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Title: **Comparison of changes in water status and photosynthetic parameters in wild type and abscisic acid-deficient *sitiens* mutant of tomato (*Solanum lycopersicum* cv. Rheinlands Ruhm) exposed to sublethal and lethal salt stress**

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Comparison of changes in water status and photosynthetic parameters in wild type and abscisic acid-deficient *sitiens* mutant of tomato (*Solanum lycopersicum* cv. Rheinlands Ruhm) exposed to sublethal and lethal salt stress

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Highlights

- ABA-deficient *sitiens* mutant of tomato was more sensitive to salt stress than WT
- *Sitiens* mutant exhibited severe osmotic and moderate ionic stress under salt stress
- Mutants displayed higher decrease in net CO_2 assimilation rate under high salinity
- Cyclic electron transport was severely reduced under salt stress in *sitiens* mutants
- Proline could alleviate salt stress injury at sublethal salt stress in the mutants

Abstract

Abscisic acid (ABA) regulates many salt stress-related processes of plants such as water balance, osmotic stress tolerance and photosynthesis. In this study we investigated the responses of wild type (WT) and the ABA-deficient *sitiens* mutant of tomato (*Solanum lycopersicum* cv. Rheinlands Ruhm) to sublethal and lethal salt stress elicited by 100 mM and 250 mM NaCl, respectively. *Sitiens* mutants displayed much higher decrease in water potential, stomatal conductance and net CO_2 assimilation rate under high salinity, especially at lethal salt stress, than the WT. However, ABA deficiency in *sitiens* caused more severe osmotic stress and more moderate ionic stress, higher K^+/Na^+ ratio, in leaf tissues of plants exposed to salt stress. The higher salt concentration caused irreversible damage to Photosystem II (PSII) reaction centres, severe reduction in the linear photosynthetic electron transport rate and in the effective quantum yields of PSII and PSI in *sitiens* plants. The cyclic electron transport (CET) around PSI, which is an effective defence mechanism against the damage caused by photoinhibition in PSI, decreased in *sitiens* mutants, while WT plants were able to increase CET under salt stress. This suggests that the activation of CET needs active ABA synthesis and/or signalling. In spite of ABA deficiency, proline accumulation could alleviate the stress injury at sublethal salt stress in the mutants but its accumulation was not sufficient at lethal salt stress.

Keywords

Abscisic acid-specific changes in photosynthesis; cyclic electron flow; ionic stress; proline; salt stress; *sitiens* mutant

Abbreviations

A, net CO_2 fixation rate; ABA, abscisic acid; CAB, chlorophyll *a*/*b* binding protein; CET, cyclic electron transport around PSI; DW, dry weight; FW, fresh weight; ETR, linear electron transport rate; g_s, stomatal conductance; NDH, NAD(P)H-dependent dehydrogenase complex; Pro, proline; *sit* mutant, *sitiens* mutant; P5CS, Δ'-pyrroline-5-carboxylate synthetase; P5CR, Δ'-pyrroline-5-carboxylate reductase; PPFD, photosynthetic photon flux density; PSII and PSI, photosystem II and I; RH, relative humidity; RUBISCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RBCL and RBCS, RUBISCO large and small subunits, RWC, relative water content; WT, wild type; Ψw, water potential

1. Introduction

Soil salinity caused by excess of Na^+ is one of the major abiotic stress factors that limits sustainable development in agriculture (Rengasamy, 2006). Salt stress disrupts plant ion homeostasis resulting in the accumulation of toxic Na^+ in the cytoplasm with a concomitant deficiency of K^+, which induces osmotic, ionic and oxidative stress in plant tissues. Since the inhibition of photosynthesis and the degradation of the photosynthetic apparatus are among the first targets of salt stress, high salinity reduces growth and productivity of crop plants (Chaves et al., 2009). The initial growth reduction by salt stress seems to be driven by water relations and can be prevented by keeping the plants at full turgor, however, after several hours the cell elongation remains reduced and shoot growth is hormonally regulated. Later the growth is mostly constrained by high foliar Na^+ accumulation (Munns and Tester, 2008).

The phytohormone abscisic acid (ABA) plays an important role in alleviating salt stress injury in many ways (Javid et al., 2011). ABA is newly synthesized from xanthophylls under water-deficit conditions e.g. under drought or salinity stress and regulates plant water balance and osmotic stress tolerance (Zhu, 2002). Several tomato mutants expressing mutant alleles of various ABA biosynthesis genes have already been characterized. These ABA-deficient wilty mutants, such as *sitiens* (*sit*), *flacca* and *notabilis* can serve as a model system for studying the role of ABA in developmental processes or in stress acclimation. *Sit* mutants impaired in ABA-aldehyde oxidation to ABA possessed about 15-30 % of the wild type (WT) ABA content in the leaves under control conditions (Mäkelä et al., 1998; Hlavinka et al., 2012; Muñoz-Espinoza et al., 2015) and the increase in ABA level was also much higher in WT (from 400 to 660 ng g^{-1} FW) than in *sit* mutants (from 80 to 100 ng g^{-1} FW) under salt stress (Mäkelä et al., 1998). The same authors investigated and compared the growth and water status parameters of WT and *sit* mutants exposed to 75 mM NaCl and proved that the mutant plants were more sensitive to high salinity at 70 % relative humidity (RH) (Mäkelä et al., 2003).

Salt stress triggers the production of ABA in the root, which then is immediately transported to the shoot causing stomatal closure. ABA can also be synthesized in leaf vascular tissues and after entering the xylem sap, it may diffuse out to the leaf apoplast and may control stomatal aperture (Fricke et al., 2004; Cabot et al., 2009; Dodd, 2013; Kohli et al., 2013). Therefore, the salinity-induced ABA can influence the assimilation of CO_2 by stomatal closure that decreases the availability of CO_2 (Li et al., 2015).

Previously it was observed that short- and long-term ABA treatments had no effects on Photosytem II (PSII) photochemistry but induced a decrease in CO_2 assimilation and stomatal conductance in maize (Jia and Lu, 2003). In another study exogenous ABA at 10^{-7}-10^{-5} M concentration range inhibited net CO_2 assimilation rate and decreased mesophyll conductance to CO_2 in the leaves of pea and wheat plants, which was most prominent one day after the treatment, but the quantum yields of photosystems and the linear electron transport rate were not affected by ABA (Sukhov et al., 2017). The same authors found increased cyclic electron transport (CET) upon 10^{-5} M ABA treatment in pea leaves, while there were no changes in CET in wheat plants under the same experimental conditions. CET around PSI transfers electrons from PSI to $cytb_6/f$ complex via reduced ferredoxin and through the plastoquinone cycle, and it contributes to lumen acidification. It serves only to support the proton motive force across thylakoid membranes, thus ATP generation without net formation of reductants such as NADPH. CET in higher plants consists of two pathways mediated by PGR5/PGRL1 (PROTON GRADIENT REGULATION5 and PGR-LIKE1) and NAD(P)H-dependent dehydrogenase (NDH) complexes. Both of them are ferredoxin-dependent, form supercomplexes with PSI and $cytb_6/f$ and redirect electrons from reduced ferredoxin to plastoquinone pool. Moreover, NDH complex pumps protons directly to the lumen and increases the proton motive force across thylakoid membranes leading to excess ATP production (reviewed by Li et al., 2018). Thus CET provides possibility to adjust the ratio of ATP and NADPH production to the demand of tissues in changing environment (reviewed by Foyer et al., 2012).

Although light is a driving force of photosynthesis, excess light energy can cause a fast degradation of D1 protein in the PSII reaction centres and the imbalance between photodamage and the repair mechanisms results in photoinhibition. Under high light, CET is thought to be essential for protecting PSI and PSII from photodamage via acidification of the thylakoid lumen, which down-regulates the electron flow from PSII to PSI and activates regulated non-photochemical quenching (NPQ). At low light intensity, however CET plays an important role in optimizing photosynthetic CO_2 assimilation probably via the supply of extra ATP (Huang et al., 2015). It was also found that CET was significantly stimulated at low light after chilling-induced photoinhibition of PSII and the authors hypothesized that this CET stimulation mainly enhanced the synthesis of ATP for the fast repair of PSII (Huang et al., 2010; Huang et al., 2018). $Cytb_6/f$ complex, which is needed for both linear electron transport and CET is also very sensitive to photoinhibition. The PetD subunit of $cytb_6/f$ complex and

the PGRL1 protein show much faster turnover rate than the other proteins involved in CET, and the repair mechanisms need extra ATP generated by cyclic electron transport (Li et al., 2018).

Exogenous ABA also decreased the CO_2 assimilation and transpiration rates in WT tomato (*S. lycopersicum* cv. Moneymaker) and in corresponding *sit* mutants (Herde et al., 1997). In accordance with the above-mentioned findings, the steady state stomatal conductance (g_s) and the net CO_2 assimilation rate (A) were significantly higher in *sit* mutants in Moneymaker background than in WT (Hlavinka et al., 2012). The higher g_s of mutant plants could be explained not only by the reduced ABA content but also by a higher number of stomata per unit leaf area, which is a morphological feature of the mutants (Tal, 1966; Nagel et al., 1994).

However, the effect of ABA on the photosynthetic activity in plants exposed to salt stress is often contradictory. Gomez-Cadenas et al. (2002) found that exogenously applied ABA did not modify the water potential in citrus plants under salt stress but improved the net CO_2 fixation rate and g_s and decreased the 100 mM NaCl-induced Cl^- accumulation.

Nevertheless, the most important role of ABA in salt stress acclimation is the control of osmotic adaptation by inducing the synthesis of compatible osmolytes (Zhu, 2002). In response to different stresses plants accumulate large quantities of different types of compatible solutes. These solutes provide protection to plants during stress conditions by contributing to cellular osmotic adjustment, detoxification of reactive oxygen species (ROS), maintenance of membrane integrity and enzyme/protein stabilization.

A large body of data suggests a positive correlation between proline (Pro) accumulation and plant stress. Besides acting as an excellent osmolyte, the amino acid Pro plays a role during stress as a metal chelator, an antioxidative defence and a signalling molecule. It can also function in photosynthetic electron transport by maintaining appropriate $NADP^+$/NADPH ratio in chloroplasts since the enzymes (Δ'-pyrroline-5-carboxylate synthetase (P5CS) and Δ'-pyrroline-5-carboxylate reductase (P5CR)) participating in Pro biosynthesis from glutamate consume NADPH. This pathway functions predominantly under osmotic stress, contributes to the maintenance of the oxidized $NADP^+$ pool and prevents over-reduction of $NADP^+$ and thus photoinhibition in chloroplasts exposed to abiotic stress (Hayat et al., 2012).

Some of the ABA-mediated effects in stressed plants are well documented, but the role of ABA in the activity and cooperation of Photosystem II (PSII) and I (PSI) under salt stress

in WT and ABA-deficient mutants is not known. Moreover, little is known about the effects of salt stress in correlation with changes in photosynthetic activity and Pro accumulation in the presence or absence of ABA and we have little information about the differences between the effects of tolerable (100 mM) or cell death-inducing (250 mM) concentrations of NaCl in ABA-deficient *sit* mutants.

In this work, short-term changes in water status, ion accumulation and the most important photosynthetic and fluorescence induction parameters were studied in WT tomato and in ABA-deficient *sit* mutants exposed to tolerable or cell death-inducing concentrations of NaCl.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of wild type tomato (*Solanum lycopersicum* L. cv. Rheinlands Ruhm) and ABA-deficient *sitiens* mutants in Rheinlands Ruhm background were germinated for 4 days in the dark and transferred to perlite for 2 weeks (Seeds obtained from C. Rick, University of California, Davis). Plants were grown in a controlled environment under 200 μmol m^{-2} s^{-1} light intensity (F36W / GRO lamps, Sylvania, Germany), 12 h light/12 h dark period, 24/22 ^{o}C day / night temperature and 55–60 % relative humidity for 8 weeks in hydroponic culture containing 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 0.5 mM KCl, micronutrients (0.001 mM $MnSO_4$, 0.005 mM $ZnSO_4$, 0.0001 mM $CuSO_4$, 0.0001 mM $(NH_4)_6Mo_7O_{24}$, 0.01 mM H_3BO_4) and 0.02 mM Fe(III)-EDTA at pH 6.8 (Poór et al., 2011). The nutrient solution was changed every second day.

Plants were treated with 100 mM or 250 mM NaCl for 6 h through the root system in the hydroponic culture solution. Salt concentrations were chosen on the basis of our earlier experiments (Poór et al., 2014; Takács et al., 2015). It was found that WT plants were able to acclimate to 100 mM NaCl but they show the symptoms of cell death at 250 mM NaCl. The samples were prepared from the second, fully expanded young leaves in three replicates. The experiments were performed at 9 o'clock a.m. and repeated 3-4 times in independent experiments.

2.2. Water status parameters

The leaf water potential (Ψw) was measured on the second fully expanded leaf with a pressure chamber (PMS Instrument Co., Corvallis, WA, USA), as described earlier by Gallé

et al. (2013). Relative water content (RWC) of the leaves was determined according to Corrêa de Souza et al. (2013). RWC was calculated as follows: RWC (%) = 100 x (fresh weight – dry weight)/(turgid weight – dry weight).

2.3. Stomatal conductance and gas exchange

Stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) and CO_2 assimilation rate (A; μmol CO_2 m^{-2} s^{-1}) were measured on the second, fully expanded leaves with a portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE, USA), as described by Poór et al. (2011). Measurements were carried out on a 2 cm^2 leaf area, with the controlled CO_2 flow at the concentration of 400 μmol mol^{-1}. Photon flux density (PPFD) was the same as in the growth chamber (200 μmol m^{-2} s^{-1}) and the leaf temperature was also controlled (25 ^oC).

2.4. Chlorophyll a fluorescence measurements and PSI activity

Chlorophyll *a* fluorescence and P700 redox state were analysed with Dual-PAM-100 (Heinz-Walz, Effeltrich, Germany) (Klughammer and Schreiber, 1994). Leaves were dark-adapted for 20 min before the determination of the minimal fluorescence (F_0), using weak measuring light. The maximal fluorescence (Fm) was measured by applying 800 ms pulse of saturating light (12000 μmol m^{-2} s^{-1}). Leaves were then illuminated continuously with 220 μmol m^{-2} s^{-1} actinic light. After 20 min the light-adapted steady-state fluorescence (Fs) was recorded and the maximum fluorescence level (Fm') in the light-adapted state was determined with the help of extra saturating pulses. The actinic light was next turned off and the minimum fluorescence level in the light-adapted state (F_0') was determined by illuminating the leaf with far-red light (5 μmol m^{-2} s^{-1}) for 3 sec. The following chlorophyll fluorescence parameters were calculated: $Fv/Fm=(Fm–F_0)/Fm$, $Y(II)=(Fm'–Fs)/Fm'$, $Y(NO)=Fs/Fm$, and $Y(NPQ)=1–Y(II)–Y(NO)$, ETR II=0.84x0.5xPPFDxY(II) and ETR I=0.84x0.5xPPFDxY(I) (Huang et al., 2018). Fv/Fm is the maximal quantum yield of PSII in dark-adapted and Y(II) and Y(I) are the effective quantum yields of PSII and PSI in light-adapted state, respectively. Y(NO) is the non-regulated quantum yield and Y(NPQ) is the regulated quantum yield of non-photochemical energy dissipation. ETR II and ETR I represent the photosynthetic electron flow through PSII or PSI, respectively (Genty et al., 1989; Kramer et al., 2004).

The quantum yield of PSI [Y(I)] is defined by the proportion of the overall P700, which is reduced at a given state and not limited by the acceptor side. It was calculated from the complementary PSI quantum yields of non-photochemical energy dissipation, Y(ND) and

Y(NA). Y(I)=1–Y(ND)–Y(NA), where Y(ND) and Y(NA) are the quantum yields of non-photochemical energy dissipation in PSI due to donor and acceptor side limitations, respectively. Y(ND)=1–$P700_{red}$ and Y(NA)=(Pm–Pm')/Pm. Pm was determined by applying a saturation pulse after pre-illumination with far-red light and it represents the level where P700 is fully oxidized. Pm' was defined in the same way as the fluorescence parameter Fm' and was determined in a given state with the help of a saturation pulse using actinic light instead of far-red light (Klughammer and Scheiber, 1994; Schreiber and Klughammer, 2008; Zhang et al., 2014). Cyclic electron transport around PSI is calculated as (PSI ETR)–(PSII ETR) (Laisk et al., 2010).

2.5. Photosynthetic pigments

Twenty-five mg of leaf samples were homogenized in 1 ml of 100% acetone and extracted for 24 h, then samples were centrifuged (12000 g for 15 min at 4 °C). The pellet was extracted again with 1 ml of 80% (v/v) acetone for 24 h. After centrifugation (12000 g, 15 min, 4 °C), the supernatants were collected and the pigment content was measured by a spectrophotometer (KONTRON, Milano, Italy) according to Wellburn and Lichtenthaler (1984).

2.6. RUBISCO content

Leaf protein content was determined according to the method of Bradford (Bradford, 1976). Separation of proteins occurred by SDS-PAGE (6% stacking gel, 20% separating gel, 200 V, 60 min), then the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) protein was detected by Western blot using rabbit polyclonal antibody (Agrisera, Vännäs, Sweden) based on Meng et al. (2016).

2.7. RNA extraction, expression analyses by qRT-PCR

Quantitative real-time reverse transcription-PCR (qRT-PCR; Piko Real-Time qPCR System, Thermo Scientific, Waltham, MA, USA) was used to detect the expression pattern of the selected tomato RUBISCO large subunit (*RBCL*) gene mined from Sol Genomics Network (SGN; http://solgenomics.net; accessed 23 September 2018) (Horváth et al., 2015). Primers were designed using NCBI and Primer 3 software (http://frodo.wi.mit.edu/; accessed 23 September 2018). The qRT-PCR reaction consisted of 10 ng cDNA template, 400-400 nM forward and reverse primers (R: 5'-CTGCGTGATGATTTTGTTGAA-3'; F: 5'-

TGAATACCTCCTGAAGCCACA-3'), 5 µL of Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, Waltham, MA, USA), and nuclease-free water in a total volume of 10 µL. After denaturation at 95 ^{o}C for 7 min, followed by 40 cycles of denaturation at 95 ^{o}C for 15 s and annealing extension at 60 ^{o}C for 60 s, a melting curve analysis of the products was performed (increasing the temperature from 55 to 90 ^{o}C (0.2 ^{o}C s^{-1})) to determine the specificity of the reaction. Data analysis occurred by PikoReal Software 2.2 (Thermo Scientific, Waltham, MA, USA). Tomato 18S rRNA and elongation factor-1α subunit genes were applied as the reference genes and $2^{(-\Delta\Delta Ct)}$ formula was used to calculate data from the qRT-PCR. Each reaction was repeated at least three times.

2.8. Sugar and starch content

Carbohydrate (soluble sugars and starch) analysis was performed according to Hansen and Møller (1975). Briefly, soluble sugars were extracted from 100 mg of grinded leaf samples with 1 ml of 80% ethanol at 80 ^{o}C for 30 min. The homogenate was centrifuged at 2600 g for 10 min and the supernatant was used for determination of sugar content at 630 nm after reaction with anthrone (Normapur, VWR Int., Leuven, Belgium) dissolved in 72% sulphuric acid. Then, the pellet was cleaned with 1 ml of deionized water, digested with 1 ml of 1.1% HCl at 100 ^{o}C for 30 min, and centrifuged for 10 min at 2600 g. Starch concentration was also determined spectrophotometrically at 630 nm with anthrone reagent using starch (Normapur, VWR Int., Leuven, Belgium) dissolved in 1.1% HCl as a standard.

2.9. Proline accumulation

Pro content was determined with the modified acid-ninhydrin method (Bates et al., 1973). Pro was estimated spectrophotometrically (KONTRON, Milano, Italy) at 520 nm after extraction with 3% sulfosalicylic acid and reaction with acid ninhydrin reagent (3% ninhydrin in 6 M phosphoric acid and 60% acetic acid). The calibration curve was prepared with standard Pro (Sigma-Aldrich, St. Louis, MO, USA).

2.10. Macroelement content

Macroelements of the leaf tissues were determined by XSeries II ICP-MS (Thermo Scientific, Bremen, Germany) according to Tari et al. (2013). 6 mL of 70 % HNO_3 (Reanal, Budapest, Hungary) and 2 mL of 30 % H_2O_2 (Reanal, Budapest, Hungary) were added to 100 mg dried leaf samples for 20 h. The samples were digested in microwave destructor (MarsXpress CEM,

Matthews NC, USA) at 200 ºC for 25 min and after cooling they were diluted with 12 mL double distilled water.

2.11. Statistical analysis

Data presented are mean values of at least three independent experiments. Statistical analysis was carried out with Sigma plot 11.0 software (Systat Software Inc., Erkrath, Germany). After analysis of variance (ANOVA), Duncan's multiple comparisons were performed. Differences were considered significant if $P \leq 0.05$.

3. Results

3.1. Salt stress induced changes in water homeostasis

To examine the role of ABA in salt-induced stress responses in the leaves of WT and ABA-deficient *sit* tomato, the water status was determined after treatments with different concentrations of NaCl. The relative water content (RWC) was lower in the leaves of *sit* than in the WT plants (Fig. 1A) and it significantly decreased both in WT and *sit* leaves as a function of increasing NaCl concentration, but the change was more pronounced in the mutant (Fig. 1A).

Salt stress also reduced the water potential (Ψ_W) of leaf tissues in WT and *sit* plants after 6 hours (Fig. 1B). However, the reduction of Ψ_W was much stronger in the leaves of *sit* mutants after both NaCl treatments (Fig. 1B).

3.2. Salt stress-induced ionic stress

Salt stress induced significant Na^+ accumulation in concentration-dependent manner in the leaves of the two tomato genotypes (Table 1). Surprisingly, the basic K^+ level was significantly higher in the leaves of ABA-deficient plants than in those of WT. Although potassium content was reduced as a function of increasing NaCl concentrations, the leaf tissues of the mutants contained much more K^+ even at 250 mM NaCl. The accumulation of Na^+ increased during salt exposure in both genotypes and was significantly higher in the mutants, nevertheless, K^+/Na^+ ratio remained higher in *sit* leaf tissues. This suggests that ABA-deficient mutants were exposed to much stronger osmotic (Fig. 1) but less severe ionic stress than WT plants (Table 1).

3.3. Effects of salt stress on photosynthetic activity

In parallel with the decrease in RWC and Ψ_W, changes in stomatal response were rapid and considerable. Basically, g_s was higher under the control condition in *sit* plants than in WT, which implied that the stomata in ABA-deficient plants were much open. Salt stress induced a decrease in g_s in both genotypes, but this parameter remained higher in *sit* mutants after exposure to NaCl (Fig. 2A). While mutant plants had almost two times higher transpiration rate than the WT under control conditions and transpired stronger under salt stress, the transpiration rate declined also significantly upon salt treatments in both genotypes (Fig. 2B).

Since stomatal opening influences the uptake of CO_2 into the mesophyll cells and photosynthetic activity depends on CO_2 availability, the net CO_2 assimilation rate was also reduced under salt stress in both genotypes (Fig. 2C). It has to be mentioned that in spite of higher g_s, the decline in net CO_2 assimilation rate at 250 mM NaCl was much dramatic in *sit* mutants than in WT. At the same time, the intercellular CO_2 concentration decreased significantly only in the WT leaves under salt stress (Fig. 2D).

Interestingly, the level of RUBISCO large subunit (RBCL), which is a part of the key enzyme in Calvin-cycle, decreased after treatment with 250 mM NaCl in WT leaves but was not reduced upon salt exposure in *sit* plants (Fig. 3A). In contrast to protein level, the expression of *RBCL* gene did not change under salt stress either in the presence or in the absence of an effective ABA biosynthesis (Fig. 3B).

Chlorophyll *a* fluorescence induction parameters are useful tools to detect salt stress-induced damage in the photosynthetic apparatus. The maximal quantum efficiency of PSII (Fv/Fm) decreased significantly in ABA biosynthetic mutant plants exposed to lethal salt stress, while it remained constant in WT plants (Fig. 4).

The effective quantum yield of PSII (YII) exhibited a steep decline (Fig. 5A) and the regulated Y(NPQ) and especially the non-regulated non-photochemical energy dissipation, Y(NO) increased at much higher rate in *sit* leaves under high salinity than in the WT (Fig. 5C, E). Similar tendencies were observed in the effective quantum yield of PSI (YI), which decreased significantly only in *sit* leaves at 100 mM NaCl at this time point after NaCl treatments (Fig. 5B). Moreover, the non-photochemical energy dissipation originated from the limitation of PSI at both acceptor Y(NA) and donor sides Y(ND) was much higher in ABA-deficient mutants than in the WT under salt stress, while these parameters did not change in WT as a function of NaCl concentration (Fig. 5D, F). Y(NA) exhibited a maximum at the smaller salt concentration in *sit* leaves (Fig. 5F).

389 The linear electron transport (ETR II) between the two photosystems and CET around
390 PSI were calculated in the two genotypes. In contrast to mutant plants ETR II was not affected
391 in WT, but salt stress had an opposite effect on CET in mutant and WT leaves. CET was
392 significantly enhanced by 250 mM NaCl in WT but was reduced at the same concentration in
393 *sit* leaves (Fig. 6).
394 Photosynthetic pigment content can determine the efficiency of photosynthesis.
395 Chlorophyll *a*+*b* level remained constant upon salt exposure in the leaves of WT plants but
396 decreased significantly upon 250 mM NaCl treatment in the mutants (Fig. 7A), however,
397 carotenoids were significantly degraded in *sit* leaves compared to the control plants under salt
398 stress (Fig. 7B).
399 To detect the key products of photosynthesis after salt treatments, starch and sugar
400 contents were also measured. Both NaCl treatments reduced starch accumulation in the leaves
401 of both genotypes after 6 hours (Fig. 8A). However, 100 mM NaCl treatment incerased
402 soluble sugar content in WT leaves (Fig. 8B) while soluble sugar accumulation was lower in
403 control plants and at 100 mM NaCl in the mutants. High salt treatment reduced the sugar
404 accumulation in both genotypes (Fig. 8B).
405 Pro accumulation in plant tissues plays a role in osmoprotection and water stress
406 tolerance under drought and salinity stress. Both NaCl treatments significantly enhanced Pro
407 levels in *sit* leaves, but the change was much moderate at 250 mM NaCl, while in the WT
408 leaves Pro accumulation was negligible upon salt treatment (Fig. 9).
409
410 **4. Discussion**
411 Plants have developed a wide range of strategies to minimize the negative effects of high
412 salinity, among others the imbalance of ion and water homeostasis, inhibition of
413 photosynthetic activity and generation of oxidative stress (Munns and Tester, 2008). The
414 complex network of this defence system is mediated by hormonal interactions, where ABA
415 plays an important role in the adaptive responses (Ismail et al., 2014). Thus, the study of ABA
416 effect during the early stage of salt stress in WT and ABA-deficient mutants could help to
417 distinguish between common and ABA-specific responses.
418 The changes in growth parameters, water relations and ion content are well
419 documented in ABA-deficient *sit* tomato mutants in Moneymaker (Nagel et al., 1994) and in
420 Rheinlands Ruhm (Mäkelä et al., 1998; 2003) backgrounds. The deficiency in ABA
421 influenced the relative growth rate, which was 22% lower than that of the WT, but the net

CO_2 assimilation rate was not affected. The *sit* mutant showed a much higher transpiration rate and lower hydraulic conductance in the roots and consequently a significantly lower leaf water potential and turgor relative to the WT (Nagel et al., 1994). Mäkelä et al. (2003) found similar changes in growth rate under moderate salt stress at 70% RH. Moreover, they did not find significant differences between the WT and mutant plants grown at 95% relative humidity in Na^+ accumulation and only slight differences in potassium accumulation of old and young leaves under salt stress. Moreover, chloride accumulation was well below the level likely to affect enzyme activities. We found similar differences between RWC and Ψ_W of WT and mutant plants, which were severely reduced further at both salt concentrations. Surprisingly, the ion accumulation of plants grown in hydroponic culture for 8 weeks, then exposed to salt stress for 6 h, exhibited an interesting change. Mutant plants with high g_s accumulated much more potassium than the WT, which was maintained in the early period of salt stress. So in spite of higher Na^+ accumulation, the K^+/Na^+ ratio, which is a measure of ionic stress, was lower in *sit* leaves than in WT plants. Thus ABA deficiency caused more severe osmotic stress (decrease in Ψ_W) and more moderate ionic stress in *sit* mutants than in WT when exposed to high salinity.

It is well-known that after imposition of salinity the osmotic stress dominates, which is a consequence of the Na^+ accumulation in the apoplast of root and shoot tissues. During this phase higher cytoplasmic K^+ concentrations in *sit* cells may alleviate the intracellular effects of Na^+, since the two cations compete with the same binding sites. Ionic stress develops later with increasing Na^+ accumulation in the cytoplasm, which results in ion imbalance, disintegration of proteins structure, oxidative damage of cell constituents and disturbance of metabolic processes such as limitation of photosynthesis (Pérez-Alfocea et al., 2010).

Based on our results, it can be concluded that salt stress-induced imbalance in water homeostasis is more significant in the case of ABA-deficient plants. ABA plays pivotal role in controlling the fast closure of stomata, which is one of the first physiological response of stressed plants (Bright et al., 2006). Both NaCl treatments decreased g_s and promoted closure of stomata in WT tomato plants, but induced stomatal closure in ABA-deficient *sit* plants, too, where the disturbance in ABA biosynthesis should weaken ABA-controlled closure. This suggests that other factors, e.g. severely reduced water potential or accumulation of reactive oxygen species in mutant plants may also contribute to stomatal closure under salt stress.

Since closure of stomata limits not only transpiration but also net CO_2 assimilation rate, as it was expected, salt stress reduced photosynthetic activity in parallel with the decline

in g_s in both genotypes. However, in spite of higher g_s, this decline was significantly higher in mutant leaves exposed to 250 mM NaCl.

ABA may directly affect CO_2 assimilation since it could directly bind to RUBISCO protein and could cause a weak inhibition of its catalytic activity and a more potent inhibition of RUBISCO activation in *Arabidopsis* leaves (Galka et al., 2015). Thus higher net CO_2 assimilation rate is expected in the mutant plants in the absence of ABA, as it was observed under control conditions.

In tomato the inhibiting effect of ABA on photosynthesis was closely related with down-regulation of important photosynthetic genes encoding small subunit of RUBISCO (*RBCS*) and chlorophyll *a*/*b* binding (*CAB*) proteins (Bartholomew et al., 1991). In this species RBCS is encoded by five nuclear genes and the large subunit (RBCL) by one chloroplast gene. ABA-mediated down-regulation of *RBCS* genes was observed in WT plants, but only minor effects could be detected in *RBCS* expression in *sit* mutants under water deficit in this system (Sugita and Gruissem, 1987). However, water stress and ABA had little effect on *RBCL* transcript level in the same tomato genotypes. We found a significant decrease in the amount of RBCL in WT plants but not in the mutants at lethal salt stress. It is in accordance with the results of Nakano et al. (2006) who found that the direct degradation of RBCL by reactive oxygen species occurred in intact leaves under abiotic stress. The explanation for the higher stability of RBCL protein in the mutants is not clear but it can be a consequence of slower activation of specific proteases. However, we did not find changes in the expression of *RBCL* gene in the two genotypes during early stages of salt stress.

The decline in CO_2 assimilation has been coupled with an increase in intercellular CO_2 concentration in *sit* mutants under salt stress indicating that CO_2 assimilation was severely inhibited and/or CO_2 generating processes increased in the mutants. This higher intercellular CO_2 level can also influence stomatal behaviour, leading to much higher reduction in stomatal pores in the absence of ABA as it was detected in *sit* leaves.

According to the significant reduction in Fv/Fm, the higher salt concentration caused irreversible damage to the reaction centres of PSII in *sit* mutants, while in WT plants they remained intact during this early period of salt stress. To best of our knowledge, the comparison of the photochemical efficiency of the two photosystems in WT and *sit* mutants under salt stress has not been performed yet. Surprisingly, we found that PSI was more sensitive to 100 mM NaCl in mutant plants than in WT since the effective quantum yield of PSI decreased by more, than 60% at sublethal salt stress while that of WT plants was not

affected. At the same time, the effective quantum yield of PSII decreased step by step with increasing NaCl concentration. A very pronounced increase in Y(NO) also suggests a significant damage to photosynthetic apparatus in the mutant plants at 250 mM NaCl, while Y(NPQ) efficiently developed at sublethal salt stress. Similarly, the non-photochemical energy dissipation due to donor side limitation of PSI, Y(ND) increased as a function of NaCl concentration, but Y(NA) exhibited a maximum at 100 mM NaCl in *sit* plants. These parameters remained unchanged under salt stress in WT plants. Similar results were described by Huang et al. (2010), who found that in a chilling-sensitive tropical tree *Dalbergia odorifera* chilling induced photoinhibition, depressed electron flow from PSII to PSI and increased the oxidation ratio of P700, which was manifested in higher Y(ND).

These observations are in accordance with the changes in electron transport rate (ETRII) between PSII and PSI in our system. It was much more inhibited in the mutant plants than in the WT under salt stress, and WT plants were able to increase CET in order to prevent photoinhibition at 250 mM NaCl. In contrast to this activation, CET was reduced in the mutants, which led to photoinhibition-caused damage manifested in the decline in Fv/Fm or increase in Y(NO). However, the activation of CET in WT plants could not prevent the death of plants exposed to 250 mM NaCl.

The molecular background of the changes in photosynthetic efficiency in *sit* mutants is far from clear. Carotenoid biosynthesis in ABA biosynthesis mutants of tomato is seriously affected. *High pigment 3 (hp3*) mutant, which has a mutation in zeaxanthin epoxidase (ZEP) gene, lacks violaxanthin and neoxanthin in the leaves and the tissues contain by 75% lower ABA than the WT. Significant reduction was also found in zeaxanthin pool in the fruits of *sit* and *flacca* mutants in mature green stage (Galpaz et al., 2008). The reduced pool of the xanthophyll cycle components, such as violaxanthin or zeaxanthin, may restrict the development of NPQ under high salinity. Surprisingly, the lycopene content increased in fruits of these ABA biosynthesis mutants on fresh mass basis. However, on dry mass basis we found lower total carotenoid level in *sit* leaves in our experiments. Since carotenoids are effective non-enzymatic antioxidants, this may determine the oxidative stress response of these tissues.

In spite of their significantly lower catalase activity, *sit* mutants in Micro-Tom background did not show the symptoms of oxidative stress, e.g. increased H_2O_2 and malondialdehyde content under control conditions (Monteiro et al., 2012), however, they can increase H_2O_2 accumulation and antioxidant enzyme activities during Cd-induced abiotic

stress (Pompeu et al., 2017) or the accumulation of reactive oxygen species (ROS) during high salinity (Kovács, 2017; Kovács et al., 2017). However, the non-enzymatic antioxidants (e.g. carotenoids, ascorbate or glutathione) may be temporarily exhausted in tomato tissues exposed to salt stress (Flors et al., 2007).

Since salt stress is one of the abiotic stress factors, which generates considerable amount of ROS and induces intensive oxidative stress, the enzymatic and non-enzymatic antioxidant capacity of tomato genotypes will determine the acclimation of plants. It was found that the expression of the genes encoding proteins involved in CET are regulated by redox signals (Queval and Foyer, 2012) and most of them are over-expressed under photorespiratory conditions (Foyer et al., 2012). However, the transcriptome pattern of the leaves of catalase (*cat2*), ascorbate (*vtc1* and *vtc2*) or glutathione deficient (*rml*) *Arabidopsis* mutants showed only partial overlap. Low ascorbate decreased the expression of β-carotene hydroxylase which participates in the biosynthesis in carotenoids but violaxanthin de-epoxidase, which catalyzes the synthesis of zeaxanthin, the intermediate of xanthophyll cycle was down-regulated by low GSH. Low ascorbate or low GSH also decreased the expression of NDH subunits, NDF5 and NDHJ or *CCR1* and *CCR3* (*CHLORORESPIRATORY REDUCTION1* and *2*), respectively. One essential subunit of $cytb_6/f$ complex, *PETG* was also down-regulated in *vtc1* mutant (Queval and Foyer, 2012). However, the direct role of ascorbate in photoprotection has recently been questioned (Plumb et al., 2018).

We have only few data about the effect of ABA on the linear or cyclic electron transport components at protein level. The quantitative proteomic analysis of the ABA-deficient maize mutants *vp5* and its WT counterpart VP5 grown under control conditions and under osmotic stress, was performed by Zhao et al. (2016). The CAB protein and the ε chain of the chloroplastic ATP synthase were up-regulated by osmotic stress in WT but it did not take place in the mutants, while the amount of $cytb_6/f$ complex subunit 6 protein decreased in the WT but not in the mutant plants 8 h after the exposure to -0.7 MPa PEG6000-induced osmotic stress. This suggests that the photosynthetic electron transport may be regulated at the level of ATP-ase protein in ABA biosynthesis *vp5* mutant.

Moreover, the ABA-insensitive ABI4 transcription factor, a component of ABA signalling has been implicated in the retrograde signal transduction, which conveys information from the chloroplast to the nucleus and coordinates the expression of photosynthetic genes encoded by the nuclear genome with chloroplast functions

553 (Koussevitzky et al., 2007). This means that the communication between chloroplast and
554 nuclear gene expression needs active ABA signalling.

555 It can be concluded that ABA deficiency may control the components of linear and
556 cyclic electron transport at transcriptional level or at the level of protein stability, which can
557 lead to the absence of the activation of CET under high salinity.

558 It has to be mentioned that the biosynthesis of other stress hormones, jasmonic acid
559 and salicylic acid was induced in ABA-deficient *flacca* mutants under water stress, these
560 hormones may also contribute to the regulation of photosynthesis (Muñoz-Espinoza et al.,
561 2015).

562 These processes suggest that the efficiency of Calvin-cycle is limited by the rate of
563 both linear and cyclic electron transport in ABA-deficient mutants under salt stress. The result
564 is that total soluble sugar content is lower in the mutant under control conditions and at 100
565 mM NaCl, while starch accumulation exhibited more steep decline with the severity of salt
566 stress. This deficiency of soluble carbohydrates may contribute to the decline in water
567 potential of tissues under salt stress.

568 ABA activates the biosynthesis of compatible osmolytes such as Pro and chaperones
569 that protect proteins and membranes under water-deficit conditions (Shinozaki and
570 Yamaguchi-Shinozaki, 2007). Since the main enzymes in Pro biosynthesis are induced by
571 ABA (reviewed by Szabados and Savouré, 2009), it was expected, that Pro content will be
572 reduced in *sit* mutant under salt stress. However, we found that Pro accumulated under salt
573 stress in the mutants, but not in the WT during the first 6 h of salt exposure, but this increase
574 was much higher at sublethal stress. This suggests that the biosynthesis of Pro can be induced
575 by other factors than ABA (e.g. by reactive oxygen species) during early stage of salt stress
576 acclimation or at the same time the degradation of Pro is inhibited. It is well documented that
577 in *Arabidopsis* a stress-specific isoform of the *P5CS1* protein is localized to the chloroplast
578 and the *P5CS1* gene is induced by osmotic and salt stress, and is activated by ABA-dependent
579 and ABA-independent regulatory pathways. Pro synthesis from glutamate generates not only
580 $NADP^+$ for the electron transport chain in the chloroplasts but also consumes ATP and
581 produces ADP for the chloroplastic ATP synthase (reviewed by Szabados and Savouré,
582 2009). Thus Pro accumulation may contribute to the maintenance of the electron transport
583 between photosystems and may serve as an osmoprotectant in chloroplast during stress
584 conditions. This alleviating effect of Pro accumulation was much moderate at 250 mM NaCl
585 treatment in *sit* mutants.

5. Conclusions

It can be summarized that ABA deficiency in *sit* mutants caused more severe osmotic stress and more moderate ionic stress in salt stressed tomato plants than in the WT. However, the mutants displayed severe salt stress symptoms such as high decrease in water potential, g_s and net CO_2 assimilation rate under high salinity, especially at lethal salt stress. Exposure of mutants to 250 mM NaCl caused irreversible damage to PSII reaction centres, severe reduction in the photosynthetic electron transport rate and effective quantum yields of PSII and PSI. These parameters were less affected in WT plants under salt stress. The CET, which is an effective defence mechanism for the two photosystems to avoid the damage caused by photoinhibition, was severely reduced under salt stress in *sit* plants. This suggests that the activation of cyclic electron transport around PSI needs active ABA synthesis and/or signalling. In spite of ABA deficiency Pro accumulation could alleviate the stress injury at sublethal salt stress in the mutant but its accumulation was not sufficient at lethal salt stress.

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References

Bartholomew, D.M., Bartley, G.E., Scolnik, P.A., 1991. Abscisic acid control of rbcS and cab transcription in tomato leaves. Plant Physiol. 96, 291–296.

Bates, L.S., Waldren, R.P., Teare, I. D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bright, J., Desikan, R., Hancock, J.T., Weir, I.S., Neill, S.J., 2006. ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. Plant J. 45, 113–122.

619 Cabot, C., Sibole, J.V., Barceló, J., Poschenrieder, C., 2009. Abscisic acid decreases leaf Na^+
620 exclusion in salt-treated *Phaseolus vulgaris* L. J. Plant Growth Regul. 28, 187–192.
621 Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress:
622 regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560.
623 Corrêa de Souza, T., Magalhães, P.C., de Castro, E.M., de Albuquerque, P.E.P., Marabesi,
624 M.A., 2013. The influence of ABA on water relation, photosynthesis parameters, and
625 chlorophyll fluorescence under drought conditions in two maize hybrids with
626 contrasting drought resistance. Acta Physiol. Plant. 35, 515–527.
627 https://doi.org/10.1007/s11738-012-1093-9
628 Dodd, I.C., 2013. Abscisic acid and stomatal closure: a hydraulic conductance conundrum?
629 New Phytol. 197, 6–8.
630 Flors, V., Paradis, M., Garcia-Andrade, J., Cerezo, M., Gonzalez Bosch, C., García-Agustín,
631 P., 2007. A tolerant behavior in salt-sensitive tomato plants can be mimicked by
632 chemical stimuli. Plant Signal. Behav. 2, 50-57.
633 Foyer, C.H., Neukermans, J., Queval, G., Noctor G., Harbinson, J., 2012. Photosynthetic
634 control of electron transport and the regulation of gene expression. J. Exp. Bot. 63,
635 1637–1661.
636 Fricke, W., Akhiyarova, G., Veselov, D., Kudoyarova, G., 2004. Rapid and tissue-specific
637 changes in ABA and in growth rate in response to salinity in barley leaves. J. Exp. Bot.
638 55, 1115–1123.
639 Galka, M.M., Rajagopalan, N., Buhrow, L.M., Nelson, K.M., Switala, J., Cutler, A. J., et al.
640 2015. Identification of interactions between abscisic acid and ribulose-1,5-bisphosphate
641 carboxylase/ oxygenase. PLoS ONE 10(7):e0133033.
642 https://doi.org/10.1371/journal.pone.0133033
643 Gallé, Á., Csiszár, J., Benyó, D., Laskay, G., Leviczky, T., Erdei, L., Tari, I., 2013. Isohydric
644 and anisohydric strategies of wheat genotypes under osmotic stress: biosynthesis and
645 function of ABA in stress responses. J. Plant Phys. 170, 1389–1399.
646 Galpaz, N., Wang, Q., Menda, N., Zamir, D., Hirschberg, J., 2008. Abscisic acid deficiency in
647 the tomato mutant *high-pigment* 3 leading to increased plastid number and higher fruit
648 lycopene content. Plant J. 53, 717-730.
649 Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of
650 photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim.
651 Biophys. Acta (BBA)-General Subjects 990, 87–92.

652 Gomez-Cadenas, A., Arbona, V., Jacas, J., Primo-Millo, E., Talon, M., 2002. Abscisic acid
653 reduces leaf abscission and increases salt tolerance in citrus plants. J. Plant Growth Reg.
654 21, 234–240.
655 Hansen, J., Møller, I.B., 1975. Percolation of starch and soluble carbohydrates from plant
656 tissue for quantitative determination with anthrone. Anal. Biochem. 68, 87–94.
657 Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. Role of
658 proline under changing environments. Plant Signal. Behav. 7, 1456–1466.
659 Herde, O., Peña-Cotrés, L., Willmitzer, L., Fisahn, J., 1997. Stomatal responses to jasmonic
660 acid, linolenic acid in wild-type and ABA-deficient tomato plants. Plant, Cell Environ.
661 20, 136–141.
662 Hlavinka, J., Nožková-Hlaváčková V., Floková, K., Novák, O., Nauš, J., 2012. Jasmonic acid
663 accumulation and systemic photosynthetic and electrical changes in locally burned wild
664 type tomato, ABA-deficient s*itiens* mutants and *sitiens* pre-treated by ABA. Plant
665 Physiol. Biochem. 54, 89–96.
666 Horváth, E., Brunner, Sz., Bela, K., Papdi, Cs., Szabados, L., Tari, I., Csiszár, J., 2015.
667 Exogenous salicylic acid-triggered changes in the glutathione transferases and
668 peroxidases are key factors in the successful salt stress acclimation of *Arabidopsis*
669 *thaliana*. Funct. Plant Biol. 42, 1129–1140.
670 Huang, W., Zhang, S. B., Cao, K. F., 2010. Stimulation of cyclic electron flow during
671 recovery after chilling-induced photoinhibition of PSII. Plant Cell Physiol. 51, 1922–
672 1928.
673 Huang, W., Yang, Y. J., Hu, H., Zhang, S. B., 2015. Different roles of cyclic electron flow
674 around photosystem I under sub-saturating and saturating light intensities in tobacco
675 leaves. Front. Plant Sci. 6:923. https://doi.org/10.3389/fpls.2015.00923
676 Huang, W., Yang, Y-J., Zhang, S-B., Liu, T., 2018. Cyclic electron flow around photosystem
677 I promotes ATP synthesis possibly helping the rapid repair of photodamaged
678 photosystem II at low light. Front. Plant Sci. 9:239.
679 https://doi.org/10.3389/fpls.2018.00239
680 Ismail, A., Takeda, S., Nick, P., 2014. Life and death under salt stress: same players, different
681 timing? J. Exp. Bot. 65, 2963–2979.
682 Javid, M.G., Sorooshzadeh, A., Moradi, F., Sanavy, S.A.M.M., Allahdadi, I., 2011. The role
683 of phytohormones in alleviating salt stress in crop plants. Aust. J. Crop Sci. 5, 726-737.

Jia, H., Lu, C., 2003. Effects of abscisic acid on photoinhibition in maize plants. Plant Sci. 165, 1403–1410.

Kovács, J., 2017. Comparative study of salt stress-induced physiological and molecular responses in tomato (*Solanum lycopersicum* L.). PhD Thesis, pp. 77. Repositorium of the Biological Doctoral School, University of Szeged, Hungary, doktori.bibl.u-szeged.hu

Kovács, J., Poór, P., Kaschani, F., Chandrasekar, B., Hong, T.N., Misas-Villamil, J.C., Xin, B.T., Kaiser, M., Overkleeft, H.S., Tari, I. and Van Der Hoorn, R.A., 2017. Proteasome activity profiling uncovers alteration of catalytic β2 and β5 subunits of the stress-induced proteasome during salinity stress in tomato roots. Front. Plant Sci., 8:107. https://www.frontiersin.org/article/10.3389/fpls.2017.00107

Klughammer, C., Schreiber, U., 1994. Saturation pulse method for assessment of energy conversion in PS I. Planta 192, 261–268.

Kohli, A., Sreenivasulu, N., Lakshmanan, P., Kumar, P.P., 2013. The phytohormone crosstalk paradigm takes center stage in understanding how plants respond to abiotic stresses. Plant Cell Rep. 32, 945–957.

Koussevitzky, S. Nott, A., Mockler, T.C., Hong, F., Sachetto-Martins, G., Surpin, M., Lim, J., Mittler, R., Chory J., 2007. Signals from chloroplasts converge to regulate nuclear gene expression. Science 316, 715-719.

Kramer, D.M., Johnson, G., Kiirats, O., Edwards, G.E., 2004. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth. Res. 79, 209–218.

Laisk, A., Talts, E., Oja, V., Eichelmann, H., Peterson, R.B., 2010. Fast cyclic electron transport around photosystem I in leaves under far-red light: a proton-uncoupled pathway? Photosynth. Res. 103, 79–95.

Li, H., Wang, Y., Xiao, J., Xu, K., 2015. Reduced photosynthetic dark reaction triggered by ABA application increases intercellular CO_2 concentration, generates H_2O_2 and promotes closure of stomata in ginger leaves. Environ. Exp. Bot. 113, 11–17.

Li, L., Aro, E-M., Millar, A.H., 2018. Mechanisms of photodamage and protein turnover in photoinhibition. Trends Plant Sci. 23, 667-676. https://doi.org/10.1016/j.tplants.2018.05.004

Mäkelä, P., Munns, R.., Colmer, T.D., Condon, A.G., Peltonen-Sainio, P., 1998. Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute

717 concentrations in leaves of salt- or drought-stressed tomato. Funct. Plant Biol. 25, 655–
718 663.
719 Mäkelä, P., Munns, R., Colmer, T. D., Peltonen-Sainio, P., 2003. Growth of tomato and an
720 ABA-deficient mutant (*sitiens)* under saline conditions. Physiol. Plant. 117, 58-63.
721 Meng, F., Luo, Q., Wang, Q., Zhang, X., Qi, Z., Xu, F., Sun, G., 2016. Physiological and
722 proteomic responses to salt stress in chloroplasts of diploid and tetraploid black locust
723 (*Robinia pseudoacacia* L.). Sci. Rep. 6, 23098.
724 https://www.nature.com/articles/srep23098.pdf
725 Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59,
726 651–681.
727 Muñoz-Espinoza, V.A., López-Climent, M.F., Casaretto, J.A., Gómez-Cadenas, A., 2015.
728 Water stress responses of tomato mutants impaired in hormone biosynthesis reveal
729 abscisic acid, jasmonic acid and salicylic acid interactions. Front. Plant Sci. 6, 997.
730 https://doi.org/10.3389/fpls.2015.00997
731 Monteiro, C.C., Rolão, M.B., Franco, M.R., Peters, L.P., Cia, M.C., Capaldi, F.R., Carvalho,
732 R.F., Gratão, P.L., Rossi, M.L., Martinelli, A.P., Peres, L.E.P., Azevedo, R.A., 2012.
733 Biochemical and histological characterization of tomato mutants. An. Acad. Bras.
734 Ciênc. (Annals of the Brazilian Academy of Sciences) 84, 573-585.
735 Nagel, O.W., Konings H., Lambers H., 1994. Growth rate, plant development and water
736 relations of the ABA-deficient tomato mutant *sitiens.* Physiol. Plant. 92, 102–18.
737 Nakano, R., Ishida, H., Makino, A., Mae, T., 2006. *In vivo* fragmentation of the large subunit
738 of ribulose-1,5-bisphosphate carboxylase by reactive oxygen species in an intact leaf of
739 cucumber under chilling-light conditions. Plant Cell Physiol. 47, 270–276.
740 Pérez-Alfocea, F., Albacete, A., Ghanem, M. E., Dodd, I. C., 2010. Hormonal regulation of
741 source-sink relations to maintain crop productivity under salinity: a case study of root-
742 to-shoot signalling in tomato. Funct. Plant Biol. 37, 592-603.
743 Plumb, W., Townsend, A.J., Rasool, B., Alomrani, S., Razak, N., Karpinska, B., Ruban, A.V.,
744 Foyer, C.H., 2018. Ascorbate-mediated regulation of growth, photoprotection, and
745 photoinhibition in *Arabidopsis thaliana*. J. Exp. Bot. 69, 2823–2835.
746 Pompeu, G.B., Vilhena, M.B., Gratão, P.L., Rogério F., Carvalho, R.F., Rossi, M.L.,
747 Martinelli, A.P., Azevedo, R.A., 2017. Abscisic acid-deficient *sit* tomato mutant
748 responses to cadmium-induced stress. Protoplasma 254, 771-783.

749 Poór, P., Gémes, K., Horváth, F., Szepesi, A., Simon, M. L., Tari, I., 2011. Salicylic acid
750 treatment via the rooting medium interferes with stomatal response, CO_2 fixation rate
751 and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent
752 salt stress. Plant Biol. 13, 105–114.
753 Poór, P., Borbély, P., Kovács, J., Papp, A, Szepesi, Á., Takács, Z., Tari, I., 2014. Opposite
754 extremes in ethylene/nitric oxide ratio induce cell death in cell suspension culture and in
755 root apices of tomato exposed to salt stress. Acta Biol. Hung. 65, 428-438.
756 Queval, G., Foyer, C. H., 2012. Redox regulation of photosynthetic gene expression. Phil.
757 Trans. R. Soc. B. 367, 3475-3485.
758 Rengasamy, P. (2006). World salinization with emphasis on Australia. J. Exp. Bot. 57, 1017–
759 1023.
760 Schreiber, U., Klughammer, C., 2008. Non-photochemical fluorescence quenching and
761 quantum yields in PS I and PS II: analysis of heat-induced limitations using Maxi-
762 Imaging-PAM and Dual-PAM-100. PAM Application Notes 1, 15–18.
763 Shinozaki, K., Yamaguchi-Shinozaki, K., 2007. Gene networks involved in drought stress
764 response and tolerance. J. Exp. Bot. 58, 221–227.
765 Sugita, M., Gruissem, W., 1987. Developmental, organ-specific and light-dependent
766 expression of the tomato ribulose-1,5bisphosphate carboxylase small subunit gene
767 family. Proc. Natl. Acad. Sci. USA 84, 7104–7108.
768 Sukhov, V.S., Gaspirovich, V.V., Gromova, E.N., Ladeynova, M.M., Sinitsyna, Yu.V.,
769 Berezina, E.V., Akinchits, E.K., Vodeneev, V.A., 2017. Decrease of mesophyll
770 conductance to CO_2 is a possible mechanism of abscisic acid influence on
771 photosynthesis in seedlings of pea and wheat. Biochemistry (Moscow), Supplement
772 Series A: Membrane and Cell Biology 11, 237–
773 247.https://doi.org/10.1134/S1990747817030096
774 Szabados, L., Savouré, A., 2009. Proline: a multifunctional amino acid. Trend Plant Sci.15,
775 289–97.
776 Takács, Z., Poór, P., Szepesi, Á. and Tari, I., 2017. In vivo inhibition of polyamine oxidase by
777 a spermine analogue, MDL-72527, in tomato exposed to sublethal and lethal salt stress.
778 Funct. Plant Biol. *44*, 480-492.
779 Tal, M. 1966. Abnormal stomatal behavior in wilty mutants of tomato. Plant Physiol. 41,
780 1387–1391.

781 Tari, I., Poór, P., Ördög, A., Székely, Á., Laskay, G., Bagi, I., 2013. Enhanced biomass
782 production in sudangrass induced by co-treatment with copper and EDTA. Environ.
783 Exp. Biol. 11, 151–157.
784 Wellburn, A.R., Lichtenthaler, H.K., 1984. Formulae and program to determine total
785 carotenoids and chlorophylls A and B of leaf extracts in different solvents. in: Sybesma
786 C. (Ed.), Advances in photosynthesis research. Advances in agricultural biotechnology,
787 Martinus Nijhoff/Dr W. Junk Publishers, The Hague/Boston/Lancaster, pp. 10–12.
788 Zhang, G., Liu, Y., Ni, Y., Meng, Z., Lu, T., Li, T., 2014. Exogenous calcium alleviates low
789 night temperature stress on the photosynthetic apparatus of tomato leaves. PloS One,
790 9(5), e97322, https://doi.org/10.1371/journal.pone.0097322.
791 Zhao, Y., Wang, Y., Yang, H., Wang, W., Wu, J., Hu, X., 2016. Quantitative proteomic
792 analyses identify ABA-related proteins and signal pathways in maize leaves under
793 drought conditions. Front. Plant Sci. 7:1827. https://doi.org/10.3389/fpls.2016.01827
794 Zhu, J. K., 2002. Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biol.
795 53, 247–273.
796

Fig. 1. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the relative water content (A) and on the water potential (B) in the leaf tissues of wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=6. Means denoted by different letters are different at $P<0.05$ level (Duncan's multiple range test).

Fig. 2. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the stomatal conductance (A), transpiration rate (B), net CO_2 assimilation rate (C), and intercellular CO_2 concentration (D) in the leaf tissues of wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at $P<0.05$ level (Duncan's multiple range test).

Fig. 3. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the protein content of RUBISCO large subunit (RBCL) (A) and the relative expression levels of *RBCL* (B) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=3 (Duncan's multiple range test; ns: not significant).

Fig. 4. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the maximal quantum efficiency of PSII (Fv/Fm) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at $P<0.05$ level (Duncan's multiple range test).

Fig. 5. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the effective quantum yield of PSII (A), on non-regulated (C) and regulated (D) energy dissipation as well as on the effective quantum yield of PSI (B), on non-photochemical quenching originated from the limitation of PSI at donor (D) and acceptor sites (F) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at $P<0.05$ level (Duncan's multiple range test).

Fig. 6. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the linear (A, ETR II) and cyclic electron transport (B, CET) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at $P<0.05$ level (Duncan's multiple range test).

Fig. 7. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the chlorophyll *a*+*b* (A) and carotenoids content (B) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at P<0.05 level (Duncan's multiple range test).

Fig. 8. Effect of salt stress elicited by 100 mM or 250 mM NaCl on the starch (A) and total sugar content (B) in wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=5. Means denoted by different letters are different at P<0.05 level (Duncan's multiple range test).

Fig. 9. Effect of salt stress elicited by 100 mM or 250 mM NaCl on proline content in the leaf tissues of wild type (black columns) and *sitiens* mutants (open columns) of tomato. Means ± SE, n=6. Means denoted by different letters are different at P<0.05 level (Duncan's multiple range test).

Table 1. Changes in K^+ and Na^+ contents and the K^+/Na^+ ratio in the leaves of wild type (WT) and *sitiens* tomato after exposure to 100 or 250 mM NaCl.

Elements (μmol gDW^{-1})	**Treatments**					
	Control		100 mM NaCl		250 mM NaCl	
	WT	*Sitiens*	WT	*Sitiens*	WT	*Sitiens*
K^+	626.82±4.93^c	1086.39±5.53^a	651.37±29.75^c	1133.96±5.53^a	584.88±2.94^d	1022.09±28.17^b
Na^+	50.50±0.63^f	85.51±0.39^e	90.79±0.46^d	128.69±2.26^c	151.06±0.23^b	213.19±0.55^a
K^+/Na^+	12.41±0.05^a	12.70±0.03^a	7.17±0.30^c	8.81±0.19^b	3.87±0.03^e	4.79±0.14^d

(Means ± SD, n=6). Bars with different letters are significantly different at 0.05 levels (Duncan's multiple range test).