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Analysis of high temperature stress on the dynamics of antenna size and reducing side heterogeneity of Photosystem II in wheat leaves (*Triticum aestivum*)

Sonal Mathur [a], Suleyman I. Allakhverdiev [b,c], Anjana Jajoo [a,*]

[a] School of Life Science, Devi Ahilya University, Indore 452017, M.P., India
[b] Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia
[c] Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia

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ABSTRACT

This study demonstrates the effect of high temperature stress on the heterogeneous behavior of PSII in Wheat (*Triticum aestivum*) leaves. Photosystem II in green plant chloroplasts displays heterogeneity both in the composition of its light harvesting antenna i.e. on the basis of antenna size (α, β and γ centers) and in the ability to reduce the plastoquinone pool i.e. the reducing side of the reaction centers (Q_B-reducing centers and Q_B-non-reducing centers). Detached wheat leaves were subjected to high temperature stress of 35 °C, 40 °C and 45 °C. The chlorophyll *a* (Chl *a*) fluorescence transient were recorded *in vivo* with high time resolution and analyzed according to JIP test which can quantify PS II behavior using Plant efficiency analyzer (PEA). Other than PEA, Biolyzer HP-3 software was used to evaluate different types of heterogeneity in wheat leaves. The results revealed that at high temperature, there was a change in the relative amounts of PSII α, β and γ centers. As judged from the complementary area growth curve, it seemed that with increasing temperature the $PSII_\beta$ and $PSII_\gamma$ centers increased at the expense of $PSII_\alpha$ centers. The reducing side heterogeneity was also affected as shown by an increase in the number of Q_B-non-reducing centers at high temperatures. The reversibility of high temperature induced damage on PSII heterogeneity was also studied. Antenna size heterogeneity was recovered fully up to 40 °C while reducing side heterogeneity showed partial recovery at 40 °C. An irreversible damage to both the types of heterogeneity was observed at 45 °C. The work is a significant contribution to understand the basic mechanism involved in the adaptation of crop plants to stress conditions.

1. Introduction

Photosynthesis is one of the most important metabolic processes in plants and its study provides information about the general "health" of plants. The main protein components of the photosynthetic electron transport chain are Photosystem II (PSII), Photosystem I (PSI), the cytochrome ($Cytb_6f$) complex, and ATP synthase. The majority of PS II reaction centers (RC) with their main light harvesting complex (LHC) II are located in the grana. The structural and functional aspects of PS II are interrelated. Photosystem I (PSI) is localized in stroma-exposed thylakoid membranes [1]. Photosystem II is a multi-subunit complex consisting of several different types of chlorophyll binding components whose function is to organize the chlorophylls for light harvesting and harbor the electron transport cofactors needed for the oxidation of water [2].

Global warming, accompanied by an increased frequency of periods with exceptionally high temperatures, is one of the most important characteristics of accelerated climatic changes. High temperatures adversely affect plant growth and survival but the impact of high temperature on the photosynthetic apparatus is considered to be of particular significance because photosynthesis is often inhibited before other cell functions are impaired [3]. Extensive research over the last years has focused on the organization and structure of the photosynthetic complexes in response to high temperature stress. There are at least three major stress-sensitive sites in the photosynthetic machinery the PSII, the ATPase and the carbon assimilation process [4]. Rubisco activase, the protein that facilitates the release of sugar phosphates from Rubisco ceases to function properly at high temperatures, inactivating rubisco [5–8]. In the photosynthetic machinery, Photosystem II has been found to be one of the sensitive components for thermal denaturation [9–11]. Heat stress also affects processes related to membrane stacking, membrane integrity, ion conductivity, and phosphorylation activity [9]. Heat induces the dissociation of the manganese-stabilizing 33 kDa protein from the PSII reaction center complex followed by a release of

Abbreviations: ABS, absorbance; Chl, chlorophyll; CS, cross-section; $Cytb_6f$, cytochrome; DCMU, 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea; DIo, dissipation; ETo, electron transport flux beyond Q_A; FI, fluorescence induction curve; Fm, maximal Chl *a* fluorescence; Fo, minimal Chl *a* fluorescence; FR, fluorescence rise; Fv, variable fluorescence; K, J, I, intermediate steps of Chl *a* fluorescence rise between Fo and P; LHC, light harvesting complex; OEC, oxygen evolving complex; PEA, plant efficiency analyzer; PS II, Photosystem II; PQ, plastoquinone; Q_A, primary plastoquinone; Q_B, secondary plastoquinone; RC, reaction center; TR, trapping

* Corresponding author. Tel.: +91 731 2477166; fax: +91 731 4263453.
E-mail address: anjanajajoo@hotmail.com (A. Jajoo).

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the manganese atoms [12,13]. Heat inactivation of PS II may be accompanied with the aggregation and subsequent dissociation of the LHC II [11].

It is well established that PSII of higher plants is not homogenous in nature [14]. The PSII varies in its structure and function both and this diverse nature of PSII is known as Photosystem II heterogeneity. The concept of PSII heterogeneity was introduced to explain the biphasic nature of the kinetics of primary PSII activity. Two main aspects of PSII heterogeneity have been studied widely i.e., PSII antenna heterogeneity and PSII reducing side heterogeneity. On the basis of the differences in the antenna size α, β and γ centers have been defined while on the basis of acceptor/reducing side function, Q_B-reducing and Q_B-non-reducing centers have been defined. Extent and nature of PSII heterogeneity may vary under different physiological conditions [14] i.e. salinity stress, temperature stress etc.

Analysis of the biphasic data suggested the presence of two distinct populations of PSII centers in the chloroplast, termed as PSIIα and $PSII_\beta$ [15–17]. Later by using kinetic analysis of fluorescence induction curve of DCMU poisoned chloroplast, PSII was resolved into three components i.e. PS II α, PS II β and PS II γ [18]. Four types of PSII heterogeneity have been also reported [19] but the later studies have emphasized only on three major types of heterogeneity. In a step-wise process in the development of PSII units, the addition of LHC II-inner portion first augments the antenna of $PSII_\gamma$ (contains ~50 Chl) by about 80 Chl to yield $PSII_\beta$ (~130 Chl). In a second step, the addition of LHC II-peripheral part increases the antenna size by another 80chl to yield $PSII_\alpha$ [20]. The dominant form, PSII α, is localized in the grana partition regions [1] and is responsible for the majority of the water oxidation activity and plastoquinone reduction. These centers possess a Chl *a* core complex, an accessory Chl *a*–*b* light harvesting inner antenna (LHC II-inner), and a peripheral antenna (LHC II-peripheral) containing a combined total of about 210–250 Chl a and Chl b molecules [21]. These have a higher absorption cross-section area due to association with the peripheral Chla/b LHCs. $PSII_\alpha$ are characterized by a large light harvesting antenna and possibility of excited states transfer between PSII units that is reflected in a sigmoidal fluorescence rise when measured with DCMU. On the other hand $PSII_\beta$ are mainly located in stromal region of thylakoid membranes and are characterized by about 2.5 times smaller light harvesting antenna of $PSII_\alpha$ and impossibility of the excited states transfer between PS IIs that is reflected in an exponential fluorescence rise when measured with DCMU. Smaller antenna size has been ascribed to the absence of peripheral LHC II in PSII. The intrinsic trapping and fluorescence property of α and β centers are considered to be similar [22]. As proposed by Black et al. [17] $PSII_\alpha$ and $PSII_\beta$ differs also on the level of the primary acceptors; α-centers possess an acceptor Q_1 which is reduced nearly completely by a single flash and remains stable for a long time in presence of DCMU and hydroxylamine [23–25] while some of β-centers possess a less stable acceptor Q_2 which requires a series of saturating flashes for its stabilization in the reduced form [26]. Differences were also found in the recombination rate (reopening) in the α and β centers [15,27]. Apart from their lateral location the two types of PSII are thought to differ in terms of their kinetic properties, apparent mid-point potential of their primary electron acceptors, connectivity to the plastoquinone pool, as well as their DCMU sensitivity [28]. The O to I rise in the fluorescence induction curve demonstrated by Strasser (1981) [29] was shown to represent an increase in the variable fluorescence yield due to which represents the inactive centers [30]. The $PSII_\gamma$ is localized in stroma lamellae region [20], has the smallest antenna size among the three components and has the maximum lifetime. $PSII_\alpha$ is believed to be the major 'normal' PSII centers whereas $PSII_\beta$ and $PSII_\gamma$ represent the two minor groups of 'abnormal' PSII centers with low quantum efficiencies due to their slow electron donation systems. The $PSII_\beta$ and PSII γ have slow rate as compared to the α center. The slow rate might be due to slow electron donation to their reaction center, which might undergo many turnovers via back reaction under continuous excitation, until their reduced primary acceptors were stabilized by the electron donation into the system [20]. PSIIα is the only phase that responds to depletion of Mg^{2+} while $PSII_\beta$ and PSII γ are not sensitive to the addition of ferricyanide, Mg^{2+}, and light intensity but are accelerated by the addition of electron donors like hydroxylamine. The $PSII_\beta$ and PSII γ centers also have defects in their quinine-iron complexes and thus are unable to reduce plastoquinone [18].

In addition to heterogeneity on the antenna size, PSII centers also display heterogeneity related to the reducing side of Q_A^- with respect to electron flow to the plastoquinone pool. Several investigators have shown that a number of PS II centers, though photochemically competent, are unable to transfer electrons efficiently from electron acceptor Q_A^- to secondary electron acceptor Q_B [24,27,30–32]. Using Lavergne's nomenclature [24] these centers are termed as PS II Q_B-non-reducing. In such centers Q_A^- can only be reoxidized by a back reaction with the donor side of PSII [33]. Q_B-non-reducing differs from Q_B-reducing center in being incapable of reducing the PQ pool. Q_B-non-reducing is normally either equal to $PSII_\beta$ or a subset of it [32]. With QB-non-reducing centres are located in the non-appressed regions while Q_B-reducing centres are present in the appressed regions of the thylakoids [34]. It is found that, in the inactive centers, the electron transfer from the primary acceptor Q_A to PQ is more than 1000 times slower than the active centers [35]. In spinach chloroplasts, the pools of PS II_β and Q_B-non-reducing centers are identical [30].

The biological processes in plants that are involved in adaptation to stress are complex. Several biological macromolecules including proteins and lipids are involved in these adaptive mechanisms. In case of high temperature stress, adaptive mechanisms include change in the composition and degree of saturation of fatty acids [36], changes in the levels of protective antioxidants [37,38] and treinoic fatty acids [39], changes in membrane permeability and increased cyclic electron flow [9,40]. In addition to these, synthesis of heat-shock proteins [41], activation of synthesis of new form of Rubisco activase [6,7], rapid dephosphorylation of PS II facilitating repair of PS II [42] also play very important roles to enable plants to tolerate heat stress. PS II rearrangements seem to be an adaptive mechanism of plants to tolerate stress conditions.

Earlier studies have used chl *a* induction kinetics to study high temperature and salinity induced changes on the function of PSII [43,44]. In the present study we have determined the changes in different types of heterogeneity of PS II in response to high temperature stress in wheat leaves. The effect of high temperature on wheat leaves on the two types of PSII heterogeneity (including α, β, γ and Q_B-reducing and Q_B- non-reducing type of heterogeneities) simultaneously has never been explored so extensively till yet. It is necessary to understand the physiological basis of high temperature stress tolerance in crop plants. We want to investigate the probable mechanism by which PSII may respond to high temperature stress through manipulation of its heterogeneous structure and function. It will be a significant contribution to elucidate the mechanism involved in the adaptation of crop plants to stress condition.

2. Materials and methods

2.1. Plant material: wheat (Triticum aestivum)

Lok-1 cultivar of wheat was used for experiments. Wheat seeds were allowed to germinate and then transferred to petriplates containing Knop solution with a photosynthetically active photon flux density (PPFD) of 300 μmol m^{-2} s^{-1} in 18 h of white light/6 h dark condition at 22 °C The plantlets were grown up to two leaf stages and then the high temperature treatment was given to them in dark.

2.2. High temperature treatment

The wheat leaves were immersed in a water bath (Julabo F10-UC, Germany) for 15 min at temperatures of 25 °C, 35 °C, 40 °C, 45 °C. The

temperature stress was given in complete dark. The measurements were performed two inches away from the tip and the base i.e. in the middle portion on the ventral surface of the leaves i.e. the abaxial surface.

2.3. Measurement of fluorescence induction kinetics

The chlorophyll *a* (Chl *a*) fluorescence induction kinetics was measured at room temperature using a Plant Efficiency Analyzer (PEA), (Hansatech England). Excitation light of 650 nm (peak wavelength) from array of three light-emitting diodes is focused on the surface of the leaf to provide a homogenous illumination. Light intensity reaching the leaf was 3000 μmol m^{-2} s^{-1} which was sufficient to generate maximal fluorescence for all the treatments. The fluorescence signal is received by the sensor head during recording and is digitized in the control unit using a fast digital converter. Energy pipeline model were deduced using the Biolyzer HP 3 software (the chlorophyll fluorescence analyzing program by Bioenergetics Laboratory, University of Geneva, Switzerland) and specific energy fluxes were calculated from it.

Leaves incubated at 25 °C exhibit a polyphasic rise called O–J–I–P chlorophyll *a* fluorescence transient; the O to J phase (ends at ~2 ms), the J to I phase (ends at ~30 ms) and I to P phase (ends at ~500 ms). The JIP test is named after the basic steps in the fluorescence transient when plotted on a logarithmic time scale [45]. The O to J phase is due to the net photochemical reduction of Q_A to Q_A^-. The intermediate I step and the final P step have been proposed to be due to existence of fast and slow reducing plastoquinone (PQ) pool, as well as due to different redox states of the reaction centers (RC) of PS II which reduces the PQ pool [44]. Also O = Fo for dark-adapted sample, P = Fm for a dark-adapted sample, J and I = are the shoulder and K = an additional step resulting under heat stress which appears at 200–400 μs.

The energy fluxes were calculated using Biolyzer HP3 software. The following fluxes were measured. ABS/RC, TRo/RC, ETo/RC, DIo/RC. The ABS/RC represents the total number of photons absorbed by chl molecules of all RCs divide by the total number of active RCs. TRo/RC represents the maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A. ETo/RC depicts the reoxidation of reduced Q_A via electron transport in an active RC. It reflects the activity of only the active RCs. DIo/RC represents the ratio of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs [45].

The connectivity for different centers was calculated according to Lazar et al. (2001) [46]. The curves are presented by means of relative variable fluorescence (rFv(t)), which is defined as (F(t) − Fo)/(F_M − Fo), where Fo, F_M, and F(t) are the minimal and maximal measured fluorescence intensity at time t respectively.

2.4. Determination of Q_B-reducing and Q_B-non-reducing centers

The double hit method of Strasser [47] was followed for the calculation of Q_B-reducing and Q_B-non-reducing centers. In this method two fluorescence transients were induced by two subsequent pulses (each of 1 s duration). The first pulse (denoted as 1st hit) was conducted after a dark period long enough to ensure the reopening of all reaction centers, followed by a second pulse (2nd hit). The duration of the dark interval between two hits was 500 ms (Fig. 1).

$$Fv = Fm–Fo, Fv^* = Fm^*–Fo^*$$

Fv: variable fluorescence of 1st hit, Fm: maximal fluorescence of 1st hit,

Fv*: variable fluorescence of 2nd hit, Fm *: maximal fluorescence of 2nd hit

Fo: minimal fluorescence of 1st hit; Fo*: minimal fluorescence of 2nd hit.

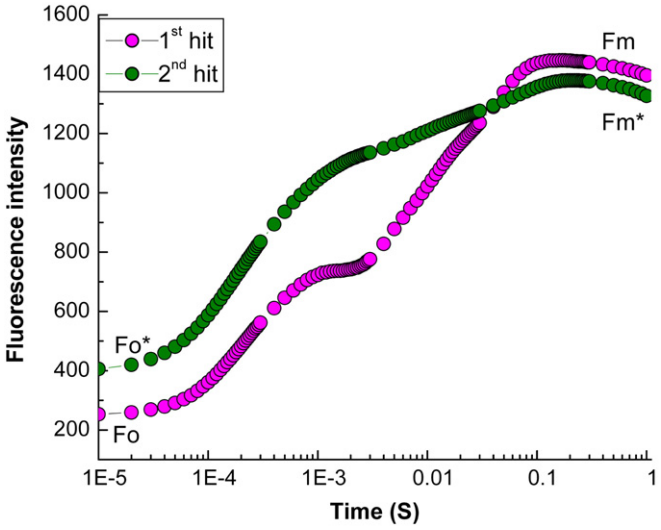

Fig. 1. The O–J–I–P transient provided by numerical simulations on estimation of Q_B-non-reducing RCs. The fluorescence measurement were induced by two subsequent pulses (each of 1 s). The first pulse (denoted as 1st hit) was conducted after a dark period long enough to ensure the reopening of all reaction centers, followed by a second pulse (2nd hit). The duration of the dark interval between two hits was 500 ms.

Q_B-non-reducing centers were calculated by the following equation:

$$Vo(Bo) = [(Fv / Fm)–(Fv^* / Fm^*)] / (Fv / Fm)$$

where Bo = Relative amount of Q_B-non-reducing PSII centers

2.5. Determination of antenna heterogeneity

Determination of the heterogeneity of PSII from fluorescence rise (FR) curve measured with DCMU was first introduced by Melis and Homann [15,48]. For calculation of antenna heterogeneity DCMU poisoning method was used [29,49]. The method is described below.

The detached leaves were put into small tray filled with 100 ml DCMU solution overnight and in complete darkness (the DCMU concentration was 200 μM, [50] and the solution contained 1% ethanol, which was used to dissolve the DCMU). The leaves were removed from the DCMU solution (in darkness), wiped and left in the air for ~1 h to avoid possible effects of anaerobiosis. Following this, high temperature stress was given to the leaves in complete darkness and recorded.

Alpha (α), beta (β) and gamma (γ) centers were calculated from the complementary area growth curve [15,30,48]. It involved the calculation of growth of normalized complementary area, defined by the fluorescence induction curve and the line parallel with the maximum level of fluorescence (Fm), with time. Kinetics of complementary area of the dark-adapted sample was fitted with three exponentials phases (corresponding to α, β and γ). Based on the lifetimes (τ) of each of the fraction (the lifetime (τ) of the fastest α component was ~0.37 ms, the β component was about 3.8-fold slower (τ ~1.44 ms) the γ component being slowest was (τ ~9.14 ms), their contribution to the total amplitude (A) of the kinetics of complementary area has been indicated as percentage of α, β and γ centers [29,49]. The kinetics of Q_A-accumulation was obtained by the calculation of the kinetics of complementary area [B = ∫(Fm − Ft)dt], where B is the double normalized (between 0 and 1) kinetics of complementary area [51] and the B kinetics of the first light pulse were fitted with three exponentials that correspond to α, β and γ type centers.

3. Results and discussion

Electron transport in PSII can easily be probed by fluorescence measurements. Fig. 2 shows fluorescence induction curve in wheat

leaves treated with high temperature. When dark-adapted plants are illuminated by strong actinic light, fluorescence rises from its minimal level (Fo or O) to its maximal level (Fm or P) in three distinct phases separated by two shoulders named J and I. This O–J–I–P fluorescence transient is called as fluorescence induction (FI) curve [51]. When a photosynthetic organism is exposed to strong heat stress, the oxygen evolving capacity decreases due to a blockage between the OEC and PSII before tyrosine Yz. Under the same conditions, an additional step, denoted as K-step appears 300 μs in the fluorescence transient (now OKJIP). The K step is very specific for high temperature. The K step indicates that the OEC has been damaged completely and at 45 °C a permanent K step was observed. The heat treatment induced irreversible damage to the photosynthetic apparatus. Thus, injury to the OEC due to heat stress induces the K-step by inhibiting efficient electron donation to the RC [44,52]. The physiological status of PSII donor side influences the OJIP curve particularly the J–I rise [53].

3.1. Thermal effects on the antenna heterogeneity

The antenna heterogeneity involves antenna size heterogeneity as well as energetic connectivity between PS IIs [46]. The antenna size heterogeneity deals with the relative amounts of α, β and γ centers. It is usually studied in plants with Q_A^- reoxidation inhibited by a herbicide. By using a herbicide (DCMU in this study) the linear flow of electron is inhibited causing rapid antenna reorganization (e.g. state transition or Violaxanthin/Zeaxanthin conversion) thus allowing the measurement of antenna dynamics [54]. The presence of DCMU inhibits PSII at the quinone B (Q_B) binding sites.

By analyzing the kinetics of the complementary area growth curve three components were distinctly observed (α, β and γ) [29,49]. These components were ascribed to different populations of PSII units that differ in antenna size. The antenna size of a β center is supposed to be 2–3 fold smaller than that of α center and does not demonstrate exciton migration between different units [55] and the antenna size of γ center is supposed to be smaller than the alpha center. The kinetics of Q_A-accumulation was obtained by the calculation of the kinetics of complementary area [$B = \int (Fm - Ft)dt$], where B is the double normalized (between 0 and 1) kinetics of complementary area [51] and the B kinetics of the first light pulse were fitted with three exponentials that correspond to α, β and γ type centers. The results obtained from complementary area growth curve method (Fig. 3A–D) prompted a direct measurement of the relative amounts of $PSII_\alpha$, $PSII_\beta$ and $PSII_\gamma$ in leaves kept at different temperatures. The curves shown in Fig. 3A–D give us information about the lifetime and the relative proportions of α, β and γ in high temperature treated wheat leaves. In control leaves (Fig. 3A), the lifetime (τ) of the fastest α component was ~0.37 ms and it represented ~72% of the total amplitude, the β component was about 3.8-fold slower (τ ~1.44 ms) and it was responsible for ~25% of the total amplitude and the γ component being slowest was (τ ~9.14 ms) ~3% of the total amplitude. Subsequent increase in temperature from 25 °C to 45 °C led to a decrease in the proportion of α centers while β and γ centers showed an increase. The β and γ centers seemed to increase at the cost of α centers and thus these components probably are interconvertable depending on the environmental conditions. The proportion of α:β:γ centers was 72:25:3 in control, 61:26:14 at 35 °C, 59:32:8 at 40 °C and 45:42:13 at 45 °C. These results suggest that the $PSII_\alpha$ centers have been converted into $PSII_\beta$ and $PSII_\gamma$ centers or in other words the active centers have been converted into inactive one. Changes in antenna organization following a heat treatment involve the dissociation of $PSII_\alpha$ into free LHC II and $PSII_\beta$ and that the latter migrates from the grana to the non-appressed thylakoid membranes [28]. The fast α component of the fluorescence induction curve decreased upon heating leaves at various high temperatures (25–45 °C) and the slow β component became dominant at the expense of α component. After incubation at 45 °C it was revealed that there were 42% β centers which was only 25% in control leaves and 13% γ centers which were only 3% in control leaves. The α phase represents a group of PSII reaction centers, each of which are associated with a large number of pigment molecules. Once the high temperature stress begins, there is a dissociation of the pigment molecules (which are probably bound to proteins) from these α PSII centers, leaving behind smaller complexes which are probably detected as an increase in the number of β and γ centers [56]. The inhibition of OEC and the variations in the complementary areas seems to bring about a partial conversion of PSII $_\alpha$ to the β and γ center [18].

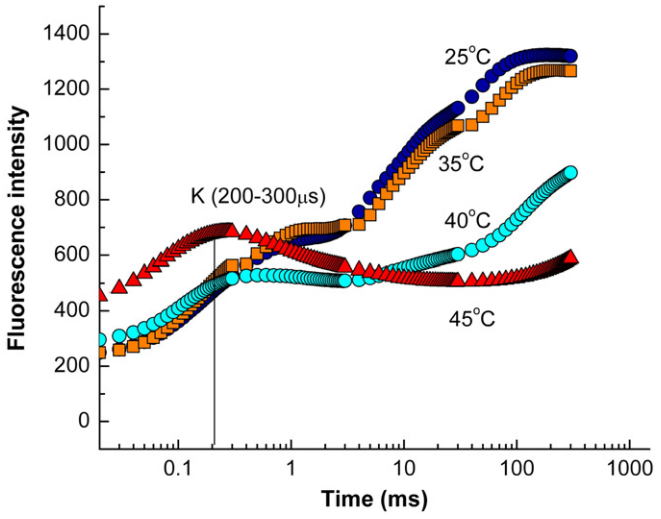

Fig. 2. Changes in Chl *a* fluorescence induction curves in wheat leaves treated with elevated temperature 25 °C, 35 °C , 40 °C and 45 °C.

The antenna size heterogeneity was also calculated on the basis of connectivity (Fig. 4). According to the concept of connectivity (also called grouping) closed PSII reaction centers (RC) may transfer their excitation energy to the open neighboring PSII units that results in sigmoidal fluorescence rise instead of exponential rise [47]. It was suggested that the three populations of PSII units (α, β and γ) may be different in their connectivity properties i.e. the α-centers are supposed to be grouped. These centers are capable of exchanging excitation energy with each other [57], whereas the two others are not, and the trapping efficiency of the γ-centers is thought to be lower. $PSII_\beta$ were characterized by an exponential rise if the time course of complementary area (CA), whereas $PSII_\alpha$ showed a non-exponential (sigmoidal) rise [15]. The exponential shape of this rise for $PSII_\beta$ was suggested to reflect mutual energetic separation of these PSII which are unable to exchange excitation energy [57]. On the other hand, the non-exponential fluorescence rise of $PSII_\alpha$ is generally believed to reflect energetic connectivity between these PSIIs. The fluorescence rise (FR) curves measured with DCMU and high temperatures treated wheat leaves is shown in Fig. 4. The curves are presented by means of relative variable fluorescence (rFv(t)), which is defined as $(F(t) - Fo)/(F_M - Fo)$, where Fo, F_M, and F(t) are the minimal and maximal measured fluorescence intensity at time t respectively [46]. As compared to 25 °C (control) a gradual loss of connectivity was observed in the FR of 40 °C but the FR of 45 °C showed no grouping at all. As the temperature was increased the sigmoidal component of the curve decreased suggesting a decrease in the connectivity between antenna molecules and an increase in the number of inactive centers. Temperature at 45 °C shows an additional positive L step. The presence of this L step indicates that the PSII units in high temperature treated samples are less grouped or less energy was being exchanged between independent PS II units [10]. Loosing cooperativity (ungrouping) indicates that the PSII units of high temperature treated samples lost stability and became more fragile.

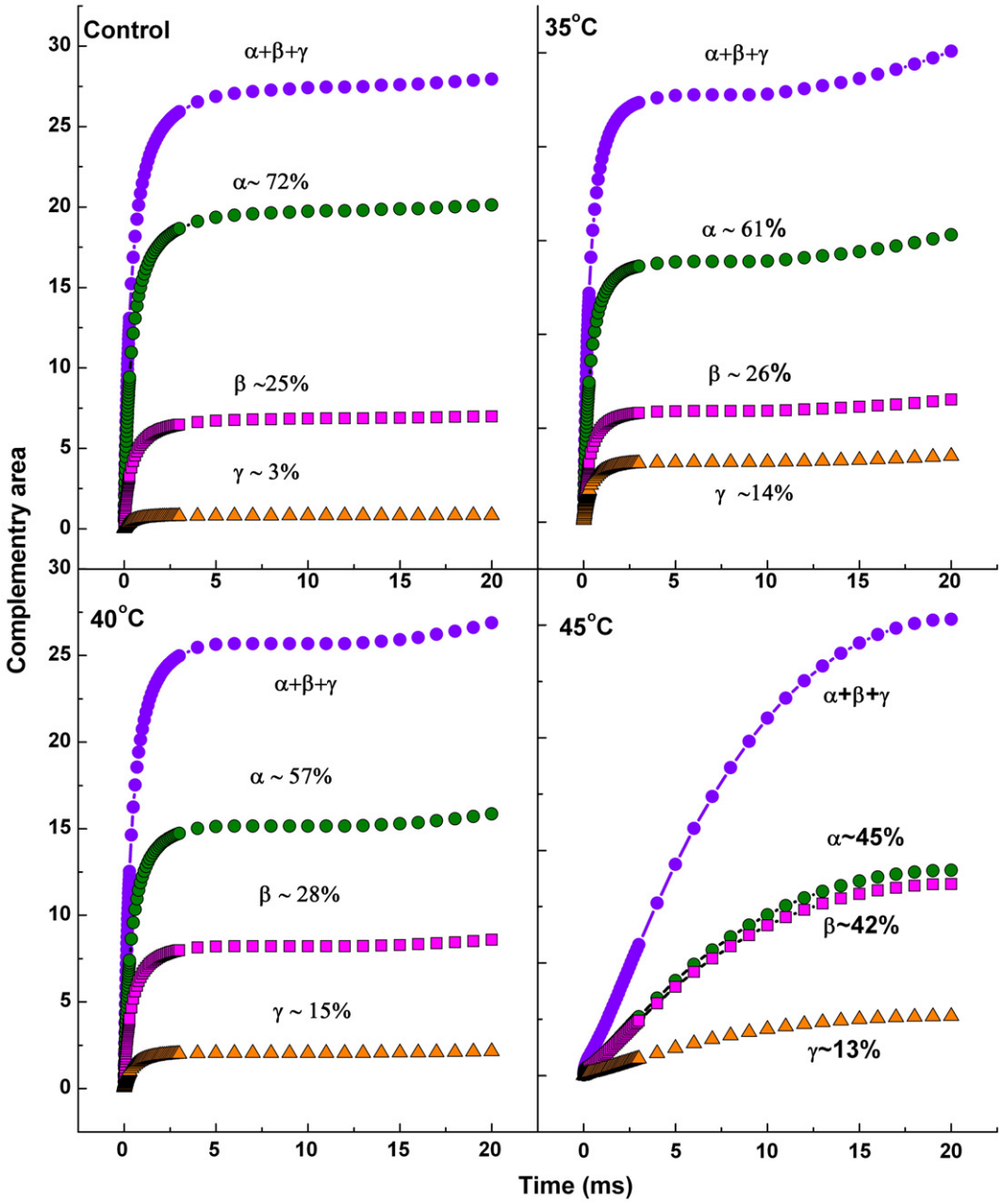

Fig. 3. Complementary area growth curve (A Control (25 °C), B 35 °C, C 40 °C D, 45 °C) showing percentage of α, β and γ centers with increasing temperature.

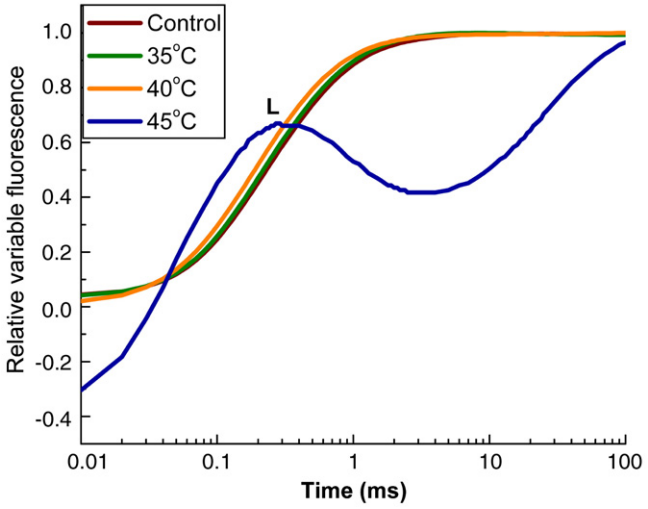

Fig. 4. Time course of the DCMU–FR curves in wheat leaves treated with different temperature (25 °C, 35°, 40 °C and 45 °C). The curves are presented in terms of rFv(t) starting from 10 μs (Fo) and finishing at 10 ms.

The appearance of K-step may also reflect the changes in the energetic connectivity between the PSII units. A loss in connectivity also indicates that the fraction of closed RCs i.e. Q_B-non-reducing centers has also increased [58].

3.2. Thermal effects on the reducing side heterogeneity

Reducing side heterogeneity was estimated by measuring relative amounts of Q_B-reducing and Q_B-non-reducing centers as described in Materials and methods. The population of Q_B-non-reducing centers was quantified (Table 1) to about 18–20% (control) of total PSII at temperatures below 40 °C. Exposure of leaves to temperature up to 35 °C does not affect much the relative fractions of Q_B-non-reducing

Table 1
Relative amounts (normalized) number of Q_B-reducing and Q_B-non-reducing centers as a result of high temperature treatment in detached wheat leaves. Q_B-non-reducing centers were calculated using the following equation Vo (Bo) = (Fv/Fm) − (Fv*/Fm*) / (Fv/Fm). Five repeats were done for each treatment.

Temperature in °C	Amount of Q_B-reducing centers$(1-B_0)$.	Amount of Q_B-non-reducing centers (B_0)
25 °C	82 ± 4	18 ± 3
35 °C	80 ± 3	20 ± 2
40 °C	61 ± 2	39 ± 2
45 °C	50 ± 3	50 ± 3
Recovery		
35 °C	79 ± 4	21 ± 2
40 °C	69 ± 3	31 ± 3
45 °C	50 ± 3	50 ± 3

PSII centers but as the temperature was raised to 40 °C a gradual change in the fractions of Q_B-non-reducing PSII centers was observed while at temperature greater than 40 °C i.e. at 45 °C the fraction of Q_B-non-reducing increased drastically up to 50% (Table 1). At high temperatures the fractions of Q_B-non-reducing centers increased which imply that these centers were unable to reduce Q_A to PQ pool and also that the active Q_B-reducing centers were converted into inactive Q_B-non-reducing centers. The O–J–I–P fluorescence induction curves were next evaluated for the determination of Q_B-reducing and Q_B-non-reducing centers. The I–P phase in O–J–I–P represents the reduction of acceptor side of PSII or more specifically to the reduction of two distinct PQ pools. Above 40 °C, a prominent decline in the reduction of PQ pool was observed which may be because of the retardation of Q_A oxidation step. This could occur as a result of increased formation of Q_B-non-reducing centers which is evident from Table 1. The Fv/Fm ratio in control plants (25 °C) was found to be 0.832 while at 35 and 45 °C, this ratio was 0.819 and 0.331 respectively and was also accompanied by a substantial increase in the proportion of the Q_B-non-reducing PSII RC. This suggests that the decreased Fv/Fm was a result of the increased proportion of Q_B-non-reducing PSII RC. Thus Table 1 shows that the electron transfer has been blocked from Q_A to Q_B as a result of considerable increase in the number of Q_B-non-reducing centers. The reduction in the ratios of Q_A- and Q_B-reducing reaction centers of the heat-treated demonstrated that high temperature treatment not only decreased the electron transfer from PSII reaction centers to Q_A but also decreased the possibility of electron transfer from Q_A to Q_B (second quinone electron acceptor of PSII. The Fo to F_I rise seen in the Chl *a* fluorescence induction has been suggested due to the presence of 'PS II inactive centers' [59]. Thus 'PS II inactive centers' display heterogeneity on the reducing side of PS II with respect to electron transfer from Q_A to Q_B [24,27,30,32].

The antenna size and reducing side heterogeneity was also studied by dynamic energy pipeline models of photosynthetic apparatus [51,60] and specific energy fluxes were calculated from these models derived from OJIP curves (Fig. 5). The flux ratios ABS/RC, TRo/RC, ETo/RC and DIo/RC increased as a result of high temperature stress. The ABS/RC represents the total number of photons absorbed by chl molecules of all RCs divide by the total number of active RCs. It is influenced by the ratio of active/inactive RCs and as the number of inactive centers increased, the ratio ABS/RC also increased [44]. Increase in antenna size also represents that the active centers have decreased but since the inactive centers have also been added in the total antenna size, apparently the ratio of ABS/RC appears to be increased. The change in antenna size of PSII can be due to the change in the number of LHC complexes per RC. However, these changes could also be due to inactivation of RCs [61] and conversion of PSII units into heat sinks units. TRo/RC represents the maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A. An increase in this ratio indicates that all the Q_A has been reduced but

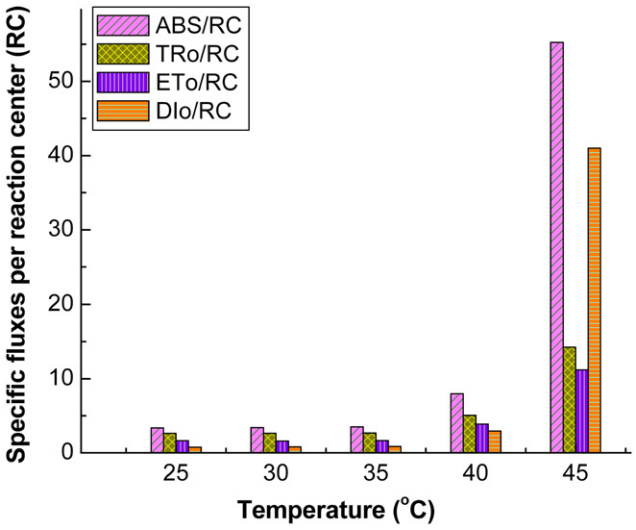

Fig. 5. Changes in ABS/RC (Absorption flux per reaction center (RC)), TRo/RC (Trapped energy flux per RC), ETo/RC (Electron transport per RC) and DIo/RC (Dissipated energy flux per RC) ratio after high temperature treatment.

it is not able to oxidize back due to temperature stress i.e. the reoxidation of Q_A is inhibited so that Q_A cannot transfer electrons efficiently to Q_B and also that maximum energy is lost in dissipation. Although trapping has increased but it is not the efficient trapped photon that can help in electron transport. ETo/RC depicts the reoxidation of reduced Q_A via electron transport in an active RC. It only reflects the activity of active RCs. Fig. 5 shows an increased electron transport per active reaction center due to a thermal activation of the dark reactions. As ETo/RC is represented only by active centers the increased ratio indicates that the inactive centers has increased and the Q_A cannot transfer electrons efficiently to Q_B, and thus the ratio has increased but the electron efficiency has decreased. DIo/RC represents the ratio of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs. Dissipation can be thought of as the absorption of photons in excess of what can be trapped by the RCs. Dissipation occurs as heat, fluorescence and energy transfer to other systems. It is also influenced by the ratios of active/inactive RCs. The ratio of total dissipation to the amount of active RCs increased (DIo/RC) due to the high dissipation ratio of the active RCs. As the inactive centers increased, the DIo/RC also increased because the inactive centers were unable to trap the photon so the untrapped amount of photons increased. If JIP test is applied then an increase in the effective dissipation of an active RC (DIo/RC) also reflects the loss of connectivity between PSII heterogeneous units [45] which is in evidence with the results presented in Fig. 4.

4. Recovery

In order to know whether the high temperature induced changes caused in PS II heterogeneity are permanent or temporary, recovery studies were carried out. For recovery, the temperature stressed wheat leaves were kept in distilled water in dark for 2 h and then the fluorescence induction curves were measured in dark.

4.1. Recovery of antenna size heterogeneity

Recovery of antenna size heterogeneity was observed at 35 °C and 40 °C but not at 45 °C. During the recovery process the α and β centers recovered completely but the γ centers could not be recovered completely (35 °C α: β: γ 71: 26: 1, 40 °C α: β: γ 70: 28: 1).When the leaves treated with 45 °C were kept for recovery no recovery was

observed (45 °C α: β: γ 44: 42: 14) in any of the centers indicating that the temperature 45 °C caused a permanent irreversible damage.

4.2. Reducing side heterogeneity

The recovery study (Table 1) showed that the leaves treated with 35 °C showed complete recovery in reducing side heterogeneity and the number of Q_B-non-reducing centers became equal to that of control. The leaves treated with 40 °C showed partial recovery while at 45 °C no recovery was observed suggesting a permanent damage and the number of Q_B-non-reducing centers remained almost the same as in the 45 °C treated leaves.

5. PS II heterogeneity as an adaptive mechanism

We suggest that certain environmental stimuli like high temperature stress evoke interconversions of α centers into β and γ centers and the active Q_B-reducing centers into inactive Q_B-non-reducing centers. The plant adapts to high temperature stress by temporary interconversions of PSII heterogeneity up to temperature of 40 °C. This may be attributed to some reversible structural rearrangements in PS II. It is likely that structural rearrangement functions as an adaptive mechanism that allows plants to maintain maximum quantum yield of PS II. However at 45 °C an irreversible loss occurs to PSII and no recovery was observed both the types of heterogeneity. This may be because of thermal denaturation and irreversible dissociation of various PS II components. The changes in energy flux in response to high temperature leads to increased dissipation of energy and untrapped photons which may protect the plant from oxidative stress transiently.

6. Conclusions

The changes in different types of heterogeneity of PS II in response to high temperature stress in wheat leaves has been investigated in order to understand the physiological basis of stress tolerance. The response of PSII to high temperature stress by manipulating its heterogeneous structure and function has been reported. Both antenna size and reducing side heterogeneity are greatly affected with increasing temperature. Thus, high temperature stress led to changes in PS II heterogeneity which may be one of the adaptive mechanisms to cope with high temperature stress.

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References

[1] J.M. Andersson, A. Melis, Localization of different photosystems in separate regions of chloroplast membranes, Proc. Natl Acad. Sci. USA 80 (1983) 745–749.
[2] T.J. Wydrzynski, Water splitting by Photosystem II—where do we go from here? Photosynth. Res. 98 (2008) 43–51.
[3] P. Haldimann, U. Feller, Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens*) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1, 5-bisphosphate carboxylase/oxygenase, Plant, Cell Environ. 27 (2004) 1169–1183.
[4] S. Allakhverdiev, V. Kreslavski, V. Klimov, D. Los, R. Carpentier, P. Mohanty, Heat stress: an overview of molecular responses in photosynthesis, Photosynth. Res. 98 (2008) 541–550.
[5] S.J. Crafts-Bradner, M.E. Salvucci, Rubisco avtivase constrains the photosynthetic potential of leaves at high temperature and CO_2, Proc. Natl. Acad. Sci. USA 97 (2000) 13430–13435.
[6] I. Kurek, T.K. Chang, S.M. Bertain, A. Madrigal, L. Liu, M.W. Lassner, G. Zhu, Enhanced thermostability of Arabidopsis Rubisco activase improves photosynthesis and growth rates under moderate heat stress, Plant Cell 19 (2007) 3230–3241.
[7] M.E. Salvucci, S.J. Crafts-Bradner, Realtionship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments, Plant Physiol. 134 (2004) 1460–1470.
[8] R.R. Wise, A.J. Olson, S.M. Schrader, T.D. Sharkey, Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature, Plant, Cell Environ. 27 (2004) 717–724.
[9] R. Zhang, T.D. Sharkey, Photosynthetic electron transport and proton flux under moderate heat stress, Photosynth. Res. 100 (2009) 29–43.
[10] L.S. Chen, L. Cheng, Photosystem 2 is more tolerant to high temperature in apple (*Malus domestica* Borkh.) leaves than in fruit peel, Photosynthetica 47 (1) (2009) 112–120.
[11] P. Li, L. Cheng, H. Gao, C. Jiang, T. Peng, Heterogeneous behavior of PSII in Soybean (*Glycine max*) leaves with identical PSII photochemistry efficiency under different high temperature treatments, J. Plant Physiol. 166 (2009) 1607–1615.
[12] I. Enami, M. Kitamura, T. Tomo, Y. Isokawa, H. Ohta, S. Katoh, Is the primary cause of thermal inactivation of oxygen evolution in spinach PSII membranes release of the extrinsic 33 kDa protein or of Mn? Biochim. Biophys. Acta 1186 (1994) 52–58.
[13] Y. Yamane, Y. Kashino, H. Koike, K. Satoh, Effects of high temperatures on the photosynthetic systems in spinach: oxygen-evolving activities fluorescence characteristics and the denaturation process, Photosynth. Res. 57 (1998) 51–59.
[14] J. Lavergne, J.M. Briantais, Photosystem II heterogeneity, in: R.D. Ort, C.F. Yocum (Eds.), Oxygeneic Photosynthesis: The Light Reactions, Kluwer Publishers, Dordrecht, The Netherlands, 1996, pp. 265–287.
[15] A. Melis, P.H. Homann, Heterogeneity of the photochemical centers in system II of chloroplasts, Photochem. Photobiol. 23 (1976) 343–350.
[16] A. Melis, L.M.N. Duysens, Biphasic energy conversion kinetics and absorbance difference spectra of photosystem II of chloroplasts. Evidence for two different photosystem II reaction centers, Photochem. Photobiol. 29 (1979) 373–382.
[17] M.T. Black, T.H. Brearley, P. Horton, Heterogeneity in chloroplast photosystem II, Photosynth. Res. 89 (1986) 193–207.
[18] B.D. Hsu, J.Y. Lee, A study on the fluorescence induction curve from DCMU-poisoned choloroplast, Biochim. Biophys. Acta 1056 (1991) 285–292.
[19] J. Sinclair, S.M. Spence, Heterogeneous photosystem 2 activity in isolated spinach choloroplast, Photosynth. Res. 24 (1990) 209–220.
[20] B.D. Hsu, J.Y. Lee, The Photosystem II heterogeneity of chlorophyll b-deficient mutants of rice: a fluorescence induction study, Aust. J. Plant Physiol. 22 (1995) 195–200.
[21] P.J. Morrissey, R.E. Glick, A. Melis, Supramolecular assembly and function of subunits associated with the chlorophyll *ab* light-harvesting complex II (LHC II) in soybean chloroplasts, Plant Cell Physiol. 30 (1989) 335–344.
[22] A. Melis, Dynamics of photosynthetic membrane composition and function, Biochim. Biophys. Acta 1058 (1991) 87–106.
[23] A. Joliot, M. Joliot, Evidence for a double hit process in photosystem II based on fluorescence studies, Biochim. Biophys. Acta 462 (1977) 558–574.
[24] J. Lavergne, Two types of primary acceptors in chloroplasts photosystem II, Photobiochem. Photobiophys. 3 (1982) 257–285.
[25] J. Lavergne, Mode of action of 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea. Evidence that the inhibitor competes with plastoquinone for binding to a common site on the acceptor side of photosystem II, Biochim. Biophys. Acta 682 (1982) 345–353.
[26] I.R. Vasilev, A.I. Komarov, D.N. Matorin, V.N. Verkhoturov, P.S. Venediktov, Two types of PSII centers as manifested by light saturation of delayed fluorescence from DCMU-treated chloroplasts, Photosynth. Res. 26 (1990) 101–107.
[27] A.P.G.M. Thielen, H.J. Van Gorkom, Electron transport properties of photosystems IIα and II_β, in: G. Akoyunogtou (Ed.), Photosynthesis, Proceedings of the 5th International Congress, Balaban International Science Services, Philadelphia, PA, 1981, pp. 57–64.
[28] C. Sundby, A. Melis, P. Mäenpää, B. Andersson, Temperature-dependent changes in the antenna size of photosystem II. Reversible conversion of Photosystem II_α to Photosystem II_β, Biochim. Biophys. Acta 851 (1986) 475–483.
[29] R.J. Strasser, The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids Primary reactions of photochemistry in higher plants, in: G. Akoyunoglou (Ed.), Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Apparatus, Balaban International Science Services, Philadelphia, PA, 1981, pp. 727–737.
[30] A. Melis, Functional properties of PS II_β in spinach chloroplasts, Biochim. Biophys. Acta 808 (1985) 334–342.
[31] T. Graan, D.R. Ort, Detection of oxygen-evolving photosystem II centers inactive in plastoquinone reduction, Biochim. Biophys. Acta 852 (1986) 320–330.
[32] J.E. Guenther, J.A. Nemson, A. Melis, Photosystem stoichiometry and chlorophyll antenna size in *Dunaliella salina* (green algae), Biochim. Biophys. Acta 934 (1988) 108–117.
[33] G. Schanker, R.J. Strasser, Quantification of non-Q_B-reducing centers in leaves using a far-red pre-illumination, Photosynth. Res. 84 (2005) 145–151.
[34] E. Tyystjarvi, E.-M. Aro, Temperature-dependent changes in PSII heterogeneity support a cycle of PSII during photoinhibition, Photosynth. Res. 26 (1990) 109–117.
[35] B.D. Hsu, The active photosystem II centers can make a significant contribution to the initial fluorescence rise from Fo to Fi, Plant Sci. 81 (1992) 169–174.
[36] P.G. Quinn, W.P. Williams, Environmentally induced changes in chloroplast membranes and their effects on photosynthetic function, in: G. Barber, N.R. Baker

(Eds.), Photosynthetic mechanisms and the environment, Elsevier Science Publishers, Amsterdam, 1985, pp. 1–47.
[37] M. Almeselmani, P.S. Deshmukh, R.K. Sairam, S.R. Kushwaha, T.P. Singh Protective, role of antioxidant enzymes under high temperature stress, Plant Sci. 171 (2006) 382–388.
[38] J.L. Snider, D.M. Oosterhuis, E.M. Kawakami, Genotypic differences in thermotolerance are dependent upon prestress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*, Physiol. Plant. 138 (2010) 268–277.
[39] Y. Murakami, M. Tsuyama, Y. Kobayashi, H. Kodama, K. Iba, Trienoic fatty acids and plant tolerance of high temperature, Science 287 (5452) (2000) 476–479.
[40] T.D. Sharkey, R. Zhang, High temperature effects on electron and proton circuits of photosynthesis, J. Int. Plant Biol. 52 (2010) 712–722.
[41] J.J. Burke, Identification of genetic diversity and maturation in higher plant acquired thermotolerance, Physiol. Plant. 112 (2001) 167–170.
[42] A. Rokka, E.M. Aro, R.G. Herrmann, B. Andersson, A.V. Vener, Dephosphorylation of photosynthetic membranes as an immediate response to abrupt elevation of temperature, Plant Physiol. 123 (2000) 1525–1535.
[43] P. Mehta, A. Jajoo, S. Mathur, S. Bharti, Chlorophyll *a* fluorescence study reveling effects of high salt stress on photosystem II in wheat leaves, Plant Physiol. Biochem. 48 (2010) 16–20, doi:10.1016/j.plaphy.2009.10.006.
[44] S. Mathur, A. Jajoo, P. Mehta, S. Bharti, Analysis of elevated temperature-induced inhibition of photosystem II by using chlorophyll *a* fluorescence induction kinetics in wheat leaves (*Triticum aestivum*), Plant Biol. (2009), doi:10.1111/j.1438-8677.2009.00319.x.
[45] L. Force, C. Critchley, J.J.S. van Rensen, New fluorescence parameters for monitoring photosynthesis in plants 1. The effect of illumination on the fluorescence parameters of the JIP-test, Photosynth. Res. 78 (2003) 17–33.
[46] D. Lazar, P. Tomek, P. Ilík, J. Naus, Determination of the antenna heterogeneity of PSII by direct simultaneous fitting of several fluorescence rise curves measured with DCMU at different light intensities, Photosynth. Res. 68 (2001) 247–257.
[47] R.J. Strasser, M. Tsimilli-Michael, A. Srivastava, Analysis of chlorophyll *a* fluorescence transient, in: G. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration: Chlorophyll *a* Fluorescence: A Signature of Photosynthesis, Springer Publishers, Dordrecht, The Netherlands, 2004, pp. 321–362.
[48] A. Melis, P.H. Homann, Kinetic analysis of the fluorescence induction in 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea poisoned chloroplasts, Photochem. Photobiol. 21 (1975) 431–437.
[49] B.-D. Hsu, Y.-S. Lee, Y.-R. Jang, A method for analysis of fluorescence induction curve from DCMU-poisoned chloroplasts, Biochim. Biophys. Acta 975 (1989) 44–49.
[50] S.Z. Toth, G. Schansker, R.J. Strasser, In intact leaves, the maximum fluorescence level (FM) is independent of the redox state of the plastoquinone pool: a DCMU-inhibition study, Biochim. Biophyis. Acta 1708 (2005) 275–282.
[51] R.J. Strasser, A. Srivastava, M. Tsimilli-Michael, The fluorescence transient as a tool to characterize and screen photosynthetic samples, in: M. Yunus, U. Pathre, P. Mohanty (Eds.), Probing Photosynthesis: Mechanisms, Regulation and Adaptation, Taylor & Francis, London, 2000, pp. 443–480.
[52] B.J. Strasser, Donor side capacity of PS II probed by chlorophyll *a* fluorescence transients, Photosynth. Res. 52 (1997) 147–155.
[53] D. Joly, E. Jemâa, R. Carpentier, Redox state of the photosynthetic electron transport chain in wild-type and mutant leaves of *Arabidopsis thaliana:* impact on photosystem II fluorescence, J. Photochem. Photobiol. B Biol. 98 (2010) 180–187.
[54] L. Nedbal, M. Trtilek, D. Kaftan, Flash fluorescence induction: a novel method to study regulation of photosystem II, J. Photochem. Photobiol. B Biol. 48 (1999) 154–157.
[55] N.G. Bukhov, R. Carpentier, Heterogeneity of photosystem II reaction centers as influenced by heat treatment of barley leaves, Physiol. Plant. 110 (2000) 279–285.
[56] J. Sinclair, S.M. Spence, The analysis of fluorescence induction transients from dicholoro-phenyldimehtylurea-poisoned chloroplasts, Bichim. Biophys. Acta 935 (1988) 184–194.
[57] A. Joliot, M.P. Joliot, Etude cinetique de la reaction photochimique liberant l oxygene au cours de la photosynthese, C. R. Acad. Sci. Paris 258 (1964) 4265–4622.
[58] R.J. Strasser, M. Tsimilli-Michael, Activity and heterogeneity of PSII probed *in vivo* by the Chlorophyll *a* fluorescence rise O-(K)-J-I-P, in: G. Garab (Ed.), Photosynthesis: Mechanisms and Effects, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998, pp. 4321–4324.
[59] J. Cao, Govindjee, Chlorophyll a fluorescence transients as an indicator of active and inactive photosystem II in thylakoid membranes, Biochim. Biophys. Acta 1015 (1990) 180–188.
[60] R.J. Strasser, Energy pipeline model of the photosynthetic apparatus, in: J. Biggins (Ed.), Progress in Photosynthesis Research, Matinus Nijhoff Publishers, Dordrecht, The Netherlands, 1987, pp. 717–720.
[61] J. Lavergne, E. Leci, Properties of inactive photosystem II centers, Photosynth. Res. 35 (1993) 323–343.