Environmental and Experimental Botany 73 (2011) 64–72

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces☆

Hazem M. Kalaji[a,*], Govindjee[b], Karolina Bosa[c], Janusz Kościelniak[d], Krystyna Żuk-Gołaszewska[e]

[a] Department of Plant Physiology, Warsaw University of Life Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland
[b] Departments of Biochemistry, and Plant Biology, and Center of Biophysics & Computational Biology, University of Illinois at Urbana-Champaign, 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801-3707, USA
[c] Department of Pomology, Faculty of Horticulture and Landscape Architecture, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland
[d] Department of Plant Physiology, Agricultural University, Podluzna 3, 30-239 Krakow, Poland
[e] Department of Agrotechnology and Crop Production Management, University of Warmia and Mazury in Olsztyn, Oczapowskiego 8, 10-718 Olsztyn, Poland

ARTICLE INFO

Keywords:
Barley
Chlorophyll *a* fluorescence
OJIP transient
Photosynthesis
Photosystem II
Salinity

ABSTRACT

The photosynthetic activity of two Syrian barley landraces, Arabi (A.) Aswad and A. Abiad, grown under 120 mM NaCl, was studied, using gas exchange and chlorophyll (Chl) *a* fluorescence transient (OJIP) measurements. Salt treatment of barley seedlings decreased both the rates of photosynthesis and photosystem II (PSII) activity, as evaluated from chlorophyll fluorescence data. However, the noted decrease was dependent on the duration of the salt treatment and the barley cultivar. Several parameters (e.g., light absorption flux per cross section of leaf; time to reach maximum chlorophyll a fluorescence intensity; plastoquinone pool size; yield of heat loss; rate of reaction center closure; and the so-called Performance Index), calculated and inferred from Chl fluorescence measurements, and related to PSII activity, were affected after 24 h of salt application, but these changes were much more pronounced after 7 days of salt treatment. Similar changes were found for measured gas exchange parameters: CO_2 uptake (photosynthetic) rate and stomatal conductance. The photosynthetic apparatus of the cultivar variety (c.v.) Arabi Aswad was found to be much more tolerant to salt treatment, compared with c.v. Arabi Abiad. After 7 days of salt treatment, the latter showed a very high value of the initial (minimal) fluorescence (F_o) and then essentially almost flat fluorescence transient curve; this result may be due to several causes that include structural changes as well as changes in the rate constants of different dissipative processes. The parameters that were most affected, by salt treatment, were: the time needed to reach the maximal chlorophyll fluorescence (F_m), and the inferred oxygen evolving complex activity (F_v/F_o, where F_v, is $F_m - F_o$), and the calculated Performance Index (PI_{ABS}) that depends on the efficiency and the yield of energy transfer and primary photochemistry. We suggest that the early reactions of the photosynthetic apparatus of barley plants could play a key role in their tolerance to salt stress. Further, we found that the first stage of salinity effect on photosynthesis of barley plants is related to stomatal conductance limitation rather than to PSII activity reduction. Thus, on the basis of our results on the two barley landraces, we recommend the use of a combination of gas exchange measurements along with the analysis of the OJIP fluorescence transient for the detection of salt stress-induced changes in plants.

1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop in the world and, according to Jiang et al. (2006), it is one of the most salt tolerant crop species. However, salinity limits barley production and it is one of the major abiotic stresses, especially in arid and semi-arid regions where salt concentration can be close to that in the seawater (Shannon, 1998; Kalaji and Nalborczyk, 1991; for general information on abiotic stress in plants, see Pareek et al., 2010). The negative effect of salinity on plant growth is a result of the low osmotic potential of soil solution, changes in nutrient uptake and specific sodium and chloride ion effects, and the effect depends on the salt concentration as well as on the growth conditions (Kalaji and Pietkiewicz, 1993; Marschner, 1995). These changes affect plant growth and development at different levels of plant organization (Munns, 2002), e.g., they may reduce photosynthetic carbon gain and leaf growth rate (Munns, 1993).

☆ This paper is submitted for inclusion in the Special Issue devoted to the Workshop of fluorescence at Pisa, Italy "Chlorophyll fluorescence: from theory to the (good) practice".

* Corresponding author. Tel.: +48 22 608079998; fax: +48 22 6425923.
E-mail address: hazem@kalaji.pl (H.M. Kalaji).

0098-8472/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2010.10.009

Nomenclature

ABS/RC	light absorption flux (for PSII antenna chlorophylls) per reaction center (RC)
DI/ABS	dissipated energy flux per PSII ABS
DI/RC	dissipation energy flux per PSII reaction center (RC)
ET/ABS	electron transport flux per PSII ABS
ET/RC	maximum electron transport flux (further than Q_A^-) per PSII reaction center (RC)
TR/ABS	trapped (maximum) energy flux per PSII ABS
TR/RC	trapped (maximum) energy flux (leading to Q_A reduction) per reaction center (RC)
RC/ABS	density of reaction centers per PSII antenna chlorophyll
Area	the area above the chlorophyll fluorescence curve between F_o and F_m (reflecting the size of the plato-quinone pool)
$\Delta V/\Delta t_o$	the initial slope of the relative variable fluorescence which directly describes the trapping flux TR_o/RC; thus, this ratio also expresses the rate of accumulation of closed reaction centers; $\Delta V/\Delta t_o = \{[\Delta F/(F_m - F_o)]/\Delta t\}_0 = [\Delta(Q_A^-/Q_{A(total)})/\Delta t]_0$
F_m or F_{max}	maximal chlorophyll fluorescence intensity measured when all photosystem II (PSII) reaction centers are closed
F_J	fluorescence intensity at the J-step during fluorescence induction (at 2 ms)
F_I	fluorescence intensity at the I-step during fluorescence induction (at 30 ms)
F_o	chlorophyll fluorescence intensity measured when all PSII reaction centers are assumed to be open; however, the measured value may be affected by several other parameters (at $t = 0$)
F_v	variable chlorophyll fluorescence ($F_m - F_o$)
F_v/F_m	a value that is related to the maximum quantum yield of PSII
F_v/F_o	a value that is proportional to the activity of the water-splitting complex on the donor side of the PSII
g_s	stomatal conductance
k_N	the non-photochemical de-excitation rate constant in the excited antennae for non-photochemistry
k_P	the photochemical de-excitation rate constant in the excited antennae of energy fluxes for photochemistry
N	the number indicating how many times Q_A is reduced while fluorescence reaches its maximal value (number of Q_A redox turnovers until F_m is reached)
O–J–I–P	transient fluorescence induction defined by the names of its intermediate steps (O is for 'origin' and 'P' is for peak, whereas, J and I are intermediate steps)
PI_{ABS}	the performance index that is calculated as: $(RC/ABS) \times (\varphi_{Po}/(1-\varphi_{Po})) \times (\psi_o/(1-\psi_o))$, where, RC is for reaction center; ABS is for absorption flux; φ_{Po} is for maximal quantum yield for primary photochemistry; and ψ_o is for the quantum yield for electron transport
P_N	net photosynthetic rate (measured as CO_2 uptake/exchange)
PSII	photosystem II
Q_A	the primary quinone acceptor of PSII
SFI_{ABS}	an indicator of PSII 'structure and functioning', calculated as $(RC/ABS) \times \varphi_{Po} \times \psi_o$
S_M	$(Area)/(F_m - F_o)$, representing energy necessary for the closure of all reaction centers
S_M/T_{FM}	the ratio representing the average redox state of Q_A in the time span from 0 to T_{FM} and, concomitantly, the average fraction of open reaction centers during the time needed to complete their closure
SumK	the sum of photochemical rate constant k_P and non-photochemical rate constant k_N (Havaux et al., 1991), where, $k_N = k_H$ (rate constant of heat dissipation) + k_F (rate constant of fluorescence emission) + k_x (rate constant of energy migration to PSI)
T_{FM}	time needed to reach Fm
V_j	relative variable fluorescence at time J (relative variable fluorescence at phase J of the fluorescence induction curve
φ_{Do}	thermal dissipation quantum yield
φ_{Eo}	electron transport quantum yield
$\varphi_o/(1-\varphi_o)$	a 'conformation' term for primary photochemistry
$\psi_o/(1-\psi_o)$	'conformation' term for thermal reactions (non-light dependent reactions)

The mechanisms of salt tolerance are very complex (see e.g., Kalaji and Pietkiewicz, 1993). The metabolic sites of salt influence have not been fully investigated, in particular in the photosynthetic apparatus; thus, there are no reliable indicators of plant tolerance to salinity that could be used by plant breeders to improve salinity tolerance in agricultural crops (Shannon, 1998; Kalaji and Pietkiewicz, 2004). Crop yield and growth parameters have been commonly used by breeders to screen for salinity tolerance (Cramer et al., 1990; Noble and Rogers, 1992; Munns, 1993; Kalaji and Guo, 2008). Plant responses to salinity stress are determined by many factors (including gene expression), particularly by those responsible for osmoprotectant production and accumulation in chloroplasts. The osmoprotectant glycinebetaine protects the photosystem II (PSII) complex by stabilizing the association of extrinsic PSII complex proteins under salt stress (Murata et al., 1992; Papageorgiou and Murata, 1995), while glycinebetaine and proline are responsible for osmotic adjustment and they protect subcellular structures in stressed plants (Hare et al., 1998; Yamaguchi-Shinozaki, 2001). Plant, or algal, responses to salinity depend on PSII response to this stress (Mohanty et al., 1974; Belkhodja et al., 1994; Kalaji and Guo, 2008) and on the increased accumulation of zeaxanthin which is believed to be involved in the energy dissipation mechanism that protects the photosynthetic apparatus (Ashraf and Harris, 2004). Enhancement of superoxide dismutase (SOD) and ascorbate peroxidase activities may also play an important role in plant tolerance to salt stress which keeps PSII activity at a high level (Tanaka et al., 1999).

Chlorophyll *a* fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis (see e.g., Kalaji and Nalborczyk, 1991; Strasser et al., 2000; Fricke and Peters, 2002; Kalaji and Rutkowska, 2004; Kalaji et al., 2004; see chapters in Papageorgiou and Govindjee, 2004 (reprinted 2010); Papageorgiou et al., 2007; Stirbet and Govindjee, 2011). It is important to state that the simplest and the most accepted hypothesis is that the major determinant of chlorophyll fluorescence is the redox state of Q_A, the first quinone electron acceptor of PSII: when it is in the oxidized state, fluorescence is low, and when it is in the reduced state, it is high; thus, the net concentration of Q_A^- is related to chlorophyll fluorescence yield (Duysens and Sweers, 1963; Govindjee, 1995, 2004). Further, the "O" level (or F_o) is the minimal fluorescence when all Q_A is in the oxidized

state, the reaction centers II are open; and the primary photochemistry is maximal, whereas the "P" level (or F_{max}) is the maximal fluorescence when all Q_A is in the reduced state (Q_A^-); here, the reaction centers II are closed, and there is a traffic jam of electrons on the electron acceptor side photosystem I and the primary photochemistry is minimal (Munday and Govindjee, 1969a,b; Govindjee, 1995, 2004). The basics of the OJIP chlorophyll transient are as follows. When dark-adapted photosynthetic sample is illuminated with high-light, chlorophyll *a* fluorescence rises from its minimal fluorescence level "O" to a "J" level (or F_J) at around 2 ms, due to the reduction of Q_A by PSII; this is followed by a fluorescence rise to the "I" level (or F_I) around 30 ms, due to the filling up of the plastoquinone pool; and finally, there is a rise from the I level to the "*P*" level, due to traffic jam on the electron acceptor side of PSI (see chapters in Govindjee et al., 1986; Papageorgiou and Govindjee, 2004, reprinted in 2010).

Among various approaches to the analysis of chlorophyll *a* fluorescence signals, the so-called 'JIP-test', as pioneered by Strasser et al. (2000, 2004), is frequently employed in different areas of plant biology to understand the responses of the photosynthetic apparatus to different physiological, genetic and environmental conditions (see e.g., Govindjee et al., 1998; Yusuf et al., 2010). The JIP-test is based on the theory of energy flow in thylakoid membranes (see e.g., Force et al., 2003). This kind of analysis enables us to understand the relationships between PSII activity, fluorescence signals and their analytical expressions (Strasser, 1978, 1981; Strasser et al., 2000; Bussotti et al., 2007). This analysis offers simple equations expressing the equilibrium between the inflow and outflow of the entire energy flux within PSII. Moreover, it provides relevant information about the probable fate of the absorbed energy. Although indirect, the JIP-test allows us to obtain information about the structure and function of the photosynthetic apparatus (mostly related to PSII). Some of the parameters calculated using the JIP-test are related to energy fluxes for light absorption (ABS), trapping (TR) of excitation energy and electron transport (ETR) per reaction center (RC) or per sample area called cross-section (CS). For convenience, those per RCs are referred to as *specific* and those per CS as *phenomenological* energy fluxes. Their estimates are based on the analysis of several groups of measured and calculated parameters. The JIP-test includes the analysis of the fraction of reactions centers which cannot reduce the secondary quinone electron acceptor Q_B, and it also estimates the probability of complete energy flow among PSII components (Strasser et al., 2000).

It is essential to recognize that the main advantage of this analysis is that it is quick, and is based on measurements by a non-invasive and a highly sensitive and accurate method. Parallel measurements with other methods are necessary to obtain a complete picture. Thus, in this paper, we have included measurements on photosynthesis (CO_2 assimilation), but further direct measurements on electron flow in both PS II and PSI are suggested for future research.

The negative effect of salinity on photosynthesis process has been known for a long time, but it has not yet been fully understood (Kalaji and Łoboda, 2009). A number of examples illustrate the effects of short- and long-term exposure to salinity on photosynthetic activity expressed by gas exchange parameters and chlorophyll *a* fluorescence measurements. However, only few publications have provided simultaneous measurements of both photosynthesis and chlorophyll *a* fluorescence (see reviews in Govindjee, 1995; Baker and Oxborough, 2004). Moreover, it is not yet clear whether there is one common salt tolerance mechanism in barley cultivars; further, we do not know which physiological parameter, related to photosynthetic apparatus functioning, can be recommended as a reliable indicator (biomarker) for its tolerance. Therefore, the goal of the present study was to assess net photosynthesis rate, stomatal conductance and specific chlorophyll fluorescence parameters in barley cultivars to improve our knowledge of barley photosynthetic apparatus responses to salinity stress.

2. Materials and methods

Two barley (*Hordeum vulgare* L.) cultivars, Arabi Abiad (A. Abiad) and Arabi Aswad (A. Aswad), were grown in a computer-controlled greenhouse in 1liter dark glass pots filled with a modified Hoagland nutrient solution. The average temperature for day/night was 26/18 °C, respectively, relative humidity was 50–60%, the photoperiod for the day/night cycle was 16/8 h, respectively, and the maximum photosynthetically active radiation was about 1400 μmol (photons) m^{-2} s^{-1}. After 7 days of growth, the seedlings were subjected to salinity stress. Sodium chloride was added to the nutrient solution to obtain a final concentration of 120 mM. Plant gas exchange (net photosynthetic (CO_2) rate – P_N and stomatal conductance – g_s) and chlorophyll *a* fluorescence measurements were performed directly after stress application (24 h; 8 days after emergence) to monitor prompt reactions of photosynthetic apparatus, and 7 days after stress application (14 days after emergence) to allow observations of further stress application effects before the senescence of first, second and third leaves occurs.

Gas exchange parameters were measured by CIRAS-2 *photosynthesis measurements system* (PP Systems International, Inc., Amesbury, MA, USA). Chlorophyll fluorescence parameters were measured using the *plant efficiency analyzer* (HandyPEA fluorimeter, Hansatech Instruments Ltd., Pentney, King's Lynn, Norfolk, England).

Barley seedlings were pre-darkened for 45–60 min at room temperature. Chlorophyll *a* fluorescence induction transients were measured when leaves were exposed to a strong light pulse (3500 μmol (photons) m^{-2} s^{-1}); these data were analyzed and the so-called JIP-test was conducted using Biolyzer v.3.0.6 software (both developed in the Laboratory of Bioenergetics, University of Geneva, Switzerland) (Strasser et al., 2000).

Chlorophyll fluorescence measurements and gas exchange were performed on the 1st, 2nd and 3rd leaves of barley plants. However, only the average values are shown in this paper. Measurements of chlorophyll fluorescence were done on 30 plants from each treatment and we had 3 replicates for each plant ($n = 90$) while the gas exchange measurements were performed on 3 plants from each treatment and we had 3 replicates for each plant($n = 9$). The data in tables are shown as percent of control to make the obtained results readable and easier for interpretation.

3. Results

We present here data under 2 extreme situations: (1) when the salinity effect was rather small (24 h after 120 mM NaCl treatment), we could see only small differences in a couple of parameters between A. Aswad and A. Abiad; and (2) when the salinity effect was large and obvious (7 days after 120 mM NaCl treatment), the sensitive A. Abiad showed remarkably large reductions in almost all the PSII parameters and in photosynthetic rates, whereas A. Aswad was highly tolerant. Further research is needed to investigate the time dependence of the salinity stress in these and other varieties.

3.1. After 24 h of salinity stress

The time needed to reach the maximal fluorescence value (T_{FM}) by barley plants grown under salt (120 mM NaCl) conditions for 24 h and the area over the normalized fluorescence induction curve

Table 1
Chlorophyll *a* fluorescence and gas exchange parameters (net photosynthetic (CO_2 exchange) rate, stomatal conductance) of two barley cultivars (Arabi Aswad and Arabi Abiad) grown under 120 mM NaCl. Numbers are given as percentage of control after 24 h of salt application (see list of abbreviations).

Parameter	Arabi Abiad	Arabi Aswad
T_{FM}	100	86
Area	99	85
F_v/F_m	99	99
F_v/F_o	95	96
P_N	72	98
g_s	79	94

(*Area*) decreased to ca. 85% in the A. Aswad cultivar, but it remained unchanged in the A. Abiad cultivar (Table 1). The maximal quantum efficiency of PSII (calculated from F_v/F_m) and the efficiency of the water-splitting complex on the donor side of PSII (as inferred from F_v/F_o) did not significantly decrease. However, gas (CO_2) exchange measurements showed a decrease of net photosynthetic rate and stomatal conductance values in A. Abiad plants (ca. 30 and 20%, respectively) (Table 1), but not in A. Aswad. No evident changes in chlorophyll fluorescence transient (O–J–I–P) curves were observed in either of the studied cultivars after 24 h of salt application (Fig. 1). Data plotted after normalization, both at F_o (O) and at F_{max} (P), did not show any differences (data not shown).

After salt treatment the values of most parameters (see list in the table of Nomenclature) characterizing PSII functioning, shown in what we call a "spider plot" (Strasser et al., 2000), were similar to those of control plants. Only in A. Aswad, the ratio expressing the fractional rate of accumulation of closed reaction centers ($\Delta V/\Delta t_o$) increased (about 20%) and the *area* above the O–J–I–P curve, representing energy necessary for the closure of all the reaction centers (S_M) and the performance index (PI_{ABS}) decreased (about 20%) as compared to the control plants (Fig. 2). The control plants of both cultivars showed similar values of energy flux parameters (Fig. 3), calculated on the basis of equal absorbance parameter, ABS (trapped energy flux: TR/ABS; electron transport flux: ET/ABS; and dissipated energy flux: DI/ABS) while under salt stress, plants of A. Aswad showed that they had ca. 10% inactive reaction centers as compared to those in A. Abiad. However, this change was not statistically significant (Fig. 3).

3.2. After 7 days of salinity stress

After 7 days of growth under salinity stress, time to reach F_m (T_{FM}) in A. Aswad increased significantly (ca. 200%), whereas it decreased (ca. 70%) in A. Abiad, relative to control plants (Table 2).

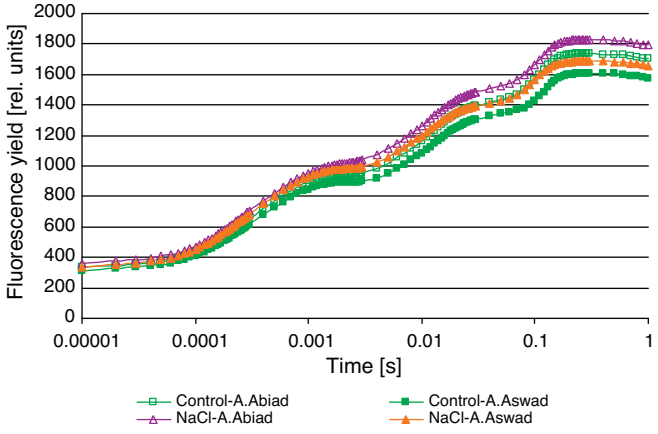

Fig. 1. Chlorophyll *a* fluorescence induction curve of barley seedlings of two cultivars (Arabi Aswad and Arabi Abiad) grown under 120 mM NaCl for 24 h.

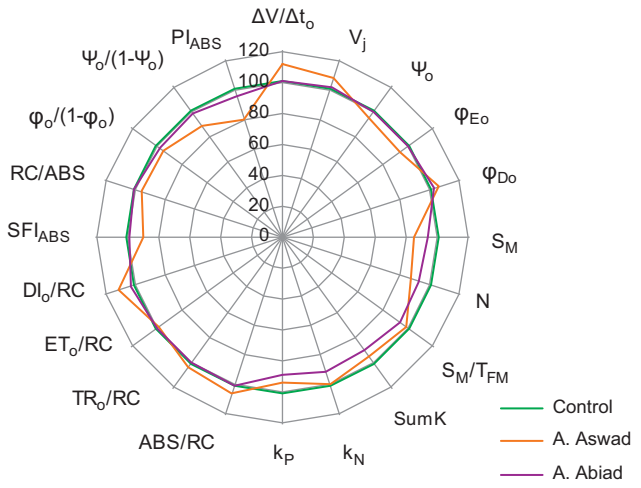

Fig. 2. A 'spider plot' of selected parameters characterizing behavior of Photosystem II of barley leaves exposed 24 h to 120 mM NaCl. (See the table of Nomenclature for the meaning of the symbols and the parameters.) All values are shown as percent of control (control plants = 100).

Table 2
Chlorophyll *a* fluorescence and gas exchange parameters (net photosynthetic (CO_2 exchange) rate, stomatal conductance) of two barley cultivars (Arabi Aswad and Arabi Abiad) grown under 120 mM NaCl. Numbers are given as percentage of control after 7 days of salt application (see list of abbreviations).

Parameter	Arabi Abiad	Arabi Aswad
T_{FM}	68	214
Area	9	65
F_v/F_m	25	90
F_v/F_o	7	66
P_N	21	77
g_s	35	81

The value of the *Area* parameter (the area above the chlorophyll fluorescence curve between F_o and F_m) of A. Aswad plants decreased to only ~65% of the value determined in control treatments; in contrast, the sensitive A. Abiad had a very low value (ca. 10%). Further, the maximal efficiency of PSII (calculated from F_v/F_m) decreased only slightly in A. Aswad, but drastically to ~25% in A. Abiad. Similarly, F_v/F_o values were reduced upon salinity treatment: A. Aswad had still a value that was ~70% of control; however, this value was drastically low, only ~7% of control, in A. Abiad. In agreement with the above trend, a large reduction (80%) of net photosynthetic rate and stomatal conductance was observed in A. Abiad (~20% of that in the control), but A. Aswad was tolerant; it had lost only 23% of its photosynthetic activity (the activity was 77% of control) (Table 2).

The O–J–I–P chlorophyll fluorescence curves obtained from the 120 mM NaCl-treated A. Aswad cultivar showed much slower fluorescence rise, and reached a much lower "*P*" level (cf. orange curve with the green curve, Fig. 4). (For interpretation of the references to color in text, the reader is referred to the web version of the article.) On the other hand, salt-treated fluorescence transient curve for A. Abiad was almost flat and showed much higher F_o and drastically lower F_m values (Fig. 4). When we normalize salt-treated and control A. Abiad curves at F_o, we observe a drastic reduction in variable fluorescence. There is clearly a large increase in F_o and a drastic decrease in the variable ($F_v = F_m - F_o$) fluorescence. These data indicate multiple large effects of salt-stress on A. Abiad. It could include, among other things, a structural damage leading to decreased excitation energy transfer from the antenna to the reaction center, leading to high F_o, and a damage to the reaction center leading to drastic reduction in photochemistry, and, thus, to very low variable fluorescence. However, when the salt-treated

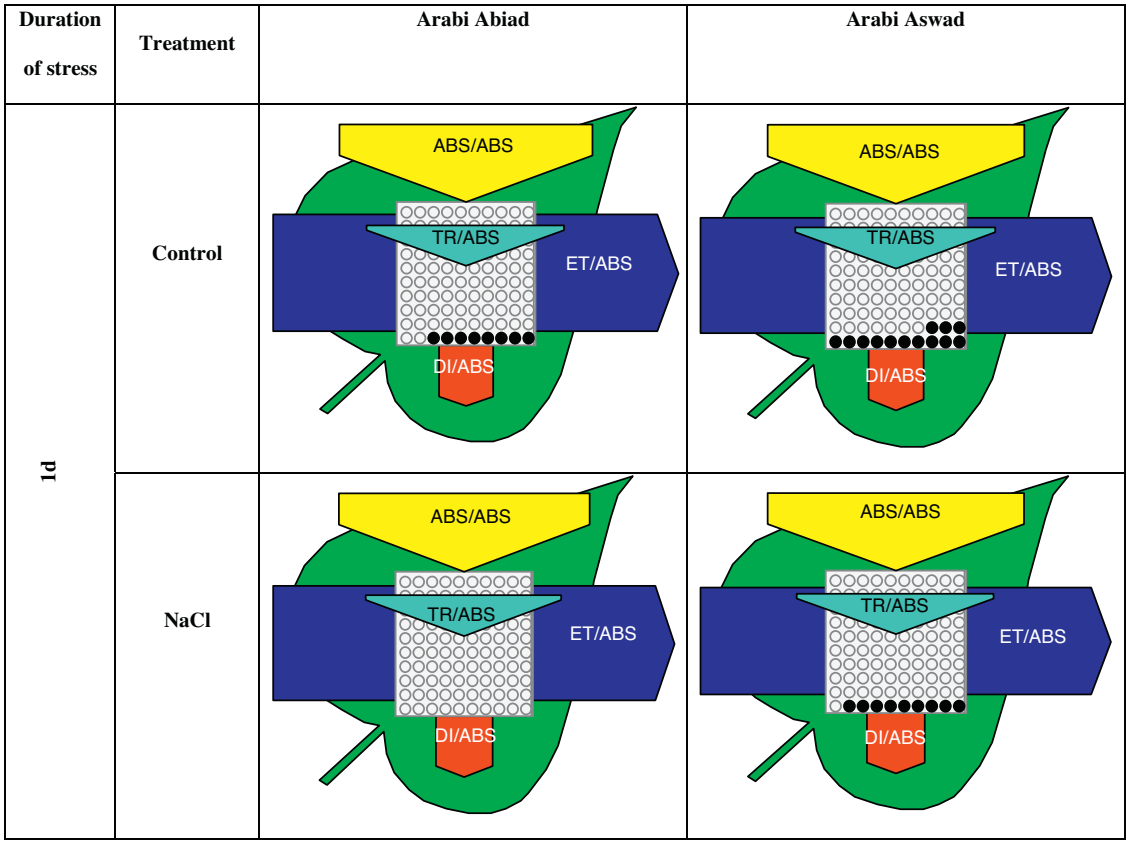

Fig. 3. Leaf pipeline model showing the proportion of phenomenological energy flux parameters within a leaf, calculated per equal chlorophyll absorption (ABS)/(ABS) = 1. Shown are results for barley cultivars Arabi Abiad and Arabi Aswad controls and after 1 day of 120 mM NaCl application. TR/ABS, trapped energy flux per chlorophyll. ET/ABS, electron transport flux per chlorophyll, DI/ABS, dissipated energy flux per light absorbed by chlorophylls (ABS). Each relative value is drawn by the width of the corresponding arrow, standing for a parameter. Empty and full black circles indicate, respectively, the percentage of active (Q_A reducing) and non active (non Q_A reducing) reaction centers of Photosystem II. Models are drawn with Biolyzer software (ronald@fluoromatics.com) on the basis of the equations of the JIP-test (Strasser et al. 2004). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

and control curves of A. Abiad are normalized both at F_o and at F_m, no obvious large difference in the kinetics of fluorescence rise is observed (data not shown) suggesting that the few reaction centers that are left untouched behave almost normally; however, there is a large increase in number of inactive reaction centers.

Pronounced changes are observed in the 'spider plot' diagram in many of the estimated parameters (see Fig. 5 and the list of abbreviations in the table of Nomenclature) of both the cultivars when compared to control plants. In A. Aswad, the values of several parameters per reaction center: DI_o/RC (dissipated energy flux per reaction center), $\Delta V/\Delta t_o$ (a ratio expressing the rate of accumulation of closed reaction centers), φ_{Do} (thermal dissipation yield), N (number of Q_A redox turnover before F_m is reached), k_N (non-photochemical de-excitation rate constant) and ABS/RC

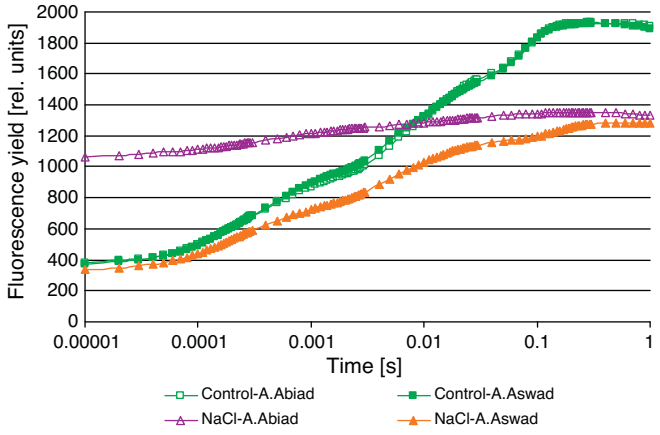

Fig. 4. Chlorophyll *a* fluorescence induction curve of barley seedlings of the two cultivars (Arabi Aswad and Arabi Abiad) grown under salinity stress (120 mM NaCl) for 7 days.

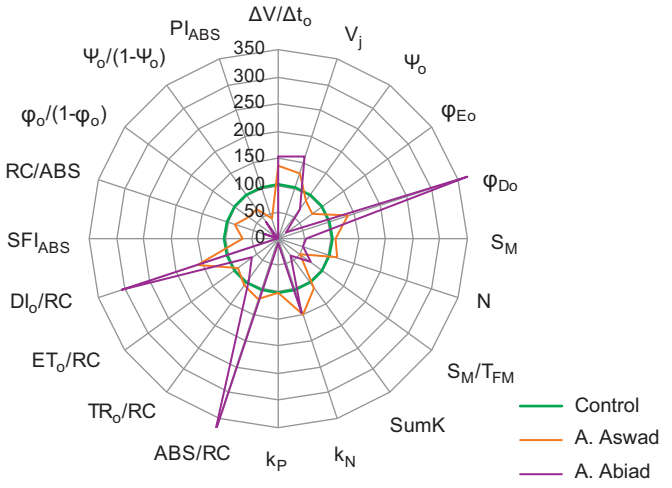

Fig. 5. A 'spider plot' of selected parameters characterizing behavior of Photosystem II of barley leaves exposed for 7 days to 120 mM NaCl. (See the abbreviation list for the meaning of the symbols and the parameters.) All values are shown as percent of control (control plants = 100).

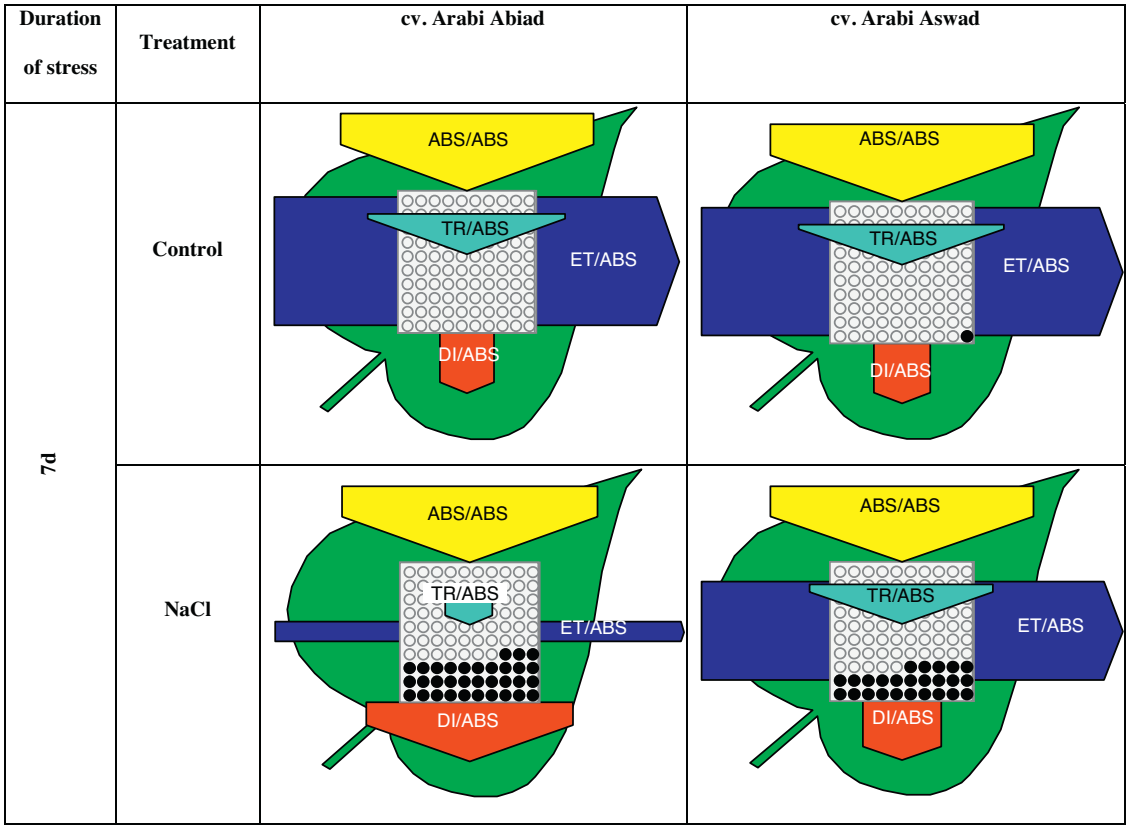

Fig. 6. Leaf pipeline model showing the proportion of phenomenological energy flux parameters within a leaf, calculated per equal chlorophyll absorption (ABS)/(ABS) = 1. Shown are results for barley cultivars Arabi Abiad and Arabi Aswad controls and after 7 days of 120 mM NaCl application. TR/ABS, trapped energy flux per chlorophyll. ET/ABS, electron transport flux per chlorophyll, DI/ABS, dissipated energy flux per light absorbed by chlorophylls (ABS). Each relative value is drawn by the width of the corresponding arrow, standing for a parameter. Empty and full black circles indicate, respectively, the percentage of active (Q_A reducing) and non active (non Q_A reducing) reaction centers of Photosystem II. Models are drawn with Biolyzer software (ronald@fluoromatics.com) on the basis of the equations of the JIP-test (Strasser et al. 2004). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(absorption flux per reaction center) were higher while those of φ_{Eo} (electron transport yield), S_M/T_{FM} (the average redox state of Q_A in the time span from 0 to T_{FM}), SFI_{ABS} (an indicator of PSII structure and functioning), RC/ABS (density of reaction centers per antenna chlorophyll), $\varphi_o/(1-\varphi_o)$ (a 'conformation' term for primary photochemistry) and $\psi_o/(1-\psi_o)$ (a 'conformation' term for thermal reactions) were lower, compared with the control plants. However, much more pronounced and dramatic changes in the analyzed and calculated PSII parameters were noted in A. Abiad grown under salinity stress.

The largest decrease, upon salt treatment, was in the so-called performance index (PI_{ABS}); as noted in the table of Nomenclature, it is a complex parameter that is related to the ratio of reaction center per (light) absorption flux, the maximal quantum yield for primary photochemistry; and the quantum yield for electron transport. As compared to A. Aswad, where it had dropped to ~40%, A. Abiad had an extremely low value of ~1% (Fig. 5). On the other hand, the values of DI_o/RC (dissipative energy flux per reaction center), ABS/RC (the absorption flux per reaction center) and φ_{Do} (thermal dissipation yield) were higher (~250%) and the values of $\Delta V/\Delta t_o$ (ratio expressing the rate of accumulation of closed reaction centers), V_j (relative variable fluorescence at time J) and k_N (non-photochemical rate) were close to (or higher than) 150%, compared with the control plants. The values of several remaining parameters were lower than those determined in control plants.

A. Abiad had much lower calculated ET/ABS (electron transport flux) and k_P (photochemical deexcitation rate constant) than the control plants, and it had increased number of non-active reaction centers. Further, the salt-stressed A. Abiad had much higher DI/ABS (dissipated energy flux) than the control plants. On the other hand, salt-stressed A. Aswad was somewhat closer to the control. Although the pipeline model (Fig. 6) showed changes in the inactive centers, in both cultivars under salt stress, the damage was drastic in A. Abiad. This is obvious from the larger changes in the sizes of DI/ABS, of ET/ABS and of increased inactive centers (Fig. 6).

4. Discussion

Salinity affects plant growth due to changes in many physiological processes including photosynthesis (Kalaji and Pietkiewicz, 1993; Kalaji and Guo, 2008). A reduction in chlorophyll content, stomatal conductance, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and an increase in the chlorophyll a/b ratio had been observed earlier (Kalaji and Nalborczyk, 1991; Delfine et al., 1999).

Syrian barley landraces are exclusively two-row types, and known as either A. Abiad (white-seeded), common in slightly better environments (250–350 mm of annual rainfall) or A. Aswad (black-seeded), common in harsher environments (<250 mm of annual rainfall). Considerable phenotypic and genotypic heterogeneity exists among landraces collected in different farmers' fields, as well as among individual plants within the same farmer's field (Ceccarelli et al., 2000). Farmers in dry areas consider that grain and straw quality of the black-seeded landrace is better than the white-seeded one. However, this has never been tested either in the field or under laboratory conditions and the relationships between desirable qualities and specific use purposes are unclear (Ceccarelli et al., 2000).

Our results show that A. Aswad cultivar of barley is clearly much more resistant to salinity stress than A. Abiad cultivar. This was clearly obvious after 7 days of stress application, based on the maximal photochemical activity of PSII (calculated from F_v/F_m), the size of the reduced plastoquinone pool (*Area* over the Chl fluorescence induction curve), efficiency of the water-splitting complex on the donor side of PSII (inferred from F_v/F_o) (Schreiber et al., 1994) and net photosynthetic rate (Table 2). The efficiency of the water-splitting complex on the donor side of PSII (F_v/F_o) is the most sensitive component in the photosynthetic electron transport chain. A decrease in this ratio results from photosynthetic electron transport impairment (Pereira et al., 2000). According to Fricke and Peters (2002), an inhibition of osmotically driven uptake of water is also observed under salinity stress. This can explain the lower values of F_v/F_o of both the cultivars after 7 days of stress application. During stress treatment, the area above the chlorophyll fluorescence curve between F_o and F_m decreases as a result of the inhibition of electron transport from reaction centers to the plastoquinone pool (Strasser et al., 2000). A. Aswad had a higher number of active PSII reaction centers able to reduce electron acceptors (Fig. 6).

In most higher plants, F_v/F_m is close to 0.83 (Björkman and Demmig, 1987) and under controlled conditions this parameter is often proportional to photosynthetic rate. The F_v/F_m values in A. Abiad, grown under salinity stress for 7 days, was only 25% of that noted in control plants; this indicates that in the plants that had salt-stress, reaction centers are damaged (photochemically inactive), thus reducing electron transport capacity in PSII (cf. Basu et al., 1998). Changes in F_v/F_m also could be caused by non-photochemical quenching (Maxwell and Johnson, 2000). After 7 days of salinity stress, the photochemical efficiency (F_v/F_m) of PSII in dark-adapted barley seedlings was much lower than in the control plants, but it was similar as in *Phaseolus* and *Brassica* grown under 0.1–0.3 M NaCl solutions for 3 days (Misra et al., 2006). Netondo et al. (2004) found that F_v/F_m decreased by about 9 and 10%, and electron transport rate decreased by 20 and 25% in two sorghum varieties grown under 250 mM NaCl, and that the photosynthesis rate of sorghum grown under salinity stress was affected primarily by stomatal closure, while stress effects on the photosynthetic apparatus were less important. Our experiments revealed similar results. Changes in net photosynthetic rate were observed directly after stress application (after 24 h) in A. Abiad, while no change in the PSII efficiency was observed (Table 1).

The high F_o values observed in A. Abiad seedlings grown under salinity stress for 7 days may have several causes, one of which may be increased number of inactive reaction centers where electrons cannot be transferred out of reduced Q_A and thus higher measured F_o; another possibility is low energy transfer from LHC II to PSII reaction center and thus higher fluorescence from LHCII; this may have been caused by the dissociation of LHCII from the PSII core (Havaux, 1993; Murkowski, 2002). In addition, the very low F_m values determined in A. Abiad plants grown under salinity stress for 7 days are indicative of the accumulation of inactive PSII reaction centers (also considered for higher F_o). In the present study, changes observed in A. Abiad grown under salinity stress for 7 days may also be due to D1 protein degradation and the inactivation of PSII reaction centers (Rintamäki et al., 1995).

After 7 days of salinity stress, both light and dark reactions of photosynthesis were impaired (much more in A. Abiad than in A. Aswad), as the performance indices of photochemical $[\varphi_o/(1-\varphi_o)]$ and non-light-dependent $[\psi_o/(1-\psi_o)]$ reactions were much lower in stress-exposed plants than in control plants (Fig. 5). This was confirmed by the decrease of net photosynthetic rate, much larger decrease in A. Abiad than in A. Aswad (Table 2). The inhibition of light and dark photosynthetic reactions due to salinity, observed in both the studied barley cultivars, resembles the changes occurring under photoinhibition (Osmond, 1994; also see Demmig-Adams et al., 2005, reprinted in 2008). It seems that the higher susceptibility of barley A. Abiad cultivar to salinity stress may result from the reduced capacity of non-assimilatory electron pathways (as the rate constant of PSII photochemistry and electron transport flux per cross section were dramatically reduced in this cultivar, in comparison with control plants). One of those pathways could be the Mehler reaction (the water–water cycle) (Asada, 1999), which has a protective function in the case of limited water supply to PSII and which supports ATP formation and enables efficient cycling of water in the cell in plants suffering from osmotic stress (Osmond and Grace, 1995; Lovelock and Winter, 1996). Further experiments are needed to test this hypothesis. Moreover, it is well documented that a severe interruption of the Calvin–Benson cycle may inhibit the repair of PSII after damage (Takahashi and Murata, 2005). Thus, we speculate that under salt stress, PSII repair is inhibited to a much higher extent in A. Abiad than in A. Aswad, while the non-photochemical rate constant (k_N) is similar in both the cultivars and is higher than in control plants. Further experiments are needed to test this hypothesis also.

The model, presented in Fig. 6, revealed that salt-stressed A. Abiad had a lower level of calculated electron transport (ET/ABS) and much more inactive reaction centers (cf. Delfine et al., 1999). We suggest that the accumulation of inactive reaction centers is associated with the increased efficiency of dissipation of absorbed light as heat, as shown by the significantly higher values of those parameters indicating the efficiency of non-photochemical de-excitation processes (DI/RC, DI/ABS, φ_{Do}, etc. Figs. 2, 3, 5 and 6).

Salt stress significantly affected the chlorophyll *a* fluorescence parameters and net photosynthetic rate of both the studied barley cultivars after 7 days of NaCl application, and it is possible that salinity did not affect PSII solely through the reduction of water potential, proved by reduction of stomatal conductance (Table 2). According to Shangguan et al. (2000), PSII is relatively resistant to both water deficits and low water potential. The higher salt tolerance of A. Aswad could also result from the higher activity of antioxidant enzymes. Mittova et al. (2002) found that salt tolerance was higher in the wild tomato (*Lycopersicon pennellii*) than in the cultivated tomato (*L. esculentum*) due to the increased activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (POD). The so-called *Radar* and *Spider* plots (Figs. 2 and 5) show the group of parameters that first undergo changes (in time), and enable us to compare the shifted values in stress-exposed barley cultivars. Under salt stress, the specific flux of energy (DI_o/RC and ABS/RC), quantum efficiency (φ_{Do}) and energy flux rate ($M_o = \Delta V/\Delta t_o$) parameters were much higher in A. Abiad than in control plants and in A. Aswad. It seems that prompt reaction of photosynthetic machinery to stress application could be a key factor in barley tolerance towards salinity (Kalaji and Nalborczyk, 1991).

5. Conclusions

Salinity stress negatively influenced PSII activity in barley plants, and its effect was dependent on the duration of stress application and on the cultivar used. Primary reactions of photosynthetic apparatus to salt stress of barley plants could play a key role in their tolerance to that stress. Our experiments allowed us to determine as to which chlorophyll *a* fluorescence parameters were most significantly altered under salinity stress. The value of the deactivation rate, calculated on the basis ABS, related to absorption of light section is significantly increased, whereas the electron transfer rates decreased after salt stress application, while energy trapping remained almost constant (Fig. 6).

Both the measured and the calculated values of the analyzed fluorescence parameters indicate that the photosynthetic apparatus of A. Aswad cultivar of barley is more tolerant to salinity, compared with the A. Abiad cultivar.

The results of the so-called JIP test, that analyzes quantitatively the OJIP chlorophyll fluorescence transient, has contributed to a better understanding of the responses of different barley cultivars to salinity stress, or for that matter many other plants of economic importance. Apart from the commonly applied fluorescence parameters, such as F_v/F_m, that measure PSII efficiency, our research shows that it is also important to consider other key parameters, including the PSII performance index, oxygen evolving complex activity and the time needed to reach maximal chlorophyll fluorescence. Simultaneous measurements of chlorophyll fluorescence and plant gas exchange allowed a better understanding of the mechanism of salinity effect on photosynthetic apparatus during early stages of plant growth.

Acknowledgement

We thank Reto J. Strasser for his valuable suggestions at the proof stage of this paper.

References

Asada, K., 1999. The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639.

Ashraf, M., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.

Baker, N., Oxborough, K., 2004. Chlorophyll *a* fluorescence as a probe of photosynthetic productivity. In: Papageorgiou, G., Govindjee (Eds.), Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Springer, Dordrecht, pp. 65–82 (reprinted 2010).

Basu, P.S., Sharma, A., Sukumaran, N.P., 1998. Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress. Photosynthetica 35, 13–19.

Belkhodja, R., Morales, F., Abadia, A., Gomez-Aparisi, J., Abadia, J., 1994. Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). Plant Physiol. 104, 667–673.

Björkman, O., Demmig, B., 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489–504.

Bussotti, F., Strasser, R.J., Schaub, M., 2007. Photosynthetic behaviour of woody species under high ozone exposure probed with the JIP-test: a review. Environ. Pollut. 147, 430–437.

Ceccarelli, S., Grando, S., Tutwiler, R., Baha, J., Martini, A.M., Salahieh, H., Goodchild, A., Michae, M., 2000. A methodological study on participatory barley breeding I. Selection phase. Euphytica 111, 91–104.

Cramer, G.R., Epstein, E., Läuchli, A., 1990. Effects of sodium, potassium and calcium on salt-stressed barley. Physiol. Plant 80, 83–88.

Delfine, S., Alvino, A., Villani, M.C., Loreto, F., 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. Plant Physiol. 119, 1101–1106.

Demmig-Adams, B., Adams III, W.W., Mattoo, A., 2005. Photoprotection, photoinhibition, gene regulation, and environment. In: Govindjee (Series Ed.,), Advances in Photosynthesis and Respiration, vol. 21. Springer, Dordrecht (reprinted 2008).

Duysens, L.N.M., Sweers H.E., 1963. Mechanisms of two photochemical reactions in algae as studied by means of fluorescence. In: Japanese Society of Plant Physiology, Studies on Microalgae and Photosynthetic Bacteria. University of Tokyo Press, Tokyo, pp. 353–372.

Force, L., Critchley, C., van Rensen, J.J.S., 2003. New fluorescence parameters for monitoring photosynthesis in plants. 1. The effect of illumination on the fluorescence parameters of the JIP-test. Photosynth. Res. 78, 17–33.

Fricke, W., Peters, W.S., 2002. The biophysics of leaf growth in salt-stressed barley. A study at the cell level. Plant Physiol. 129, 374–388.

Govindjee, 1995. Sixty-three years since Kautsky: chlorophyll *a* fluorescence. Aust. J. Plant Physiol. (now Funct. Plant Biol.) 22, 131–160.

Govindjee, 2004. Chlorophyll *a* fluorescence: a bit of basics and history. In: Papageorgiou, G., Govindjee (Eds.), Chlorophyll *a* fluorescence: a signature of photosynthesis. Springer, Ordrecht, pp. 1–42 (reprinted 2010).

Govindjee, Amesz, J., Fork, D.C. (Eds.), 1986. Light emission by plants and bacteria. Academic Press, Orlando.

Govindjee, Srivastava, A., Strasser R.J., 1998. The "Oxygen Clock" in greening gea leaves as probed by the period four oscillations in the fluorescence intensity at 50 micro-seconds and 2 milli-seconds after pre-flashing during the OJIP transient. In: Garab, G. (Ed.), Photosynthesis: Mechanisms and Effects. Kluwer Academic Publishers (now Springer), Dordrecht, The Netherlands, pp. 1467–1450.

Hare, P.D., Cress, W.A., Staden, J.V., 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ. 21, 535–553.

Havaux, M., 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. Plant Cell Environ. 16, 461–467.

Havaux, M., Strasser, R.J., Greppin, G., 1991. A theoretical and experimental analysis of the qP and qN coefficients of chlorophyll fluorescence quenching and their relation to photochemical and nonphotochemical events. Photosynth. Res. 27, 41–55.

Jiang, Q., Roche, D., Monaco, T.A., Durham, S., 2006. Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. Field Crop Res. 96, 269–278.

Kalaji, M.H., Nalborczyk, E., 1991. Gas exchange of barley seedlings growing under salinity stress. Photosynthetica 25, 197–202.

Kalaji, M.H., Pietkiewicz, S., 1993. Salinity effects on plant growth and other physiological processes. Acta Physiol. Plant. 143, 89–124.

Kalaji, M.H., Pietkiewicz, S., 2004. Some physiological indices to be exploited as a crucial tool in plant breeding. Plant Breed. Seed Sci. 49, 19–39.

Kalaji, M.H., Rutkowska, A., 2004. Photosynthetic machinery response of maize seedlings to salt stress. Zesz. Probl. Post. Nauk Roln. 496, 545–558.

Kalaji, M.H., Wołłejko, E., Łoboda, T., Pietkiewicz, S., Wyszyński, Z., 2004. Chlorophyll fluorescence: new tool for photosynthetic performance evaluation of barley plants grown under different nitrogen rates. Zesz. Probl. Post. Nauk Roln. 496, 375–383.

Kalaji, M.H., Guo, P., 2008. Chlorophyll fluorescence: a useful tool in barley plant breeding programs. In: Sanchez, A., Gutierrez, S.J. (Eds.), Photochemistry Research Progress. Nova Publishers, NY, USA, pp. 439–463.

Kalaji, M.H., Łoboda, T., 2009. Chlorophyll fluorescence to in plants' physiological state researches. Warsaw University of Life Sciences—SGGW, Warsaw (in Polish).

Lovelock, C.E., Winter, K., 1996. Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical tree species. Planta 198, 580–587.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Maxwell, K., Johnson, N.G., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659–668.

Misra, A.N., Latowski, D., Strzalka, K., 2006. The xanthophyll cycle activity in kidney bean and cabbage leaves under salinity stress. Russ. J. Plant Physiol. 53, 102–109.

Mittova, V., Guy, M., Tal, M., Volokita, M., 2002. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: increased activities of antioxidant enzymes in root plastids. Free Radic. Res. 36, 195–202.

Mohanty, P., Govindjee, Wydrzynski, T., 1974. Salt-induced alterations of the fluorescence yield and of emission spectra in chlorella. Plant Cell Physiol. 15, 213–224.

Munday Jr., J.C.M., Govindjee, 1969a. Light-induced changes in the fluorescence yield of chlorophyll *a* fluorescence *in vivo*. III. The dip and the peak in fluorescence transient of *Chlorella pyrenoidosa*. Biophys. J. 9, 1–21.

Munday Jr., J.C.M., Govindjee, 1969b. Light-induced changes in the fluorescence yield of chlorophyll *a* fluorescence *in vivo*. IV. The effect of preillumination on the fluorescence transient of *Chlorella pyrenoidosa*. Biophys. J. 9, 22–35.

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16, 15–24.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239–250.

Murata, N., Mohanty, P.S., Hayashi, H., Papageorgiou, G.C., 1992. Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. FEBS Lett. 296, 187–189.

Murkowski, A., 2002. Oddziaływanie czynników stresowych na luminescencję chlorofilu w aparacie fotosyntetycznym roślin uprawnych. Monografia 61, Instytut Agrofizyki im. Bohdana Dobrzańskiego PAN, Lublin.

Netondo, G.W., Onyango, J.C., Beck, E., 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop. Sci. 44, 806–811.

Noble, C.L., Rogers, M.E., 1992. Arguments for the use of physiological criteria for improving the salt tolerance in crops. Plant Soil 146, 99–107.

Osmond, C.B., 1994. What is photoinhibition? Some insights from comparison of sun and shade plants. In: Baker, N.R., Boyer, J.R. (Eds.), Photoinhibition: Molecular Mechanisms to the Field. Bios Scientific Publications, Oxford, UK, pp. 1–24.

Osmond, C.B., Grace, S.C., 1995. Perspectives on photoinhibition and photorespiration in the field—quintessential inefficiencies of the light and dark reactions of photosynthesis. J. Exp. Bot. 46, 1351–1362.

Papageorgiou, G.C., Govindjee (Eds.), 2004. Chlorophyll *a* fluorescence: a signature of photosynthesis. Advances in Photosynthesis and Respiration, vol. 19 (Series Editor: Govindjee), Springer, Dordrecht (reprinted 2010).

Papageorgiou, G.C., Murata, N., 1995. The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving photosystem complex. Photosynth. Res. 44, 243–252.

Papageorgiou, G.C., Tsimilli-Michael, M., Stamatakis, K., 2007. The fast and slow kinetics of chlorophyll a fluorescence induction in plants, algae and cyanobacteria: a viewpoint. Photosynth. Res. 94, 275–290.

Pareek, A., Sopory, S.K., Bohnert, H.J., Govindjee (Eds.), 2010. Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation. Springer, Dordrecht.

Pereira, W.E., de Siqueira, D.L., Martínez, C.A., Puiatti, M., 2000. Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. J. Plant Physiol. 157, 513–520.

Rintamäki, E., Salo, R., Lehtonen, E., Aro, E.M., 1995. Regulation of D1 protein-degradation during photoinhibition of photosystem-II in-vivo—phosphorylation of the D1 protein in various plant groups. Planta 195, 379–386.

Schreiber, U., Bilger, W., Neubauer, C., 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D., Caldwell, M.M. (Eds.), Ecophysiology of Photosynthesis, Ecol. Stud., vol. 100. pp. 49–70.

Shangguan, Z., Shao, M., Dyckmans, J., 2000. Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. J. Plant Physiol. 156, 46–51.

Shannon, M.C., 1998. Adaptation of plants to salinity. Adv. Agron. 60, 75–119.

Stirbet, A., Govindjee, 2011. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: basics and applications. J. Photochem. Photobiol. B: Biol. 104, 236–257.

Strasser, R.J., 1978. The grouping model of plant photosynthesis. In: Akayunoglou, G., Argyroudi-Akoyunoglou, J. (Eds.). Chloroplast Development. Elsevier/North-Holland, Bio-medical, Amsterdam, pp. 513–524.

Strasser, R.J., 1981. The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids. In: Akoyunoglou, G. (Ed.), Photosynthesis. III. Structure and Molecular Organisation of the Photosynthetic Apparatus. Balaban International Science Services, Philadelphia, Pennsylvania, pp. 727–737.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Taylor & Francis, London, pp. 445–483.

Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou, G., Govindjee (Eds.), Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Springer, Dordrecht, pp. 321–362 (reprinted 2010).

Takahashi, S., Murata, N., 2005. Interruption of the Calvin cycle inhibits the repair of photosystem II from photodamage. Biochem. Biophys. Acta 1708, 352–361.

Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Kishitani, S., Takabe, T., Yokota, S., Takabe, T., 1999. Salt tolerance of transgenic rice overexpressing yeast mitochondrial Mn-SOD in chloroplasts. Plant Sci. 148, 131–138.

Yamaguchi-Shinozaki, K., 2001. Biological functions of proline osmotolerance revealed in antisense transgenic plants. JIRCAS Newslett., 27.

Yusuf, M.A., Kumar, D., Rajwanshi, R., Strasser, R.J., Tsimilli Michael, M., Govindjee, Sarin, N.B., 2010. Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll fluorescence measurements. Biochim. Biophys. Acta 1797, 1428–1438.