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EFFECT OF TEMPERATURE ON THE LUMINESCENT CHARACTERISTICS IN LEAVES OF *ARABIDOPSIS* MUTANTS WITH DECREASED UNSATURATION OF THE MEMBRANE LIPIDS

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Summary. The luminescent characteristics of *Arabidopsis* wild type and two mutants, JB67 and LK3, deficient in lipid fatty acid desaturation are investigated as a function of temperature. The data reveal that lipid unsaturation exerts stronger effect on the delayed compared with on the prompt fluorescence. Some differences in the form of the induction curves of the delayed fluorescence and the kinetics of the delayed fluorescence dark relaxation between the wild type and the mutants were found. They are attributed to acceleration of the linear electron transfer, earlier activation of PSI and faster light-induced generation of proton gradient in the mutants, particularly in LK3, as compared to the wild type. The temperature course of the fluorescence stationary level indicates higher thermal stability of the *Arabidopsis* mutants than that of the wild type. The splitting of the slow phase of the induction curves of DF into two maxima at low temperature in LK3 mutant suggests that the mutation renders structural rearrangements similar to those induced by heat treatment of the wild type. The importance of the membrane lipids unsaturation for the structural organization and photosynthetic activity of thylakoid membranes is discussed.

Key words: *Arabidopsis*, delayed fluorescence, electron transport, high and low temperature, lipid unsaturation, mutants, variable chlorophyll fluorescence

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Abbreviations: IC – induction curves; LHC – light-harvesting complex; PF, DF – prompt and delayed chlorophyll florescence; PS – photosystem; Q_A – primary quinone electron acceptor of PSII; Q_B – secondary quinone electron acceptor of PSII; PQ – plastoquinone; RC – reaction centre; $I_{1\text{-}5}$ and D_2 – parameters of the delayed fluorescence induction curves; I_{max1} and I_{max2} – maxima of DF intensities in fast and slow phases of induction curves; MGDG – monogalactosyl-diacylglycerol; DGDG – digalactosyl-diacylglycerol.

Introduction

The photosynthetic membranes in higher plants are characterized by unusually high content of polyunsaturated fatty acids (Murphy, 1986). In order to investigate their functional significance, different approaches have been used including analysis of mutants with altered fatty acids membrane composition. Useful objects in this field are the *Arabidopsis* mutants, due to the relatively simple genom, which enables their easier isolation. As a result of single-locus mutations in two nuclear genes (*fad b* and *fad c)* the *Arabidopsis* mutants JB67 and LK3 were obtained (Somerville and Browse, 1988). Both are characterized by altered leaf lipid acyl composition. JB67 is deficient in the activity of the chloroplast ω-9 desaturase, which attacks $C_{16:0}$ (palmitic acid) at position sn-2 in MGDG and converts it in *cis*-$C_{16:1}$ (Kunst et al., 1989a). As a consequence about 28% of the chloroplast MGDG contain at least one molecule $C_{16:0}$ whereas 97% of the fatty acids are polyunsaturated in the wild type; the remaining of the lipids are almost unchanged (Kunst et al., 1989b). LK3 mutant is deficient in the activity of the chloroplast ω-6 desaturase, which normally desaturates $C_{16:1}$ and $C_{18:1}$ (Browse et al., 1989; Hugly et al., 1989). The polyunsaturated fatty acids are reduced at about 51% in this mutant as compared to the wild type.

During the transition of the photosynthetic apparatus from dark adapted to stationary "light" state typical changes in the intensity of both the prompt (PF) and the delayed (DF) chlorophyll fluorescence are observed (Kautski and Hirsch, 1931; Lavorel, 1975). The PF intensity at the beginning of the induction period follows the well known O-I-J-P course (Papageorgiou, 1975). In the same period DF shows quite different transient with well-pronounced maxima, denoted with the letter I and an index, which reflects its subsequent number. Minimums between the maxima are denoted with the letter D and the corresponding index. The maxima observed in the DF intensity at the millisecond time range (denoted as I_1 and I_2) coincide with the moment of maximal rate of F_o–F_i and of F_i–F_p increase in PF, and their amplitudes reflect the redox transitions in the acceptor side of PSII (Goltsev and Yordanov 1997). The appearance of the late maxima in DF (I_3 and I_4 according to Goltsev and Yordanov, 1997) in the second time range of the IC can be related to the photoinduced proton gradient as well as to the initiation of the photosynthetic dark reactions (Wraight and Crofts, 1971; Gaevski and Morgun, 1993). A well pronounced deep, D_2, is usually observed

between the fast (I_1–I_2) and slow (I_4–I_5) phases of the DF induction curves. It may be a result of closure of the RCs of PSII during the PQ pool reduction (Goltsev and Yordanov, 1997).

The induction curves of PF and DF, the kinetics of the dark relaxation of DF and the stationary levels of both types of emitted light are tightly connected with the photosynthetic activity. These are convenient parameters for *in vivo* investigations of the function of thylakoid membranes. The aim of the present study was to examine how the lipid content of thylakoid membranes influences the functional activity and temperature resistance of PSII reactions by means of luminescence characteristics of plants differing in polyunsaturated lipid content.

Materials and Methods

Plants (*Arabidopsis thaliana* wild type and its mutants JB67 and LK3) were grown for 1 month on soil in a climatic chamber at controlled conditions of temperature (25 °C/20 °C, day/night) and light (50 μmol photons $m^{-2}.s^{-1}$, 16 h d^{-1}).

Prompt and delayed chlorophyll fluorescence (PF and DF) induction curves were recorded simultaneously using a fluorometer Fl-2006 (manufactured by "Test", Russia) as described by Goltsev and Yordanov (1997). The maximal intensity of modulated light at the level of the object was 1200 μmol.$m^{-2}.s^{-1}$. PF values were registered every 11 ms as an integral emission during 5.5 ms illumination period. DF signal was read by an analog-digital converter every 50 μs during registration period. DF values of the induction curve situated *via* time interval of 11 ms represent a sum of two fast kinetic components ($\tau_1 \sim 200 \div 800$ μs and $\tau_2 \sim 1.5 \div 3.5$ ms) and a "tail" of slow components ($\tau > 20$ ms). PF and DF induction curves were registered using a phosphoroscope whose working cycle includes alternative periods of illuminating the sample, when the PF is detected, and dark periods of DF registration. The registration of DF starts about 440 μs after each light interval. During the next 5.5 ms about 110 points are collected every 50 μs. Within this time interval the DF intensity exponentially decreases. Each point of the DF induction curves is a mean value of all registered values during the DF registration period. Before measurements plants were kept in darkness for 1 h and then the detached leafs were transferred to a measuring chamber. The samples were placed in a thermostabilized holder for 3 min at the required temperature and the chlorophyll fluorescence induction curves were recorded at the same temperature. The temperature range of 5 ÷ 45 °C was explored with step of 5 °C.

Temperature curves of stationary levels of PF and DF were registered after 2 min preillumination inside the sample holder with light intensity 1200 μmol $m^{-2}.s^{-1}$. Levels of DF and PF intensities were scanned in a temperature range of 5 ÷ 65 °C, recording a value per each degree. The temperature was automatically changed at about 3 °C/min. The temperature of the object was maintained with precision of ±0.2%.

Results

The DF is assigned to charge recombination in the PSII reaction center. The $Z^+P_{680}Q_A^-$ states, generated during the last period of sample illumination, have a major contribution to the DF emitted in the millisecond time range. Thus, the DF intensity in each moment of the IC is proportional to the relative concentration of the "open" RC $ZP_{680}Q_A$ which are capable to produce "light-emitting" states upon illumination as well as to the radiative recombination quantum efficiency. On the other hand, the quantum efficiency of the radiative recombination depends both on the thylakoid membrane energization and on the redox state of the subsequent electron carrier Q_B. Therefore, one can consider $ZP_{680}Q_AQ_B$, $ZP_{680}Q_AQ_B^-$ and $ZP_{680}Q_AQ_B^=$ as "light-emitting" states, whereas $ZP_{680}Q_A^-Q_B^=$ can be marked as "non-light-emitting". The earlier changes in the DF intensity are connected with transitions between these states (Goltsev and Yordanov, 1997).

The DF induction curves registered at 25 °C in *Arabidopsis* leaves of wild type and mutants JB67 and LK3, are shown in Fig. 1. The curves are presented on a logarithmic time scale since the changes in both types of light emission intensities are much faster at the beginning of illumination and the first registered maxima appear in the millisecond time range. The intensity of the DF maxima I_1 and I_2, which is thought to reflect the dynamics of the electron transfer of the PSII acceptor side (Goltsev and Yordanov, 1997), was lower in the mutants than in the wild type. One of the differences between the three plants is that the DF intensity starts to increase earlier from D_2 to I_4 levels for the mutants. This may be the reason for the observed splitting of the slow peak in the mutants. In leaves from the wild type plants I_4 and I_5 overlap, in those from JB67 I_4 was more pronounced as a shoulder of I_5, and in those from LK3 two maxima can be well distinguished. This effect may be due to earlier PSI

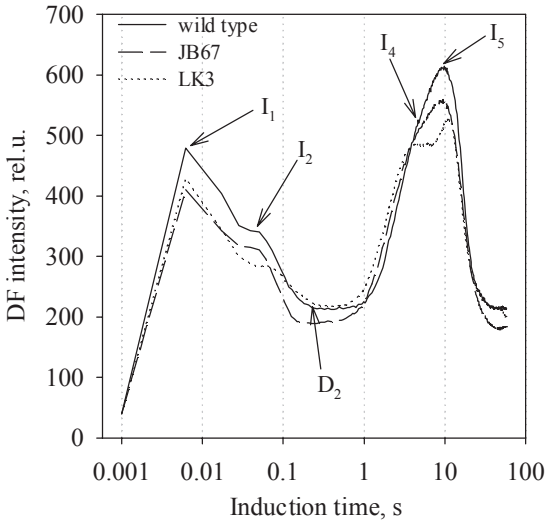

Fig. 1. Induction curves of delayed chlorophyll fluorescence of wild type and JB67 and LK3 mutants of *Arabidopsis*. Detached leaves were dark adapted for 1 h before the measurements. The chlorophyll fluorescence induction curves were measured at 25 °C. Values are mean of a minimum 3 replicates. DF induction kinetics parameters are denoted: I_1-I_5 – DF intensities at local maxima; D_2 – DF intensity at local minimum after second maximum I_2.

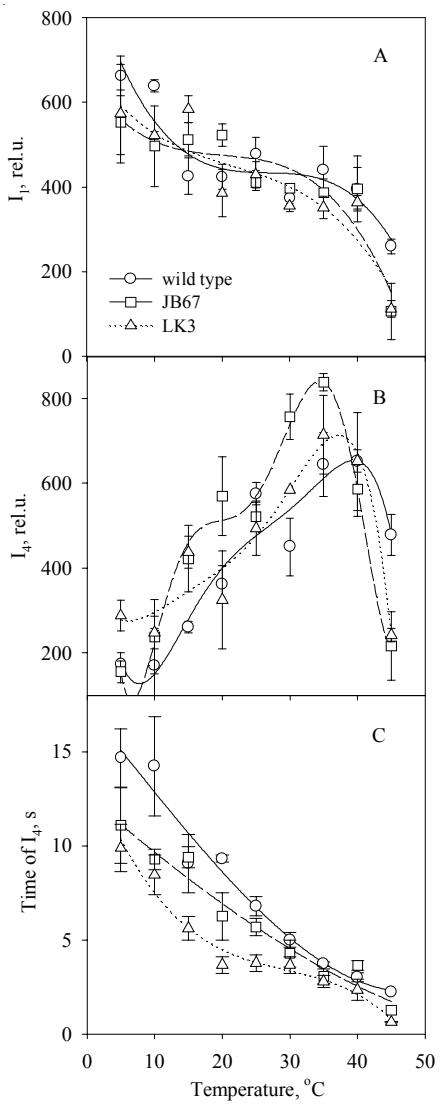

Fig. 2. Amplitudes of DF maxima I_1 (A) and I_4 (B) and time for reaching of I_4 (C) in leaves of wild type and JB67 and LK3 mutants of *Arabidopsis* as a function of the measuring temperature. The samples were dark adapted for 1 h at room temperature and for 3 min at different temperatures in the range 5÷60 °C, and induction curves were recorded at the same temperature. Data are means ±SD of three independent experiments.

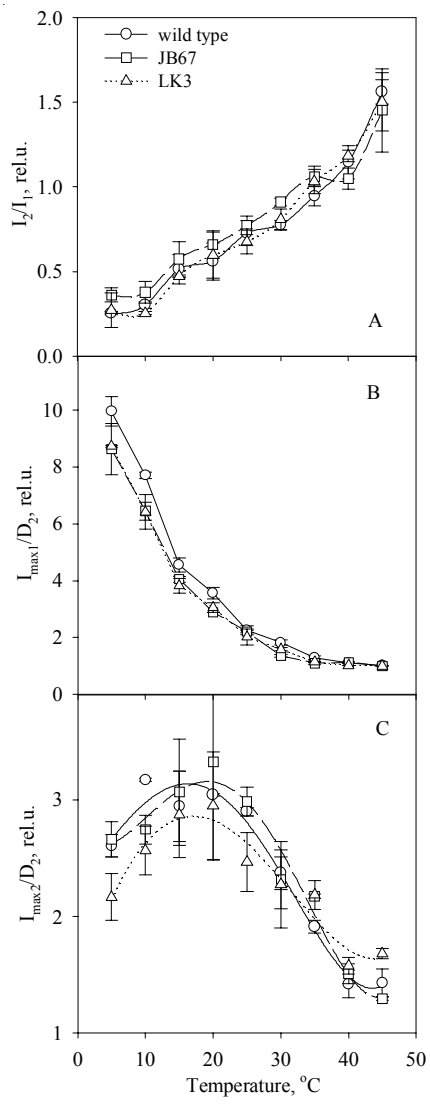

Fig. 3. Dependence of the relative DF parameters of IC (I_2/I_1 – A, I_{max1}/D_2 – B, I_{max2}/D_2 – C) on the temperature for wild type and JB67 and LK3 mutants of *Arabidopsis*. The conditions of the measurements were as in Fig. 2.

photoactivation as well as to some delay of the secondary ion transfer in the mutants (especially in LK3).

Both the intensity and the shape of DF induction curves depend on temperature. The temperature dependencies of some parameters of DF for the wild type and the mutants are shown in Fig. 2 and Fig. 3. Common trends were observed with the temperature increase for all plants studied: (i) a decrease of I_1 which may result from acceleration of the electron flow in the Q_A-Q_B section of the electron-transport chain (Zaharieva et al., 1999) (Fig. 2A); (ii) an increase of the emitted light to a certain temperature followed by a decrease at higher temperatures, known to inhibit the electron flow (Bukhov and Karapetyan, 1987; Bukhov and Mohanty, 1999), was observed in all other points of the DF induction curves (Fig. 2B). This temperature course has been already registered in leaves from other plant species – pea (Lambrev and Goltsev, 1999), barley wild type and chlorophyll b-less *chlorina f2* mutant (Zaharieva et al., 1999); (iii) all maxima of DF induction except for I_1 were reached faster with the temperature increase, as shown for I_4 in Fig. 2C, which well agrees with previous results on pea and barley leaves (Lambrev and Goltsev, 1999, Zaharieva et al., 1999). In line with the temperature-induced changes in DF induction curves described above, F_p as well as the PF intensity in the time range under study decrease (data not shown). This testifies to an acceleration of electron flow, intersystem electron transfer and a relative decrease of quasistationary PQ pool reduction.

Besides the common feature in the temperature-induced changes for all three *Arabidopsis* types investigated, some differences between the wild type and the mutants were observed. An increased thermal stability of the luminescent characteristics of the wild type as compared to the mutants was observed: the I_4 maximum reached its highest value at different temperatures for the wild type and the mutants. Inactivation for the wild type was observed at higher temperatures (above 45 °C) than for the mutants (30–35 °C); it is evident from the I_1 temperature dependence that at 45 °C this parameter declines less for the wild type than for the mutants (Fig. 2A, B).

The increase of the I_2/I_1 ratio (Fig. 3A) is an indication for electron flow acceleration through the PSII acceptor side (Goltsev and Yordanov 1997). The electron transfer in the Q_AQ_B complex was speeded up with elevating the temperature from 5 to 45 °C, but there were no differences in the values of I_2/I_1 ratio for the three plant types. There was no difference in the temperature dependence of the ratio of the maximal DF intensity in the fast phase to the minimum D_2 (I_{max1}/D_2) as well (Fig. 3B). As we have already mentioned, the I_1-I_2-D_2 transition in the fast phase correlates with the appearance and disappearance of "light-emitting" states of PSII reaction centers. These states disappeared by the PQ pool reduction and the creation of "non-light-emitting" $Q_A^-Q_B^=$ states. Accordingly, the I_{max1}/D_2 ratio could be used for estimation of the quasistationary PQ pool reduction state at the beginning of the illumination period. As a consequence, PQ pool reduction level is determined by the rate of PQ oxidation by PSI. The decrease of the I_{max1}/D_2 ratio with elevating the temperature indicates the inter-

system electron flow acceleration in the three *Arabidopsis* types. The ratio of the slow DF maximum to the D_2 minimum (I_{max2}/D_2) is indicative for the generation of the photoinduced proton gradient (Fig. 3C). Considering this parameter, the proton gradient reaches maximal values at about 15–20 °C for the three plants types. Moreover, there was no significant difference between the three plants types investigated for this parameter.

The delayed fluorescence intensity is proportional to the fluorescence quantum yield (Lavorel 1975) which varies during the induction period. Simultaneous registration of both prompt and delayed chlorophyll fluorescence allows one to analyze the ratio of both fluorescence types (DF/PF) and to qualitatively evaluate the photoinduced changes of the electron carriers' redox states and the rate constant of radiative recombination reaction in PSII reaction centers. The time courses of the DF/PF ratio for wild type, JB67 and LK3 registered during the induction period at different temperatures are presented in Fig 4. The values are normalized to the corresponding I_1 values of each curve. The response of the photosynthetic apparatus to different temperatures was practically similar for the three *Arabidopsis* plant species. The following common features were observed with the increase of the registration tempera-

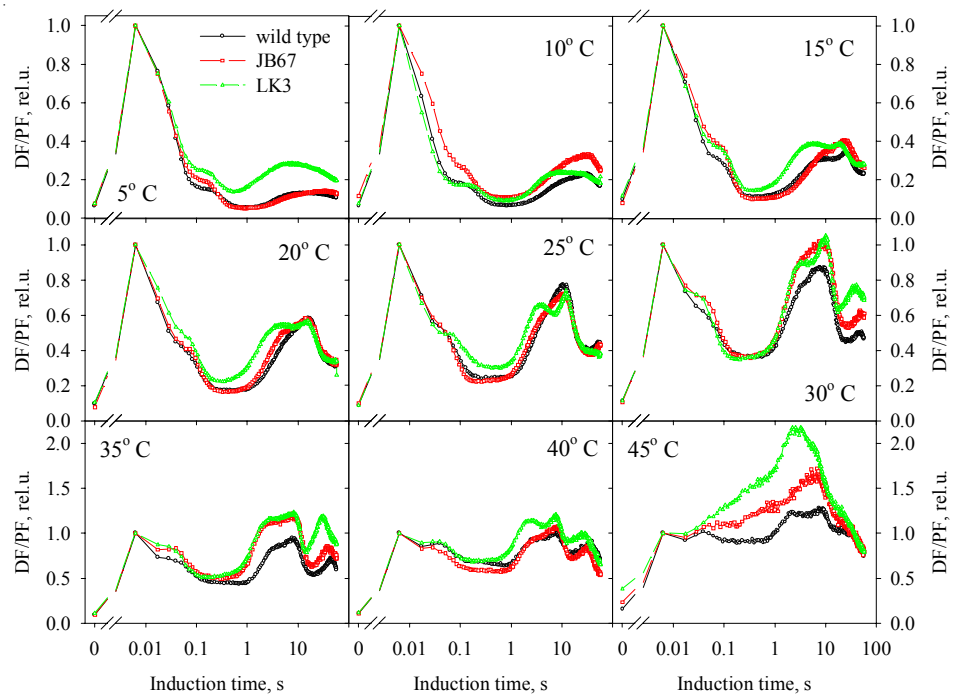

Fig. 4. Induction curves of DF/PF ratio of wild type and JB67 and LK3 mutants of *Arabidopsis*, registered at different temperatures. The conditions of the measurements were as in Fig. 2. Values are normalized to the I_1 value for each sample.

ture: (i) the relative part of I_2 in the fast DF phase increased; (ii) the slow phase of the induction curves (maximums I_4 and I_5) rose and the fast phase (I_1 and I_2) diminished; (iii) the D_2 level grew up; (iv) the observed induction maxima were shifted toward earlier moments of the induction curves; (v) a new maximum, I_6, appeared in the slow phase at temperatures above 30 °C. It can be supposed that I_6 is a result of activation of the Calvin cycle that is initiated by ATP and NADP.H accumulation. The Calvin cycle as a terminal acceptor of reduced equivalents opens the electron transfer chain, i.e. accelerates the electron transport. It leads to a rise and decline in DF and PF intensities, respectively. Thus, the maximum I_6 which is well pronounced in DF induction curves, becomes more evident if the ratio DF/PF is plotted.

Besides the common effects, some differences between the three lines in respect of the thermal-induced response were found. The maxima of the induction curves were reached earlier in both mutants than in the wild type, the effect being more pronounced for the LK3 mutant. In addition, at temperatures below 25 °C the D_2–I_4 rise was accelerated in LK3 suggesting earlier PSI activation as compared with the wild type and the JB67 mutant. These changes reflect accelerated electron transport and increased PSI activity, as previously shown by Hugly et al. (1989). Nevertheless, the DF/PF ratio was the same for the three types *Arabidopsis* species below 25 °C. The DF/PF ratio was higher for the mutants than for the wild type above 25 °C, this tendency was kept up to 45 °C. The I_4 and I_5 DF maxima were well distinguished at all temperatures above 10 °C for LK3. For JB67 this effect was less pronounced and for the wild type the splitting appeared around 45 °C. Splitting of the slow-phase maxima suggests some structural differences between the thylakoid membranes of the mutants and the wild type (more pronounced for LK3). Similar changes are likely to be induced in the wild type by high temperatures.

The DF intensity during the dark period of registration exponentially decreases and can be presented as a sum of three exponentials – a sub-millisecond component with amplitude L_1 and lifetime $\tau_1 \approx 0.4 \div 0.8$ ms, a millisecond component with amplitude L_2 and lifetime $\tau_2 \approx 1.5 \div 3.5$ ms, and a third component with lifetime much greater than the time of the registration period (which is presented as a constant). The parameters obtained from fitting the exponential decay, using the equation $L = L_1 e^{-t/\tau_1} + L_2 e^{-t/\tau_2} + L_3$, reflect the kinetics of PSII electron transport reaction. The amplitudes and the lifetimes of the three components during the induction period at 25 °C are shown (Fig. 5). The amplitudes of DF sub-millisecond and millisecond components (L_1 and L_2) in the millisecond time range of IC (about time of appearance of I_1 and I_2) are possibly determined by quasistationary concentration of PSII reaction center in $Q_A Q_B^-$ and $Q_A Q_B^=$ states, respectively. The sub-millisecond relaxation kinetics with lifetime τ_1 is determined by rate of dark dissipation of $Z^+ Q_A^- Q_B^-$ reaction center states. For the millisecond component (lifetime τ_2) it depends on the rate of disappearance of $Z^+ Q_A^- Q_B^=$ states. The reaction center states $Z^+ Q_A^- Q_B$ disappeared within ca. 100 μs and could not be registered by the equipment used in this study.

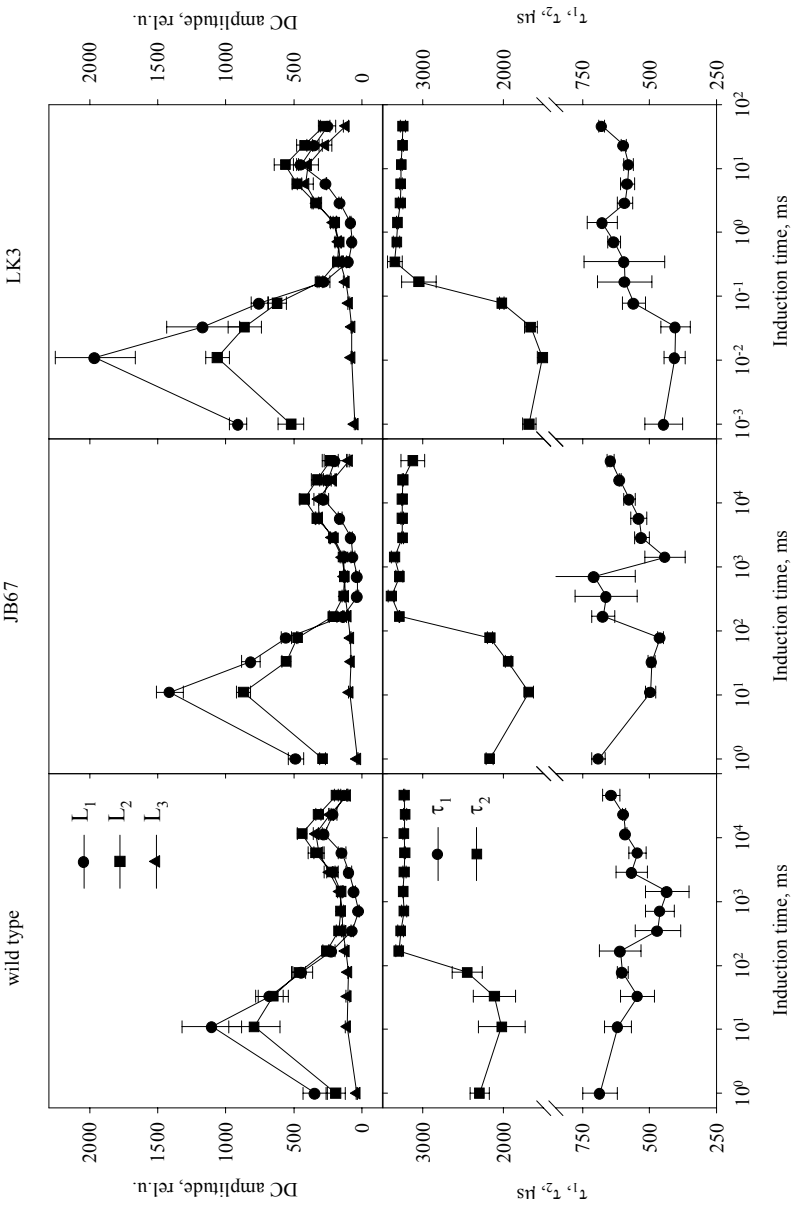

Fig. 5. Dynamic of the photoinduced changes in the amplitudes (above) and the characteristic times (bellow) of delayed fluorescence decay (DC) in wild type and JB67 and LK3 mutants of *Arabidopsis*. DF decay kinetics was measured at 25 °C. Measuring conditions in Fig. 1.

If the rate of electron transfer from $Q_B^{=}$ to PQ slows down, the relative amount of the low light emitting states $ZP_{680}Q_A^{-}Q_B^{=}$ will increase at the expense of the $ZP_{680}Q_AQ_B$ and $ZP_{680}Q_AQ_B^{-}$ states. In JB67 and LK3 leaves the relative part of the fast (L_1) DF component compared to that of the wild type was higher which pointed to an increased electron flow through the Q_B site in the former.

The lower values of τ_2 at the beginning of the induction period might be connected with a decreased quasistationary level of PQ pool reduction during the first one second after turning on the light (Zaharieva et al., 1998). This suggestion was confirmed by higher D_2 values, particularly for LK3 mutant (Fig. 4). Comparison of the τ_1 values of the three plant species during the first 100 ms of sample illumination showed considerable decrease of τ_1 mainly for LK3 mutant. This means that the direct and back electron transport reactions at PSII acceptor side are accelerated in the mutant.

The parameters of the DF relaxation kinetics are temperature dependent. Most typical were the changes in the millisecond DF component. Its temperature course had the same pattern for the three *Arabidopsis* plants. The temperature dependencies of the photoinduced changes in the lifetime (τ_2) and the amplitude (L_2) of millisecond DF component for *Arabidopsis* wild type are shown in Fig. 6. The lifetime of this component during the first 1 s of the induction period increased with the temperature increase (Fig. 6B). This could be due to a decreased affinity of the Q_B binding site in D1 protein to PQ molecules (Zaharieva et al. 1998). The increase of the initial values of τ_2 with the temperature increase could be explained also by a smaller electrical gradient which is more rapidly compensated at elevated temperatures and retains higher values at 5 °C. At higher electrical potential the light emitting recombination increases (Fleishman 1971), which results in a rise of the L_2 (Fig. 6A) and the

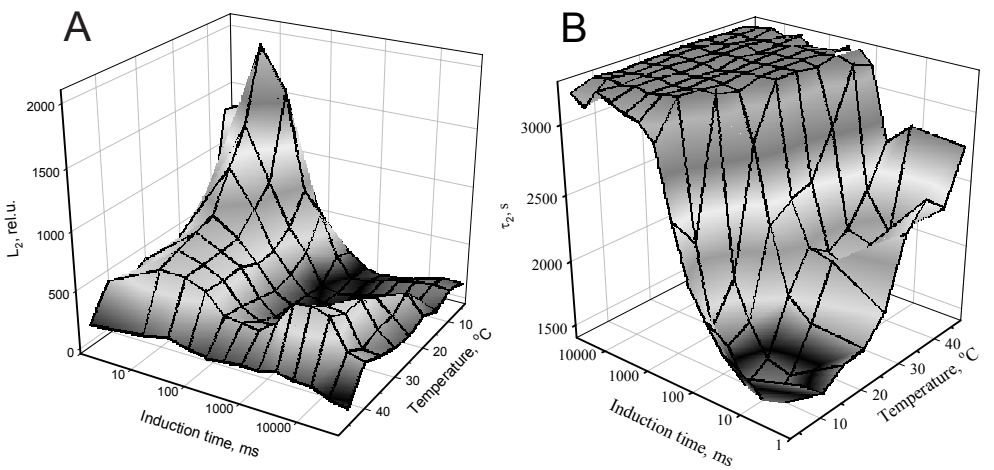

Fig. 6. Temperature dependence of photoinduced changes in the lifetime, τ_2 (A) and the amplitude, L_2 (B) of the millisecond component of DF decay for wild type *Arabidopsis*. The conditions of induction curves measurement were as in Fig. 2.

rate of non-radiative recombination with decreasing τ_2 (Venediktov et al. 1980). Another effect provoked by elevating the temperature was the faster reaching of maximal τ_2 values probably due to a faster photoinduced reduction of the PQ pool. After the first second of the induction period, when the PQ pool was reduced, τ_2 reached its maximal values within about 3.2 ms, and after that it does not change during the induction time and is independent of the temperature.

At lower temperatures the DF intensity as well as the relative part of the millisecond DF component were increased during the fast phase while the level of L_2 was diminished during the slow phase (Fig. 6A). The two phases change in opposite directions with the temperature increase, namely the relative part of the fast phase decreased and that of the slow phase increased. This fact might be related to acceleration of the acceptor side electron transport and to an increase of the relative part of the transmembrane proton gradient.

The temperature dependence of the stationary levels of both types of chlorophyll fluorescence was studied, too. The temperature curves of the stationary values of DF and PF intensities in the temperature range 5–65 °C are presented in Fig. 7 for the three types of *Arabidopsis*. The DF intensity increased with heating the sample from 5 to ca. 35 °C, then decreased and reached zero above 50 °C. The decrease of DF intensity at temperatures above 35 °C is related to inactivation of the oxygen-evolving system (Veselovskii and Vesselova, 1990). The temperature of half inactivation of the DF (reflecting the oxygen-evolving system stability) was about 45 °C, 47 °C and 48 °C for wild type *Arabidopsis*, JB67 and LK3, respectively. This suggests somewhat higher thermal stability of the mutants.

In the temperature range from 5 to 40 °C the PF intensity slightly decreases (Melcarek and Brown, 1977), which is probably due to acceleration of the electron flow. In our study we registered the thermal-induced changes of PF stationary level at actinic light intensity 1200 $\mu mol.m^{-2}.s^{-1}$. In contrast to the dead fluorescence, the PF stationary level depends not only on the antennae pigment fluorescence and the efficiency of the energy migration toward PSII reaction center, but also on the electron transport rate between the two photosystems and the reduction level of the PQ pool. A rapid increase of PF was observed at ca. 43.8 °C for the wild type and at 49.3 and 48.8 °C for JB67 and LK3, respectively. It was followed by a drop of PF at temperatures higher than 55 °C, 54 °C and 60 °C for the wild type JB67 and LK3, respectively. The fast increase of PF intensity is a result of physical separation of the light-harvesting chlorophyll-protein complexes from PSII core complex (Schreiber and Berry, 1977; Armond et al., 1978) which blocks the energy transfer and the emission of absorbed light as fluorescence. The fluorescence stationary value is found to decrease in the temperature range 50–65 °C, where structural changes in the chiral macrodomains take place (Cseh et al., 2000). The chiral macrodomains in intact thylakoid membranes have been shown to gradually disassemble between 50 and 60°C (Cseh et al., 2000). Our data on the temperature inactivation of the photosynthetic electron

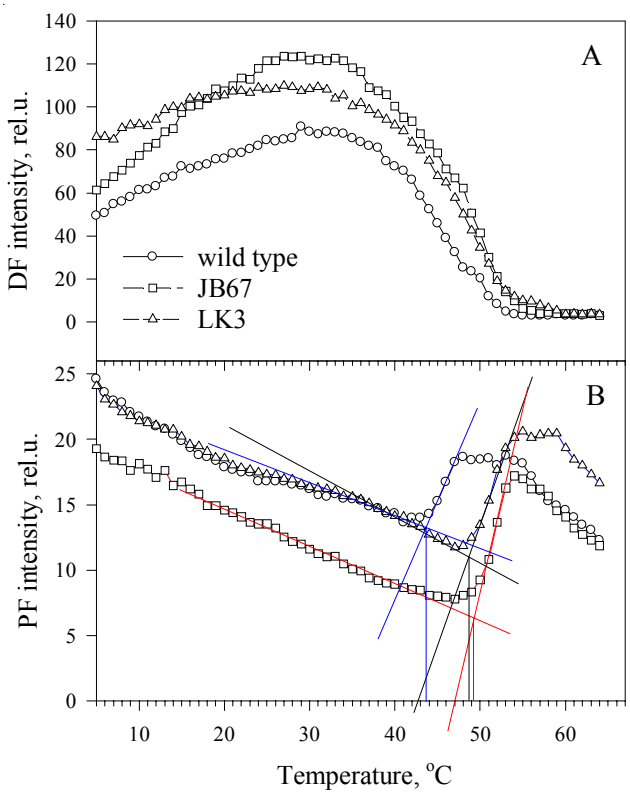

Fig. 7. Thermal-induced changes in the stationary levels of DF (A) and PF (B) in different *Arabidopsis* leaves. Detached leaves were preilluminated for 2 min with actinic light (1200 μmol.m^{-2}.s^{-1}) before measuring. The steady-state prompt fluorescence was recorded at temperatures from 5 to 65 °C.

flow agree with those obtained previously by Kunst et al. (1989b) for JB67 and Hugly et al. (1989) for LK3.

From the breakpoints of PF temperature curves presented on semilogarithmic scale from 5 to 50 °C, two transition temperatures were determined for each type of *Arabidopsis*, low (I) and high (II) (Table 1). The temperature of the first transition is slightly shifted toward higher temperatures in the mutants as compared to the wild type. The transition II in LK3 was detected at temperature somewhat higher than that in the wild type. This transition was observed at significantly lower temperature in JB67 (Table 1).

Discussion

The alteration of the lipid composition in the two *Arabidopsis* mutants (decreased content of polyunsaturated fatty acids) is accompanied by changes in the structure

and function of the lipid-protein complexes assembling the photosynthetic apparatus. The *fad b* mutation results in a 15–20% chlorophyll content reduction, a decrease of LHC II relative content, less effective energy transfer from LHC toward both photosystems, lower values of F_m and F_v/F_o (Kunst et al., 1989b). The *fad c* mutation is also accompanied by: a) a decrease of the growth rate as compared to the wild type, b) lower protein/lipid ratio, c) lower PSII activity, d) changes in chloroplast ultrastructure, expressed by diminishing of the relative part of stacked regions in thylakoid membranes and by a 36% decrease of total membranes in chloroplasts (Hugly et al., 1989). A change in the membrane lipid geometry in mutants that leads to a change in membrane topology has been reported (Hugly et al., 1989). Tsvetkova et al. (1994) using electron microscopy have shown a significant difference in thylakoid membrane structure between *Arabidopsis* wild type and JB67 and LK3 mutants. Dobrikova et al. (1997) have shown that the structure of the electrical double layer is altered and the transversal charge asymmetry is decreased in the mutants. Recently, different amounts of oligomer and monomer forms of LHCII in the mutants (JB67 and LK3) have been found (Busheva et al. 2000). The authors have also shown a higher order of LHC II aggregation in both mutants, arising from the lipid modification, which influences the organization of supramolecular complex of PSII and energy transfer between chlorophyll protein complexes in thylakoid membranes.

Comparison of the luminescent characteristics of leaves from the wild type and both mutants confirms the existence of some differences in the photosynthetic apparatus between the three *Arabidopsis* types. The transition temperatures observed (Table 1) reflect changes not only in the lipid bilayer structure but mostly conformational changes in the lipid-protein complexes. As a result of these changes alterations of the luminescent characteristics of the mutants are observed which reflect changes in the mutants functional properties.

More significant differences between the wild type and the mutants are observed in IC of the delayed than of prompt fluorescence. Earlier appearance of DF induction maxima and the splitting of the slow phase in I_4 and I_5 peaks (Fig. 1) show accel-

Table 1. Phase transition temperatures in leaves from *Arabidopsis* wild type and JB67 and LK3 mutants, calculated from PF stationary level termogramms. Plants were grown for 30 days on soil. Detached leaves were preilluminated for 2 min with actinic light (1200 μmol m^{-2} s^{-1}) before measuring. The steady-state prompt fluorescence was recorded at temperatures from 5 to 65 °C.

Strain	Temperature of the phase transition, °C	
	I	II
wild type	13	34.5
JB67	14	27
LK3	14	36

eration of the electron transfer in the whole electron-transport chain, earlier PSI activation, faster light-induced generation of proton gradient in the mutants, particularly LK3, as compared to the wild type. The fact that the I_4 and I_5 peaks in LK3 mutant are already well distinguished at low temperatures (T = 10 °C) suggests that the mutation renders structural rearrangements similar to those induced by heat treatment of the *Arabidopsis* wild type (T = 45 °C). These observations confirm the data, obtained earlier by Hugly et al. (1989), which suggest accelerated electron transport, decreased PSII activity and unchanged PSI activity in LK3 mutant, i.e. as a result of *fad c* mutation the antenna chlorophyll level is more reduced than the chlorophyll of the RCs. Kunst et al. (1989b) have shown that the PSI and PSII activity are affected to a similar extent in JB67 as a result of *fad b* mutation. Correspondingly, a reduction in the levels of antenna chlorophyll and the chlorophyll associated with the RCs has been observed (Kunst et al., 1989b). Another characteristic of the mutants is the lower quasistationary level of PQ pool reduction at the beginning of the induction period as compared to the wild type (Fig. 5).

Elevated temperature as a factor affecting the functional characteristics of higher plants initiates some common changes in all plant species studied: acceleration of the electron transport in the $Q_A - Q_B$ part of the electron transport chain (Fig. 2A, 3A); a relative decrease in the quasistationary level of the PQ pool reduction (Fig. 2B–C, 3B, 4); faster photo-induced filling of PQ pool (Fig. 6B); activation of Calvin cycle enzymes (Fig. 4).

Besides, our results show that the mutants are more sensitive to higher temperatures than the wild type (Fig. 2A and 2B). With temperature increase the electron flow and PSI activity are more strongly modified, especially in LK3 (Fig. 4, 5). As can be seen from Fig. 2A and 2B the wild type is more stable at higher temperatures. On the other hand, the parameters characterizing physical rather than functional properties of thylakoid membranes, indicate an increase thermal stability of the mutants as compared to the wild type. As shown in Fig. 7 by thermograms, thermo-induced physical dissociation of 33 kDa protein of oxygen-evolving system (Enami et al., 1994) and LHCII from PSII (Schreiber and Berry, 1977; Armond et al., 1978) occurred at lower temperatures in the wild type than in the mutants.

The increased content of polyunsaturated fatty acids enhances the high-temperature resistance of the eukaryotic cells (Raison et al., 1982). Studies of Kunst et al. (1989b) and Hugly et al. (1989) confirm that this is valid for JB67 and LK3 *Arabidopsis* mutants, too. However, as it has been previously shown, some mutations leading to deficiency in activity of two other chloroplast fatty acid desaturases do not enhance the thermal stability of chloroplast membranes of *Arabidopsis* (McCourt et al., 1985; 1987). So, it is not simply the number of double bonds in membrane fatty acids that is the critical factor for the high temperature resistance of plants (Kunst et al., 1989b). The relatively small differences found in the induction curves between mutants and wild type as well as the similar changes induced by elevated temperatures, could be

explained by development of adaptation adjustments in the mutants. Supportive of this assumption is the fact that the electron flow acceleration and splitting of the slow phase DF maxima observed in the mutants are found also in the wild type, but at temperatures significantly exceeding the optimal for *Arabidopsis*. This suggests that in order to adapt to environmental conditions, plants with a decreased content of polyunsaturated fatty acids should have changed the structure of the lipid-protein complexes participating in the photosynthetic process in order to ensure equalizing of the conformational mobility of these complexes or of some functional groups as compared to normal plants. As a result of these changes mutants are adapted mainly to conditions optimal for their growth. From thermodynamic point of view, we could speculate that in the mutants the capacity for adaptation to environmental changes, i.e. for transition between different physiological states, might be "exhausted" and they cannot answer effectively to other stress factors – for instance, to high temperature. Similarly, during the photosynthetic apparatus transition from dark adapted state to working state, the "imperfections" of the photosynthetic functions in mutants, for example lower functional efficiency at sub-optimal temperatures, are expressed.

From the data presented in this paper it can be concluded that the higher degree of membrane lipid unsaturation, even not influencing directly the primary photosynthetic reaction, can affect later stages of the photosynthetic process thus leading to lower adaptability of the photosynthetic apparatus to stress conditions.

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