of *in vivo* fluorescence parameters of agronomic plants.

K e y w o r d s: induction of fluorescence, chlorophyll, agronomic plants, stress

INTRODUCTION

Photosynthesis is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. The process of de-excitation of the absorbed light energy during photosynthesis is related to heat emission and chlorophyll fluorescence. Fluorescence is the radiation process referring to the transition between electronic states of the same multiplicity. It begins at the ground vibrational state of the first electronic singlet state S_1 and continues through various vibrational levels until it reaches the ground singlet state S_0. When the ground singlet state is achieved, a photon is emitted. Light absorbed by accessory pigments (chlorophyll *b* and carotenoids) is transferred to chlorophyll *a*. This is why the primary processes of photosynthesis are reflected by chlorophyll *a* fluorescence (Hall and Rao, 1999).

It is established that about 5% of the excited light is returned by chlorophyll as fluorescence emission (Lichtenthaler, 1988a; Lichtenthaler and Rinderle, 1988). This emission is related to the total process of photosynthesis (Campbell *et al*., 2007; Daughtry *et al*., 1995; Lichtenthaler, 1988b, 1996, 1997). The most important conclusion is that chlorophyll fluorescence can be used as a tool for stress detection in agronomic plants in the laboratory and field conditions (Luedeker *et al*., 1997; McMurtrey *et al*., 2000; Stober and Lichtenthaler, 1993; Zarco-Tejada *et al*., 2002).

Particularly, the effect of such external factors and vegetation stress as nitrogen supply (Corp *et al*., 2000), high-intensity irradiance (Cajanek *et al*., 2002), ultraviolet radiation (Bilger *et al*., 2007), and water deficit (Posudin *et al*., 2007; Xu *et al*., 2008) on chlorophyll fluorescence were studied. All abovementioned investigations were performed with stationary convenctional equipment or with expensive techniques, which requires highly qualified personal. Development of simple and inexpensive devices for fluorescence analysis of agronomic plants is a preferable alternative.

The main objective of this research is the demonstration of a portable, two-wavelength fluorometer used to record chlorophyll fluorescence induction as a way to quantify the agronomic state of plants under field and laboratory conditions. The temporal behaviour of fluorescence intensity (induction of fluorescence, Kautsky effect) reflects the sum total of processes which are linked with photosynthesis activity of a plant (Kautsky and Hirsch, 1931).

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MATERIALS AND METHODS

Soybean (*Glycine soja*) (cv. *Elena*), rapeseed (*Brassica napus*) (cv. *Maria*), lettuce (*Lactuca sativa*) (cvs. *Lolla Bionda, Lolla Rossa* and *May Queen*) and bush bean (*Phaseolus compressus*) (cv. *Prisadybna*) from the collection of the National University of Life and Environmental Sciences of Ukraine were used in the experiments. The effects of fertilizers in field conditions were studied with soybean and rapeseed. Fertilizers such as $N_{15}P_{15}K_{15}$, $N_{30}P_{30}K_{30}$, $N_{45}P_{45}K_{45}$ for soybean and $N_{45}P_{30}K_{45}$, $N_{60}P_{45}K_{60}$, $N_{75}P_{60}K_{75}$, $N_{90}P_{75}K_{90}$, $N_{120}P_{75}K_{120}$, and $N_{90}P_{75}K_{120} + N_{30}$ for rapeseed were added (kg/hectare) to the soil (here numerical indices correspond to the mass fraction of each component of fertilizer).

Chlorophyll fluorescence was monitored under field and laboratory conditions for approximately two to three weeks following the start of seed filling. Under field conditions, the effect of fertilizers and high-intensity solar radiation were monitored. In the laboratory, the impacts of water deficit and artificial ultraviolet radiation were monitored.

We compared the fluorescence kinetics in green leaves with normal water supply and with increasing water stress and dehydration of detached leaves from plants 10 h after leaf abscission. The water deficit can be measured by determining the water potential. It is shown that at normal water supply conditions the values of water potential varies from –2 to –5 bar in the evening and night to –15 to –20 bar during a sunny day (Lichtenthaler and Rinderle, 1988). In detached leaves the water deficit develops faster and therefore it is accompanied with a decline of the fluorescence indices.

The chlorophyll fluorescence induction kinetics of agronomic plants in minute range was measured by a portable two-wavelength fluorometer which was developed at the Department of Biophysics of the National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine (Posudin *et al*., 2007, 2008) (Fig. 1).

The two-wavelength fluorometer is operated such that the radiation of the light diode is directed through the collimator and prism which divide optical radiation into two parts. Both parts of optical radiation excite the chlorophyll fluorescence at the same point of the sample green leaf. Then the chlorophyll fluorescence passes through two interference filters of 690 and 740 nm and is detected by photodetectors. Each photodetector generates an electric signal which is proportional to the intensity of fluorescence. These signals are analyzed by the readout system which is equipped with a display where fluorescence indices are indicated on the screen. Each 4 min of recording chlorophyll fluorescence are accompanied with an acoustic signal.

The chlorophyll fluorescence kinetics of leaves under investigation was measured as follows: the leaf of the plant was fixed into the clip of the device where the leaf was left to adapt to the darkness during 4 min; then it was illuminated by the radiation of the light diode during the next 4 min. At the end of this process an acoustic signal was transmitted and a button was pressed to read the values of fluorescence indices on the fluorometer display.

The vitality indices *Rfd*(690) and *Rfd*(740) and the stress adaptation index $A_p = 1 - [Rfd(740)+1]/[Rfd(690)+1]$ were determined with the portable fluorometer. Here $Rfd = f_d/f_s$, where $f_d = f_m - f_s$ is the fluorescence decrease; f_m – maximal fluorescence; f_s – steady-state fluorescence. The *Rfd* values were measu- red in the 690-nm [*Rfd*(690)] and in the 740-nm [*Rfd*(740)] regions.

High-intensity solar radiation is utilized by plants for photosynthesis in the region of the electromagnetic spectrum from 400 to 700 nm. This radiation, referred to as the photosynthetically active radiation (PAR), was measured as photosynthetic photon flux density (PPFD), in units of micromoles of quanta per second per square meter (μmol s^{-1} m^{-2}).

Ultraviolet (UV) radiation (irradiance 2 W m^{-2}) was generated by a UV-source OI-18 (OMO, S.-Peterbourg, Russia); it was transmitted through a wideband ultraviolet filter UFS (240-410 nm). Solar ultraviolet radiation is characterised as having a substantial impact on human health, terrestrial plants, aquatic ecosystems, and air quality. Ultraviolet range can be divided into three parts: UV-A (400-315 nm), UV-B (315-280 nm), and UV-C (< 280 nm). UV-A is safe radiation. UV-B may provoke specific but not always dangerous effects in living organisms. Solar UV-B radiation provides certain effects on physiological and developmental processes of plants, including changes in plant morphology, phenology, biomass accumulation, inhibition of photosynthesis, and DNA damage. UV-C radiation is the most hazardous for living organisms, but UV-C radiation is entirely screened out by ozone at around 35 km altitude.

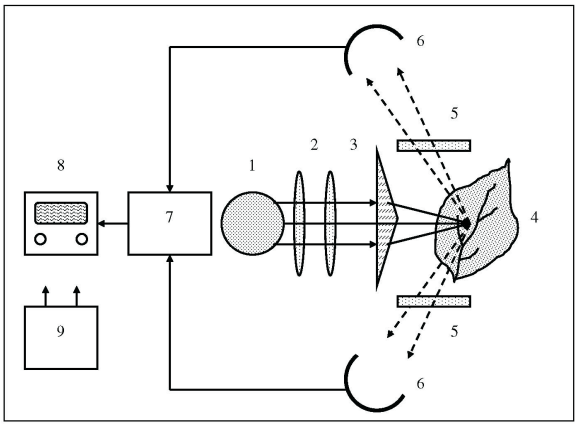

Fig. 1. Schematic diagram of the two-wavelength portable fluorometer: 1 – light diode as a source of fluorescence excitation, 2 – collimator, 3 – prism as a beam splitter, 4 – sample a green leaf, 5 – interference filters with transmittance maxima of 690 and 740 nm, 6 – photodetectors, 7 – amplifier, 8 – readout system, 9 – power supply (a rechargeable battery).

This is why the application of wideband filter UFS can simulate modelling the irradiation of terrestrial surface with UV-A and UV-B radiation in laboratory conditions. Ultraviolet irradiance was estimated by radiometer IMO-2 (Sigma, Kharkiv, Ukraine).

All measurements were repeated five times to calculate the mean values and errors.

RESULTS AND DISCUSSION

It was possible to use the portable fluorometer in the field to estimate the effect of fertilizers and high-intensity solar radiation on the chlorophyll fluorescence of dark adapted leaves of soybean, rapeseed, lettuce and bush bean, comparable to artificial UV radiation and water deficit in the laboratory.

The dependence of fluorescence indices *Rfd*(690), *Rfd*(740) and A_p on fertilization is shown in Fig. 2 for soybean (cv. *Elena*) and in Fig. 3 for rapeseed (cv. *Maria*).

Application of $N_{15}P_{15}K_{15}$, $N_{30}P_{30}K_{30}$, $N_{45}P_{45}K_{45}$ affected soybean photosynthetic activity and correspondingly the fluorescence indices. The same was observed with the rapeseed – where there was an effect of the fertilizers $N_{45}P_{30}K_{45}$; $N_{60}P_{45}K_{60}$; $N_{75}P_{60}K_{75}$; $N_{90}P_{75}K_{90}$; $N_{120}P_{75}K_{120}$; $N_{90}P_{75}K_{120} + N_{30}$ on fluorescence indices.

Application of fertilizers of different composition affected plant viability and photosynthetic activity. As shown in Figs 2,3 the most effective fertilizer combinations were $N_{15}P_{15}K_{15}$ for soybean and $N_{75}P_{60}K_{75}$ for rapeseed. Probably the effect of fertilizers on chlorophyll fluorescence of green leaves can be explained by different total chlorophyll content and values of the Chl *a/b* ratio in plants that were exposed to different combinations of fertilizers (Shangguan *et al*., 2000).

The dependence of fluorescence indices of three types of lettuce (cvs. *Lolla Bionda, Lolla Rossa* and *May Queen*) on water deficit is shown in Figs 4,5. The results of this investigation demonstrated increasing fluorescence kinetics at the first stages (3-4 h); the same behaviour of fluorescence kinetics was registered also by Lichtenthaler and Rinderle (1988). A decrease of the fluorescence induction kinetics was observed (Figs 4, 5): in cv. *Lolla Rossa* – from 1.15 to 0.55 for *Rfd*(690) and from 0.85 to 0.1 for *Rfd*(740) and; in cv. *Lolla Byonda* – from 1.9 to 1.0 for *Rfd*(690) and from 1.55 to 0.15 for *Rfd*(740); and in cv. *May Queen* – from 1.8 to 0.8 for *Rfd*(690) and from 1.45 to 0.05 for *Rfd*(740). Values of the *Rfd*(690) index exceed the values of the *Rfd*(740) index. This can be explained by re-absorption of the shorter wavelength fluorescence by the leaf chlorophyll (Lichtenthaler and Rinderle, 1988).

Such behaviour of chlorophyll-fluorescence induction kinetics can be explained by the dehydration of the cytoplasm and the chloroplast stroma. Water deficit induces the desiccation of the cytoplasm and greater density of the chloroplasts in a cell. This is why re-absorption of the emitted fluorescence is higher in such leaves than in controls. The reflection properties of the leaves are changed also by water deficit which influences the chlorophyll fluorescence. The decreasing fluorescence indices can be explained by the decline of photosynthetic quantum conversion with increasing dehydration of leaves (Lichtenthaler and Rinderle, 1988).

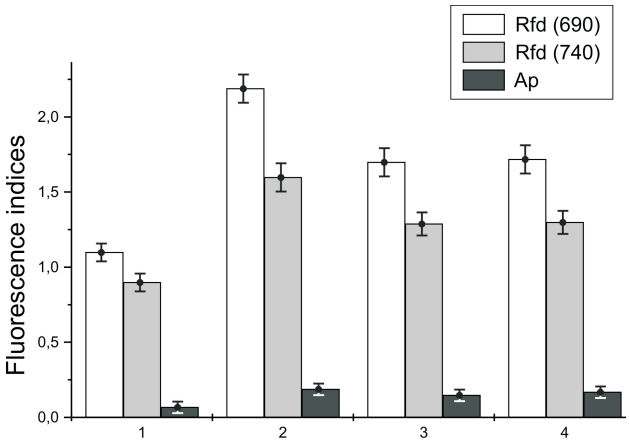

Fig. 2. Dependence of fluorescence indices *Rfd*(690), *Rfd*(740) and A_p on type of fertilizer applied to soybean (cv. *Elena*); 1 – control (no fertilizer), 2 – $N_{15}P_{15}K_{15}$, 3 – $N_{30}P_{30}K_{30}$, 4 – $N_{45}P_{45}K_{45}$.

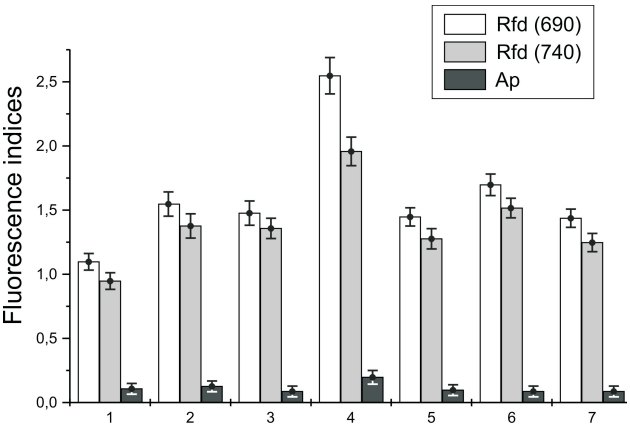

Fig. 3. Dependence of fluorescence indices *Rfd*(690), *Rfd*(740) and A_p on type of fertilizer applied to rapeseed (cv. *Maria*); 1 – control (no fertilizer), 2 – $N_{15}P_{15}K_{15}$, 3 – $N_{30}P_{30}K_{30}$, 4 – $N_{45}P_{45}K_{45}$, 5 – $N_{90}P_{75}K_{90}$, 6 – $N_{120}P_{75}K_{120}$, 7 – $N_{90}P_{75}K_{120} + N_{30}$.

Leaf dehydration substantially influences net photosynthetic rate and stomatal conductance (Bukhov and Carpentier, 2004), the electron transport rate (Xu *et al*., 2008), and chlorophyll *a, b*, and total chlorophyll content in leaves (Nyachiro *et al*., 2001), processes that are related to the chlorophyll fluorescence kinetics of green leaves.

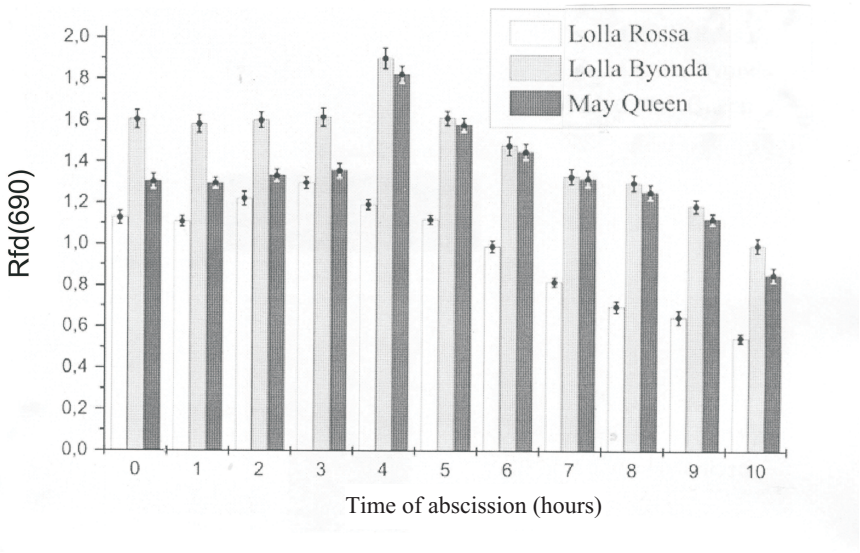

Fig. 4. Dependence of fluorescence indices *Rfd*(690) of three types of lettuce on water deficit.

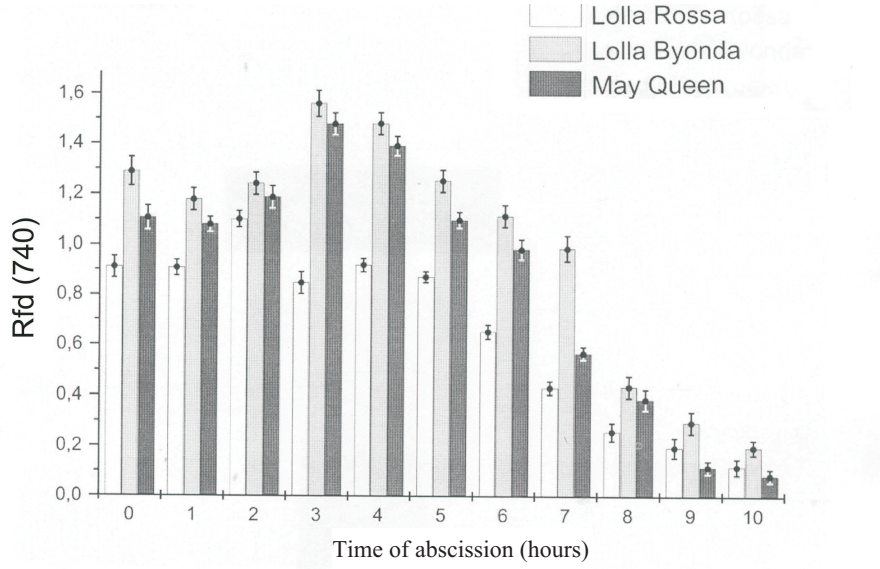

Fig. 5. Dependence of fluorescence index *Rfd*(740) of three types of lettuce on water deficit.

Abscisic acid (ABA), a stress hormone, plays an important role in plant response to water stress at both the whole-plant and the cellular level (Seo and Koshiba, 2002). The basis of ABA as a stress hormone is its rapid and massive accumulation under water deficit conditions. ABA induces partial stomatal closure which is the main reason for decreased photosynthesis in response to water deficit. Stomatal limitation is generally accepted as the main cause of reduced photosynthesis under water deficit.

The results of the effect of high-intensity solar radiation are shown in Figs 6, 7. Exposure of photosynthetic organs to high irradiance reduced photosynthetic capacity. This is called photoinhibition. Photoinactivation of the PSII reaction centre can occur by two independent mechanisms, associated with the acceptor and donor sides of PSII respectively, that both result in inhibition of electron transfer through PSII and subsequent degradation of the D1 protein (Baker, 1996). The transfer of excitation energy along the electron transport chain is associated with the process of quenching of chlorophyll fluorescence which occurs due to acceptor oxidation. The main mechanisms of quenching are energy-dependent *qE*-quenching which depends on the presence of a proton gradient across the thylakoid membrane and *qI*-quenching which occurs with excess radiation; this type of quenching provokes photoinhibition. It is possible to suggest an effect of protein structural change in chlorophyll fluorescence quenching. Protein aggregation prevents high levels of non-radiative energy dissipation and leads to quenching.

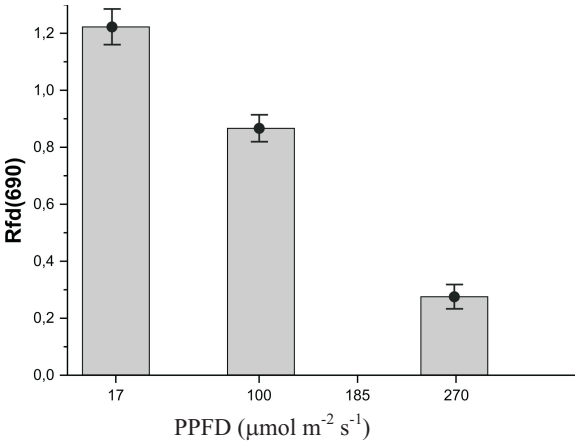

Fig. 6. Dependence of *Rfd*(690) on photosynthetic photon flux density level.

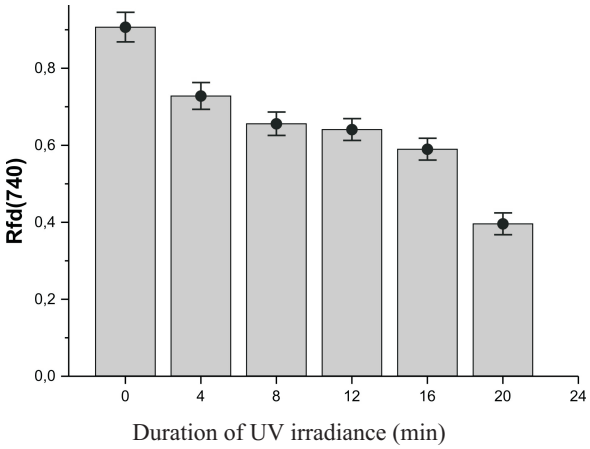

Fig. 8. Dependence of *Rfd*(690) on duration of UV irradiation at 2 W m^{-2}.

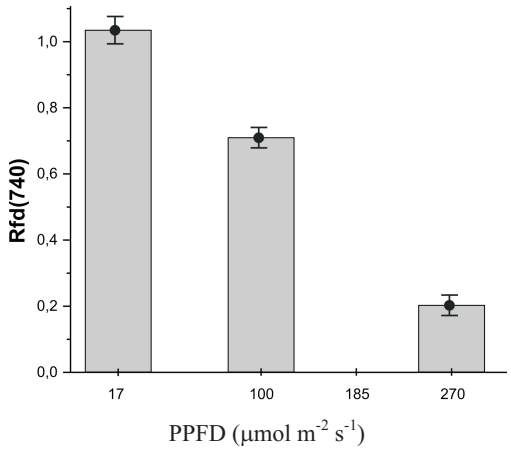

Fig. 7. Dependence of *Rfd*(740) on photosynthetic photon flux density level.

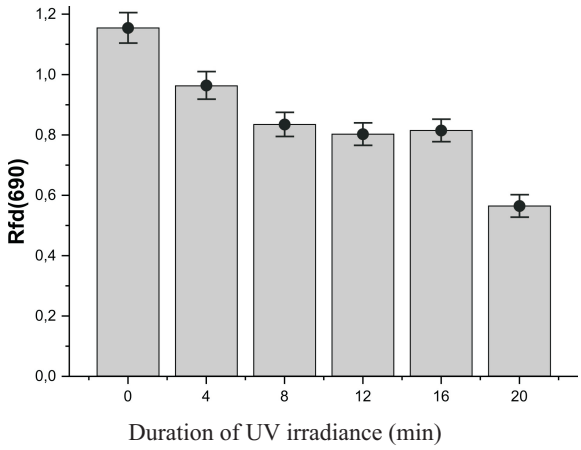

Fig. 9. Dependence of *Rfd*(740) on duration of UV irradiation at 2 W m^{-2}.

Usually, most plants grow faster when available light increases, but further increase of light intensity leads to light saturation where plants receive more light than they can utilize. The decrease in the fluorescence indices of bush bean (up to 0.2) was reached under PPFD 270 μmol s^{-1} m^{-2}.

The effect of artificial ultraviolet (UV) radiation on fluorescence indices is shown in Figs 8, 9. Such UV-irradiation of bush bean for 20 minutes induces a decrease of the fluorescence indices up to 43-48 % of control values. Enhanced UV (especially UV-B) radiation can have many direct and indirect effects on plants, including inhibition of photosynthesis, DNA damage, changes in morphology, phenology, and biomass accumulation (Caldwell *et al*., 1995). UV-B radiation predominantly damages DNA which absorbs in this part of spectrum. The main molecular alteration in UV-B-irradiated DNA is the formation of dimer photoproducts - pyrimidine dimers of cyclobutane structure which are responsible for disrupting the genetic code and damaging the photosynthetic apparatus of algae. PS II inhibition could be a main factor for UV inhibition of photosynthesis and, therefore, of chlorophyll fluorescence.

CONCLUSIONS

1. The recording chlorophyll fluorescence induction is a possible method of agronomic plants analysis during development and while under stress conditions.

2. The results of field and laboratory application of a portable two-wavelength fluorometer showed that the instrument has a number of advantages compared with stationary devices *ie* lower expense, independent power supply, compactness, high sensitivity and robust structure.

3. It can be used for measuring fluorescence parameters *in vivo* in agronomic plants in the field and in the laboratory.

REFERENCES

Baker N.R., 1996. Photoinhibition of photosynthesis. In: Light as an Energy Source and Information Carrier in Plant Physiology. Proc. NATO Adv. Study Institute, September 26 – Ocotober 6, 1994.

Bilger W., Rolland M., and Nybakken L., 2007. UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem. Photobiol. Sci., 6(2), 190-195.

Bukhov N.G. and Carpentier R., 2004. Effects of water stress on the photosynthetic efficiency of plants. Advances in Photosynthesis and Respiration, 19, 623-635.

Cajanek M., Navratil M., Kurasova I., Kalina J., and Spunda V., 2002. The development of antenna complexes of barley (*Hordeum vulgare* cv. Akcent) under different light conditions as judged from the analysis of 77 K chlorophyll a fluorescence spectra. Photosynthesis Res., 74(2), 121-133.

Caldwell M.M., Teramura A.H., Tevini M., Bornman J.F., Bjorn L.O., and Kulandaivelu G., 1995. Effects of increased solar ultraviolet radiation on terrestrial plants. Ambio, 24(3), 166-173.

Campbell P.K.E., Middleton E.M., McMurtrey J.E., Corp L.A., and Chappelle E.W., 2007. Assessment of vegetation stress using reflectance or fluorescence measurements. J. Environ. Quality, 36(3), 832-845.

Corp L.A., Chappelle E.W., McMurtrey J.E., Mulchi C.L., Daughtry C.S.T., and Kim M.S., 2000. Advances in fluorescence sensing systems for the remote assessment of nitrogen supply in field corn. In: Advances in Laser Remote Sensing for Terrestrial and Hydrographic Applications, SPIE Press, Orlando, FL, USA.

Daughtry C.S.T., McMurtrey, III, J.E., Chappelle E.W., Dulaney W.P., Irons J.R., and Satterwhite M.B., 1995. Potential for discriminating crop residues from soil by reflectance and fluorescence. Agron. J., 87, 165-171.

Hall D.O. and Rao K.K., 1999. Photosynthesis. Cambridge University Press, Cambridge, UK.

Kautsky H. and Hirsch A., 1931. Neue Versuche zur Kohlenstoffassimilation. Naturwissenschaften, 19, 964-971.

Lichtenthaler H.K. (Ed.), 1988a. *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. In: Application of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing. Kluwer Press, Dordrecht, the Netherlands.

Lichtenthaler H.K., 1988b. The stress concept in plants: an introduction. Annals of the New York Academy of Sciences, 851, 187-98.

Lichtenthaler H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant Physiol., 148(1/2), 4-14.

Lichtenthaler H.K. (Ed.), 1996. Vegetation stress. An introduction to the present state of the art of plant stress. Fischer Press, Stuttgart, Germany.

Lichtenthaler H.K. and Rinderle U., 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytic Chem., 19, 29-85.

Luedeker W., Guenther K.P., and Dahn H-G., 1997. Laser induced leaf fluorescence. A tool for vegetation status and stress monitoring and optical aided agriculture. Proc. SPIE Conf., April 21, Orlando, FL, USA.

McMurtrey J.E., Corp L.A., Kim M.S., Chappelle E.M., and Daughtry C.S.T., 2000. Fluorescence techniques in agricultural applications. In: Optics in Agriculture 1999-2000 (Eds J.A. DeShazer, G.E. Meyer). SPIE Critical Review, CR80, 37- 64.

Nyachiro J.M., Briggs K.G., Hoddinott J., and Johnson-Flanagan A.M., 2001. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. Cereal Res. Comm., 29(1-2), 135-142.

Posudin Yu., Gural T.I., Milutenko V.M., and Sobolev O.V., 2007. Device for registration of fluorescence induction (in Ukrainian). Patent of Ukraine, N 20668.

Posudin Yu., Melnychuk M., Kozhem'yako Ya., Zaloilo I., and Godlevska O., 2008. Portable fluorometer for fluorescence analysis of agronomic plants under stress conditions. Proc. Pittsburgh Conf. Analytical Chemistry and Applied Spectroscopy, March 2-7, New Orlean, LU, USA.

Seo M. and Koshiba T., 2002. Complex regulation of ABA biosynthesis in plants. Trends in Plant Sci., 7, 41-48.

Shangguan Z., Shao M., and Dyckmans J., 2000. Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. J. Plant Physiol., 156(1), 46-51.

Stober F. and Lichtenthaler H.K., 1993. Characterization of the laser-induced blue, green and red fluorescence signatures of leaves of wheat and soybean grown under different irradiance. Physiologia Plantarum, 88(4), 696-704.

Xu Z.Z., Zhou G.S., Wang Y.L., Han G.X., and Li Y.J., 2008. Changes in chlorophyll fluorescence in maize plants with imposed rapid dehydration at different leaf ages. J. Plant Growth Regulation, 27(1), 83-92.

Zarco-Tejada P.J., Miller J.R., Mohammed G.H., Noland T.L., and Sampson P.H., 2002. Vegetation stress detection through chlorophyll $a+b$ estimation and fluorescence effects on hyperspectral imagery. J. Environ. Quality, 31(5), 1433-41.