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Fluorescence parameters as early indicators of light stress in barley

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ABSTRACT

Photosynthetic efficiency of two Syrian barley landraces Arabi Aswad and Arabi Abiad grown under different light intensities were studied by the application of qualitative and quantitative analysis of chlorophyll *a* fluorescence. Different values of fluorescence parameters, quantum efficiencies, specific and phenomenological energy fluxes were obtained for each cultivar. Both low and high light stresses decreased photosystem II (PSII) activity in barley seedlings depending on the stress type and its duration. Cultivar Arabi Aswad was more tolerant to high light while Arabi Abiad was more tolerant to low light stress. The results allowed us to select chlorophyll *a* fluorescence parameters related to energy flux within PSII which were specifically affected under low or high light stress. We found that the performance index parameter is a sensitive indicator to explore the effect of light changes on PSII activity immediately after stress application, while maximal quantum yield of PSII and phenomenological parameters were only modified after a long period of stress application indicating PSII damage. Thus, we recommend the former parameter for early detection of light stress.

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1. Introduction

Plant growth is a dynamic process which is continuously shaped by environmental conditions. Light is one of the main factors affecting photosynthesis and plant growth as it is the source of energy for carbon fixation [1]. During the day, the quality and quantity of photosynthetically active radiation (PAR) changes frequently and plants try to keep a balance between the conversion of light energy and protection of the photosynthetic apparatus from photoinhibition or repair of eventual damage [2]. Usually, plants grown under high light intensity show a decrease in the quantum yield of photosystem II (PSII), the capacity of photosynthetic electron transport and photochemical quenching, and an increase in non-photochemical quenching as consequence of the photoinhibition of PSII. Photosystem I (PSI) proved to be more stable against photoinhibition than PSII in plants exposed to strong light treatments, due to the cyclic electron flow [3]. The negative action of light stresses on photosynthesis has been known for a long time. In particular, specific negative effects of light stress on gas exchange [1], chlorophyll content [4], chloroplast ultrastructure, enzyme activities, physiological and photochemical processes [5,6] have been established. However, a comprehensive view of the overall response of photosynthesis to both low and high light stresses is absent. On the other hand, although the main role of PSII in photosynthetic machinery performance has been fully demonstrated [7–9], there remains a lack of literature about energy flux and its destination within PSII, especially under low light intensity conditions.

Chlorophyll (Chl) *a* fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis [5,10]. It is one of the main methods to investigate the function of PSII and its reactions to changes in the environment and growth conditions [11–14]. However, only few reports illustrate the effects of exposure to low or high light intensity on photosynthetic activity [15,16] expressed as Chl *a* fluorescence parameters and do not deal with energy flux and its destination within PSII.

A powerful and popular tool to study PSII reactions is Chl fluorescence induction. The first seconds of the induction curves are characterized by three apparent kinetic steps denoted OJ, JI, and IP. These phases of fluorescence induction were correlated with peak accumulation of different reduced species on the acceptor side of PSII [17]. It was confirmed that OJ and JI phases are related to the accumulation of Q_A^- (reduced primary quinone acceptor of photosystem II) and the PSII reaction center status including photochemical and photoelectrochemical events and that IP is associated with PQ pool reduction [18–21]. The so-called JIP-test is used in different areas of plant biology to distinguish the responses of

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the photosynthetic apparatus to different stresses. It is based on the theory of energy flow in thylakoid membranes and enables an understanding of the relationships between the biophysical side of photosynthesis and various fluorescence parameters [22]. Part of the calculated parameters are related to energy fluxes for absorption (ABS), trapping (TR_O) and electron transport (ET_O) per reaction center (RC) or measured area of sample which is called cross section (CS) [22]. Additionally, this test also considers the fraction of centers that cannot reduce the secondary quinone acceptor Q_B and also estimates the entire probability of the flow of energy among components of PSII. In this work, we considered changes in quantum efficiencies, specific and phenomenological energy fluxes as they are very informative about the vitality of PSII after stress application [13]. The quantum efficiencies is equal to quantum yields or flux ratios, where the denominator is ABS i.e. TR/ABS = quantum efficiency from light absorbed for primary photochemistry to reduce Q_A, ET/ABS = quantum yield for energy transport from light absorbed (ABS) to energy transport (ET) between PSII and PSI and RE/ABS = quantum yield for energy transport from light absorbed (ABS) to the reduction of end acceptors (RE). The specific flux is the energy flux in biomembranes calculated per the excited reaction centres (RCs) of the tested sample (ABS/RC, TR_O/RC and ET_O/RC), while phenomenological flux is based on energy flux in the excited cross-section (CS) of the tested sample (ABS/CS, TR_O/CS and ET_O/CS) [13,22].

Barley (*Hordeum vulgare L.*) originates from the Eastern Mediterranean region where plants experience many abiotic stresses in the field. Its production has become more intense and complex in recent years and crop managers have to get a better understanding of the yielding of this plant. This will depend upon providing trials to estimate responses to different unfavorable conditions such as low and high light intensities which affect the photosynthetic process [3,16]. The present work presents an initial trial of chlorophyll *a* fluorescence measurements in barley plants aiming for early stress detection and to find out PSII specific reactions of different cultivars towards low and high light stresses.

2. Materials and methods

Two barley (*Hordeum vulgare* L.) landraces Arabi Aswad and Arabi Abiad (predominant in Syria- 99% of the area) were used. They are exclusively two-row types (Two row barley has lateral spikelets that bear a sterile floret, so the mature spike appears to have only two rows of kernels). Arabi Abiad (white-seeded) is common in slightly better environments (250–350 mm of rain) compared to Arabi Aswad (black-seeded) (<250 mm of rain). Considerable phenotypic and genotypic heterogeneity exists both among landraces collected in different farmers' fields (even if designated by the same name) and among individual plants within the same farmer's field. Farmers in dry areas consider the grain and straw quality of the black-seeded landrace is the best [23].

Plants were grown in 1 l dark glass pots filled with modified Hoagland nutrients solution under greenhouse conditions. The average temperature for day/night was 26/18 °C, the relative humidity was 50–60%, and photoperiod for the day/night cycle was 16/8 h. After 7 days of growth, two sets of seedlings were subjected to light stresses. Thus, seedlings were grown under 3 light intensities i.e. under 1400 (control plants), 200 (low PAR stress), or 1800 (μmol (photon) m^{-2} s^{-1}) (high PAR stress) using sodium lamps (Philips High pressure sodium, 600 W/230 V, 90.000 Lm, Gavita, Norway). The level of low and high light stresses were established in a preliminary experiment in which several light intensities were checked and two of them which caused an approximate 50% reduction of seedling growth after 14 days from germination were finally chosen.

Chlorophyll *a* fluorescence was measured using Plant Efficiency Analyzer (Handy-PEA fluorimeter, Hansatech Instruments Ltd., Pentney, King's Lynn, Norfolk, England) after 1 day (8 days after emergence) and after 7 days of stress application (14 days after emergence) on the middle region of mature leaves. Before measurement, barley seedlings were dark adapted for 45–60 min at 26 °C. Thereafter, Chl *a* fluorescence signals were analyzed with the Biolyzer v.3.0.6 software (developed by Laboratory of Bioenergetics, University of Geneva, Switzerland). The average values from 30 measurements done on 1st, 2nd and 3rd leaves for each treatment are shown. All data were analyzed using Statistica 8.0 software (Statsoft Inc., Tulsa, OK, USA). Appropriate numbers of replications and tests used are indicated in table and its description.

3. Results

3.1. Chlorophyll fluorescence induction curve

Fluorescence induction of cv. A. Abiad grown 7 days under low light resembled that of control plants although the IP phase was strongly attenuated (Fig. 1). Conversely, the transient fluorescence curve of cv. A. Aswad grown 7 days under low light was dramatically changed as compared with control plants. The initial fluorescence was much higher and all the induction phases were strongly declined showing a much flatter curve than for control plants (Fig. 1). In contrast with low PAR, transient fluorescence curve of cv. A. Aswad plants grown at high PAR was similar to the control plants but for cv. A. Abiad it was flat and almost all OJIP phases disappeared (Fig. 1).

3.2. Tf(max)

Time to reach maximal fluorescence Tf(max) of barley plants grown 1 day under low PAR stress increased for cv. A. Aswad to about 140% and decreased for cv. A. Abiad to about 86% of control plants (Table 1). Tf(max) for cv. A. Aswad grown 1 day under high PAR did not change but it dropped to 64% for cv. A. Abiad seedlings in comparison with control plants (Table 1). After 7 days of growing under low PAR Tf(max) was only slightly elevated for cv. A. Aswad but increased to about 140% for cv. A. Abiad as compared with control plants while under high PAR, this parameter was almost unchanged for cv. A. Aswad and increased to about 286% for cv. A. Abiad (Table 1).

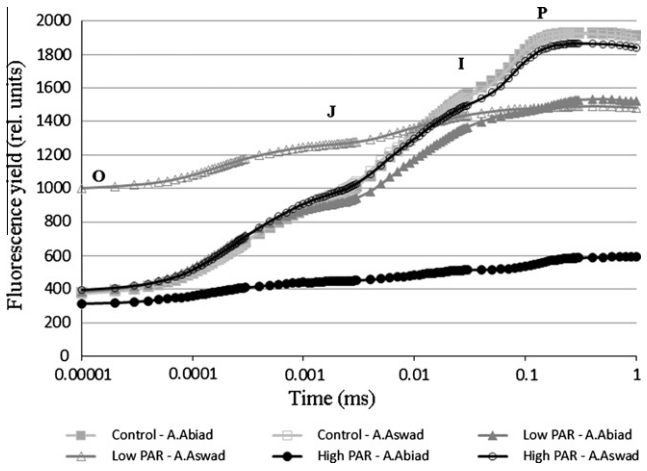

Fig. 1. Transient chlorophyll *a* fluorescence induction curves of two Syrian landraces (Arabi Abiad and Arabi Aswad) grown under low and high light stresses for 7 days.

Table 1
Some chlorophyll fluorescence parameters (% of control) of two barley cultivars (cv. A. Aswad and cv. A. Abiad) grown under low and high light stresses after 1 and 7 days of stress application.

Stress type	Stress duration	cv. Arabi Aswad					cv. Arabi Abiad				
		Tf(max)	Area	Fv/Fm	Fv/Fo	PI_{ABS}	Tf(max)	Area	Fv/Fm	Fv/Fo	PI_{ABS}
Low PAR	1 d	142.86[a]	69.95[a]	94.16[a]	78.04[a]	63.81[a]	85.71[b]	71.97[a]	99.21[a]	96.40[a]	84.48[b]
	7 d	107.14[a]	17.34[a]	38.91[a]	12.23[a]	2.12[a]	142.86[b]	61.79[b]	91.20[b]	69.78[b]	37.39[b]
High PAR	1 d	103.57[a]	94.83[a]	97.09[a]	88.04[a]	70.21[a]	64.29[b]	63.99[b]	95.92[a]	83.33[a]	49.33[b]
	7 d	96.43[a]	100.51[a]	97.35[a]	88.92[a]	80.62[a]	285.71[b]	40.32[b]	55.93[b]	22.05[b]	6.32[b]

Means followed by the same letter in the same row do not differ significantly and by the different letter in the same row differ significantly (for example, value of Area after 1 day of low PAR for A. Aswad (69.95) and for A. Abiad (71.97), do not differ significantly. However, value of Area after 1 day of high PAR for A. Aswad (94.83) and for A. Abiad (61.99) differ significantly). According to Duncan's multiple range test ($p = 0.05$; $n = 90$).

3.3. Area

After 1 day Area parameter (total complementary area between fluorescence induction curve and Fm) was lower for both cultivars under low light (decrease to ca. 70%) as compared with control plants (Table 1). For plants grown 1 day under high PAR a decrease of Area parameter to about 64% was observed only for cv. A. Abiad (Table 1). Areas of both cultivars grown for 7 days under low PAR were lower than those for control plants and this decrease was much more pronounced for cv. A. Aswad (decrease to ca. 17%) than for cv. A. Abiad (to about 60%). When plants were grown 7 days under high PAR Area parameter of cv. A. Aswad was the same as that for control plants but decreased to about 40% in cv. A. Abiad (Table 1).

3.4. Fv/Fm

Maximal quantum yield of PSII (Fv/Fm) almost did not change for plants of both cultivars grown 1 day under both low and high PAR (Table 1). Fv/Fm of cv. A. Aswad grown 7 days under low PAR was lowered to about 40% of control plants (Table 1) but for cv. A. Abiad it was decreased only to about 90% (Table 1). Fv/Fm of cv. Abiad grown 7 days under high PAR was lowered to about 56% of control plants but Fv/Fm of cv. A. Aswad was almost the same as that for control plants (Table 1).

3.5. Fv/Fo

After 1 day of stress application Fv/Fo, a parameter that accounts for the simultaneous variations in Fm and Fo in determinations of the maximum quantum yield of PSII [24], decreased to ca. 80% in plants of A. Aswad grown under low PAR and cv. A. Abiad plants grown under high PAR in comparison with control plants (Table 1). Generally, after 7 days of both PAR stresses application barley seedlings showed low Fv/Fo values. Under low PAR, Fv/Fo decreased to about 10% for cv. A. Aswad and to about 70% for cv. A. Abiad (Table 1). Reverse situation was observed under high PAR. Values of this parameter decreased more for cv. A. Abiad (to about 20%) than for cv. A. Aswad (to about 90%) (Table 1).

3.6. PI_{ABS}

Low and high PAR treatment lowered the performance index calculated on energy absorption basis PI_{ABS} of both cultivars after 1 day. When plants were grown under low PAR this drop was to about 64% for cv. A. Aswad and 84% for cv. A. Abiad as compared to control plants. Opposite changes were observed when plants were grown under high PAR – PI_{ABS} dropped only to about 70% for cv. A. Aswad and to 50% for cv. A. Abiad when weighed against control plants (Table 1). After 7 days of low PAR application PI_{ABS} in both cultivars was much lower in stressed than control plants. However, much higher decrease was found for cv. A. Aswad (ca. 2%) than for cv. A. Abiad (ca. 40%) (Table 1). Under high PAR treatment PI_{ABS} of cv. A. Aswad was about 80% that of control plants but for cv. A. Abiad it dropped to about 6% (Table 1).

3.7. Phenomenological energy flux (leaf model)

After 1 day phenomenological energy fluxes i.e. ABS/CSo, TRo/CSo, ETo/CSo and DIo/CSo for both cultivars of barley plants grown without stresses and under both light stresses were pretty similar (data not shown). After 7 days of low light application plants of cv. A. Abiad showed higher values of DIo/CSo and amount of inactive reaction centers when matched with control plants. However, higher values of these parameters were observed in plants of cv. A. Aswad. In addition, electron transport per cross section (TRo/CSo) was much lower. Finally, the situation was reversed under high PAR (Fig. 2).

4. Discussion

Generally, under low light intensity most of the absorbed light can be used in photosynthesis (high photosynthetic efficiency), but under relatively high light intensity only part of the absorbed light can be used [25]. Nevertheless, plants possess different mechanisms to cope with light stress e.g. barley plants grown at different light intensities showed changes of Chl fluorescence parameters that related to photosystem II antenna size [26]. Commonly, an increase in photosynthetic capacity reduces photodamage, whilst changes in photosystem stoichiometry serve to optimize light utilization [27].

Our experiments showed that changes in PSII were usually much lower for plants grown 1 day under a given stress than after 7 days. Using Chl *a* fluorescence we were able to capture some changes in PSII bioenergetics immediately after stress application (after 1 day). The main changes were denoted in the case of the time to reach Fm value (Tf(max)), reduced plastoquinone pool size (Area) and the vitality index of PSII (PI_{ABS}). While after 7 days of stress application, besides a greater extent of the above mentioned changes, the decline of maximal quantum yield of PSII measured as Fv/Fm or Fv/Fo clearly indicated damage caused by light stress. However, the above mentioned changes differentiated each cultivar. Cv. A. Abiad was more sensitive to 7 days under high light than cv. A. Aswad while cv. A. Aswad was more sensitive than cv. A. Abiad to low light (Table 1 and Fig. 1). These results were supported by phenomenological energy flux expressed by leaf models (Fig. 2).

Under high light, plants cope with excess light energy by means of different mechanisms of adaptation/acclimation which are mainly directed towards photosynthetic machinery defense. Recent literature reveals that plants growing under high light intensities have a smaller antenna size than those growing under low-light conditions that can protect plants against photoinhibition. Usually, the composition in major peripheral antenna proteins

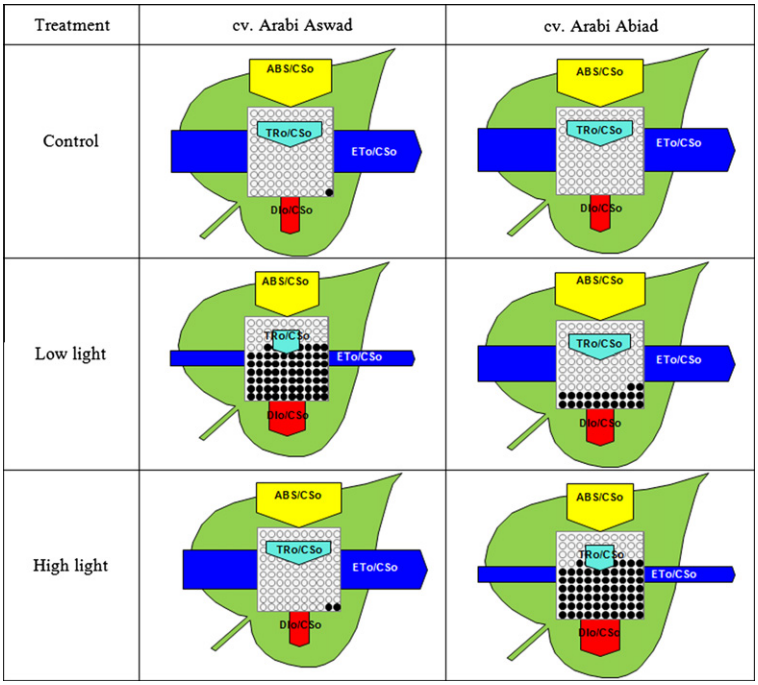

Fig. 2. Leaf model showing phenomenological energy fluxes per excited cross section (CS) of barley cvs. A. Aswad and A. Abiad grown without stress (control) and after 7 days low and high light treatment. ABS/CSo – absorption flux per CS approximated by Fo, TRo/CSo – trapped energy flux per CS, ETo/CSo – electron transport flux per CS, DIo/CSo – dissipated energy flux per CS. Each relative value is represented by the size of proper parameters (arrow), empty circles represent Q_A-reducing reaction centers (active), full black circles – non-Q_A-reducing reaction centers (inactive or silent).

is modified in response to light conditions, while the core antenna proteins and the inner peripheral antenna proteins do not change [28]. An increase of Chl *a*/*b* ratios, photosynthetic apparatus components such as PSII, cytochrome *b*/*f* complex, ATP synthase and in components of the Calvin cycle (especially RuBisCO) and a reduction in the level of LHCII leads to increased capacities for oxygen evolution, electron transport and CO_2 consumption [4]. Moreover, under high light conditions, the antenna system brings about excessive influx of photons into the photosystem II reaction center (RC) [29]. This causes a reduction of electron transport as a protection mechanism which causes photoinhibition at the acceptor side of PSII [30]. During this process the reduction in the pool of plastoquinones contributes to the recombination of the charge separated state $P680^+Pheo^-$. This creates P680 triplet ($3Chl^*$) which forms reactive singlet oxygen ($^1O_2$) when combined with molecular oxygen. This reactive species directly or by means of secondary radicals, attacks protein D1 and causes its degradation [25,31]. Some experiments revealed that, plants acclimated to low light also showed lower capacity to scavenge reactive oxygen species (ROS) compared with plants grown in full sun [32–34]. However, recent studies *in vivo*, in which photoinhibition (photodamage) was examined separately from repair, demonstrated that ROS act primarily by inactivating the repair of PSII and not by damaging PSII directly with the resultant apparent enhancement of the extent of photoinhibition. In cyanobacterial cells, the *de novo* synthesis of the D1 protein was markedly suppressed by elevated intracellular levels of ROS when the repair of PSII was inactivated [35–37]. Not only the synthesis of the D1 protein but also the synthesis of almost all other proteins was suppressed at elevated levels of ROS [35–38]. Suppression of protein synthesis under oxidative stress was also observed in mutants that were deficient in carotenoids [39] and in α-tocopherol [40]. The global suppression of protein synthesis suggested that the protein-synthesis machinery might be a specific target of inactivation by ROS under high light illumination [10,37,38,41,42].

In addition, in the current scheme, photodamage occurs via a two-step process: the first step is the light-dependent destruction of the manganese cluster of the oxygen-evolving complex of PSII and the second step is the inactivation of the photochemical reaction center of PSII by light that has been absorbed by Chl [6]. For details of this mechanism of photoinhibition, the reader is referred to articles [10,37,38,41,42].

Fv/Fm for most plants grown without stress is close to 0.83 [43]. Values lower than 0.83 suggest that plants are growing under stress and that PSII reaction centers are damaged which, in turn, is connected with reduced effectiveness of electron transport such as when plants are grown under excess light [44]. This parameter is usually considered as a suitable indicator characterizing photoinhibition [44–46]. However, our results did not support this idea as Fv/Fm of barley seedlings grown under both radiation stresses did not show any measurable change after 1 day of stress application. Changes were only observed when severe changes or damage to PSII structure have taken place such as after 7 days of stress application (Table 1) [16]. Our results revealed that the performance index (PI_{ABS}) which is related to the general vitality of PSII was the most sensitive parameter to capture the effect of PAR changes in the short time scale (Table 1). Our results support the work of Force et al. [8] who reported that, among all the JIP-test parameters, TRo/ABS (representing Fv/Fm) was the least sensitive to changes under stress application.

An analysis of Chl *a* fluorescence parameters by application of the leaf model (Fig. 2) shows that in shade plants of cv. A. Aswad electron transport (ETo/CSo) decreased. That was due to lower energy absorption by antenna pigments (ABS/CSo), energy trapping by reaction centers (TRo/CSo) and higher energy loss as heat (DIo/CSo). On the other hand, this cultivar proved to be more tolerant to excess light stress when grown at high light intensity (1800μmol (photon) m^{-2} s^{-1}) (Fig. 2). This suggests that the photosynthetic machinery of this cultivar has the ability to cope with or uses excess light energy.

One attribute which this cultivar could possess is the involvement of photorespiration which seems to play an important role under high light stress. It accepts electrons when CO_2 assimilation is low e.g. under excess light conditions and it protects electron transport components between PSII and PSI against over-reduction [47]. The Mehler reaction also acts as a dissipating energy mechanism allowing the maintenance of electron flow [48]. Another possibility for cv. Arabi Aswad to deal with excess light could be by enhancing cyclic electron transport activity which plays an important role in photoprotection and this transport increases under photoinhibitory conditions [49,50].

An increase of pH-dependent energy dissipation i.e. nonphotochemical quenching, protects the photosynthetic electron transport chain against over-reduction and is another photo-protective mechanism which could be involved under light stress. Thermal dissipation in photosynthetic pigments in the antenna system is also regulated through the xanthophyll cycle where zeaxanthin from thylakoid membranes plays a photo-protective role through non-photochemical quenching [30,51] and this quenching is faster than electron transport and photochemical reactions. Under high light, zeaxanthin and antheraxanthin content increases which leads to energy dissipation as heat as a result of de-epoxidation of violaxanthin to antheraxanthin and further to zeaxanthin in the xanthophyll cycle. These changes were shown to be much lower in shade plants [24,52,53]. Carotenoids also can play a direct role in photoprotection as they can prevent the formation of radicals derived from oxygen or other reactive molecular species [54] stimulating the transition of singlet oxygen into triplets and dispersing the absorbed energy as heat [25,48]. However, heat dissipation was unexpectedly higher in the case of low light treatment in the case of cv. A. Aswad (Fig 2) which is very difficult to interpret. We propose that heat dissipation, expressed by higher minimal fluorescence value (Fo) (Fig. 1) and heat dissipation calculated per cross section (DIo/CSo) (Fig. 2), is not only an indicator of the ability of plants to cope with excess light but also could be a kind of need or cost to enhance light absorption and energy flux within PSII antenna under low light.

Conversely, the opposite was observed in the case of cv. A. Abiad which only showed the ability to use low light efficiently when compared with cv. A. Aswad [54,55]. This aptitude could be related to an increase in light-harvesting complexes (LHCII), grana thylakoids [1,27] and amount of PSI [4]. On the other hand, for the quantitative analysis of the mechanism of photoinhibition of PSII, it is essential to monitor the rate of photodamage and the rate of repair separately and, also, to examine the respective effects of various perturbations on the two processes. Above mentioned all early studies of photoinhibition suggested that all of these factors and mechanisms protect PSII against photodamage. However, re-evaluation by the strategy mentioned above has indicated that, rather than protecting PSII from photodamage, they stimulate protein synthesis, with resultant repair of PSII and mitigation of photoinhibition (see [10,37,38,41,42]).

In summary, low and high light stresses negatively influenced PSII activity of barley plants and these effects were dependent on the duration of the stress and the tested cultivar since Chl *a* fluorescence parameters characterizing PSII activity changed in different manners. The results reported here allowed us to determine the Chl *a* fluorescence parameters related to energy flux within PSII which were mostly changed under specific stress. It seems to be that only the performance index of PSII (PI_{ABS}) could be considered as a good indicator to unveil light changes effect on PSII activity immediately after stress application (after 1 day). The time to reach maximal fluorescence (Tf(max)) and the size of reduced pool of plastoquinone (Area) could also help to identify early light stress effects on the photosynthetic machinery of barley. In contrasts, the PSII maximal efficiency (Fv/Fm) and the phenomenological parameters related to energy absorption, trapping and electron transport calculated from the JIP-test on the basis of cross section were shifted only after a long period of stress application (7 days). Thus, Fv/Fm cannot be recommended as an early detection tool of light stress.

Both cultivars used here originated from the same geographical region (Fertile Crescent in Syria). Despite the fact they were cultivated for a long time in the same agricultural expanse as local landraces and proved to be well adapted to high light conditions in Mediterranean basin, cultivar A. Aswad seems to be more tolerant to high PAR while cv. A. Abiad was more tolerant to low PAR stress. This suggests that each cultivar has different strategies and mechanisms to cope with light stress. Our results partially explain why cv. Abiad is typically grown in more favorable environments and cv. A. Aswad has been preferred to be cultivated in that region.

5. Abbreviations

ABS/CSo*	Absorption flux per cross section (CS) at $t = 0$, approximated by Fo
Area	The area above chlorophyll fluorescence curve between Fo and Fm (total complementary area between fluorescence induction curve)
Chl	Chlorphyll
CS*	Cross section of tested sample
DIo/CSo*	Dissipated energy flux per cross section (CS) at $t = 0$
ETo/CSo*	Electron transport flux per cross section (CS) at $t = 0$
Fo	Chlorophyll fluorescence intensity measured when all photosystem II reaction centers are open
Fm	Maximal chlorophyll fluorescence intensity measured when all photosystem II reaction centers are closed
Fv	Variable chlorophyll fluorescence (Fm − Fo)
Fv/Fm	Maximum quantum yield of PSII
Fv/Fo	Efficiency of the water-splitting complex on the donor side of PSII
OJIP	transient* – fluorescence induction defined by the names of its intermediate steps
PAR	Photosynthetically active radiation
PI^*_{ABS}	Performance index on absorption basis where: $PI_{ABS} = \frac{RC}{ABS} \cdot \frac{\varphi_{Po}}{1-\varphi_{Po}} \cdot \frac{\psi_o}{1-\psi_o}$
Q_A	The primary quinone acceptor of photosystem II
RC	Reaction center
Tf(max)	Time needed for reaching Fm (ms)
TRo/CSo*	Trapped energy flux per cross section (CS) at $t = 0$
φ^*_{Po}	Maximal quantum yield of primary photochemistry
ψ^*_o	Exciton transfer efficiency to electron transport chain
*	Calculations of these parameters are derived from Schindler and Lichtenthaler [54]

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References

[1] R.G. Walters, P. Horton, Acclimation of *Arabidopsis thaliana* to the light environment: changes in composition of the photosynthetic apparatus, Planta 195 (1994) 248–256.
[2] S. Bailey, P. Horton, R.G. Walters, Acclimation of *Arabidopsis thaliana* to the light environment: the relationship between photosynthetic function and chloroplast composition, Planta 218 (2004) 793–802.
[3] M.J. Quiles, N.I. López, Photoinhibition of photosystems I and II induced by exposure to high light intensity during oat plant growth. Effects on the chloroplast NADH dehydrogenase complex, Plant Sci. 166 (2004) 815–823.
[4] S. Bailey, R.G. Walters, S. Jansson, P. Horton, Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses, Planta 213 (2001) 794–801.
[5] H. Clijsters, F. Van Assche, Inhibition of photosynthesis by heavy metals, Photosynth. Res. 7 (1985) 31–40.
[6] N. Ohnishi, S.I. Allakhverdiev, S. Takahashi, S. Higashi, M. Watanabe, Y. Nishiyama, N. Murata, Two-Step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center, Biochemistry 44 (2005) 8494–8499.
[7] U. Schreiber, Detection of rapid induction kinetics with a new type of high frequency modulated chlorophyll fluorescence, Photosynth. Res. 9 (1986) 261–272.
[8] 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, Photosynthesis Res. 78 (2003) 17–33.
[9] M.H. Kalaji, A. Rutkowska, Photosynthetic machinery response of maize seedlings to salt stress, Zesz. Probl. Post. Nauk Roln. 496 (2004) 545–558 (In Polish).
[10] S.I. Allakhverdiev, N. Murata, Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage-repair cycle of photosystem II in *Synechocystis* sp. PCC 6803, Biochim. Biophys. Acta 1657 (2004) 23–32.
[11] M.H. Kalaji, K. Rykaczewska, S. Pietkiewicz, E. Kotlarska-Jaros, The use of chlorophyll a fluorescence to study the effect of foliage application of nitrogenous-sulphuric fertilizer on photosynthetic activity and growth of very early potato cultivar, Zesz. Probl. Post. Nauk Roln. 496 (2004) 367–374 (In Polish).
[12] M.H. Kalaji, E. Wolejko, T. Loboda, S. Pietkiewicz, Z. Wyszynski, Chlorophyll a fluorescence. A convenient tool for photosynthetic performance evaluation of barley plants grown under different nitrogen rates, Zesz. Probl. Post. Nauk Roln. 496 (2004) 375–383 (In Polish).
[13] A. Oukarroum, R.J. Strasser, Phenotyping of dark and light adapted barley plants by the fast chlorophyll a fluorescence rise OJIP, South African J. Bot. 70 (2004) 277–283.
[14] N.G. Bukhov, R. Carpentier, Effects of water stress on the photosynthetic efficiency of plants, in: Chlorophyll a Fluorescence. A Signature of Photosynthesis, Springer, Dordrecht, The Netherlands, 2004. pp. 623–635.
[15] L.T. Evans, in: R.O. Slatyer (Ed.), Plant Response to Climatic Factors, UNESCO, Paris, 1973. pp. 21–35.
[16] D. Bruce, S. Vasil'ev, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration, Springer, Dordrecht, 2004, pp. 497–523.
[17] X.G. Zhu, Govindjee, N. Baker, E. deSturler, D. Ort, S. Long, Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II, Planta 223 (2005) 114–133.
[18] S. Boisvert, D. Joly, R. Carpentier, Quantitative analysis of the experimental O-J-I-P chlorophyll fluorescence induction kinetics. Apparent activation energy and origin of each kinetic step, FEBS J. 273 (2006) 4770–4777.
[19] A. Gauthier, D. Joly, S. Boisvert, R. Carpentier, Period-four modulation of photosystem II primary quinone acceptor (Q_A) reduction/oxidation kinetics in thylakoid membranes, Photochem. Photobiol. 86 (2010) 1064–1070.
[20] W. Vredenberg, M. Durchan, O. Prášil, Photochemical and photoelectrochemical quenching of chlorophyll fluorescence in photosystem II, Biochim. Biophys. Acta 1787 (2009) 1468–1478.
[21] D. Joly, J. Essemine, R. Carpentier, Redox state of the photosynthetic electron transport chain in wild-type and mutants leaves of *Arabidopsis thaliana*: Impact on photosystem fluorescence, J. Photochem. Photobiol. B: Biol. 98 (2010) 180–187.
[22] R.J. Strasser, in: G. Akoyunoglou (Ed.), Structure and Molecular Organisation of the Photosynthetic Apparatus, Balaban International Science Services, Philadelphia, Pennsylvania, 1981. pp. 727–737.
[23] S. Ceccarelli, S. Grando, R. Tutwile, J. Bah, A.M. Martini, H. Salahieh, A. Goodchild, A methodological study on participatory barley breeding. I. Selection phase, Euphytica 111 (2000) 91–104.
[24] S. Govindachary, N.G. Bukhov, D. Joly, R. Carpentier, Photosystem II inhibition by moderate light under low temperature in intact leaves of chilling-sensitive and – tolerant plants, Physiol. Plant 121 (2004) 322–333.
[25] S.P. Long, S. Humphries, P.G. Falkowski, Photoinhibition of photosynthesis in nature, Annu. Rev. Plant Physiol. Plant Mol. Biol. 45 (1994) 633–662.
[26] P. Chernev, V. Goltsev, R.J. Strasser, A highly restricted model approach quantifying structural and functional parameters of photosystem II probed by the chlorophyll a fluorescence rise, Ecol. Eng. Ecol. Protect. 6 (2006) 19–29.
[27] R.G. Walters, Towards understanding of photosynthetic acclimation, J. Exp. Bot. 56 (2005) 435–447.
[28] R. Tanaka, A. Tanaka, Chlorophyll b is not just an accessory pigment but a regulator of the photosynthetic antenna, Porphyrins 9 (2000) 240–245.
[29] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants, Annu. Rev. Plant Physiol. 47 (1996) 655–684.
[30] I. Baroli, A. Melis, Photoinhibitory damage is modulated by the rate of photosynthesis and by the photosystem II light-harvesting chlorophyll antenna size, Planta 205 (1998) 288–296.
[31] N. Adir, H. Zer, S. Shokhat, I. Ohad, Photoinhibition – a historical perspective, Photosynth. Res. 76 (2003) 343–370.
[32] E.H. Murchie, P. Horton, Acclimation of photosynthesis to irradiance and spectral quality in british plant species: chlorophyll content, photosynthetic capacity and habitat preference, Plant Cell Environ. 20 (1997) 438–448.
[33] B.A. Logan, B. Demmig-Adams, W. Adams, W.S. Grace, Antioxidant and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. Acclimated to four growth PPFDs in the field, J. Exp. Bot. 49 (1998) 1869–1879.
[34] D.J. Burritt, S. MacKenzie, Antioxidant metabolism during the acclimation of begonia x erythrophylla to high light levels, Ann. Bot. 91 (2003) 783–794.
[35] Y. Nishiyama, Yamamoto, S.I. Allakhverdiev, M. Inaba, A. Yokota, N. Murata, Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery, EMBO J 20 (2001) 5587–5594.
[36] Y. Nishiyama, S.I. Allakhverdiev, H. Yamamoto, H. Hayashi, N. Murata, Singlet oxygen inhibits the repair of photosystem II by suppressing the translation elongation of the D1 protein in *Synechocystis* sp. PCC 6803, Biochemistry 43 (2004) 11321–11330.
[37] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, Inhibition of the repair of photosystem II by oxidative stress in cyanobacteria, Photosynth. Res. 84 (2005) 1–7.
[38] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II, Biochim. Biophys. Acta 1757 (2006) 742–749.
[39] O. Sozer, J. Komenda, B. Ughy, I. Domonkos, H. Laczkó-Dobos, P. Malec, Z. Gombos, M. Kis, Involvement of carotenoids in the synthesis and assembly of protein subunits of photosynthetic reaction centers of *Synechocystis* sp. PCC 6803, Plant Cell Physiol. 51 (2010) 823–835.
[40] S. Inoue, K. Ejima, E. Iwai, H. Hayashi, J. Appel, E. Tyystjarvi, N. Murata, Y. Nishiyama, Protection by α-tocopherol of the repair of photosystem II during photoinhibition in *Synechocystis* sp. PCC 6803, Biochim. Biophys. Acta 2011 (1807) 236–241.
[41] N. Murata, S. Takahashi, Y. Nishiyama, S.I. Allakhverdiev, Photoinhibition of photosystem II under environmental stress, Biochim. Biophys. Acta 1767 (2007) 414–421.
[42] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II, Physiol. Plant 142 (2011) 35–46.
[43] O. Björkman, B. Demmig, Photon yield of o_2 evolution and chlorophyll fluorescence characteristics at 77 k among vascular plants of diverse origins, Planta 170 (1987) 489–504.
[44] P.S. Basu, A. Sharma, N.P. Sukumaran, Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress, Photosynthetica 35 (1998) 13–19.
[45] J. He, C.W. Chee, C.J. Goh, "Photoinhibition" of *Heliconia* under natural tropical conditions: the importance of leaf orientation for light interception and leaf temperature, Plant Cell Environ. 19 (1996) 1238–1248.
[46] M.M. Seppänen, Characterization of Freezing Tolerance in Solanum Commersonii (dun.) with Special Reference to the Relationship Between Freezing and Oxidative Stress, Academic dissertation. Publ. no. 56, Univ. of Helsinki, Dept. of Plant Production, Section of Crop Husbandry, Helsinki, 2000.
[47] A. Kozaki, G. Takeba, Photorespiration protects C_3 plants from photooxidation, Nature 384 (1996) 557–560.
[48] S.B. Powles, Photoinhibition of photosynthesis induced by visible light, Annu. Rev. Plant Physiol. 35 (1984) 15–44.
[49] H.B. Teicher, H.V. Scheller, The NAD(P)H dehydrogenase in barley thylakoids is photoactivatable and uses NADPH as well as NADH, Plant Physiol. 117 (1998) 525–532.
[50] S. Govindachary, C. Bigras, J. Harnois, D. Joly, R. Carpentier, Changes in the mode of electron flow to photosystem I following chilling-induced photoinhibition in a C_3 plant, *Cucumis sativus L.* Photosynth. Res. 94 (2007) 333–345.
[51] K.K. Niyogi, Photoprotection revisited: genetic and molecular approaches, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 333–359.
[52] B. Demmig-Adams, D.L. Moeller, B.A. Logan, W.W. Adams, Positive correlation between levels of retained zeaxanthin + antheraxanthin and degree of photoinhibition in shade leaves of *Schefflera arboricola*, Planta 205 (1998) 367–374.
[53] R.C. Bugos, S.H. Chang, H.Y. Yamamoto, Developmental expression of violaxanthin de-epoxidase in leaves of tobacco growing under high and low light, Plant Physiol. 121 (1999) 207–213.
[54] C. Schindler, H.K. Lichtenthaler, Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field-grown maple trees in the course of a sunny and a cloudy day, J. Plant Physiol. 148 (1996) 399–412.
[55] G.H. Krause, Photoinhibition of photosynthesis. – An evaluation of damaging and protective mechanisms, Physiol. Plant. 74 (1988) 566–574.