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Salinity and Photochemical Efficiency of Wheat

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Abstract: The aim of the present study was to understand the effect light intensity on the fluorescence parameters of PSII \((F_s/F_m)\) and the linear photosynthetic Electron Transport Rate (ETR) of leaf sections exposed to high salt stress. Estimation of Chl a concentration and accumulation during greening of dark-grown leaf sections within different salt gradients been used as a tool to support the Chl fluorescence parameters in light adapted plants entainment to Chl accumulation. Since the Chlorophyll (Chl) content is index of the photosynthetic fluorescence parameters in plants, the accumulation of Chl a was investigated in dark-adapted leaf. In this research, the impact of salt concentration and light intensity on photosynthetic fluorescence parameters was studied. Added to that, the regulation occurred in the parameters value was mainly species-dependent but also was perturbed according to the growth conditions. A marked increase in the ETR value was obtained following the short recovery of 1 h. Salt concentration in combination with the light intensity affected the photosynthetic parameters. In addition, the change in Chl fluorescence found to be species-dependent and also specific to plant growth conditions. The \(F_s/F_m\) ratio has decreased in leaf sections after incubation for 2 h in 600 mM under light intensity of 40 \(\mu\text{mol}/\text{m}^2/\text{sec}\).

Key words: Electron transport rate, \(F_s/F_m\), Chlorophyll, salt stress, \textit{Triticum aestivum}

INTRODUCTION

Abiotic stress, especially high salinity, has a major influence on crop growth and development and can be responsible for substantial losses in economic yield. Over 6% of arable land is salt-affected and this proportion is increasing in response to land clearance and the use of irrigation (Munns, 2005). Soil salinity stresses plants in two ways. High concentrations of salts in the soil make it harder for roots to extract water and high concentrations of salts within the plant can be toxic. Salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants before they affect plant function. We discuss the physiology and molecular biology of mechanisms that allow plants to adapt to these stresses (Munns and Tester, 2008). Salt stress can influence different aspect of the plants such as gene expression and physiological activities (Shan et al., 2008). Chlorophylls in normally functioning biological systems are bound to proteins with specific binding location (Van Amerongen et al., 2000). Other substances are also bound to proteins, e.g., carotenoids and electron transport components. Light harvesting complexes enclose carotenoids and Chl. On the other hand, the core-complex contains electron transport components like quinones and iron- sulphur complexes (Ort and Youum, 1996). The orientation inside the pigment-protein complex is necessary for the complex function. As the proper organization allows the energy transfer from the carotenoids or Chl b to the Chl a, then between Chl a molecules till reach the reaction center of where a photochemistry occurs (Govindjee et al., 1995). Chl complexes are associated to thylakoids membranes in the chloroplast and the pigment-protein complexes are organized into two main supercomplexes, photosystems I and II (PSI and II). The electron transport system in the reaction center is in connection to other electron transport components. This link is crucial in transporting electrons from water at the oxidizing end of PSII to ferredoxin and finally NADP at the reducing PSI side. Plastoquinol and plastocyanin are two components in the electron chain and highly mobile. Chl fluorescence is mainly originated from the PSII (Krause and Weis, 1991) and is used as an index for Chl concentrations. Fluorescence emission per unit Chl varies according to photosynthesis and light intensity. When the light source is low, a low fluorescence from the pigment is also measured (Kromkamp and Forster, 2003). The primary electron acceptor QA is oxidized under dark conditions. Therefore the fluorescence emission in this case is described as F0. When QA is partially reduced upon illumination the fluorescence emission is termed F. If single reduction occurs to QA, the fluorescence emission increase and reach the maximum (Fm). This particularly occurs after dark-adapted sample receive flash light. \(F_s/F_m\) is not
always an indication of the status of photosynthesis. It was reported the decrease in the F/F_\text{m}' even with highest rates of electron transport (Ensminger et al., 2001). On the other hand, F/F_\text{m}' was constant over generations of phytoplanktons exposed to nutrient stress (Parkhill et al., 2001).

Electron Transport Rate (ETR) is an actual rate of electron flow and is derived from the quantum yield of PSII (Schreiber et al., 1994). Few methods are developed for ETR calculation (Kromkamp and Forster, 2003). Under drought stress ETR found to be species-dependent. For example, no changes in ETR values were determined in E. multiflora plant whereas a significant lower ETR was detected in G. alypum plant under drought conditions than the controls (Lirorenz et al., 2003). Salt stress, a serious abiotic stress problem in the soil adversely affecting plant growth and metabolism (Allakhverdiev et al., 1998). Salt stress lead to destruction of chloroplast fine structure and instability of pigment-protein complexes (Zaman et al., 2002, Abdelkader et al., 2007a). In addition, salinity affects both Chl content and Chl fluorescence parameters. It was reported that salt stress promotes the increase of F_\text{m}' and decrease of F_\text{P} and F_\text{F_\text{m}}'. High salt stress affects the contents of Chl a and b and reduces Chl a and b ratio (Netondo et al., 2004; Ranjbarfardoei et al., 2006). During salt stress quantum yield of PSII photochemistry is decreased which can refer to a decrease in repairing the damaged PSII (Allakhverdiev et al., 2002).

Dark-grown plants lack Chl upon illumination in normal conditions Chl is spontaneously accumulated with the increase of the irradiation period. High salt stress causes retardation in Chl accumulation and inhibition of PSI fluorescence even after prolonged irradiation to continuous white light (Abdelkader et al., 2007b).

In the present study, an assessment of the effect of various light intensities on the fluorescence parameters i.e., the maximum photochemistry yield of PSII (F/F_\text{m}) and the linear photosynthetic Electron Transport Rate (ETR) of leaf sections exposed to high salt stress (600 mM) was carried out in wheat plants with variable tolerance capabilities to salt stress. The ETR was additionally measured in leaf sections treated with moderate salt stress (300 mM) under low light intensity. Estimation of Chl a concentration and accumulation during greening of dark-grown leaf sections within different salt gradients been used as a tool to support the Chl fluorescence parameters in light adapted plants entailment to Chl accumulation. We focused in detecting among the two wheat varieties submitted to different growth programs the unique variant which often shows higher Chl fluorescence values and hence better acclimation to the salt stress.

**MATERIALS AND METHODS**

This project was conducted at Arak University (Iran) during year 2009.

**Plant material and growth conditions:** Wheat grains (Triticum aestivum, cv. Giza 168 and tolerate salt up to 400 ppm salt and Seds 1, tolerate salinity up to 600 ppm were brought from the National Research Institute, Cairo, Egypt). Grains were soaked under running tap water for 16 h at room temperature in darkness. The grains were sown on a stainless grid fixed on the top of a plastic box (22×10 cm). Hoagland's nutrient solution was used as a liquid culture medium (Hoagland and Arnon, 1950). Seedlings were grown for 8 days at room temperature in a growth chamber under continuous white light (light intensity 35 μmol/m²/sec). While other set of grains were grown in darkness for 8 days.

The primary leaf was cut 1 cm from the tip and the following 2 cm were used for measurements. The leaf sections were floated in Petri dishes on Hoagland's solution or on Hoagland's solution supplied with different concentrations of salt (200, 300 and 600 mM). The salt added was a mixture of NaCl and KCl in a 1:1 Na:K ratio on a molar basis. Before illumination the dark-grown leaves were pre-treated by floating on salt solutions in the dark for 1.5 h. Dark-grown leaf sections were irradiated with continuous light (light intensity 35 μmol/m²/sec) for various periods of time during incubation. Light-grown leaf sections were incubated in 600 mM in Hoagland under different light intensities as indicated under results.

**Chl fluorescence:** Three leaf sections from light-grown wheat were placed using the LI-Cor PAM-2000 adapter chamber and coupled to a portable modulated fluorometer (PAM-2000, Walz, Effeltrich). Leaves were placed in dark-adapted state (DAS) for 30 min (Genty et al., 1989) in light isolating clips. DAS allow the reaction centers to re-oxidize. The Chl fluorescence parameters measured were: maximum photochemical efficiency of PSII (F/F_\text{m}) of dark-adapted samples and the rate of linear electron transport (ETR). F/F_\text{m}' ratio is the average from 12 replicates while ETR is the average from 6-10 separate measurements.

**Pigment determination:** Pigments were extracted using 80% acetone as described by Brouers and Michel.
Wolwertz (1983). Briefly, 0.1 g of wheat leaves was ground together with 4 mL of 80% acetone using a glass homogenizer. Acetone extracts were centrifuged at 3000 g for 7 min (Labofuge, 200) and the supernatant was used to measure the absorption spectra in room temperature with a Perkin Elmer Lambda 900 UV/VIS spectrophotometer. The amount of pigments was calculated according to Brouers and Michel-Wolwertz (1983). Chlorophyll measured in plants with different age and treatment.

RESULTS

Salt concentration under variable light intensities; a factor affecting ETR: The variable light intensity exposed to wheat leaf sections during floating on 600 mM salt play a role in altering the rate of linear electron transport (ETR). At 35 μmol/m²/sec.

The highest ETR value detected after salt (600 mM) treatment for 2 h was found in the tolerant grown using both growth programs, especially in the leaf sections grown in 200 mM salt than in leaf grown without salt stress (Fig. 1). A marked increase in the ETR value was obtained following the short recovery of 1 h. The ETR of the susceptible leaf grew in 200 mM was not different after the recovery period although the change in ETR in the susceptible leaf grown without salt stress was higher after the recovery (Fig. 1B). Referrred to the previous experiment, increase of light intensity till 40 μmol/m²/sec lead to an increase of ETR in the tolerant cultivar. The increase was significant and observable both during incubation in 600 mM salt or after the short recovery (Fig. 2). The susceptible leaf preceded a decrease of ETR during 2 h incubation in 600 mM compared to the previous treatment whereas the value after the recovery was very much similar in the salt stressed leaf during growing but was decreased in the normal grown leaf compared to the previous experiment (Fig. 1B, 2B). At 50 μmol/m²/sec ETR decreased in all different treatments. Nevertheless the short recovery had enhanced a considerable increase in the tolerant leaf grew without salt and in the susceptible grew in salt (Fig. 3). Leaf sections incubation in 300 mM salt for 2 h resulted in similar ETR value in the tolerant grew with salt (Fig. 4A), at the same time an increase in the value of the tolerant grown without salt was achieved compared 600 mM incubation (Fig. 1A). The ETR level has not changed in the susceptible leaf sections grew under both growth programs, compared to the ETR of 600 mM at similar light intensity. However, the recovery curve showed great similarity for both kinds of leaves floated on different salt concentrations (Fig. 1B, 4B).

F/Fₐ values: The F/Fₐ average was often detected between 0.80-0.83 in the different treatments. This ratio has significantly decreased in leaf sections of both wheat varieties grown without additional salt after incubation for 2 h in 600 mM under 40 μmol/m²/sec. In this case, the F/Fₐ average reached 0.363 and 0.413 in the tolerant leaf incubated in 600 and after 1 h recovery, respectively. Similar changes in the ratio were also recorded as 0.584 and 0.704 in the susceptible after incubation with 600 mM and after 1 h recovery, respectively (Table 1).

Fig. 1: Differential ETR values detected in 8-day-old leaf sections of wheat (Triticum aestivum) after treated with 600 mM during irradiation for 2 h under 35 μmol/m²/sec light intensities (A) and after short recovery of 1 h (B): 1, tolerant grown in salt-free Hoagland; 2, susceptible grow free of salt; 3, tolerant grow in 200 mM salt; 4, susceptible grew in Hoagland’s solution supplied with 200 mM salt.
Fig. 2: Differential ETR values detected in 8-day-old leaf sections of wheat (*Triticum aestivum*) after treated with 600 mM during irradiation for 2 h under varied 40 μmol/m²/sec light intensities (A) and after short recovery of 1 h (B). 1, tolerant grown in salt-free Hoagland; 2, susceptible grow free of salt; 3, tolerant grow in 200 mM salt; 4, susceptible grow in Hoagland's solution supplied with 200 mM salt.

Fig. 3: Differential ETR values detected in 8-day-old leaf sections of wheat (*Triticum aestivum*) after treated with 600 mM during irradiation for 2 h under 50 μmol/m²/sec light intensities (A) and after short recovery of 1 h (B). 1, tolerant grown in salt-free Hoagland; 2, susceptible grow free of salt; 3, tolerant grow in 200 mM salt; 4, susceptible grow in Hoagland's solution supplied with 200 mM salt.

**Chlorophyll a accumulation in dark-grown leaf floated on different salt concentrations:** Leaf sections from 8-day-old dark-grown wheat varieties were floated over 0, 200 and 600 mM salt in Hoagland's solution. The tolerant leaf sections often exhibit higher Chl a accumulation value than the susceptible. The highest Chl a value i.e., 420 nmol g⁻¹ was determined in the salt-free incubation in the tolerant leaf grown with salt stress whilst the lowest value i.e., 250 nmol g⁻¹ was recorded in the susceptible leaf grown without salt stress (Fig. 5A). When leaf sections were floated on 200 mM salt, the highest and lowest Chl a values were approximately 225 and 190 nmol/g and detected in the tolerant and the susceptible leaf sections, respectively (Fig. 5B). Leaf incubation with 600 mM revealed highest Chl a i.e., 78 nmol/g in the tolerant and lowest value i.e., 38 nmol/g in the susceptible (Fig. 5C).
DISCUSSION

More than 800 million hectares of land throughout the world are salt affected. This amount accounts for more than 6% of the world's total land area. Most of this salt-affected land has arisen from natural causes, from the accumulation of salts over long periods of time in arid and semi-arid zones (Rengasamy, 2002). All salts can affect plant growth, but not all inhibit growth. In addition, salts do not act alone in the soil, but interact in their effects on plants; some of these interactions are simple (e.g., interactions between Na⁺ and Ca²⁺), whereas some are complex (e.g., carbonates and their effects via increased soil pH) (Tester and Davenport, 2003).

From the extracted data in this presentation, some factors are involved and interfere to define the final response of the leaf different Chl fluorescence parameters to salt stress. Salt concentration in combination with the light intensity significantly affects the photosynthetic parameters. In addition, the change in Chl fluorescence found to be species-dependent and also specific to plant...
Table 1: The maximum photochemical efficiency (Fv/Fm) of varieties under different treatments

<table>
<thead>
<tr>
<th>Variety/treatment</th>
<th>Light intensity (μmol/m²/sec)</th>
<th>Fv/Fm</th>
</tr>
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<tbody>
<tr>
<td>Tolerant (600)</td>
<td>40</td>
<td>0.365</td>
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<tr>
<td>Tolerant (recovery)</td>
<td>40</td>
<td>0.413</td>
</tr>
<tr>
<td>Tolerant + salt (600)</td>
<td>40</td>
<td>0.830</td>
</tr>
<tr>
<td>Tolerant + salt (recovery)</td>
<td>40</td>
<td>0.830</td>
</tr>
<tr>
<td>Susceptible (600)</td>
<td>40</td>
<td>0.584</td>
</tr>
<tr>
<td>Susceptible (recovery)</td>
<td>40</td>
<td>0.704</td>
</tr>
<tr>
<td>Susceptible + salt (600)</td>
<td>40</td>
<td>0.828</td>
</tr>
<tr>
<td>Susceptible + salt (recovery)</td>
<td>40</td>
<td>0.828</td>
</tr>
<tr>
<td>Tolerant (600)</td>
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<td>0.818</td>
</tr>
<tr>
<td>Tolerant (recovery)</td>
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<td>0.816</td>
</tr>
<tr>
<td>Tolerant + salt (600)</td>
<td>50</td>
<td>0.822</td>
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<tr>
<td>Tolerant + salt (recovery)</td>
<td>50</td>
<td>0.816</td>
</tr>
<tr>
<td>Susceptible (600)</td>
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<td>0.825</td>
</tr>
<tr>
<td>Susceptible (recovery)</td>
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</tr>
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<td>Susceptible + salt (600)</td>
<td>50</td>
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<tr>
<td>Susceptible + salt (recovery)</td>
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<td>0.825</td>
</tr>
</tbody>
</table>

growth conditions. Running the incubation for 2 h in salt-free Hoagland's solution was favored by leaf of both tolerant and susceptible plants grown without salt stress. For tolerant leaf grown in salt stress, the best ETR value recorded from the incubation been preceded in 600 mM under 40 μmol/m²/sec. The susceptible leaf grown with salt stress has expressed general sensitivity to all high light intensities (i.e., above 35 μmol/m²/sec) and to the incubation with 600 mM, where in these treatments the lowest ETR values been regularly obtained in the susceptible (Fig. 2A, 3A). In previous experiments, the detection of variable fluorescence parameters values due to the effect of the external environmental stresses e.g., salt, was documented (Netondo et al. 2004). The effects of freezing stress and of ozone on photosynthetic fluorescence were also examined (Meinander et al., 1996; Percival and Fraser, 2001). Regardless to the concentration of the solution of incubation, it was discovered that the tolerant plant grown under salt stress showed almost constant ETR values (Fig. 1A, 4A) under low light intensity (35 μmol/m²/sec). From here, one may conclude that if tolerant plant grew free of salt stress then the salt concentration is the limiting factor in altering ETR values but if the tolerant grown with salt stress, the light intensity is the principal cause for the varied ETR values. In the susceptible leaf as shown from the figures, the ETR value and hence the photosynthetic fluorescence parameter is very sensitive to any increase in either the salt concentration or in the light intensity. A down-regulation of ETR was substantially detected under these conditions.

Effect of light intensity on Fv/Fm: The oxidation of plastoquinol (PQH2) is the rate-limiting step in photosynthetic electron transport between PS II and PS I. Under exceed light conditions or when light quality favors absorption of light by PS II, the rate of electron transport by PS II can exceed the rate of oxidation of PQH2, thus resulting in overreduction of the PQ pool. Among the thylakoid electron carriers, the PQ pool is generally considered to be an important redox sensor for signaling imbalances between the relative excitation of the two photosystems (Li et al., 2009). As stated in other reports, the general decrease of Fv/Fm is obviously detected with the increase of salt concentration exposed to plants (Ranjbarforordoei et al., 2006). The current data reveal a lot of contradictions. In Table 1, the salt (600 mM) treated leaf sections from both varieties exhibited close similarity in their maximum photochemistry yield of PSII (Fv/Fm) during the treatment and after the short recovery. In addition, a similarly was found between the 600 mM treated leaf sections and the non treated leaf sections.

The effect of light intensity exposed to plants grown without salt stress was the reference in the regulation of Fv/Fm as deduced from Table 1. This down-regulation in the quantum yield of PSII was achieved particularly under 40 μmol/m²/sec. The reason is not yet been proposed. Further investigations are crucially required to disclose this particular performance in Fv/Fm under such typical light intensity.

Chl a accumulation in dark-grown leaf: Rates of photosynthesis per unit leaf area in salt-treated plants are often unchanged (Murns and Tester, 2008). But the chl amount can be affected by salinity. From the above data it was clear the better performance of the light-adapted tolerant leaf grew with salt or without salt in maintaining higher Fv/Fm and ETR values than in the light-adapted susceptible leaf under the same growing conditions.

To address the possibility of preserved behavioral property of dark-grown tolerant plant in processing better photosynthetic fluorescence through accumulating higher Chl during greening and to figure if the difference between both tolerant and susceptible is still persist with close or even remote similarities, the potentiality of dark-grown wheat varieties (tolerant and susceptible) were investigated in accumulating Chl under certain light intensity (35 μmol/m²/sec).

REFERENCES


