FLUORESCENCE SPECTROSCOPY OF AGRICULTURAL CROPS

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Photosynthesis is the conversion of light energy into stabilized chemical energy through light absorption by a pigment molecula, excitation energy transfer and the photochemical reaction in photosystem PSII. The process of de-excitation of the absorbed light energy is related to heat emission and chlorophyll fluorescence. Fluorescence spectroscopy or fluorometry is a type of electromagnetic spectroscopy that is based on the fluorescence analysis of the sample. *In vivo* chlorophyll fluorescence is a suitable tool for non-destructive, fast and precise estimation of healthy status of agronomic plants under different stress factors. Two modern fluorescence spectroscopic methods are discussed in this paper – Laser Spectrofluorometry and Recording of Chlorophyll Fluorescence Induction Kinetics.

The following cultures were investigated during laser spectrofluorometry: leaves of cereals (rye, barley, wheat, hybrids amphidiploid, maize). Chlorophyll fluorescence induction kinetics was monitored with 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.

A typical laser spectrofluorometer consists of a nitrogen laser as the source of excitation. The main characteristics of this laser are wavelength of generation 337 nm, frequency of pulse repetition 50 Hz, an average power output 3 mW, duration of pulse about 10 ns, divergence of beam $3 \cdot 10^{-3}$ radians.

The laser radiation is directed through the semitransparent glass plate to the sample (a single intact leaf) and to the fluorescent standard (a solution of fluorescein). Either the sample, or the cell with fluorescent standard are oriented at the angle 45^0 to the laser beam in order to escape non-desirable absorption of laser radiation by the sample (or standard) volume.

The fluorescence emission of the leaf (or the standard) is collected by a spherical mirror and focused on the entrance slit of monochromator. The dispersive element (prism) of this monochromator is linked with the electrical motor which provides the

rotation of the prism and selection of the wavelength. The intensity of fluorescence is detected by a photomultiplier; the electrical signal of it is amplified and recorded by the readout system.

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

Method of laser spectrofluorometry *in vivo* of the leaves is rather promising for laboratory investigations of the effect of different agrochemical treatments and external physical factors; it is possible to control all the stages of growth and development of the plant, to study the effects of side and segment of the leaf, its nodal position, and the age. In some cases it is possible to achieve the species identification of the plants on the basis of the relevant spectral indices analysis.

The main perspective is the possible application of the results from single leaf investigation to the monitoring of agronomic plants at the canopy level and remote sensing of stressed agronomic fields on the basis of laser-induced fluorescence measurements.

Analysis of fluorescence induction kinetics of chlorophyll provides useful information about the process of the development of a leaf. Formation of internal leaf structure, increase of pigment content and intense fixation of photosynthetic activity accompany the first phase of leaf development. The next phase of leaf development is characterized by a prevalence of degradative processes such as declination of photosynthetic quantum conversion and decrease of chlorophyll content per leaf area unit. Both these processes provoke the relative changes of spectral properties of the leaf.

The measurements of several chlorophyll fluorescence parameters such as F_1 = *430/460*, F_2 = *460/530*, F_3 = *690/735*, F_4 = *460/690*, F_5 = *430/690*, F_6 = *530/690*, F_7 = *430/735*, F_8 = *460/735*, F_9= *530/735*, F_{10} = *430/530* and the fluorescence decrease $f_d = f_m - f_s$, maximal fluorescence f_m, steady-state fluorescence f_s, vitality index $Rfd = f_d / f_s$ (*Rfd'* at 740 nm and *Rfd''* at 690 nm), stress adaptation index A_p = *1 – [Rfd(740)+1]/[Rfd(690)+1]* provide useful information about healthy status of leaf under stress conditions.

Methods of fluorescence spectroscopy are characterized by high sensitivity and give a non-destructive estimation of *in vivo* fluorescence parameters of agronomic plants during development and under stress conditions.