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FLUORESCENCE ANALYSIS OF AGRONOMIC PLANTS DURING DEVELOPMENT AND UNDER STRESS CONDITIONS

Introduction

The process of photosynthesis converts the sunlight kinetic energy into the potential energy of chemical bonds. The process of de-excitation of the absorbed light energy during photosynthesis is related to heat emission and chlorophyll fluorescence. Fluorescence is the radiative process which is based on the transition between electronic states of the same multiplicity; usually it occurs from the ground vibrational state of the first electronic singlet state S_1 to various vibrational levels in ground singlet state S_0, and is accompanied by the emission of photon. The light absorbed by the accessory pigments (chlorophyll *b* and carotenoids) is transferred to chlorophyll *a*. That's why the primary processes of photosynthesis are reflected by chlorophyll *a* fluorescence [1].

It is established that about 5% of the excited light is returned by chlorophyll as fluorescence emission [2,3]. This emission is related to the total process of photosynthesis in a complex manner. But the most important conclusion is that fluorescence of chlorophyll can be used as a tool for stress detection in agronomic plants in the field conditions.

The temporal behavior of the fluorescence intensity has a complex character. The fluorescence kinetics [4] (induction of fluorescence, Kautsky effect) reflects the sum total of processes which are linked with photosynthesis activity of a plant object.

We have reported earlier [5] about stationary two-wavelength fluorometer that made it possible to estimate induction of fluorescence of dark-adapted green leaves in laboratory conditions.

The main objective of this research was elaboration of portable two-wavelength fluorometer which was based on recording chlorophyll fluorescence induction and application of this fluorometer to estimate the state of agronomic plants during development and under stress conditions.

Materials and Methods

Soya bean (sort *Elena*), rapeseed (sort *Maria*), salads (sorts *Lolla Bionda, Lolla Rossa* and *May Queen*) and bush bean (sort *Prisadybna*) from the collection of National University of Life and Environmental Sciences of Ukraine were used in these experiments. Various variants of fertilizers such as $N_{15}P_{15}K_{15}$, $N_{30}P_{30}K_{30}$, $N_{45}P_{45}K_{45}$ for soya 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 inserted into the soil.

The chlorophyll fluorescence induction kinetics of agronomic plants in minute range was measured by portable two-wavelength fluorometer which was elaborated in the Department of Biophysics of National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine

[6,7]. Fluorometer consists of: light diode that was used as a source of fluorescence excitation; collimator and prism, beam splitter, sample (green leaf), interference filters with transmittance maxima at 690 nm and 740 nm, photodetectors, amplifier and readout system. Two last units were connected with power supply (accumulator). The device is equipped with display where fluorescence indices are indicated, and acoustic signalisation that controls the 4-minutes period of recording chlorophyll fluorescence.

The chlorophyll fluorescence kinetics of dark adapted leaves was analysed during 4 minutes. The control of fluorescence emission at two wavelengths at 690 and 740 nm was realised during these experiments. The measurements of vitality index $Rfd = f_d / f_s$ (Rfd' at 690 nm and Rfd'' at 740 nm), and stress adaptation index $A_p = 1 - [Rfd(740)+1]/[Rfd(690)+1]$ were measured with portable fluorometer.

The illuminance of the samples under solar irradiation was measured by luxmeter Yu-116; ultraviolet radiation (intensity 2 W/m^2) was generated by the UV-source OI-18 and estimated by radiometer IMO-2 (Ukraine). The measurements of each regime were repeated five times in order to calculate the mean values and errors of measurements.

Results and Discussion

It was possible to use this portable fluorometer in field conditions for the estimation of the effects of fertilizers, high-intensity solar and artificial ultraviolet radiation, water deficit and temperature on chlorophyll fluorescence of dark adapted leaves of soya bean, rapeseed, salad and bush bean.

Insertion of fertilizers $N_{15}P_{15}K_{15}$, $N_{30}P_{30}K_{30}$, $N_{45}P_{45}K_{45}$ affected the photosynthetic activity of soya and correspondingly the fluorescence indices. The same situation was observed with the rapeweed – there was the effect of fertilizers $N_{45}P_{30}K_{45}$; $N_{60}P_{45}K_{60}$; $N_{75}P_{60}K_{75}$;$N_{90}P_{75}K_{90}$, $N_{120}P_{75}K_{120}$ та $N_{90}P_{75}K_{120} + N_{30}$ on fluorescence indices.

The application of fertilizers of different composition affects the viability and photosynthetic activity of the plants correspondingly. It was established that the most effective fertilizers are: $N_{15}P_{15}K_{15}$ for soya bean and $N_{75}P_{60}K_{75}$ for rapeweed.

The results of investigation have demonstrated the sensitivity of fluorescence indices to water deficit. Water deficit doesn't affect the fluorescence indices of salad leaves during first 2-3 hours, but then the decreasing of both indices was observed: for sort *Lolla Rossa* – from 0.85 to 0.1 for *Rfd*(690) and from 0.1.2 to 0.5 for *Rfd*(740); for sort *LollaByonda* – from 1.3 to 0.0.2 for *Rfd*(690) and from 1.6 to 1.0 for *Rfd*(740); for sort *May Queen* – from 1.1 to 0.1 for *Rfd*(690) and from 1.3 to 0.85 for *Rfd*(740). It is shown that values of index *Rfd*(740) exceeds the values of index *Rfd*(690) that can be explained by process of re-absorption of emitted chlorophyll fluorescence by the leaf chlorophyll.

It is known that abscisic acid (ABA) is a stress hormone which plays an important role in the plant response to water stress at both a whole-plant level and at a cellular level [8,9]. The basis of ABA as a stress hormone is its rapid and massive accumulation under water deficit conditions. ABA induces the partial stomatal closure that is the main reason for the decrease in photosynthesis in response to water deficit. In such a way, stomatal limitation is generally accepted to be the main cause of reduced photosynthesis under water deficits: effect of water deficit on chlorophyll fluorescence of photosynthetic plants can be explained by the dehydratation of the cytoplasm and the chloroplast stroma; cytoplasm is desiccated and chloroplasts are packed more densely so that proportion of chlorophyll fluorescence is decreased due to re-absorption of the emitted fluorescence.

The effect of ambient temperature on fluorescence indices of the leaves of bush bean was investigated also. It was shown, that the fluorescence indices at the temperature 7 ^{0}C were lower than at the temperature 18 ^{0}C if the plants were kept under certain temperature regime during several days. The maximal values of fluorescence indices were observed under good water supply and the minimal ones – when the plants were grown without water and fertilizers.

Exposure of photosynthetic organisms to higher irradiances provokes a reduction of photosynthetic capacity, called photoinhibition – the process which is related with the degradation of the reaction centre protein *D1* of photosystem PSII; chlorophyll P_{680} and acceptor Q_B are related to this protein. The transfer of excitation energy along electron transport chain is associated with the process of quenching of chlorophyll fluorescence which occurs due to the oxidation of acceptor. The main mechanisms of quenching are energy-dependent *qE*-quenching which is dependent on the presence of proton gradient across the thylakoid membrane, and *qI*-quenching which occurs in excessive irradiation; this type of quenching provokes photoinhibition [10]. It is possible to suggest the effect of protein structural change in chlorophyll fluorescence quenching. The aggregation of proteins prevents high levels of nonradiative energy dissipation and leads to quenching.

According to modern views ultraviolet (especially, UV-B) radiation provides similar effects on photosynthetic activity of photosynthetic organisms, but with different molecular mechanisms. UV-B radiation predominantely damages DNA, which absorbs in this part of spectrum; the main molecular alteration formed in UV-B-irradiated DNA is formation of dimer photoproducts – pyrimidine dimers of cyclobutane structure which are responsible for disrupting of the genetic code and damage of photosynthetic apparatus of alga [11].

The effect of low temperature at which plants grown for a long period of time can be explained by damage of photosynthetic apparatus of the plant and corresponding decrease of chlorophyll content of the leaf and change of the fluorescence indices [2].

Conclusions

Our investigations have shown that recording chlorophyll fluorescence induction is a perspective method of agronomic plants analysis during development and under stress conditions. The results of field

application of portable two-wavelength fluorometer testified that this instrument possesses a number of advantages in comparison with stationary devices: it is characterised with compactness, autonomous power supply, high sensitivity and non-destructive estimation of fluorescence parameters *in vivo* of agronomic plants.

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