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Inhibition of photosystems I and II activities in salt stress-exposed Fenugreek (*Trigonella foenum graecum*)

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ABSTRACT

Fenugreek (*Trigonella foenum graecum*) seedlings were exposed to increasing NaCl concentrations in the growth medium to examine the effect of salt stress on the electron transport reactions of photosynthesis. Activities of both photosystem II (PSII), measured by chlorophyll fluorescence, and photosystem I (PSI), measured by P700 photooxidation, were decreased by salt stress. The inhibition proceeded in a two step manner. At the lower salt concentrations used and shorter exposition periods, electron transfer between the quinone acceptors of PSII, Q_A and Q_B, was strongly retarded as shown by an increased amplitude of the OJ phase of the OJIP chlorophyll fluorescence induction traces and slowed chlorophyll fluorescence relaxation kinetics following a single turn-over flash. The above indicated a disturbance of the Q_B binding site likely associated with the first step of photoinhibition. In the second step, strong photoinhibition was observed as manifested by increased F_0 values, declined F_v/F_0 and loss of photoactive P700.

1. Introduction

Salt stress constitutes a major problem in arid and semi-arid regions and in irrigation areas. Approximately 7% of the world land area, 20% of the world cultivated land, and nearly 50% of the irrigated land are affected with high salt contents [1,2]. High salinity affects plant physiology at both whole plant and cellular levels through hyperionic and hyperosmotic stress effects that negatively impacts agricultural yield by limiting productivity and eventually causes plant death [3–6]. Also the salt stress causes gradual reduction in chlorophyll, carotenoid pigment contents and chlorophyll fluorescence intensity [7]. The loss of chlorophyll is often considered as a marker of a cellular component of salt stress [8], inducing the destruction of the chloroplast structure and the instability of pigment protein complexes [8]. To survive against this stress, plants respond and adapt with complex morphological, physiological, biochemical, and photosynthetic mechanisms. Although there have been many reports on salt tolerance, most plants are non-halophytes, with either a relatively low salt tolerance or a severely inhibited growth even at low salinity levels [9,10].

Salt stress affects various plant physiological processes such as mineral distribution, ion toxicity, respiration rate, and membrane permeability [11]. Membrane instability may be observed due to calcium displacement by sodium [12]. Photosynthesis is a key metabolic pathway that plays an important role in the maintenance of growth under salt stress. The decline in net photosynthesis under salt stress could be due to both stomatal and non-stomatal limitations [13]. However, reduction in plant growth due to salt stress is often associated with decreased photosynthetic activities such as the electron transport [3–15].

The photosynthetic machinery of higher plants comprises two large membrane protein complexes, photosystems I and II (PSI and PSII) that harness incident light energy and utilize it to initiate a series of electron transfer reactions across the thylakoid membrane in a concerted manner. Both ATP and NAD(P)H produced by the electron transport from water through PSII and PSI are consumed in various metabolic reactions including CO_2 fixation. The photochemical events in PSII are initiated by the capture of incident photons by the antenna complexes. The energy absorbed is quickly transferred to the photochemical reaction center where the excited singlet state of the special Chl*a*, P680, reduces a pheophytin (Pheo) molecule. Stabilization of the charge separated state occurs with the electron transfer from $Pheo^-$ to Q_A, the primary plastoquinone acceptor of PSII, forming $P680^+Q_A^-$. The $P680^+$ radical oxidizes tyrosine Y_Z (Tyrosine 161 of D1). The latter is re-reduced by electrons originating from the Mn cluster of the oxygen evolving complex (OEC) responsible for water oxidation. At the acceptor side, Q_A^- reduces the secondary plastoquinone acceptor, Q_B, in a two step process leading to the formation of plastoquinol (PQH_2).

The PSI complex is constituted with the reaction center P700 (a special pair of Chl*a* molecules, the primary electron donor of PSI),

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A_o (another Chl*a* molecule, the primary electron acceptor), A_1 (a phylloquinone), and [4Fe–4S] iron–sulfur centers (F_X, F_A and F_B) as electron transfer components. The excitation energy in the antenna pigments of PSI is quickly transferred to P700. In its lowest electronically excited state, P700 donates an electron to A_o. Charge separation and stabilization through the secondary acceptor, A_1, and terminal iron–sulfur centers results in the reduction of ferredoxin (Fd) located at the stromal side. The cationic P700 formed after charge separation is then re-reduced by plastocyanin (PC), a mobile carrier of the lumen. In turn, the oxidized PC receives electrons from Cyt b_6/f and PSII supplies electrons for Cyt b_6/f.

The effects of salt stress on the photosynthetic electron transport process have been investigated mainly in PSII. It was shown that salt stress might enhance photodamage to PSII in several organisms such as the green algae *Chlamydomonas reinhardtii* and the cyanobacterium *Spirulina platensis*, and in leaves of barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), and rye (*Secale cereale*) whereas others have found no effect on PSII [16,17]. These studies thus suggest that the sensitivity of PSII to salt stress may depend on organism or plant species. Murata et al. [18] have examined the effects of salt stress on PSII photodamage and repair separately in *Synechocystis*. Their results suggested that salt stress inhibited the repair of photodamaged PSII but did not directly accelerate the photodamage in cyanobacteria [19]. Salt stress suppressed the synthesis of almost all proteins among which the D1 protein at the translational level [20,21]. The molecular mechanisms of such inhibition remain to be determined. It is possible that an increase in the intracellular concentration of NaCl inactivates ATP synthase and decreases the intracellular level of ATP, which is essential for protein synthesis [22].

In order to obtain more information on the effect of salt stress on the electron transport reactions of photosynthesis, Fenugreek (*Trigonella foenum graecum*), a plant known for its wide range of medicinal applications and grown in several countries [23], was submitted to salt stress. Negative effects of salt stress were shown to occur in two steps. The first step caused a reduced rate of electron transfer between Q_A^- and Q_B on the acceptor side of PSII and a decline in the available $NADP^+$ for P700 photooxidation. This was followed by a stronger inhibition in both PSI and PSII.

2. Materials and methods

2.1. Plant growth conditions and salt stress

Seeds of *T. foenum graecum* (Tunisian variety) were sown in pots containing peat and placed in culture chambers with a 14 h photoperiod (300 μmol m^2 s^1 PAR) at 25 °C/22 °C (day/night). The seedlings were irrigated with distilled water during the first 5 days and then with a nutritive Hoagland solution, pH 6.2–6.5 [24]. At day 15 after germination, plants were transferred in pots of 1 l and 50 mM NaCl was added to the nutritive solution for 3/4 of the plants. The following day, further NaCl was added to reach 100 mM in 2/4 of the plants, and 24 h later NaCl was added in 1/4 of the plants to reach 150 mM. The photosynthetic parameters were measured after 4 h of light, plants were selected each day at a specific time.

2.2. Chlorophyll fluorescence induction

Chlorophyll fluorescence induction (FI) was recorded at room temperature using a Plant Efficiency Analyser (Hansatech, King's Lynn, Norfolk, UK) and a leaf clip. The transients were induced by red light at 3000 μmol m^{-2} s^{-1} provided by an array of six light emitting diodes (peak 650 nm), which were focused on the adaxial leaf surface to give homogenous illumination over the exposed area. All the samples were dark adapted for 30 min prior to fluorescence measurements. Variable fluorescence, F_v (the difference between the initial fluorescence, F_0, and the maximal fluorescence, F_m in dark adapted leaf segments), was used to calculate the F_v/F_m and F_v/F_0 ratios. For quantitative analysis of FI traces, a non-linear regression of experimental FI traces was achieved using Sigma Plot (SSI, Richmond, California, USA). FI traces were fitted with a sum of three Chapman–Richards functions corresponding to OJ, JI, and IP rises as described elsewhere [25]:

$$F(t) = F_0 + A_{O-J}(1 - e^{-k_{O-J}t})^{s_{O-J}} + A_{J-I}(1 - e^{-k_{J-I}t})^{s_{J-I}} + A_{I-P}(1 - e^{-k_{I-P}t})^{s_{I-P}}$$

where F_0 is the fluorescence level at time zero, A is the amplitude, k is the rate constant and s is the sigmoidal factor of each phase of FI. Data points recorded before 40 μs were ascribed to artefacts due to delay in response time of the instrument and were not included in the analysis.

2.3. Flash-induced Chl fluorescence relaxation kinetics

The decay of Chl a fluorescence yield after a single-turnover flash was measured with a double-modulation fluorescence fluorometer (model FL-3500, Photon Systems Instruments, Brno, Czech Republic) [26]. The instrument red LEDs for both actinic (20 μs) and measuring (5 μs) flashes were used to measure the fluorescence decay in the time range of 100 μs–60 s. The traces were averaged to estimate the half-times and amplitudes of the fluorescence decay components using the following three exponential functions:

$$F(t) - F' = A_1\, e^{-K1t} + A_2\, e^{-K2t} + A_3\, e^{-K3t}$$

where $F(t)$ is the fluorescence value at time t, k_n is the rate constant, A_n is the amplitude of the fluorescence relaxation phases, and F' is the stable minimal fluorescence at the end of the decay [27].

2.4. Redox state of P700

The redox state of P700 was monitored in vivo as the light-induced changes in absorption at 820 nm (ΔA820) using the ED-P700 DW dual wavelength unit connected via a PAM-101 fluorometer (Walz). The ED-P700 DW dual wavelength emitter detector unit measures strictly the differential absorbance changes (810 nm − 860 nm) peaking at a single wavelength band 820 nm ascribed for the $P700^+$ cation radical absorption and removes the plastocyanin absorbance changes [28]. The difference between the amplitudes of oxidized (the signal developed upon the onset of strong FR-light) and reduced state of P700 was considered as a relative measure of PSI activity. FR-light (11 μmol photons m^{-2} s^{-1}, which is 1.5 times above the intensity for saturating photooxidation of P700) was obtained by passing the beam from a Fibre-lite light source (Microview, Thornhill, ON, Canada) thourgh a RG-9 filter (Schott, Mainz, Germany). To avoid artefacts due to state transitions, measurements were made with leaves dark-adapted for 30 min at room temperature.

3. Results

Fenugreek (*T. foenum graecum*) seedlings were submitted to increasing salt stress during 6 days of treatment. A concentration of 50 mM NaCl was added to the growth solution each day during the first 3 days of treatment to reach a maximum of 150 mM. The maximum quantum yield of the primary photochemistry of PSII measured in dark-adapted leaves, F_v/F_m (where $F_v = F_m - F_0$), remained stable in plants treated with only 50 mM and in plants subjected to 100 mM, a significant decline was perceived only after the