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# Effect of Naphthalene on Photosystem 2 Photochemical Activity of Pea Plants

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**Abstract**—The effect of a typical polyaromatic hydrocarbon, naphthalene (Naph), on photosystem 2 (PS-2) photochemical activity in thylakoid membrane preparations and 20-day-old pea leaves was studied. Samples were incubated in water in the presence of Naph (0.078, 0.21, and 0.78 mM) for 0.5-24 h under white light illumination (15 μmol photons·$m^{-2}$·$s^{-1}$). The PS-2 activity was determined by studying fast and delayed chlorophyll (Chl) *a* fluorescence. Incubation of samples in water solutions at Naph concentrations of 0.21 and 0.78 mM led to a decrease in the maximum PS-2 quantum efficiency ($F_v/F_m$), noticeable changes in the polyphasic induction kinetics of fluorescence (OJIP), and a decrease in the amplitudes of the fast and slow components of delayed fluorescence of Chl *a*. The rate of release of electrolytes from leaves that were preliminarily incubated with Naph (0.21 mM) was also increased. Significant decrease in the fluorescence parameters in thylakoid membrane preparations was observed at Naph concentration of 0.03 mM and 12-min exposure of the samples. Chlorophyll (*a* and *b*) and carotenoid content (mg per gram wet mass) was insignificantly changed. The quantum yields of electron transfer from $Q_A$ to $Q_B$ ($\varphi_{ET2o}$) and also to the PS-1 acceptors ($\varphi_{RE1o}$) were reduced. These results are explained by the increase in the number of $Q_B$-non-reducing centers of PS-2, which increased with increasing Naph concentration and exposure time of leaves in Naph solution. The suppression of PS-2 activity was partly abolished in the presence of the electron donor sodium ascorbate. Based on these results, it is suggested that Naph distorts cell membrane intactness and acts mainly on the PS-2 acceptor and to a lesser degree on the PS-2 donor side.

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*Key words*: naphthalene, photosystem 2, variable and delayed fluorescence of chlorophyll, detached pea leaves, thylakoid membranes, photosynthetic pigments

Great importance is now given to the study of environmental contamination by different pollutants. Some of the most common and toxic pollutants are polycyclic aromatic hydrocarbons (PAHs), i.e. comprising at least two benzene rings. PAHs with two or three rings are present in the atmosphere, mainly in the gas phase, whereas those with four rings or more predominate in the solid state [1].

PAHs in atmosphere, such as naphthalene (two benzene rings) and phenanthrene (three benzene rings), penetrate into the intercellular spaces of plants leaves, mainly through the stomata. On one hand, due to the lipophilicity of the molecule, PAHs may accumulate in the lipid layer of the plants membranes, mainly from precipitation from the atmosphere onto the leaf surface [2]. On the other hand, in the form of water-soluble derivatives these agents reach the cell membrane, break its intactness, and increase permeability. This may be related to the change in the ratio of $H^+$ and $Ca^{2+}$ in the membranes and to decrease in the number of sulfhydryl groups

*Abbreviations*: Asc, sodium ascorbate; Chl, chlorophyll; DFl, delayed fluorescence; $F_o$, minimum fluorescence; $F_m$, maximum fluorescence; $F_v$, variable fluorescence ($F_v = F_o - F_m$); FFl, fast fluorescence; Naph, naphthalene; PA, photosynthetic apparatus; PAHs, polycyclic aromatic hydrocarbons; PS-2, photosystem 2; $Q_A$, primary quinone acceptor of PS-2; $Q_B$, secondary quinone acceptor of PS-2; RC, reaction center; TM, thylakoid membranes.

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(SH) and a corresponding increase in membrane protein disulfide bonds, and also with the formation of membrane lipid defect regions due to the accumulation of products of lipid peroxidation [3]. It is assumed that the effect of extreme factors such as PAHs leads to disruption of membrane functions and structures. Thus, there is a disruption of the functioning of the photosynthetic apparatus (PA), primarily photosystem 2 (PS-2), which is most vulnerable to the effects of stress factors and can therefore serve as a bio-indicator of PAH action [4, 5].

Effects of PAHs depend on the duration of exposure and the physicochemical properties of the pollutant molecule [4-6]. Redox properties and lipophilicity of the agent, characterized by distribution coefficient in octanol/water, determine the efficiency of penetration of PAHs to molecular targets.

Approaches for assessing the photochemical activity of PS-2 include measurement of "variable" fast fluorescence (FFl) (OJIP-transitions) and delayed fluorescence (DFl) of chlorophyll (Chl) *a* [7-9]. In this case, based on the kinetics of induction curves of millisecond DFl and fast fluorescence, different parameters characterizing the photochemical activity of PS-2 can be calculated. FFl measurements allow evaluation of the maximum quantum efficiency of PS-2 photochemistry, the efficiency of electron transfer at different sites of the electron transport chain (ETC), and a number of other parameters [8, 10, 11]. Based on the kinetics of DFl, the value of light-induced formation of the transmembrane proton gradient ($\Delta$pH) through the thylakoid membranes and the rate of electron transfer on the PS-2 acceptor side can be estimated [7].

The solubility of almost all PAHs in water is low. Among PAHs, naphthalene (Naph) has the highest solubility in water, which at 0 and 25°C is 0.148 and 0.268 mM, respectively [12]. It is assumed that Naph, as a typical representative of PAHs, due to its low solubility in water acts primarily on lipid membranes of cells. When Naph-like PAHs damage the membrane, this results in the development of oxidative stress. This leads to a decrease in PS-2 activity and degradation of some its components. It was shown that Naph causes a decrease in PS-2 activity more rapidly than phenanthrene and fluoranthene [13]. However, the mechanisms of PS-2 activity suppression under the action of Naph still unclear in many details. The targets of Naph in PS-2 and the fast reactions of PA, when formation of metabolites of Naph is unlikely, are insufficiently studied. It should be noted that experiments with PAHs in many studies take a long time (2-6 weeks) [4, 6], which complicates the understanding of the mechanisms of their effects on the activity of the PA.

In this work we studied the effect of different concentrations of Naph on the photochemical activity of PS-2 and the content of photosynthetic pigments, as well as on the rate of Naph absorption by leaves and the electrolytes' output after a short incubation of leaves in a solution of Naph.

The data suggest that Naph damages intactness of cell membranes and acts primarily on the acceptor and to a lesser extent on the donor side of PS-2.

## MATERIALS AND METHODS

**Conditions for plant growth.** Experiments were conducted on detached pea leaves (*Pisum sativum* L. cv. Moskovskii 559). Three-day-old seedlings were placed in plastic containers (25 × 25 × 14 cm, volume 8.75 liters) filled with moist sand, in which every seven days Knop medium (one fourth of total volume) have was added. Plants were grown for 20 days under white light illumination with intensity of 360 μmol photons·$m^{-2}$·$s^{-1}$ (from lamps HPS-400) and at temperature of 25 ± 1°C.

The leaves of the second and third tiers were detached and placed in Petri dishes with moist filter paper for 1 h to adapt to the white light fluorescent lamps (15 μmol photons·$m^{-2}$·$s^{-1}$) before the measurement. Then the leaves (6-7 pieces) were placed in sealed glass jars (100 ml) containing 80 ml of Naph solution with determined concentration and incubated under the same lighting conditions at 25 ± 1°C. After incubation of the leaves in the naphthalene solution for different time periods (0.5-24 h), all necessary investigations were carried out. Preparations of thylakoid membranes (TM) from peas (35 mg Chl/ml) were prepared as described previously [13] and incubated with Naph (0.03 and 0.08 mM) for 12 min.

**Preparation of naphthalene samples.** Since Naph has low solubility in water, a 1000-fold more concentrated solution of Naph in acetone (Sigma-Aldrich, USA) was first prepared. Then, the acetone solution was poured into distilled water to obtain final concentrations of 0.078 and 0.21 mM. The volume of 0.1 ml of stock solution was added to 100 ml of distilled water. In some experiments supersaturated solutions of Naph (Naph nominal concentration is 0.78 mM) were used in which some Naph was present in solution as a stable suspension. As a control, distilled water with the indicated concentration of acetone (0.1%) was used.

**Measurements of chlorophyll fluorescence.** The photosynthetic activity of PS-2 was assessed by measuring the fast and delayed fluorescence (FFl and DFl, respectively) of Chl *a* after incubation of leaves in a solution of Naph in the light for 1, 2, and 24 h. Prior to the measurements the leaves were fixed in the measuring cell and incubated in the dark for 15 min.

The $F_o$ level and kinetics of photoinduced changes in the PS-2 chlorophyll fluorescence yield were measured with a PAM-fluorimeter (XE-PAM; Heinz Walz, Germany). Induction curves of the FFl and OJIP-transitions (increase in fluorescence yield of Chl *a* from minimum to maximum intensity – OJIP) were recorded with

a fluorimeter as described previously [13]. The data is stored and processed with a computer.

Based on the Chl *a* FFl induction curves, the following fluorescence parameters were calculated: $F_o$, $F_m$, $F_v$, where $F_o$ – the minimum level of fluorescence (F), $F_m$ – maximum of F, and $F_v$ – variable F defined as the difference between $F_o$ and $F_m$ [8]. Furthermore, based on the data the following parameters were calculated: $ET_{2o}/RC$, $ET_{2o}/ABS$, $\Psi_o$, $TR_o/RC$, and ABS/RC (Table 1), where TR is the rate of exciton flow trapped by all the reaction centers (RC) of PS-2; $TR_o$ is the maximum (initial) exciton flow captured by all PS-2 RCs. The ratio $TR_o/RC$ designates the exciton capture rate (leading to the reduction of $Q_A$), i.e. the rate of $Q_A$ reduction to $Q_A^-$ as a result of the absorption of an exciton in the PS-2 RC per RC; ABS/RC is the averaged flux of photons absorbed by PS-2 antenna chlorophyll per RC, the value of ABS/RC characterizes the size of the PS-2 antenna; $ET_{2o}$ is the maximum flow of electrons transferred in PS-2 from $Q_A$ to $Q_B$; ratio $ET_{2o}/RC$ is the flow of electrons transferred from $Q_A$ to $Q_B$ per RC [8].

Calculations were carried out in accordance with the following formulas:

$$ABS/RC = (M_o/V_J)\cdot(F_v/F_m),$$

$$ET_o/RC = (M_o/V_J)\cdot(1 - V_J),$$

$$TR_o/RC = M_o/V_J,$$

$$ET_o/ABS = F_v/(F_m\cdot(1 - V_J)),$$

$$M_o = 4\ ms^{-1}\cdot(F_{300\mu s} - F_o)/(F_m - F_o),$$

where $V_J$ is relative fluorescence level in phase J; $V_J = (F_{2ms} - F_o)/(F_m - F_o)$; $ET_{2o}/TR_o = \Psi_o = 1 - V_J$. Here ABS

**Table 1.** Parameters (OJIP-parameters) calculated based on induction curves of fast chlorophyll fluorescence

| Parameters | Characteristic of parameters | Formulas for calculating the parameters |
|---|---|---|
| $M_o$ | initial slope of the curve of the relative value of the variable fluorescence intensity of; expresses the rate of electron transfer at the initial stage | $M_o = 4\ (F_{0.3\ ms} - F_o)/(F_m - F_o)$ |
| Area | area between the OJIP curve and the line $F = F_m$; expresses the amount of reduced electron carriers of plastoquinone pool | |
| $S_m$ | normalized area; it is characterized by the number of reduced plastoquinone electron carriers in the electron transport chain of PS-2 | $area/(F_m - F_o)$ |
| $V_J$ | relative variable fluorescence at time 2 ms (J-phase) after the start of irradiation by a pulse of actinic light | $V_J = (F_{2\ ms} - F_o)/(F_m - F_o)$ |
| $V_I$ | relative variable fluorescence at time 30 ms (I-phase) after the start of the irradiation by a pulse of actinic light | $V_I = (F_{30\ ms} - F_o)/(F_m - F_o)$ |
| $\varphi_{ET2o}$ | maximum quantum yield of electron transport in the PS-2 from $Q_A$ to $Q_B$ | $\varphi_{ET2o} = F_v/F_m\ (1 - V_J)$ |
| $TR_o/RC$ | maximum (initial) energy flux absorbed by all the PS-2 RC and used for the primary charge separation based on the PS-2 RC | $TR_o/RC = M_o/V_J$ |
| $\varphi_{RE1o}$ | maximum quantum yield of electron transport to the final electron acceptors of PS-1 | $\varphi_{RE1o} = (F_v/F_m)\ (1 - V_I)$ |
| $ET_{2o}/RC$ | maximum flow of electrons transferred from $Q_A$ to $Q_B$, based on the PS-2 RC | $ET_{2o}/RC = (M_o/V_J)\ (1 - V_J)$ |
| $\Psi_{ET2o}(\Psi_o) = J_o^{ET2o}/J_o^{TR}$ | the efficiency with which the capture of exciton by PS-2 RC leads to electron transfer from $Q_A$ to $Q_B$ | $\Psi_{ET2o} = (ET_{2o}/RC)/(TR_o/RC) = 1 - V_J$ |
| ABS/RC | ABS/RC – averaged absorbed by PS-2 antenna chlorophyll flux of photons per RC; reflects the size of the PS-2 antenna | $ABS/RC = (M_o/V_J)\ (F_v/F_m)$ |
| RC/CS | number of active PS-2 RC on the absorption cross section | $RC/CS = F_m\ ((F_m - F_o)/F_m)\ (V_J/M_o)$ |
| $TR_o/ABS$ | maximum quantum efficiency of PS-2 photochemistry | $TR_o/ABS = F_v/F_m$ |

is photon flux absorbed by chlorophyll of PS-2 antenna; J is the energy flux; 1 and 2 designate PS-1 and PS-2, respectively, as previously described [8].

*Measurements DFl.* Millisecond DFl of Chl *a* was measured using a disk-based phosphoroscope setup described in detail previously [14], with some modifications. Samples were irradiated with light of wavelength 645 nm and intensity at the surface equal to 1800 μmole quanta·$m^{-2}$·$s^{-1}$ from the red LED. The duration of illumination, the dark period, and the registration was 2, 4, and 2 ms, respectively. The photomultiplier signal was converted in a PCIe-6321 ADC (L-Card) and recorded every 50 μs during the registration period. The data was stored in the computer for further processing and analysis using functions of averaging and smoothing.

Contents of Chl *a* and *b* as well as carotenoids (mg per gram wet weight) was measured in extracts of ethanol using known absorption coefficients [15].

**Naphthalene accumulation in the leaves.** The rate of Naph accumulation in the pea leaves was evaluated on the basis of lowering Naph initial concentration in the aqueous solutions after incubation of 6-7 leaves in a solution of naphthalene (80 ml) for 24 h. Decrease in Naph initial concentrations (0.078 and 0.21 mM) was determined with a Spectronic Genesis 10UV spectrophotometer (Thermo Scientific, USA) in the main Naph absorption wavelength at 219 nm. As a control (taken as 100%), data on changes in the concentration of similar Naph solutions in the absence of leaves were used.

**Rate of release of electrolytes.** The cell membrane permeability was judged by the rate of electrolyte exit (REE) from detached leaves in an aqueous solution where they were placed. The conductivity of the aqueous solution was measured by conductometry. The units of measure was microSiemens per gram fresh weight (μSm/g). REE was measured as follows. Detached pea leaves (6-7 leaves) were incubated in bidistilled water and a solution of Naph (0.078 and 0.21 mM) (volumes of solutions were 80 ml) for 2 h at light intensity of 12 W/$m^2$. After washing with distilled water, the leaves were placed in 80 ml bidistilled water and incubated for 3 h at room temperature. The conductivity was measured immediately after placing the leaves in bidistilled water and during incubation of the leaves for 3 h.

To clarify the nature of the DFl components and the effect of Naph on PS-2, experiments were conducted in the presence of (i) the inhibitor of PS-2 Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (blocking electron transfer between the primary and secondary quinone electron acceptors PS-2, $Q_A$ and $Q_B$, respectively); (ii) the PS-2 exogenous electron donor sodium ascorbate. Leaves were incubated in a solution of sodium ascorbate (3 mM) or Diuron (30-50 μM) for 30 min.

In the tables and figures, the arithmetic means of values and their standard errors (±S.E.) are presented. At least three biological and at least 4-9 analytical replicates were used. The reliability of differences between the variants was determined by Student's *t*-test at significance level $p < 0.05$.

## RESULTS

Effects of Naph on photochemical activity of PS-2 in pea leaves as well as on the activity of the PS-2 preparations of thylakoid membranes were investigated both after short-term (1 h) incubation in the presence of Naph (Table 2) and in after long-term (24 h) experiments (Figs. 1 and 2 and Tables 3 and 4).

Using the DFl method, typical DFl curves were recorded including fast components with maxima $I_1$ and $I_2$ and the slow component with maximum $I_m$ (Fig. 1). In control leaves the positions of maxima corresponded to the following time values, $I_1$ – 35 (±5) ms, $I_2$ – 100 (±15) ms, and $I_m$ – 3.4 (±1) s. The relative ampli-

**Table 2.** Effect of naphthalene (Naph) on the ratio ($F_v/F_m$) and the relative amplitude of DFl slow component (($I_m - D$)/D), as well as absorption Naph by leaves incubated for 2 h in a solution at different Naph concentrations under light intensity of 12 W/$m^2$. Naph absorption in solution without leaves was taken as 100%. Values in parentheses are standard errors

| Parameter | Naph concentration, mM | | | | |
|---|---|---|---|---|---|
| | 0 | 0** | 0.078 | 0.21 | 0.78 |
| $F_v/F_m$ | 0.815 (0.005) | 0.816 (0.006) | 0.804 (0.005)* | 0.790 (0.007) | 0.78 (0.01) |
| $(I_m - D)/D$ | 2.91 (0.25) | 2.76 (0.2) | 2.72 (0.16)* | 2.66 (0.15)* | 2.5 (0.2) |
| Naph absorption, % | 0 | 0 | 75 ± 3.5 | 31 ± 4 | – |

Note: Relationship $F_v/F_m$ are amended with inserted acetone – 0.1 and 1%, respectively, $n = 8$.
* Difference is insignificant ($p > 0.05$).
** Acetone is added: 0.1% in the measurements of variable fluorescence and 1% – for DFl.

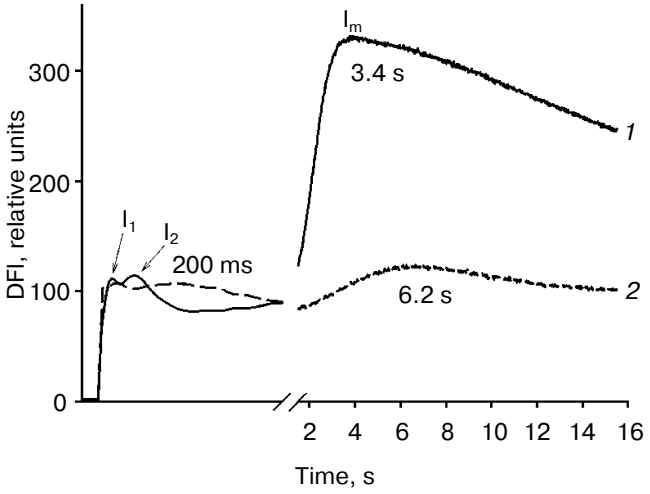


**Fig. 1.** Effect of Naph on DFl curves of detached pea leaves. Leaves were incubated in aqueous Naph solution (0.78 mM) for 24 h (*2*). Control leaves were incubated without Naph for 24 h (*1*). Maxima in the figure labeled as $I_1$, $I_2$, and $I_m$. Typical curves of nine replicates are shown.

tudes of the fast ($I_2$) and the slow component were 0.3 and 3.0 relative units, respectively. After incubation of leaves in the presence of Naph for 24 h, the relative amplitudes of the fast and slow components decreased from 0.3 to 0.1 and from 3.0 to 0.8 relative units, respectively, and their maxima were at 200 ms and 6.2 s, respectively. In preparations of TM, only the fast component (maximum at 80 ms) was detected, which was reduced by half when incubated in 0.03 mM Naph and almost to zero in 0.08 mM Naph (data not shown).

Figure 2 (curve *1*) shows a typical growth curve of FFl obtained in the absence of Naph (control), the so-called OJIP transitions – a rapid increase in the Chl *a* fluorescence from baseline (O) in a few milliseconds to a level J, and then relatively slow phases from J to I and from I to P, as shown previously [10, 11]. After incubation of the leaves in aqueous Naph solution for 24 h, the OJIP kinetics significantly changed (see Fig. 2, curve *2*). The OJ phase is characterized with the least change, whereas the amplitudes of the JI and IP phases significantly decreased and their maxima shifted to longer times.

The effects of Naph on time of the photoinduced increase in the yield of PS-2 chlorophyll fluorescence from the initial ($F_o$) to maximum ($F_m$) levels (the rate of increase $\Delta F$) were investigated. In control samples after incubation of leaves for 24 h in an acetone solution (0.1%) (which is used as a solvent for Naph), time ($\tau$½) of increase of the F yield to a level equal to 50% from the maximum level ($F_m$(½)) was 3.6 ms. In leaves incubated for 24 h in the presence of Naph, this time was reduced to 1.90 ms (0.21 mM) and 1.1 ms (0.78 mM).

Values of the maximum quantum efficiency of PS-2 photochemistry (the ratio $(F_m - F_o)/F_m$) were calculated based on induction curves. This value for the leaves before Naph treatments was 0.80 ± 0.01. After incubation of leaves for 24 h in water containing acetone (0.1%), in the absence of Naph (control), this value was reduced to 0.78 ± 0.01. Only at Naph concentrations of 0.21 and 0.78 mM, and only after 2 h of incubation, significant reduction in quantum efficiency $F_v/F_m$ was observed, while after 1 h of incubation in an aqueous solution of 0.21 mM Naph no significant effect was observed. At the same time, the contribution to this ratio of each of the variables, $F_m$ and $F_o$, was assessed. The level $F_o$ mainly increased, whereas the decrease in the maximum intensity $F_m$ was less significant (Table 3). In TM preparations, a similar trend was observed: at 0.03 mM Naph concentration $F_o$ is increased by 30% and $F_m$ was little changed.

In some experiments Diuron, an inhibitor of electron transport between the primary quinone $Q_A$ and secondary $Q_B$ electron acceptors of PS-2, and sodium ascor-

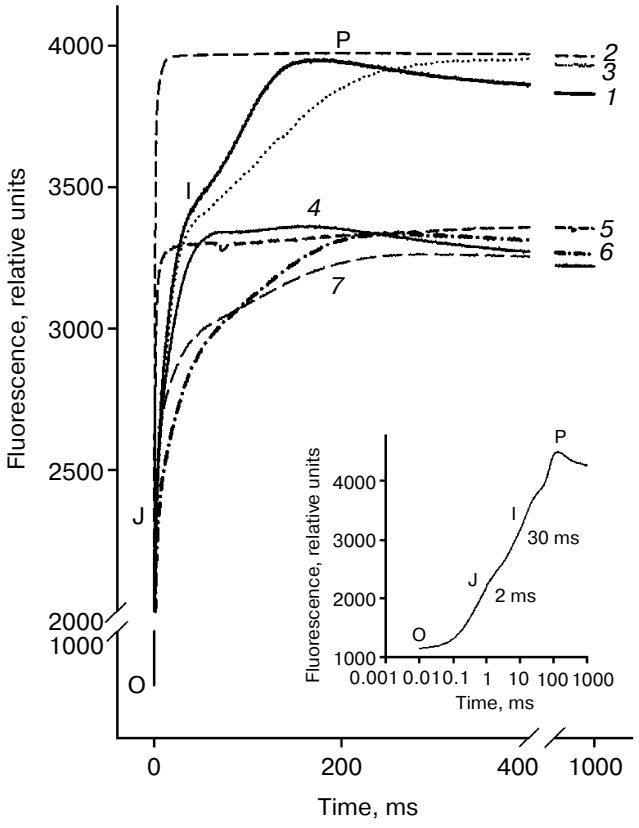


**Fig. 2.** Kinetics of chlorophyll FFl (OJIP-transitions) measured on detached leaves. Leaves were incubated with distilled water (*1*) for 24 h; then a portion of the leaves was incubated for 60 min in a solution of Diuron (*2*) or 30 min in a solution of sodium ascorbate (Asc) (*3*). Another portion of the leaves was incubated in a Naph solution (*4*) for 24 h. Then part of the leaves were incubated for 1 h in a solution of Diuron (*5*) or 30 min in a solution of Asc (*6*) as well as in the mixed solution Diuron (1 h) + Asc (30 min) (*7*). Concentrations of Naph, Diuron, and Asc were 0.21 mM, 40 μM, and 3 mM, respectively. In the lower right corner a typical OJIP curve measured on the untreated leaves is shown.

**Table 3.** Effect of naphthalene (Naph), Diuron, and sodium ascorbate (Asc) on the fluorescence parameters calculated based on induction curves of chlorophyll FFl, OJIP-transitions. Values in parentheses are standard errors

| Fluorescence parameters | W | W + Diuron | Naph (0.21 mM) | Naph (0.21 mM) + Diuron | Naph (0.21 mM) + Asc | Naph (0.78 mM) |
|---|---|---|---|---|---|---|
| τ½, ms | 3.6 (0.14) | 0.47 (0.07) | 1.9 (0.25) | 0.85 (0.06) | 3.06 (0.11) | 1.1 (0.08) |
| $\varphi_{ET2o} = (ET_{2o}/ABS)$ | 0.53 (0.03) | 0.08 (0.07) | 0.40 (0.02) | 0.14 (0.05) | 0.46 (0.02) | 0.25 (0.03) |
| $TR_o/RC$ | 1.33 (0.1) | 1.78 (0.12) | 1.55 (0.06) | 1.65 (0.2) | 1.53 (0.11) | 1.82 (0.14) |
| ABS/RC | 1.10 (0.04) | 1.21 (0.04) | 1.19 (0.06) | 1.08 (0.06) | 1.19 (0.05) | 1.32 (0.04) |
| $ET_o/RC$ | 0.97 (0.02) | 0.21 (0.02) | 0.88 (0.024) | 0.35 (0.03) | 0.98 (0.05) | 0.81 (0.025) |
| $\Psi_o = ET_{2o}/TR_o$ | 0.73 (0.02) | 0.12 (0.003) | 0.56 (0.06) | 0.21 (0.04) | 0.63 (0.06) | 0.445 (0.1) |
| $\varphi_{RE1o} = RE_{1o}/ABS$ | 0.31 (0.02) | 0.002 (0.001) | 0.15 (0.035) | 0.06 (0.01) | 0.30 (0.03) | 0.08 (0.04) |
| RC/CS | 1946 | 1519 | 1631 | 1072 | 1503 | 935 |
| $S_m$ | 1 | – | 0.40 (0.06) | | 1.05 (0.12) | 0.35 (0.09) |

Note: The value of $S_m$ in the control is taken as unity. The leaves were incubated in the light (15 μmol photons·$m^{-2}$·$s^{-1}$) in a Naph solution of two concentrations (0.21 and 0.78 mM) or distilled water (W) for 24 h. Then the leaves were kept in Diuron solution (30 μM), Asc (3 mM) for 30 min (variants: W + Diuron, Naph + Diuron, and Naph + Asc), $n = 5$.

**Table 4.** Effect of naphthalene (Naph), and sodium ascorbate (Asc) on the relative values of the fluorescent parameters ($F_o$, $F_m$ and $F_v/F_m$), and the content of photosynthetic pigments. Values in parentheses are standard errors

| Parameter | W | Naph (0.21 mM) | W + Asc (3 mM) | Naph (0.21 mM) + Asc (3 mM) | Naph (0.78 mM) |
|---|---|---|---|---|---|
| $F_v/F_m$ ($TR_o/ABS$) | 0.80 (0.008) | 0.71 (0.02) | 0.80 (0.01)* | 0.72 (0.016) | 0.65 (0.04) |
| $F_o$ | 1 (0.04) | 1.3 (0.08) | 1.04 (0.03)* | 1.16 (0.08) | 1.49 (0.12) |
| $F_m$ | 1 (0.05) | 1.05 (0.03) | 1.0 (0.045)* | 0.92 (0.06) | 0.83 (0.07) |
| Chlorophylls ($a + b$), mg/g | 1.64 (0.023) | 1.70 (0.04)* | 1.67 (0.035)* | 1.69 (0.04)* | 1.54 (0.03) |
| Carotenoids, mg/g | 0.27 (0.006) | 0.30 (0.05)* | 0.28 (0.01)* | 0.29 (0.03)* | 0.26 (0.03)* |

Note: The values of $F_o$ and $F_m$ in the control taken as unity. Leaves were exposed to light (15 μmol photons·$m^{-2}$·$s^{-1}$) in a Naph solution of two concentrations (0.21 and 0.78 mM) or distilled water (control, W) for 24 h. Then part of the leaves were kept in Asc solution (3 mM) for 30 min (variants: W + Asc and Naph + Asc), $n = 8$.

* Difference is insignificant ($p > 0.05$).

bate, an exogenous electron donor to PS-2, were used. Diuron significantly (see Fig. 2, curves *2* and *5*) and, to a lesser extent, sodium ascorbate (curve *3*) changed the appearance of the induction curve of FFl, reducing the time to achieve level $F_m$(½). In the presence of Diuron, growth of FFl from $F_o$ to $F_m$ markedly accelerated. In control leaves the increase in the quantum yield of FFl to a level $F_m$(½) occurred in 3.6 ms, whereas in leaves treated with Diuron it occurred in only 0.47 ms. Adding the electron donor sodium ascorbate had not so much influence on the time of half-growth of FFl (τ½). After 30 min of incubation in a solution of sodium ascorbate, for leaves preincubated for 24 h in the presence of Naph (0.21 mM) the τS value is increased from 1.9 to 3.06 ms. Incubation of leaves in the presence of sodium ascorbate in the absence of Naph during the same time had practically no effect on the fluorescence parameters (Table 3). The τ½ magnitude changes only slightly, from 3.6 to 4.2 ms.

Based on the FFl induction curves, other parameters characterizing the PS-2 photochemical activity were also

calculated (Table 1) including (i) flow of electrons transferred from $Q_A$ to $Q_B$ per PS-2 RC (value $ET_{2o}$/RC), (ii) maximum quantum yields of electron transport at the site $Q_A$–$Q_B$ ($\varphi_{ET2o}$, $ET_{2o}$/ABS) and from $Q_A$ to PS-1 electron acceptors ($\varphi_{RE1o}$), and (iii) $\Psi_{ET2o}$ – the efficiency with which the capture of exciton by components of the PS-2 reaction center leads to electron transfer ($ET_o$) from $Q_A$ to $Q_B$ (Table 2) [8]. All these values were decreased after incubation of leaves in Naph solution for 24 h and more so with increase in Naph concentration. The quantum yield $\varphi_{RE1o}$ was most notably decreased – 4-fold at a Naph concentration of 0.78 mM and 2-3-fold at a Naph concentration of 0.21 mM. The area ($S_m$) over the OJIP curve characterizing the size of the plastoquinone molecule pool in PS-2 was decreased by 2.5-3.0-fold. The ratio of the number of active RCs to PS-2 absorption cross-section (RC/CS) was also decreased. The following values were also calculated: $TR_o$/RC – the exciton capture rate, which reflects the rate of $Q_A$ to $Q_A^-$ reduction because of exciton absorption in PS-2 RC per RC; ABS/RC – the rate of photon absorption by PS-2 antenna chlorophyll per RC. Both values were increased under (i) incubation of leaves in 0.21 mM Naph and (ii) increase in Naph concentration to 0.78 mM. In the presence of Diuron, changes in the values of $TR_0$/RC and ABS/RC were similar to the changes observed in the presence of Naph (Table 2). An electron donor to PS-2, on the contrary, led to partial withdrawal of the effects on the parameters of the FFl of Naph treated leaves (Tables 3 and 4 and Fig. 3).

The maximum quantum efficiency of PS-2 ($F_v/F_m$) after 24 h of incubation of untreated leaves in darkness was 0.78-0.81, which corresponds to the values of $F_v/F_m$ characteristic of a healthy plant. After incubation of leaves with Naph (0.21 mM) the value of $F_v/F_m$ decreased, starting with a 2-h incubation (Table 2) at the expense of increasing $F_o$ level and decreasing $F_m$ level. Increasing the Naph concentration resulted in further reduction in the quantum efficiency of PS-2. The $F_o$ level was increased with increasing Naph concentration and incubation time (Table 4). The maximum fluorescence, $F_m$, varied little at 0.21 mM concentration and was reduced at 0.78 mM concentration.

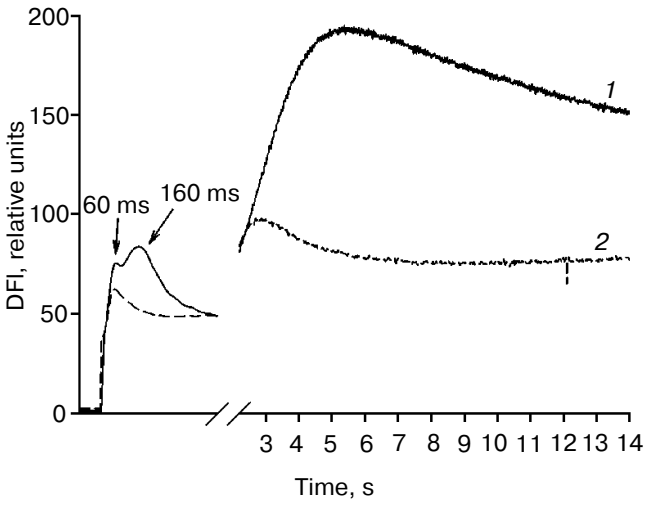


**Fig. 3.** Effect of Diuron on DFl curves of detached pea leaves. The leaves were incubated in a solution of Diuron (40 μM) for 30 min (*2*). Control leaves were incubated in distilled water with no Diuron for 30 min (*1*). Typical curves are shown.

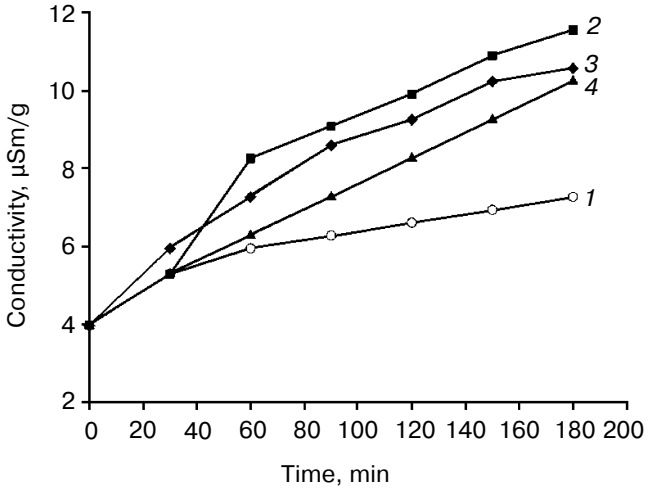


**Fig. 4.** Dynamics of conductivity of an aqueous solution in the presence of pea leaves depending on the time of preincubation in 0.21 mM naphthalene. Duration of incubation (min): *1*) 0; *2*) 15; *3*) 30; *4*) 60. Time on abscissa – the time of registration of electrical conductivity after inserting in bidistilled water of leaves incubated (*2-4*) and not incubated (*1*) in Naph solution. In the absence of the leaf conductivity varied in the range of 0.1. The standard error of the measurements did not exceed 5%.

Total Chl content ($a + b$) after detached leaves were incubated in aqueous solution of 0.78 mM Naph was reduced slightly, from 1.64 to 1.54 mg/g, whereas at 0.21 mM Naph concentration it was practically unchanged (Table 4).

We also investigated the effect of Diuron on DFl of pea leaves. In the presence of Diuron the amplitudes of the fast and slow components of the DFl induction curves were decreased. The fastest DFl component ($(I_1 - D)/D$) with maximum $I_1$ characterized by time 50 ms was the least susceptible to change compared with the slower components (150 ms and 4 s) with maxima $I_2$ and $I_m$ (Fig. 3).

Study of the conductivity of the aqueous solution in the presence of leaves depending on the time of incubation in aqueous naphthalene solution (0.21 mM) revealed that Naph increased conductivity, and this effect was observed when the leaves were preincubated for 15 min with Naph (Fig. 4). Significant reduction in the optical density of the Naph solution (0.21 mM) in the presence of leaves was also observed after 15-20 min, whereas a 50% reduction was observed after 5 h.

## DISCUSSION

Typically, levels that characterize the maximum quantum efficiency of PS-2 ($F_v/F_m$) are closely correlated with the rate of photosynthesis in intact leaves. For healthy leaves the ratio $F_v/F_m$ usually ranges from 0.75 to 0.84 [16]. This ratio for the leaves used by us for the treatments was 0.80 ± 0.01. After 24-h incubation of leaves in water containing acetone (0.1%) in the absence of Naph (control), it was reduced slightly to 0.785 ± 0.015, while after 2-h incubation it was practically unchanged, indicating the stability of the functional activity of PA in the studied leaves. Incubation of leaves in 0.21 mM Naph resulted in a decrease in this ratio after 2 h, which is in agreement with the earlier observed increase in hydrogen peroxide content in leaves after 30-min incubation at a given Naph concentration [13]. Comparing these data, one can suggest that oxidative stress precedes the reduction of the photochemical activity of PS-2. This supposition is supported by data that phenanthrene, a PAH containing three rings, decreases activity of PS-2 in *Arabidopsis* leaves, and this process is accompanied by accumulation of hydrogen peroxide [17].

PS-2 is heterogeneous in structure and in function [18, 19]. Two types of PS-2 heterogeneity are known: heterogeneity of antenna and heterogeneity of the reducing (acceptor) side [19, 20].

To investigate the possible mechanism of action of naphthalene on PS-2 activity, we used an inhibitor of electron transport on the acceptor side of PS-2, Diuron, and exogenous electron donor, sodium ascorbate. Diuron displaces the secondary quinone acceptor $Q_B$ from its binding site on the D1-protein of PS-2 [21] and thereby blocks the electron transfer from $Q_A$ to $Q_B$. Inhibition of reduced $Q_A$ reoxidation is accompanied by characteristic changes in the kinetics of induction curves (OJIP) (Fig. 2). In this case, in the curves practically only one phase, J, is observed. This phase corresponds to the reduction of the primary electron acceptor $Q_A$. The next phases after the J phase of the induction curve are mainly related to the reduction of plastoquinone at the $Q_B$-site and the accumulation of reduced plastoquinone in the membrane pool of plastoquinone.

With regard to sodium ascorbate, its concentration in chloroplasts is rather high, perhaps reaching 20 mM [22]. However, as a result of treatment with PAH, which leads to oxidative stress due to increased membrane permeability, the content of sodium ascorbate in chloroplasts can be remarkably reduced, and even its relatively low concentration (3 mM) can act on the activity of PS-2.

Based on the analysis of the kinetics of FFl induction curves, we suggest that Naph affects primarily the acceptor side of PS-2, presumably through decrease in efficiency of electron transfer from $Q_A$ to $Q_B$ and further to the ETC, but not by reducing the efficiency of electron transfer to $Q_A$, which was not reduced during treatment. This suggestion is supported by all the results obtained on the leaves after incubation in the presence of Naph, namely data on the reduction of the area over the induction curve of FFl, which characterizes the size of the pool of active plastoquinone molecules; decrease in values of $\tau$½; and that, after processing, the fast component (OJ) associated with the reduction of $Q_A$ is virtually unchanged, whereas the amplitude of the slower component is reduced.

To take into account the reduced amplitude of the DFl fast component in TM preparations, we suggest that the effect of Naph on thylakoid membranes leads to an increase in the proportion of non-reduced $Q_B$ centers of PS-2, i.e. it changes the heterogeneity of the acceptor side of PS-2. This suggestion was made previously [19] concerning effects of fluoranthene on peas leaves and some actions of PAHs on leaves of *Arabidopsis* plants [20]. The conclusion that the inhibition of electron transfer on the acceptor side is also consistent with data on the effect of Naph on the parameters calculated from the OJIP kinetics in the variant with simultaneous action on the PA of leaves of two agents, Diuron and Naph. Typical effects of Diuron in leaves treated with Naph, compared with untreated leaves, were less pronounced (Table 3). This is because Naph has an inhibitory effect on the activity of PS-2, which is identical to the influence of Diuron. On the other hand, inhibition of Naph-pretreated leaves in the presence of the exogenous electron donor sodium ascorbate resulted in a partial restoration of the shape of the OJIP curve and parameters calculated based on it. These data suggest that Naph can inhibit not only the acceptor, but also partly the donor side of PS-2. Conclusions about the action of PAHs including Naph and anthracene on both the acceptor and donor sides of PS-2 were made also in papers by Axmann and Tukaj [23] and by Kummerova et al. [24].

The redox state of the primary quinone electron acceptor of PS-2, $Q_A$, defines the fluorescence yield of Chl *a* and the intensity change in the OJ phase of OJIP-transition [25]. Under stationary conditions, measured reconstitution of the formulations of PS-2 $Q_A$ by a saturating light reaches its maximum after 2 ms ($I_{2ms}$) after turning on the light. We have shown that the rate of $Q_A$ to $Q_A^-$ reduction as a result of exciton absorption in PS-2 RC per RC ($TR_0$/RC value) and the rate of photon absorption by PS-2 antenna chlorophyll per RC (ABS/RC value) increased after incubation of leaves in Naph solution. A similar increase in $TR_0$/RC and ABS/RC values was previously observed under the influence of drought [26]. The increase in the $Q_A$ to $Q_A^-$ reduction rate observed by us may be associated with an increase in the number of inactive RCs. The increase in the ABS/RC value can be attributed to a decrease in the number of active PS-2 centers.

It was shown that Naph led to a decrease in quantum yield of electron transport at the $Q_A$–$Q_B$ site ($\varphi_{ET2o}$) and from $Q_A$ to electron acceptors of PS-1 ($\varphi_{RE1o}$) that, along with a decrease in the pool of reduced plastoquinone car-

riers (Tables 3 and 4), indicates the inhibition of the PS-2 acceptor side activity by Naph. This result is consistent with a reduction in the amplitude of the fast component DFl, $I_2$, as a result of exposure to Naph. We found a significant reduction in the magnitude $\varphi_{RE1o}$ compared with the value $\varphi_{ET2o}$ in leaves after their incubation in a Naph solution. This suggests that Naph reduces the rate of electron transport in not only the site from $Q_A$ to $Q_B$, but also elsewhere in the photosynthetic electron transport chain, probably in sites between the PS-2 and PS-1. In this case, there is a correlation between the decrease in the value of $\varphi_{ET2o}$ and ratio RC/CS with increasing concentration of Naph. Probably decrease in both quantities is due to reduction in the number of active $Q_B$-reducing centers of PS-2.

Previously, it was shown [13] that Naph acts on thylakoid membranes, causing in them certain disorders. We assume that Naph also acts on the associated PS-2 antenna complex. This can lead to disruption of conjugation between the Chl molecules in the antenna complex and as a result, enhanced antenna Chl fluorescence, which may increase the $F_o$ level. Inactivation of the PS-2 can also lead to detachment of the PS-2 antenna complex from this photosystem [24] and, thus, increase $F_o$.

The effect of Naph on PS-2 activity was also investigated by measuring DFl induction curves. A typical Chl *a* DFl curve (see Fig. 1) is characterized by several phases: fast (characterized by amplitudes $I_1$ and $I_2$ and times of the order of 30-150 ms) and slow (maximum at 3-10 s, characterized by amplitude $I_m$), which are separated by a minimum, denoted as D. The relative amplitude of the DFl $I_2$ component (ratio $(I_2 - D)/D$) reflects the rate of electron transport on the acceptor side of PS-2 depending on the redox state of $Q_A$, $Q_B$, and the plastoquinone pool PQ [27, 28]. We observed a decrease in this ratio with increasing concentration of Naph, most likely due to increased number of non-reducing $Q_B$ reaction centers of PS-2. The ratio of $(I_m - D)/D$ reflects the photoinduced formation of a proton gradient in the thylakoid membranes. Formation of ΔpH, needed for a high level of non-photochemical quenching, requires the functional integrity of thylakoid membranes [29]. Thus, Naph induces oxidative stress, leading to a significant disruption of the integrity of the thylakoid membrane, which was observed by Kreslavski et al. [13].

Depending on the measuring conditions and the functional state of the PS-2 oxygen-evolving complex (OEC), DFl of Chl *a* can be illuminated as a result of charge recombination in pairs $[Q_A^- P_{680}^+]$, $[Q_A^- Y_Z^+]$, where $P_{680}$ and $Y_Z^+$ are the primary and secondary PS-2 electron donors, chlorophyll $P_{680}$, and tyrosine 161 of D1 protein, respectively; as well as in pairs $[Q_A^- S_n^+]$, $[Q_B^- P_{680}^+]$, $[Q_B^- Y_Z^+]$, and $[Q_B^- S_n^+]$, where $S_n$ are the S-states of the PS-2 OEC [9]. In the presence of Diuron blocking electron transfer from $Q_A$ to $Q_B$, millisecond DFl arises mainly as a result of the charge recombination between $Q_A^-$ and positively charged components of the donor side of PS-2. According to our data, the relative amplitude of the fast component of DFl ($(I_1 - D)/D$) with a maximum of 50 ms (which most appropriately correlates with the $[Q_A^- Y_Z^+]$ pair lifetime) is reduced by treatment with Diuron to the least extent (see Fig. 3). Apparently, the fastest component $I_1$ is related to the recombination of charges in the $[Q_A^- Y_Z^+]$ pair to a greater extent than other components.

Membrane reactions, in particular enhanced membrane permeability, occur rather rapidly and are a primary cell response to various stress factors. The structure of the cell membrane of a plant determines its properties, physiological activity, and resistance to stress, while antioxidants, embedded in the membrane, protect it from destruction by oxidants and maintain optimum regulatory functions [30].

It is assumed that photosynthetic membranes are targets for the action of PAHs [31]. The increase in the conductivity of aqueous solutions with the introduction of the leaves incubated in Naph solution (see Fig. 4) seen by us is consistent with this idea and indicates a change in the permeability of cell membranes after incubation of leaves with Naph. Changes in conductivity was observed after 15 min and a significant increase in the pool of $H_2O_2$ was observed after 30 min. It is likely that increase in the permeability of membranes under the influence of Naph associated with subsequent changes in the content of $H_2O_2$ and other reactive oxygen species (ROS) occurs due to a decrease in PS-2 activity. Although reduction of the Chl and carotenoid content at high Naph concentrations was only slight, it may also contribute to reducing the activity of the PS-2 by prolonged Naph exposure that was demonstrated in the work of Jajoo et al. [20].

Why does an increase in ROS affect electron transfer from $Q_A^-$ and further and partly on the PS-2 donor side? It is known that ROS generated during stress caused by the influence of stress factors of different nature inhibit the synthesis of photosynthetic proteins *de novo* [32]. Proteins of PS-2 RC, D1 and D2, are the most vulnerable. Because these proteins are involved in the organization of the PS-2 RC, the structure of PS-2 is broken, which can reduce the number of active PS-2 RCs, i.e. $Q_B$-reducing RCs. In particular, disruption of D1 protein synthesis may reduce the effectiveness of electron transfer from $Q_A$ to $Q_B$.

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## REFERENCES

1. Fang, G.-C., Wu, Y.-S., Chen, J.-C., Chang, C.-N., and Ho., T.-T. (2006) Characteristic of polycyclic aromatic

hydrocarbon concentrations and source identification for fine and coarse particulates at Taichung Harbor near Taiwan Strait during 2004-2005, *Sci. Total Environ.*, **366**, 729-738.
2. Simonich, S., and Hites, R. A. (1994) Importance of vegetation in removing polycyclic aromatic hydrocarbons from the atmosphere, *Nature*, **370**, 49-51.
3. Chirkova, T. V. (2002) *Physiological Basis of Plant Resistance* [in Russian], St. Petersburg State University, St. Petersburg.
4. Kummerova, M., Krulova, J., Zezulka, S., and Triska, J. (2006) Evaluation of fluoranthene phytotoxicity in pea plants by Hill reaction and chlorophyll fluorescence, *Chemosphere*, **65**, 489-496.
5. Kummerova, M., and Vanova, L. (2007) Chlorophyll fluorescence as an indicator of fluoranthene phototoxicity, *Plant Soil Environ.*, **53**, 430-436.
6. Jajoo, A., Rao, M. N., Tomar, R. S., Grieco, M., Tikkanen, M., and Aro, E.-M. (2014) Inhibitory effects of polycyclic aromatic hydrocarbons (PAHs) on photosynthetic performance are not related to their aromaticity, *J. Photochem. Photobiol. B*, **135**, 151-155.
7. Bigler, W., and Schreiber, U. (1990) Chlorophyll luminescence as an indicator of stress-induced damage to the photosynthetic apparatus, *Photosynth. Res.*, **25**, 161-171.
8. Stirbet, A., and Govindjee (2011) On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient, *J. Photochem. Photobiol. B*, **104**, 36-57.
9. Kalaji, H. M., Golstev, V., Bosa, K., Allakhverdiev, S. I., Strasser, R. J., and Govindjee (2012) Experimental *in vivo* measurements of light emission in plants: a perspective dedicated to David Walker, *Photosynth. Res.*, **114**, 69-96.
10. Strasser, R. J., Srivastava, A., and Tsimilli-Michael, M. (2000) in *Probing Photosynthesis*: *Mechanisms*, *Regulation and Adaptation* (Yunus, M., Pathre, U., and Mohanty, P., eds.) Taylor and Francis, London, pp. 445-483.
11. Strasser, R. J., Tsimilli-Michael, M., and Srivastava, A. (2004) in *Chlorophyll a Fluorescence: a Signature of Photosynthesis*, *Advances in Photosynthesis and Respiration* (Papageorgiou, G. C., and Govindjee, eds.) Springer, Dordrecht, pp. 321-362.
12. Perlman, R. S., Yalkowsky, S. H., and Banerjee, S. (1984) Water solubilities of polynuclear aromatic and heteroaromatic compounds, *J. Phys. Chem. Ref. Data*, **13**, 555-562.
13. Kreslavski, V. D., Lankin, A. V., Vasilyeva, G. K., Lyubimov, V. Yu., Semenova, G. N., Schmitt, F.-J., Friedrich, T., and Allakhverdiev, S. I. (2014) Effects of polyaromatic hydrocarbons on photosystem II activity in pea leaves, *Plant Physiol. Biochem.*, **81**, 135-142.
14. Mehta, P., Kreslavsky, V., Bharti, S., Allakhverdiev, S. I., and Jajoo, A. (2011) Analysis of salt stress induced changes in photosystem II heterogeneity by prompt fluorescence and delayed fluorescence in wheat (*Triticum aestivum*) leaves, *J. Photochem. Photobiol. B*, **104**, 308-313.
15. Lichtenthaler, H. K., and Wellburn, A. R. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymol.*, **148**, 350-382.
16. Genty, B., Briantais, J-M., and Baker, N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochim. Biophys. Acta*, **990**, 87-92.
17. Liu, H., Weisman, D., Ye, Y. B., Cui, B., Huang, Y. H., Colon-Carmona, A., and Wang, Z. H. (2009) An oxidative stress response to polycyclic aromatic hydrocarbon exposure is rapid and complex in *Arabidopsis thaliana*, *Plant Sci.*, **176**, 357-382.
18. Bukhov, N. G., and Carpentier, R. (2000) Heterogeneity of photosystem II reaction centers as influenced by heat treatment of barley leaves, *Physiol. Plant.*, **110**, 279-285.
19. Singh-Tomar, R., and Jajoo, A. (2013) Alteration in PS II heterogeneity under the influence of polycyclic aromatic hydrocarbon (fluoranthene) in wheat leaves (*Triticum aestivum*), *Plant Sci.*, **209**, 58-63.
20. Jajoo, A., Mekala, N. R., Tomar, R. S., Grieco, M., Tikkanen, M., and Aro, E.-M. (2014) Inhibitory effects of polycyclic aromatic hydrocarbons (PAHs) on photosynthetic performance are not related to their aromaticity, *J. Photochem. Photobiol. B*, **137**, 151-155.
21. Velthuys, B. R. (1981) Electron-dependent competition between plastoquinone and inhibitors for binding to photosystem II, *FEBS Lett.*, **126**, 277-281.
22. Smirnoff, N., and Wheeler, G. L. (2000) Ascorbic acid in plants: biosynthesis and function, *Crit. Rev. Plant Sci.*, **19**, 267-290.
23. Aksmann, A., and Tukaj, Z. (2008) Intact anthracene inhibits photosynthesis in algal cells: a fluorescence induction study on *Chlamydomonas reinhardtii* cw92 strain, *Chemosphere*, **74**, 26-32.
24. Kummerova, M., Vanova, L., Krulova, J., and Zezulka, S. (2008) The use of physiological characteristics for comparison of organic compounds phytotoxicity, *Chemosphere*, **71**, 2050-2059.
25. Van Gorkom, H. J. (1986) in *Light Emission by Plants and Bacteria* (Govindjee, Amesz, J., and Fork, D. C., eds.) Academic, Orlando, pp. 267-289.
26. Rathod, D. P., Brestic, M., and Shao, H. B. (2011) Chlorophyll *a* fluorescence determines the drought resistance capabilities in two varieties of mycorrhized and non-mycorrhized *Glycine max* Linn, *Afr. J. Microbiol. Res.*, **5**, 4197-4206.
27. Goltsev, V. N., Kuzmanova, M. A., Kaladzhi, H. M., and Allahverdiev, S. I. (2014) *Variable and Delayed Fluorescence of Chlorophyll a – Theoretical Basis and Practical Application in the Study of Plants* [in Russian], Institute of Computer Science, Izhevsk-Moscow.
28. Goltsev, V., Zaharieva, I., Chernev, P., and Strasser, R. J. (2009) Delayed fluorescence in photosynthesis, *Photosynth. Res.*, **101**, 217-232.
29. Zhang, L., and Xing, D. (2008) Rapid determination of the damage to photosynthesis caused by salt and osmotic stresses using delayed fluorescence of chloroplasts, *Photochem. Photobiol. Sci.*, **7**, 352-360.
30. Chirkova, T. V. (1997) Cell membranes and plant resistance to stresses, *Soros Obrazovat. Zh.*, **9**, 12-17.
31. Duxbury, C. L., Dixon, D. G., and Greenberg, B. M. (1997) Effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*, *Environ. Toxicol. Chem.*, **16**, 1739-1748.
32. Murata, N., Takahashi, S., Nishiyama, Y., and Allakhverdiev, S. I. (2007) Photoinhibition of photosystem II under environmental stress, *Biochim. Biophys. Acta*, **1767**, 414-421.