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ORIGINAL ARTICLE

Salt stress effects on the photosynthetic electron transport chain in two chickpea lines differing in their salt stress tolerance

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Abstract The main objective of this study was to evaluate the effects of salt stress on the photosynthetic electron transport chain using two chickpea lines (*Cicer arietinum* L.) differing in their salt stress tolerance at the germination stage (AKN 87 and AKN 290). Two weeks after sowing, seedlings were exposed to salt stress for 2 weeks and irrigated with 200 ml of 200 mM NaCl every 2 days. The polyphasic OJIP fluorescence transient and the 820-nm transmission kinetics (photosystem I) were used to evaluate the effects of salt stress on the functionality of the photosynthetic electron transport chain. It was observed that a signature for salt stress was a combination of a higher J step (V_J), a smaller IP amplitude, and little or no effect on the primary quantum yield of PSII (φ_{Po}). We observed for AKN 290 a shorter leaf life cycle, which may represent a mechanism to cope with salt stress. For severely salt-stressed leaves, an inhibition of electron flow between the PQ pool and P700 was found. The data also suggest that the properties of electron flow beyond PSI are affected by salt stress.

Keywords Chickpea · Polyphasic OJIP fluorescence transient · Salt stress · 820-nm transmission · Leaf life cycle length

Abbreviations

Chl	Chlorophyll
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
dV/dt0	The initial slope (fluorescence rise between 20 and 270 µs) of the OJIP transient times 4, normalized to F_V
Fd	Ferredoxin
FNR	Ferredoxin $NADP^+$ reductase
F_{IP}	Amplitude of the I-to-P rise
F_J, F_I	Fluorescence intensity after 3 and 30 ms of illumination, respectively
F_0, F_M	Minimum and maximum fluorescence intensity emitted by dark-adapted samples, respectively
F_V	Variable fluorescence ($F_M - F_0$)
ΔI_{max}	Maximum light-inducible transmission change at 820 nm (50 times amplified)
I_{tot}	Transmission signal at 820 nm measured with amplification = 1 and no offset
JIP test	Analysis framework for the interpretation of OJIP transients developed by Bruno and Reto Strasser
LED	Light-emitting diode

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LHC, LHCI, and LHCII	Light-harvesting complex, in general, associated with PSI and mainly associated with PSII, respectively
NPQ	Non-photochemical quenching
OJIP	Fluorescence rise on a dark-to-light transition from a minimum value O via the intermediate steps J and I to the maximum value P, which is F_M if the light is saturating
P700	PSI reaction center chlorophyll dimer
PC	Plastocyanin
PI_{ABS}	Performance index of the JIP test
PSI, PSII	Photosystem I and II, respectively
Q_B, PQ	Secondary quinone electron acceptor of PSII and free plastoquinone, respectively
qP	Photochemical quenching calculated based on the puddle model
Sm	Complementary area above the fluorescence rise normalized to F_V
ΔV_{IP}	Relative contribution of the I-to-P rise to the OJIP rise
V_J, V_I	Relative position of the J and I steps between O and P
φ_{P0}, F_V/F_M	Maximum quantum yield of primary photochemistry

Introduction

Plants may encounter a range of environmental stresses, such as high or low temperature, drought, and salt stress during their growing season. All these environmental factors directly or indirectly affect the photosynthetic activity of leaves, the pigment composition and antenna size, the stoichiometry of the photosynthetic protein complexes and the redox factors connecting them, and the ultrastructure of the photosynthetic apparatus (Alia-Mohanty and Saradhi 1992; Lichtenthaler et al. 2005; Chaves et al. 2002, 2009). Changes in the Chlorophyll (Chl) *a* fluorescence kinetics reflect these modifications of the photosynthetic apparatus (Kalaji et al. 2014, 2016 and references therein). Since photosynthesis is one of the most important metabolic processes in plants (Baker 1991), negative effects of environmental stress on its performance will decrease crop productivity (Lawlor 1995).

Salinity is one of the major environmental factors limiting crop production. According to a review published in 2005, over 6% of the world's total land area is affected by salinity (Munns 2005) and an additional 1.5 million hectares of irrigated land each year pass the salt stress threshold due to salinization (Munns and Tester 2008). Salt stress affects a range of physiological processes involved in cell metabolism (Munns 2002). Salt stress also causes changes in the uptake of nutrients and ions, which affects plant growth (Kalaji and Pietkiewicz 1993; Marschner 1995). It has been reported that salt stress makes plants more sensitive to photoinhibition of photosystem II (PSII), causes osmotic stress, and leads to a higher generation of reactive oxygen species (Parida and Das 2005; Oukarroum et al. 2015; Lu et al. 2016). However, plants are able to adapt their photosynthetic activity and metabolism to cope with moderate levels of salt stress and to maintain a balance between photosynthetic activity and consumption of ATP and NADPH (Asada 2006). The effect of salinity on photosynthesis is complex (Shabala et al. 2005). Elevated, albeit low, salinity levels sometimes enhance photosynthetic performance (Greenway and Munns 1980; Çiçek and Çakırlar 2008), and at medium and high salinity leaf photosynthesis becomes severely inhibited (Seeman and Critchley 1985). Further, Çiçek and Çakırlar (2008) observed that the impact of salt stress on soybeans was stronger at 25 °C than at 30 °C.

There are also certain treatments that have been shown to reduce the negative effects of salt stress. Allakhverdiev et al. (2001) showed that in mutants of the cyanobacterium *Synechococcus* with a higher unsaturated lipid content, salt stress had less effect on the electron transport rate from PSII to P700, the reaction center chlorophylls of photosystem I (PSI). Jiang et al. (2017) demonstrated that the application of low concentrations of selenium reduces salt stress-induced chlorosis, the disruptive effects on the chloroplast ultrastructure in maize, and damage to the membranes. The authors suggest that the positive effect of selenium on the antioxidant system plays a key role in these effects. Singh et al. (2016) showed that nitrogen application also reduces the effects of salt stress, and Janda et al. (2016) demonstrated that the acclimation of wheat plants to moderate salt concentrations allows them to cope better with high salt concentrations. Acclimated plants keep their stomata longer open and have fewer chlorotic symptoms. The authors further observe that acclimation triggers the antioxidant system.

From a physiological point of view, it is also important to note that salt and osmotic stress do not induce the same responses in photosynthetic organisms. Kanesaki et al. (2002) doing a microarray study of the cyanobacterium *Synechocystis* PCC 6803 showed that the two types of stress induce/repress different sets of genes and affect the cytoplasmic volume differently.

As noted above, changes in the Chl *a* fluorescence kinetics reflect changes in the photosynthetic apparatus making it a useful, non-invasive tool for the study of different aspects of photosynthesis, and for the detection of stress in plants (Krause and Weis 1991; Strasser et al. 2004; Kalaji et al. 2014), especially when used in combination with 820-nm measurements that, in addition, give more detailed information on the electron flow through PSI (e.g., Schansker et al. 2005). Based on earlier studies (Schansker et al. 2005, 2006, 2008), we assume that ferredoxin $NADP^+$ reductase (FNR) is inactive in dark-adapted chickpea leaves and that the activation of FNR prevents to a large extent the IP rise and the re-reduction of $P700^+$ and oxidized plastocyanin (PC^+).

Çiçek and Çakırlar (2008) showed for several soybean cultivars that in response to salinity stress plants try to maintain a balance between light absorption and utilization (unchanged qP), by adjusting three parameters: Chl content, Chl *a*/*b* ratio, and non-photochemical quenching (NPQ) in a cultivar-dependent way.

In the present study, we focused on the changes in the functionality of the photosynthetic electron transport chain induced by salt stress in two chickpea (*Cicer arietinum* L.) lines (AKN 87 and AKN 290) using the extent of the salt-induced changes to judge the severity of the treatment. The two chickpea lines provided us with biological variability. Chickpea has thin leaves; pea (*Pisum sativum* L.) with its thicker leaves and a high Chl content was used as a reference plant. Experimental observations were derived from the simultaneous measurement of Chl *a* fluorescence and 820-nm transmission changes.

Materials and methods

Plant material and salt stress treatment

In this study, two chickpea (*Cicer arietinum* L.) lines (AKN 87 and AKN 290), of which the salt tolerance properties have been determined at the germination stage (AKN 290 salt tolerant, AKN 87 salt sensitive), were used (Turan et al. 2005; Kalefetoğlu Macar et al. 2009). In addition, pea (*Pisum sativum* L. cv. Ambassador) was used as a reference plant (see Schansker et al. 2005 for the growing conditions).

The seedlings were grown in the greenhouse in black pots containing about 2 L of commercial peat soil at a day temperature of about 25–30 °C. The plant density was 5 plants per pot and there were at least 4 replicate pots per treatment. During the experiment, the plants were grown under long-day conditions (16-h light, 8-h dark) by giving additional light if needed (OSRAM HQIT 400 W lamps were used). Two-week-old seedlings were exposed to salt stress for 15 days and were treated with 200 ml of 200 mM NaCl, which was dissolved in distilled water, every 2 days.

Chlorophyll *a* fluorescence measurements

Fluorescence measurements were made at room temperature with a Handy PEA fluorimeter (Hansatech Instruments Ltd., King's Lynn, UK). Leaves dark acclimated for at least 1 h were illuminated homogeneously over an area of 4 mm^2 with an array of 3 red LEDs (3000 µmol photons m^{-2} s^{-1}). Chl *a* fluorescence emission was detected by a high-performance PIN photodiode detector associated with an amplifier circuit. Fluorescence transients were recorded from 10 µs to 1 s with a time resolution of 10 µs for first 300, 100 µs between 300 µs and 3 ms, 1 ms between 3 ms and 30 ms, and 10 ms between 30 and 300 ms, with a 12-bit resolution. The fluorescence signal at 20 µs after the onset of illumination was taken as F_0 (Kalaji et al. 2014).

All photosynthetic samples in almost any physiological state [with the exception of, e.g., dried lichens (Oukarroum et al. 2012)] exhibit upon illumination a fast fluorescence rise from an initial fluorescence intensity F_0 to a maximum intensity F_P (under saturating light conditions, denoted as F_M) (Strasser et al. 2004). Between these two extrema, the fluorescence rise passes through intermediate steps at about 2–3 ms (F_J) and at about 30 ms (F_I) (Strasser et al. 1995), whereas F_M is reached after about 200–500 ms of illumination. Several basic parameters derived from the JIP test (Strasser and Strasser 1995; Strasser et al. 2004), which have been characterized experimentally, were used for the analysis: V_J, the relative position of the J step defined as $V_J = (F_J - F_0)/(F_M - F_0)$, where J is defined as the 3 ms point (Kalaji et al. 2017); Sm, the complementary area between the OJIP transient and F_M divided by the complementary area between OJ and F_J. This is a measure for the number of electron acceptors that have to be reduced before F_M is reached (see Kalaji et al. 2017 for a discussion of this parameter). The parameter dV/dt0, the initial slope, is a measure for the antenna size: 4 $(F_{270\mu s} - F_0)/(F_M - F_0)$ and PI_{ABS}: $1/(dV/dt0\ (1/V_J)\ (1/(F_V/F_M))\ ((F_V/F_M)/(1-F_V/F_M))\ ((1-V_J)/(1-(1-V_J))$, is a general stress parameter. Further, the parameters F_{IP} and ΔV_{IP} were used. F_{IP} is $F_P - F_I$, the amplitude of the IP phase, where I is the 30 ms point and F_P is F_M. ΔV_{IP} is the parameter used by Ceppi et al. (2012) as a measure for the leaf PSI content and defined as $(F_M - F_I)/(F_M - F_0)$.

820-nm transmission measurements

820-nm transmission measurements were carried out using a PEA Senior (Hansatech Instruments Ltd., King's Lynn, UK). Light-induced changes in the light transmission of photosynthetic samples at approx. 820 nm reflect redox changes of PC, P700, and ferredoxin (Fd) (Klughammer and Schreiber 1991; Schansker et al. 2003). The first reliable transmission measuring point of the PEA Senior is at 400 µs. The

maximum light intensity is 1800 µmol photons m^{-2} s^{-1} supplied by 4 red LEDs and the acquisition of measuring points is as described for the Handy PEA (see above). The measuring head also has a FR LED emitting continuous FR light used for the preferential excitation of PS I. This FR light source is a QDDH73520 LED (Quantum Devices Inc.) filtered at 720 ± 5 nm. The LED used to measure PC, P700, and Fd redox changes is modulated (33.3 kHz) and filtered at 830 ± 20 nm [OD820 LED (Opto Diode Corp.)] (Schansker et al. 2003).

Maximum 820-nm transmission amplitude

As a measure for the PC + P700 content, we used the difference between the 820-nm transmission level when all PC and P700 were oxidized and the transmission level when all PC and P700 were reduced (ΔI_{max}). To achieve both states in rapid succession, a three-pulse protocol was used [see Fig. 1 in Schansker et al. (2003) for a graphic illustration of the protocol]. It consists of a first 0.7-s pulse of red light (1800 µmol photons m^{-2} s^{-1}) to reduce the electron transport chain. In control leaves, the minimum transmission level that is reached after approximately 20 ms does not represent the state with all PC and P700 oxidized. A subsequent 15-s pulse of FR light (200 µmol photons m^{-2} s^{-1}) causes an oxidation of the electron transport chain. Finally, a second 2-s pulse of red light (1800 µmol photons m^{-2} s^{-1}) is given. During the first 20 ms of this pulse, all PC and P700 that still may have been in the reduced state are oxidized and in the subsequent few hundreds of ms all PC^+ and $P700^+$ are reduced again.

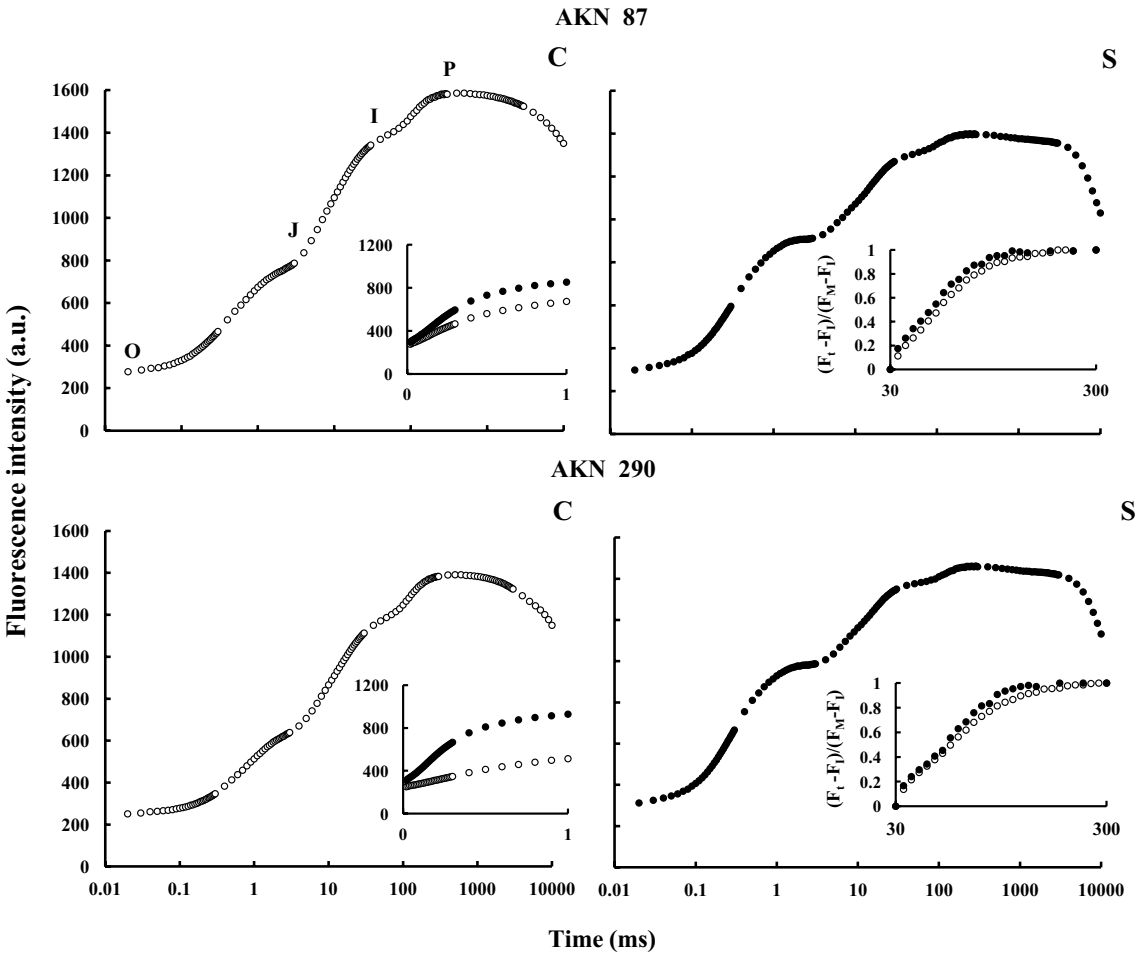

Fig. 1 OJIP fluorescence transients measured on control and salt-stressed chickpea leaves of AKN 87 and AKN 290, respectively. In the insets of the two panels on the left-hand side, the first 1 ms of the fluorescence rise is compared between measurements on control and salt-treated leaves. In the insets of the two panels on the right-hand side, the IP rise double normalized between F_I and F_P is compared. The OJIP transients were induced by 3000 µmol photons m^{-2} s^{-1} red light and the transients represent the average of 5 independent measurements

Statistics

The statistical analysis was made using Statgraphics Centurion XVII.

Results

Chlorophyll *a* fluorescence rise and 820-nm transmission measurements

As illustrated in the panels of Fig. 1, after 15 days of NaCl treatment, the Chl *a* fluorescence rise of the salt-stressed plants still displayed the same OJIP kinetics typical for control plants (Strasser et al. 1995). OJIP transients reflect a reduction of the photosynthetic electron transport chain with the three rise phases O–J, J–I, and I–P reflecting the reduction of the acceptor side of PSII, the PQ pool, and the redox factors around PSI, respectively (Schansker et al. 2005; Kalaji et al. 2014). Taking a closer look at the measurements, several salt stress-induced differences can be observed. Comparing the F_0 values of AKN 87, a slightly higher value is observed for the salt stress-treated plants. With respect to the F_M value, the salt stress treatment reduces the F_M value compared to the control. For AKN 290 plants, the amplitude of the variable fluorescence was not affected by the salt stress treatment. Looking at the rise kinetics, salt stress induces in both cases a higher and better defined J step (inset left-hand panels) and a smaller IP amplitude (insets right-hand panels). The higher and better defined J step is accompanied by a steeper initial slope. Further, when comparing the width of the P step, it is broader in the case of the salt stress treatment. In Fig. 2, the 820-nm data measured simultaneously with the OJIP fluorescence data after 5, 10, and 15 days of treatment are shown. For AKN 290, the time dependence of the changes in the amplitude during the first 20 ms of illumination is quite similar for control and salt-stressed leaves. However, in the case of the salt-stressed leaves the amplitude is systematically smaller. For AKN 87, the amplitude change during the first 20 ms of illumination after 5 and 10 days of treatment is very similar (the amplitude as well). Only after 15 days of treatment, a large amplitude difference is observed. A kinetic difference is observed around 200 ms of illumination after 10 and 15 days of treatment, where in the salt-treated plants a re-oxidation phase sets in almost immediately after reaching the maximum 820 nm value. This feature is missing in the control leaves. For AKN 87 leaves, a continuous increase of the amplitude of the initial transmission decline is observed. In contrast, for AKN 290 the maximum amplitude is already reached after 5 days and after 15 days a considerable decrease in the amplitude is observed, a pattern

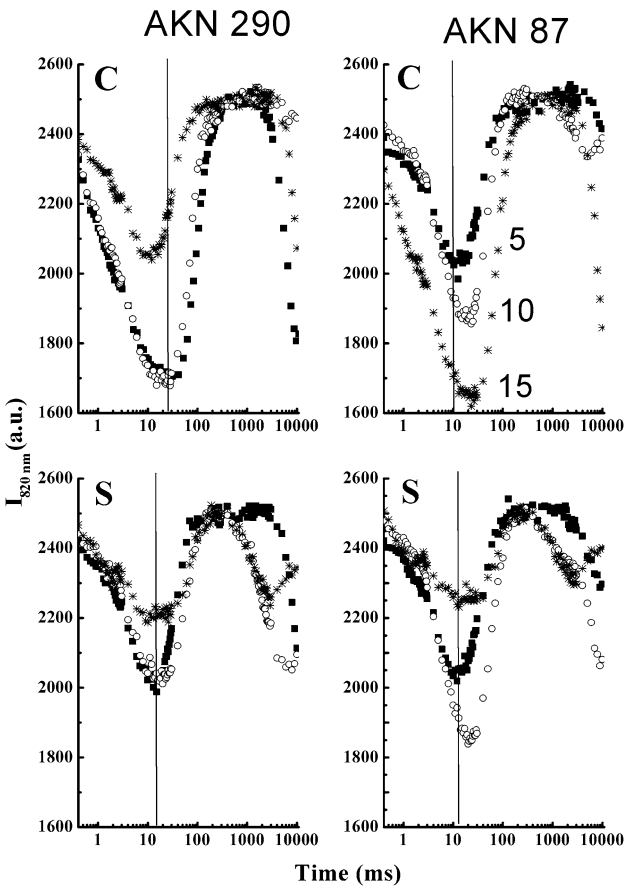

Fig. 2 820-nm transmission transients recorded during the first 10 s of illumination (1800 µmol photons m^{-2} s^{-1} red light) following a dark-to-light transition, measured on control and salt-stressed leaves of AKN 87 and AKN 290, respectively, after 5, 10, and 15 days of treatment. The transients represent the average of 4–5 independent measurements

that is also seen for the salt-treated leaves. This may suggest that AKN 290 leaves have a shorter life cycle.

PSII biophysical parameters

In Fig. 3, the changes discussed above for the OJIP measurements are captured in several parameters. V_J and dV/dt0 are considerably higher following salt stress, especially for AKN 290. The parameter F_{IP} is considerably lower for both lines following salt stress although for AKN 290 the decrease is larger. For the normalized area above the OJIP transient a very similar trend is observed indicating that the smaller Sm values are to a large extent determined by the smaller IP amplitude and in addition by the higher V_J value. All the observed kinetic changes also lead to a strong decrease of the parameter PI_{ABS}, a cumulative effect that is stronger in AKN 290 than in AKN 87. Since the salt treatment had little effect on F_0 and F_M, the parameter F_V/F_M was only slightly smaller after a salt stress treatment for 15 days.

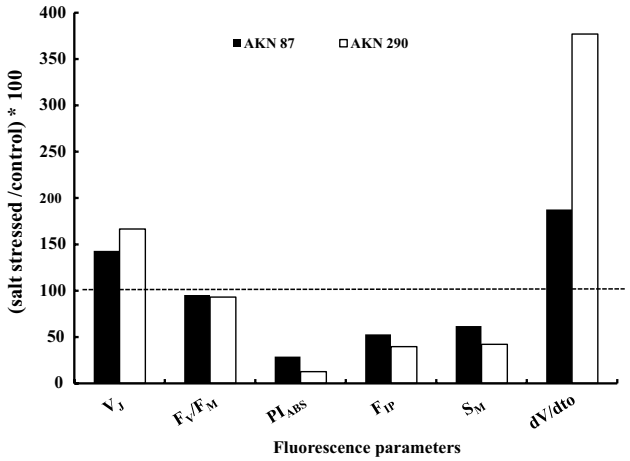

Fig. 3 Effects of salt stress on the OJIP fluorescence transients of the chickpea lines AKN 87 and AKN 290 as reflected by six biophysical parameters derived from the OJIP transients. All values are calculated relative to the control values. The calculated parameters are based on the average of 5 independent measurements. A two-way ANOVA of these parameters showed that there was no significant difference ($p < 0.05$) between the parameter values of the control plants, and there was a significant difference between control and treatment values for all parameters and for the interaction (variety × treatment) significant differences were only observed for the parameters Sm and dVt/dto

Relationship between V_{IP} and maximum 820-nm transmission change

In Fig. 4, we re-analyzed the salt stress data shown in Ceppi et al. (2012). With respect to the measurements made on the pea leaves, there was still a linear relationship between ΔV_{IP} and the transmission amplitude following the division by the total transmission I_{tot} (see panel a). In contrast, for the chickpea leaves the correlation was lost following the division by I_{tot} (compare panels b and c). It is likely that in Ceppi et al. (2012) the salt stress data were divided by the offset and not by I_{tot}.

Severe salt stress effects

The experiments were carried out several times at different times of the year and the resulting severity of the salt stress differed between experiments. In Fig. 5, an example of measurements on severely stressed chickpea leaves is shown. With respect to the fluorescence measurements, the very high V_J value and the much smaller V_{IP} amplitude are striking. Comparing the fluorescence data with the transmission data, it is also clear that this small V_{IP} amplitude is not accompanied by a comparably strong 820-nm transmission change. Further, the transmission data indicate that the re-reduction kinetics are strongly delayed. The 820-nm signal starts to increase again after 60–80 ms compared with 20 ms in the control leaves. A similar delay is also observed in

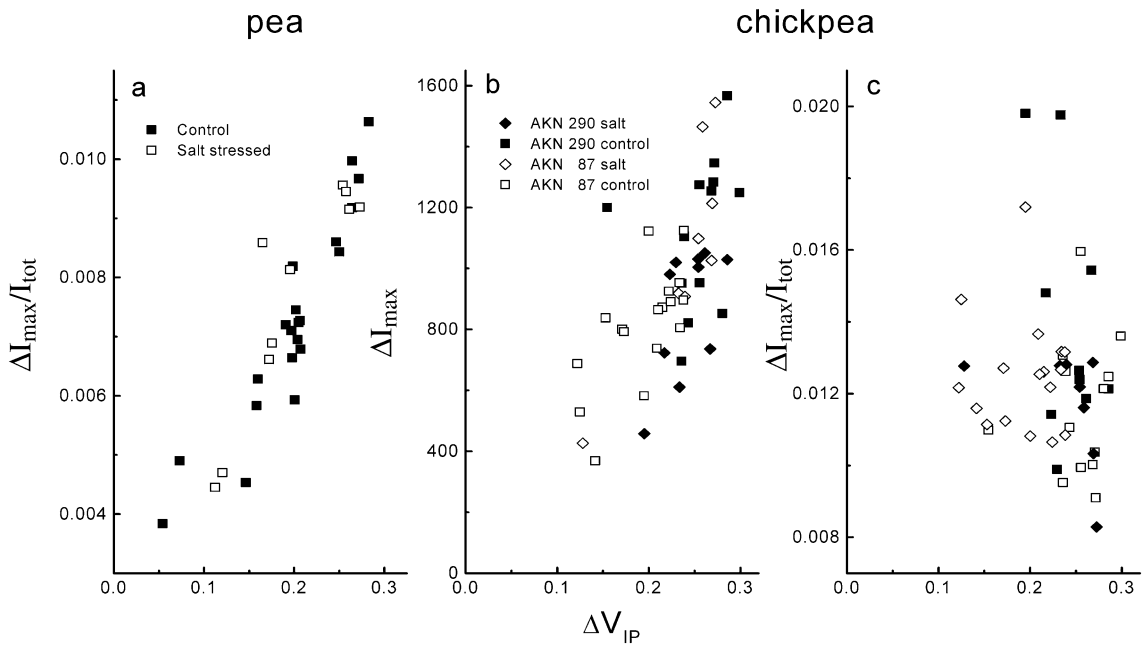

Fig. 4 Relationship between the fluorescence parameter ΔV_{IP} and the 820-nm transmission parameter ΔImax/Itot for salt-stressed pea leaves (**a**), and between ΔV_{IP} and ΔI_{max} (**b**) and between ΔV_{IP} and $\Delta I_{max}/I_{tot}$ (**c**) for salt-stressed chickpea leaves. Here ΔV_{IP} is the amplitude of the IP phase after double normalization between O and P, ΔI_{max} is the difference in the 820-nm signal between all PC, P700, and Fd oxidized and reduced, respectively, and I_{tot} is the total transmission at 820 nm

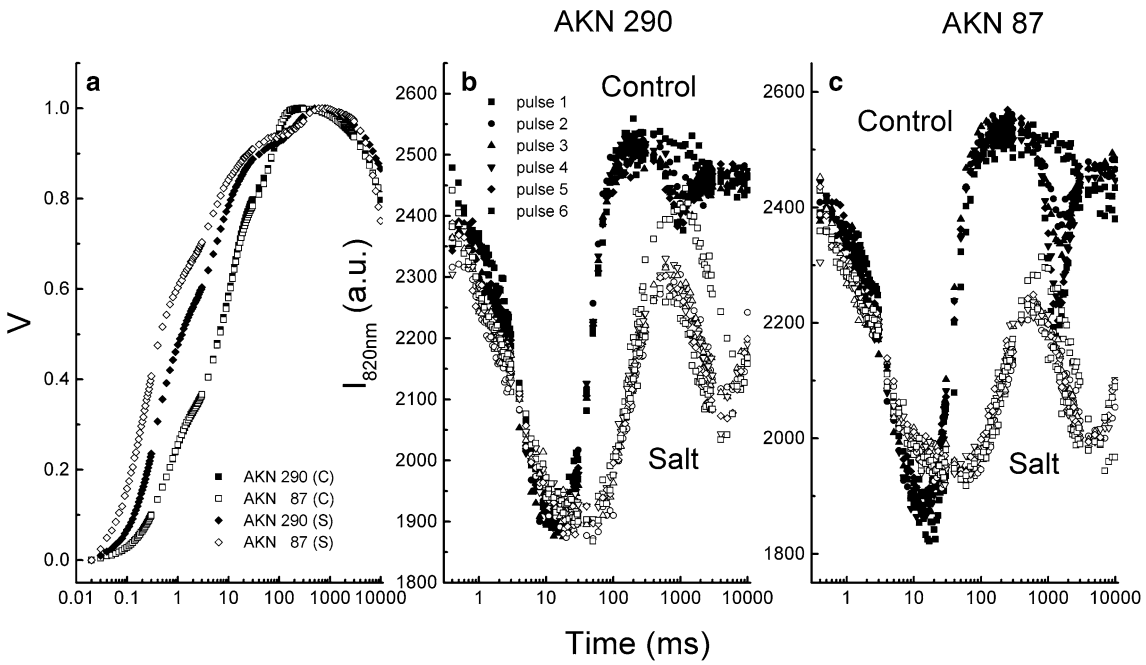

Fig. 5 Chl *a* fluorescence (**a**) and 820-nm transmission measurements (**b**, **c**) on severely salt-stressed chickpea leaves. In panel **a**, the data of the first 10-s pulse of 1800 µmol photons m^{-2} s^{-1} red light are shown, whereas in panels **b** and **c** 820-nm transmission kinetics of six 10-s pulses spaced 30 s apart are shown. The traces represent the averages of 4–5 independent measurements

the fluorescence data. Inducing photosynthesis gradually, using six 10-s pulses spaced 30 s apart, other differences are observed between 500 ms and 5 s. In the salt-treated leaves, the 820-nm signal already starts to decrease again before the maximum possible value is reached, and in terms of kinetics there is little difference between pulses 2 and 6. In the control leaves, especially in AKN 87, a dip in the 820-nm signal between 0.5 and 3 s is forming that deepens on each subsequent pulse. In the salt-treated leaves, the transmission signal starts to increase again after ~ 3 s, possibly due to a reduction of the $NADP^+$ pool before the Calvin–Benson cycle is activated. The data indicate that severe salt stress has a major impact on the electron flow around PSI.

In Fig. 6, the 820-nm transmission data of the first pulse in Fig. 5 are plotted as a function of fluorescence induction data. Such a representation is called a phase diagram. The transmission decrease during the first 20 ms is very similar in all cases. The fluorescence rise in the salt-treated leaves is steeper, due to the higher J step, and this also causes the observed shift to the right of curves of the salt-treated leaves. This phase also ends at higher V levels, because the IP phase is considerably smaller in the salt-treated leaves. The rise phase in salt-treated leaves is much steeper and the subsequent decrease is much sharper in the salt-treated leaves. In the control leaves, the slope of the rise phase becomes less steep just before reaching the maximum value and the subsequent decrease is also considerably smaller for the control leaves.

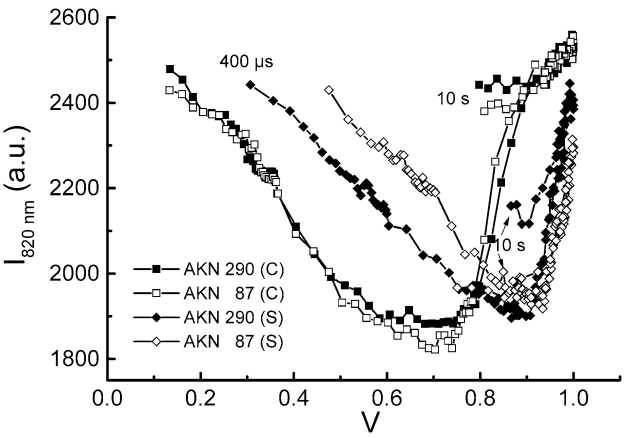

Fig. 6 Phase diagram of I_{820nm} versus V of control and severely salt-stressed chickpea leaves of AKN 87 and AKN 290, respectively. For the diagram, Chl *a* fluorescence and 820-nm transmission recorded between 400 µs and 10 s of illumination following a dark-to-light transition of the first pulse in Fig. 5 were used

Discussion

Salt stress effects on photosynthesis

Chickpea is considered a relatively salt stress-sensitive plant (Khan et al. 2015). Khan et al. (2015) studied recently 3 genotypes of chickpea differing in their salt stress tolerance under hydroponic conditions. They observed that the

accumulation of NaCl in the leaves did not differ significantly between genotypes; they concluded that salt stress tolerance is due to differences in tissue tolerance to NaCl. They further observed that the main salt stress effects are non-stomatal. Chickpea with its thin leaves may differ in this respect from other plants, because Munns (2002), reviewing salt stress effects in plants in a more general sense, concluded that the osmotic component of salt stress was responsible for a reduction in stomatal conductance, something also observed for tomato (Bacha et al. 2017). Khan et al. (2015) further observed a loss in leaf chlorophyll content, an observation also made by Seeman and Critchley (1985) in bean and Bacha et al. (2017) for tomato. In the present study, we used seedlings of two chickpea lines (AKN 290 and AKN 87) differing in their salt stress tolerance at the germination stage and subjected them to a 200 mM NaCl salt treatment for 15 days. With respect to the fluorescence measurements, we observed two clear salt stress-induced effects: a much higher and better defined J step and a smaller IP amplitude (Fig. 1). There is a set of biophysical parameters with which these changes can be captured as well (Fig. 3). The difference in the OJ rise kinetics is so large that an antenna size increase seems an unlikely explanation, especially for the severely salt-stressed leaves (Fig. 5), where a clear overall inhibition of the photosynthetic electron transport is observed. In our opinion, a more likely explanation has to do with the occupancy state of the Q_B site. If the salt stress would reduce the binding constant of PQ for the Q_B site, the PSII reaction centers would, on a short timescale, behave more like DCMU-treated centers (cf. the behavior of fluorescence transients at various times after a saturating pulse or during a gradual induction of anaerobic conditions, Schansker et al. 2005; Tóth et al. 2007). Our data do not really support the observation of several earlier studies that salt stress causes an inhibition of PSII activity (Mishra et al. 1991; Misra et al. 2001; Belkhodja et al. 1994; Soussi et al. 1998; Delfine et al. 1999; Oukarroum et al. 2015). In contrast, they are more in agreement with studies indicating that salt stress has no effect on PSII (Brugnoli and Björkman 1992; Morales et al. 1992; Lu et al. 2002). It has to be noted that this observation may be plant species and stress severity dependent, because the OJIP transients shown by Kalaji et al. (2016) for wheat do point to a possible damage/loss of PSII in response to salt stress. Our results further indicate that there are no significant differences in the maximum yield of primary photochemistry of PSII (φ_{Po}) at the end of 15 days of salt treatment. In other words, this parameter did not give information about the tolerance of the studied chickpea lines to salt stress. This may to some extent depend on the severity of the treatment or the plant species, since, e.g., Dabrowski et al. (2016) observe a specific effect on the JIP or thermal phase of the OJIP transients which would translate in lower φ_{Po} values. The effect of salt stress on the IP amplitude and the related 820-nm kinetics turned out to be more complicated. Chickpea leaves are thin and have a relatively short life cycle compared to, e.g., pea leaves that are much thicker and in which the plant has invested more. The 820-nm transmission changes shown in Fig. 2 indicate that leaf maturation and senescence occur in control leaves of AKN 290 at a faster rate than in AKN 87. For severely salt-stressed leaves (Fig. 5), it is shown that the decrease of the IP amplitude is not necessarily due to a reduced 820 nm transmission amplitude. It is also important to point out that at the minimum 820-nm signal observed in Fig. 2 not all PC and P700 molecules are in the oxidized state and in the case of the severely salt-stressed leaves the minimum value is closer to full oxidation because the hampered electron transfer between the PQ pool and P700 means that it takes longer before electrons from PSII start to reach P700 (see a discussion of this point in Ceppi et al. 2012).

Which chickpea line is the most salt tolerant?

The fluorescence data discussed in the previous paragraph allow us to define a kind of signature for salt stress effects in chickpea plants. A major salt-induced change is an increase in V_J. Comparing Figs. 2 and 5, this increase is even more pronounced in severely stressed plants. Further, we observe a smaller IP amplitude and no or little effect on φ_{Po}. These three factors together seem to define the salt stress effect. On the basis of both V_J and F_{IP}, AKN 290 seems to be more affected by salt stress than AKN 87 (Figs. 1, 3). However, maybe we should also consider Fig. 2. The faster leaf development cycle observed for AKN 290 may in itself also present a strategy to cope with stress. If leaves, in which salt has been accumulated, have a shorter life cycle, loosing these leaves and replacing them by new leaves may reduce the salt load of the plant as a whole. Sacrificing individual leaves may help AKN 290 to cope with salt stress. At the germination and early seedling stage, AKN 290 was more salt tolerant than AKN 87 (Turan et al. 2005; Kalefetoğlu Macar et al. 2009), and this is no longer the case for the somewhat older plants of the experiments discussed here.

Leaf optical properties of chickpea and 820-nm transmission measurements

In Ceppi et al. (2012), some data derived from the experiments discussed here were shown. For this article, these data were re-analyzed and it was realized that with respect to the salt stress dataset an incorrect part of the measurement protocol (the offset instead of the total transmission signal) had been used for the division by I_{tot} (the total transmission signal). As shown in Fig. 4a, for the recalculated data of the salt-stressed pea leaves the linear correlation between ΔV_{IP} and ΔI_{max} was maintained following the division by I_{tot}. In

contrast, for the very thin chickpea leaves the correlation was lost following division by I_{tot} (compare panels 4b and c). This indicates that for thin samples, like chickpea leaves, but probably also thin layers of algae, it is better to work with the parameter ΔI_{max} and not with $\Delta I_{max}/I_{tot}$.

Severe salt stress conditions

The experiments on which we report here were repeated several times and the severity of the stress induced by 15 days of treatment differed between experiments. Figure 5 shows an example of a more severe case of salt stress. This state is characterized by a strong delay of the re-reduction kinetics (starting after ~ 60 ms of illumination, instead of the 20 ms observed in control plants) as observed for the 820-nm transmission kinetics (panels 2 and 3). Further, the re-reduction kinetics are considerably slower and a second re-oxidation phase sets in before full re-reduction is achieved. In other words, in these plants the reduction of the whole electron transport chain is so slow that FNR becomes activated before the electron transport chain is completely reduced. A similar slowdown and retardation of the re-reduction kinetics has been observed by Dabrowski et al. (2017) after 10–12 days of salt stress treatment of rye grass and by Kan et al. (2017) in maize. With respect to the Chl *a* fluorescence data, a slowdown of the IP rise and a considerably smaller IP amplitude are observed. The effect on the IP amplitude is not matched by a similar strong change in the measured 820 nm transmission amplitude. To get more insight into the properties of the induction of photosynthesis in these leaves, we performed a 6-pulse experiment in which photosynthesis—at least in control plants—was induced stepwise by 10-s pulses of strong red light spaced 30 s apart. In the control leaves, especially the control leaves of AKN 87, the subsequent pulses caused on each pulse a deeper dip between 500 ms and 5 s of illumination. In contrast, in the salt-stressed leaves, there is a small difference between the first and the second pulses, but as a whole there is little difference between the induction kinetics induced by the different light pulses. Salt stress seems to have an effect on the activation kinetics of FNR (much faster than in the control leaves) and possibly as well on the properties of the electron transfer components beyond FNR.

In Fig. 6, the fluorescence and 820-nm transmission data are plotted as a function of each other (a phase diagram) to get a better insight into the question of how the salt treatment affects the relationship between the two. Taking the initial, decreasing part of the relationship, salt stress shifts the transmission decrease to higher values of fluorescence, especially in the case of AKN 87, but the slope is not that much affected. This indicates that PSII and PSI still have similar charge separating and charge transferring capacities. Also the slopes of the increasing part of the relationship are, initially, quite similar, in all cases. In the case of the control leaves, the slope becomes less steep before reaching F_M. This can be understood if we consider the reduction kinetics of PC and P700 during induction. As long as there is still oxidized P700, PC_{red} will reduce it. As a consequence, during induction, first P700 becomes completely reduced and only then PC. The extinction coefficient of PC at 820 nm is considerably lower than that of P700 (Klughammer and Schreiber 1991). In other words, just before the F_M is reached, mainly PC with a smaller extinction coefficient becomes reduced and the slope of the 820-nm signal becomes less steep. This slope change is missing in the salt-treated leaves. This may in part be due to the fact that a full re-reduction is not achieved. However, if, as we will propose below, the migration of PC is hampered by the effects of the salt stress treatment, the sharp separation in time between the reduction of PC and P700 will disappear and also in that case the sudden change in the slope, as observed for the control leaves, is expected to disappear. A more significant difference between control and salt-stressed leaves is observed beyond F_M, where in control leaves the fluorescence signal decreases more than the 820-nm transmission signal, whereas in the salt-stressed leaves a quite strong decrease in the transmission signal is accompanied by only a rather small decrease in the fluorescence signal. This indicates that the activation of FNR on the acceptor side of PSI has faster kinetics, but the subsequent (partial) re-oxidation of P700 and PC is not communicated so effectively to PSII as in the case of the control leaves. Both the mismatch between IP and transmission amplitude observed in Fig. 5 and the reduced communication between PSII and PSI observed in Fig. 6 point to salt stress-induced problems with the electron flow between the PQ pool and P700. Cruz et al. (2001) observed for hyperosmotic stress in *Chlamydomonas reinhardtii* inhibition of electron transfer from PC_{red} to $P700^+$. The authors linked this to a reduction of the thylakoid lumen volume observed by electron microscopy. As a consequence, transfer of PC between cytochrome b6f and PSI is hindered. Our observations for severely salt-stressed leaves are perfectly explained by the interpretation of Cruz et al. (2001) for the green alga *C. reinhardtii*. This may also be related to the compressed grana lamellae observed by Jiang et al. (2017) in salt-stressed maize plants. Allakhverdiev et al. (2000, 2001) had observed a similar decrease in the electron transport rate, but had interpreted this as a NaCl-induced inactivation of PSI due to a dissociation of PC or cytochrome *c553* from the PSI complex. Yang et al. (2014) used an indirect method to show that salt stress reduced electron flow to PSI, showing that photoinhibition of PSI at 4 °C was less in salt-stressed cucumber leaves compared to unstressed leaves. As observed by Ceppi et al. (2012), stress-induced kinetic changes of electron transfer through the photosynthetic electron transport chain are expected to change the relationship between the IP and the maximum 820 nm transmission amplitude.

Conclusions

As noted by Kalaji et al. (2014), it is still difficult to identify specific abiotic stress conditions on the basis of Chl *a* fluorescence measurements. Our data suggest that three signature effects of salt stress are the combination of a higher, better defined J step, a smaller IP amplitude, and little effect on φ_{Po}. As a comparison, in drought-stressed barley plants also a smaller IP amplitude and little effect on φ_{Po} are observed but in these plants V_J was not affected (Oukarroum et al. 2009). We further suggest that a shorter leaf life cycle may help AKN 290 (compared to AKN 87) to cope with salt stress. A third observation is that for the thin leaves of chickpea a correction of the maximum I_{820nm} amplitude for the total transmission amplitude does not work. And finally, in severely salt-stressed chickpea leaves an inhibition of electron flow between the PQ pool and P700 is observed, a phenomenon observed before in *C. reinhardtii* (Cruz et al. 2001).

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