Effects of Salt Stress on Photosystem II Efficiency and CO_2 Assimilation in Two Syrian Barley Landraces

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Abstract: Gas exchange and chlorophyll (Chl) a fluorescence measurements were made to study the effect of salinity stress (120 mmol NaCl) on the photosynthetic activity of two Syrian barley (*Hordeum vulgare* L.) landraces, Arabi (A.) Aswad and A Abiad. Our work provides important information on the detection of salt stress-induced changes in the two cultivars of barley used, since we measured, in parallel, both gas exchange and Chl fluorescence. Early reactions of the photosynthetic apparatus of barley plants must play a key role in their tolerance to salt stress. We expect extension of this research to be helpful in solving the salinity problem and other environmental challenges facing us.

Keywords: Barley; Chlorophyll fluorescence; Photosynthesis; Photosystem II

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 is one of the major abiotic stresses. The mechanisms of salt tolerance are very complex and the metabolic sites of salt influence have not been fully investigated, in particular in the photosynthetic apparatus (Kalaji and Nalborczyk, 1991; Kalaji and Pietkiewicz, 1993; Munns, 2002); thus, there are no reliable indicators of plant tolerance to salinity that could be used by plant breeders to improve salinity tolerance.

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. 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 minimum fluorescence level when all Q_A is in the oxidized state, the reaction centers II are open; and the primary photochemistry is maximum, whereas at the "P" level (or F_{max}), fluorescence is maximum, 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 of photosystem I and the primary photochemistry is at a minimum level (Munday and Govindjee, 1969 a, b; Govindjee, 1995, 2004).

The aim of the present study was to improve our knowledge of the responses of the barley plant photosynthetic apparatus to salinity stress through

application of the combined rapid and non-destructive fluorescence assay and gas exchange measurements. We present here some of the highlights of this research. Further details are available in Kalaji *et al*. (2010).

Abbreviations (Based on Strasser *et al*., 2000)

ABS/RC—light absorption flux (for antenna chlorophylls) per reaction center (RC),

DI_o/RC—dissipation energy flux per reaction center (RC) (at t=0),

ET_o/RC—electron transport flux (beyond Q_A^-) per reaction center (RC) (at t=0),

TR_o/RC—trapped energy flux (leading to Q_A reduction) per reaction center (RC) (at t=0),

RC/ABS—density of reaction centers per antenna chlorophyll,

Area—the area above the chlorophyll fluorescence curve (reflecting the size of the platoquinone pool),

$\Delta V/\Delta t_o$—the initial slope of the relative variable fluorescence which directly describes the trapping flux TR_o/RC,

F_o—minimum level of chlorophyll fluorescence

F_m—maximum level of chlorophyll fluorescence

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,

PI_{ABS}—the performance index calculated as: (RC/ABS) × (φ_{Po}/(1−φ_{Po})) × (ψ_o /(1− ψ_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),

SFI_{ABS}—an indicator of PSII 'structure and functioning', calculated as (RC/ABS) × φ_{Po} × ψ_o,

S_M—(*Area*) / (Fm-Fo), 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, **kn** = kh (rate constant of heat dissipation) + kf (rate constant of fluorescence emission) + kx (rate constant of energy migration to PSI),

T_{FM}—time needed to reach F_m,

Vj—relative variable fluorescence at time J (relative variable fluorescence at phase J of the fluorescence induction curve,

φ_{Do}—thermal dissipation yield,

φ_{Eo}—electron transport yield,

$\varphi_o/(1-\varphi_o)$—a 'conformation' term for primary photo-chemistry,

$\psi_o/(1-\psi_o)$—'conformation' term for thermal reactions (non-light dependent reactions).

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 ^oC, relative humidity was 50%−60%, the photoperiod for the day/night cycle was 16/8 h, and the maximum photosynthetically active radiation used was ~ 1,400 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 mmol. 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.

Gas exchange parameters were measured by CIRAS-2 *Photosynthesis Measurements System* (PP Systems International, Inc., Amesbury, MA, U.S.A.). 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 minutes at room temperature. Chlorophyll *a* fluorescence induction transients were measured when leaves were exposed to a strong light pulse (3,500 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; for a review see Stirbet and Govindjee, 2011).

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 made 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).

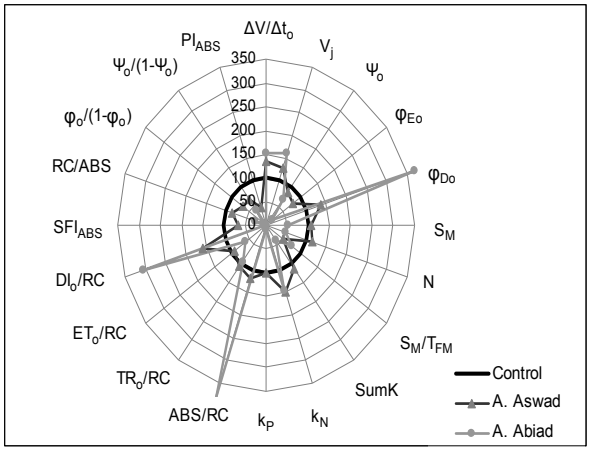

Fig. 1 A 'spider plot' of selected parameters characterizing behavior of Photosystem II of barley leaves, exposed for 7 days to 120 mmol NaCl. All values are shown as percent of control (control plants = 100).

Results and Discussion

We found that the photosynthetic apparatus of A Aswad was much more tolerant to salt treatment, compared with that of A. Abiad. Further, we found that the first stage of salinity effect on photosynthesis of barley plants is related to stomatal conductance limitation rather than to photosystem II (PSII) activity reduction (data not shown). Salinity treatment caused a decrease in both the rates of photosynthesis and PSII activity, the latter evaluated from chlorophyll (Chl) fluorescence signals. After 1 day of salt application, CO_2 uptake (photosynthetic rate - P_N) and stomatal conductance (g_s) decreased by ~20%−30% in the case of A. Abiad, whereas A. Aswad was unaffected. Surprisingly, a significant decrease of Performance Index (PI_{ABS}) was observed in A. Aswad, but less so in A. Abiad (data not shown).

The Performance Index was drastically lowered for both the cultivars after 7 days of stress application (Fig. 1). 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 1). 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 1).

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 mmol NaCl. Numbers are given as percentage of control after 7 days of salt application.

Parameter	A. Abiad	A. 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

In contrast to the tolerant A. Aswad, the sensitive A. Abiad showed a very high value of the initial (minimal) fluorescence (Fo) and a fluorescence transient curve that was essentially flat; this result may be due to several causes that include structural changes as well as changes in the rate constants of different dissipative processes (Fig. 2).

The Chl fluorescence parameters that were most affected, by salt treatment, in A. Abiad were: dissipation energy flux per reaction center (DIo/RC), related thermal dissipation yield (φDo) and light absorption flux per reaction center (ABS/RC) (Fig. 2).

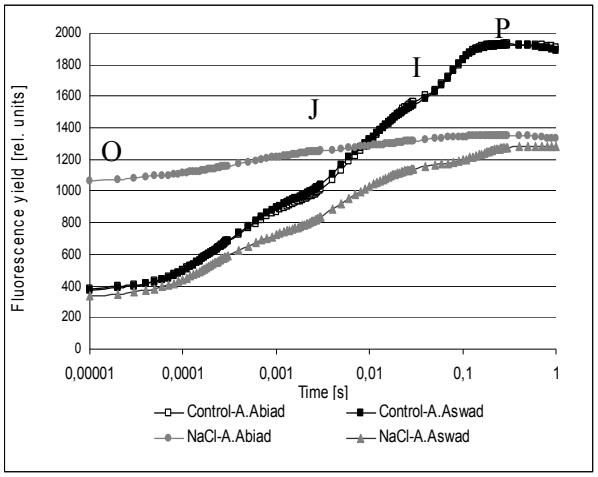

Fig. 2 Chlorophyll *a* fluorescence induction curve of barley seedlings of the two cultivars (Arabi Aswad and Arabi Abiad) grown under salinity stress (120 mmol NaCl) for 7 days.

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.

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 (see chapters in Papageorgiou and Govindjee (2004, reprinted 2010). Apart from the commonly applied fluorescence parameters, such as F_v/F_m, that measures PSII efficiency, our research, and those of many others, 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.

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