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Effects of salinity on photosynthesis in maize probed by prompt fluorescence, delayed fluorescence and P700 signals

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ABSTRACT

Salinity affects the photochemical activity of photosystems (PSs) in plants. However, it is not clear which components in the photosynthetic processes are sensitive to salt stress. In this study, simultaneous measurements of prompt chlorophyll a fluorescence (PF), delayed chlorophyll a fluorescence (DF) and modulated 820 nm reflection (MR) were employed to investigate the effect of salt stress on the entire photosynthetic electron chain in maize leaf tissues, including the PSII donor side, electron transfer between PSII and PSI, and the PSI acceptor side. For the PF transients, salt stress induced a pronounced K-band; a positive L-band; a significant reduction in PI_{ABS}, RC/CS_O, TR_O/ABS, and ET_O/TR_O; and a significant increase in ABS/RC and RE_O/ET_O. Analysis of the normalized MR kinetics showed that the re-reduction kinetics of $P700^+$ and PC^+ became slower and occurred at later times under salt treatment. For the DF signals, a decrease in the amplitude of the DF induction curve and a change in the shape of both the induction curve and the decay curve were observed under salt stress. These results suggest that salt stress decreased the number of active PSII reaction centers, impaired the connectivity between independent PSII units, destroyed the oxygen-evolving complex, and limited electron transport beyond the primary quinone acceptor Q_A^-. In contrast, the photochemical activity of PSI was largely unscathed. The results obtained from measuring three simultaneous signals were in good agreement.

1. Introduction

Salinity is one of the major abiotic stressors for agriculture in arid and semiarid regions. Soil salinity causes toxic concentrations of Na^+ to accumulate in plant leaves (Munns et al., 2002) and seriously affects crop growth at multiple stages, including germination, seedling growth, vegetative growth, flowering, and fruit setting (Javed et al., 2007). Photosynthesis is well established as a primary target of many forms of environmental challenge, including salt stress (Garcia-Sanchez et al., 2002; Liska et al., 2004; Stepien and Klobus, 2006). NaCl salinity is reported to impair the photochemical efficiency of the two photosystems (PSs), PSI and PSII, in the photosynthetic process (Gao et al., 2016; Misra et al., 1999, 2001; Qu et al., 2012).

Photochemical events in photosynthesis are initiated by the capture of incident photons by pigments in the antenna complexes of PSI and PSII. The absorbed energy is efficiently transferred to photochemical reaction centers (RCs), leading to a forward transfer of electrons along an electron transport chain from water to $NADP^+$. In this forward transfer, PSII strips electrons from water to sequentially reduce the primary quinone acceptor (Q_A), secondary quinone acceptor (Q_B), plastoquinone (PQ) pool, cytochrome b_6f complex ($cytb_6f$), and plastocyanin (PC), while PSI oxidizes the reduced PC to subsequently reduce the electron acceptors on its acceptor side. The accumulation of electrons in the electron transport chain between PSI and PSII as a result of the forward electron transfer can lead to backward electron transfer (Goltsev et al., 2009; Strasser et al., 2010). Changes at any sites in this electron transport chain can affect photosynthetic efficiency. Measurements of prompt chlorophyll a fluorescence (PF), delayed chlorophyll a fluorescence (DF) and modulated 820 nm reflection (MR) have been used to investigate changes in the three domains of the photosynthetic electron transport chain: the PSII electron donor side, electron transport between PSII and PSI, and the PSI electron acceptor side (Strasser et al., 2010; Gao et al., 2014; Oukarroum et al., 2013, 2016).

PF occurs before the utilization of the excitation energy in the primary photochemical reaction during forward electron transfer. When

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photosynthetic samples maintained in darkness are illuminated, PF intensity increases from a minimum fluorescence intensity (F_O) to a maximum fluorescence intensity (F_M or F_P) through two intermediate steps, known as J (F_J) and I (F_I). This PF induction curve, known as the OJIP transient, is determined by changes in the concentration of reduced Q_A (Q_A^-), which reflects a progressive reduction of the PQ pool located on the acceptor side of PSII (Strasser and Tsimilli-Michael, 2004). Increases in the fluorescence level from O to J, J to I, and I to P are due to the reduction of Q_A by PSII, the filling of the PQ pool, and the reduction of the PSI acceptor side, respectively. Stress-induced changes in the OJIP transient can be primarily correlated with damage to the photosynthetic electron transport chain (Chen et al., 2016; Oukarroum et al., 2013, 2016; Toth et al., 2007; Zhang et al., 2012). To further quantify the changes in the structure and function of the photosynthetic machinery, the OJIP transient data were analyzed using a procedure called the JIP-test, which was introduced by Strasser et al. (2000, 2004); Strasser et al., 2000. The JIP-test translates the shape changes of the OJIP transient into many numerical parameters (for review, see Strasser and Tsimilli-Michael, 2004; reviewed using completely different nomenclature by Stirbet and Govindjee, 2011). A summary of the formulae and an explanation of the technical data of the OJIP transient and some selected JIP-test parameters are provided in Supplemental Table S1. The JIP-test parameters can provide numerical information on changes in the redox states of PSII and/or the efficiencies of electron transfer within the photosynthetic electron transport chain.

DF occurs after electrons transfer backward in the photosynthetic chain. Backward transfer can lead to charge recombination in the PSII RCs, repopulation of the excited chlorophyll state of the PSII antenna by fast energy transfer, and finally the emission of DF (Arthur and Strehler, 1957; Goltsev et al., 2009; Strasser et al., 2010). Recently, DF measurements have been used to investigate photosynthesis in response to abiotic stresses such as high temperature and drought (Strasser et al., 2010; Oukarroum et al., 2013, 2016). One frequently used experimental approach for DF measurements is to record DF signals using light and dark cycles (Goltsev et al., 2009). The DF signals measured in each dark interval decreases polyphasically with time, and this time-dependent change in DF intensity is termed the DF decay curve. Selected DF signals from several DF decay curves, which are measured in the same time period in the dark interval, are usually chosen to construct DF induction curve (Goltsev et al., 2009).

The DF decay curve is composed of several emission components caused by several PSII redox states (Goltsev et al., 2009). From microseconds to milliseconds, DF emission components are thought to reflect the recombination between the reduced Q_A and the oxidized primary donor of PSII, such as the redox state $ZP_{680}^+Q_A^-$. DF emission components in the seconds time range are associated with the recombination of the S_2 and S_3 states of the oxygen-evolving complex (OEC) with Q_A^- and Q_B^-, such as the PSII redox state $S_3Z^+Q_A^-Q_B =$ (Joliot et al., 1971). OEC is a water-oxidizing enzyme, which exists in four redox states: S_0 to S_4 (Kok et al., 1970). Only when four oxidizing equivalents have been stored (i.e., the S_4-state) does the OEC oxidize water to release O_2 and returns to its basic S_0 state. The S_2 and S_3 states represent the storage of two and three oxidizing equivalents, respectively, by the OEC. The DF induction curve can have several maxima that are denoted by I and numbered in sequence according to their position in the DF induction curve (Goltsev et al., 2003, 2009; Goltsev and Yordanov, 1997; Van Gorkom and Donze, 1973). The amplitudes of I_1 and I_2 maxima are closely associated with the activities of PSII and PSI, respectively (Gao et al., 2014; Goltsev et al., 2009; Kalaji et al., 2012; Strasser et al., 2010).

Monitoring the modulated reflection mode at 820 nm (MR) is an efficient way to investigate the redox state of PSI under continuous light (Schansker et al., 2003; Strasser et al., 2010). The oxidation of PSI and PC can cause an increase in absorbance at 820 nm, while the reduction of PSI and PC can decrease the absorbance (Schansker et al., 2003). Recently, a technique was developed and employed for studying the energetic behavior within the photosynthetic electron transport chain based on simultaneous measurements of the kinetics of PF, DF and MR (Gao et al., 2014; Oukarroum et al., 2013, 2016; Strasser et al., 2010; Salvatori et al., 2014). This technique can provide parallel and complementary information on the structure/function of the photosynthetic machinery (Strasser et al., 2010).

Maize (*Zea mays* L.) is one of the most important food crops in the world and is considered salt susceptible (Cha-Um and Kirdmanee, 2009), particularly at the seedling stage. Previous studies have used CO_2 assimilation, the activities of enzymes involved in CO_2 assimilation, and/or chlorophyll parameters measured using pulse-amplitude-modulated fluorescence monitoring systems to investigate the effect of salt stress on photosynthesis in this species (Abbasi et al., 2015; Gao et al., 2016; Hussain et al., 2014; Kausar et al., 2014; Kaya et al., 2015; Omoto et al., 2012; Qu et al., 2012; Sharwood et al., 2014; Xie et al., 2015). However, few of these studies revealed detailed information on the damage to the photosynthetic electron transport chain during salt stress. We hypothesize that different sites in the photosynthetic electron transport chain have different sensitivities to salt stress. In the present study, PF, DF and MR signals collected simultaneously with one instrument were investigated in a maize inbred line that was identified as sensitive to salt stress. Our aim was to investigate the target site of salt stress on the photosynthetic electron transport chain and discover which components are more sensitive to salt stress in maize plants.

2. Materials and methods

2.1. Plant material, growth and treatment

Maize inbred line Zm224, which was identified as sensitive to salt stress, was used in the present study. In our previous study, 364 maize inbred lines were used for salt-tolerance screening in a greenhouse experiment (Chen, 2016). The 364 lines were grown individually in pots in a completely randomized design with six replicates (one pot per replicate). When the seedlings reached the three-leaf stage, 100 ml of 200 mM NaCl was added to each pot, and this saline solution was applied every day for the remainder of the experiment. The trait of plant survival days (PSD) of each plant was individually rated after salt treatment. The criterion of dead plant was no living tissue maintaining. Of the 364 lines, Zm224 had the shortest PSD.

The line Zm224 was grown in 40 18-cm diameter plastic pots filled to a depth of 13 cm with soil. Six seeds were sown per pot. Seedlings were grown in a greenhouse at the experimental farm of the Agricultural College of Yangzhou University, Yangzhou, Jiangsu Province, China. The greenhouse was maintained with a 16 h/8 h (day/night) photoperiod. The light radiation level was 450 μmol m^{-2} s^{-1}, the temperature was 26/30 °C (day/night), and the relative humidity was 70%. At 7 d after emergence, plants were thinned to three similar-sized plants per pot. When the seedlings reached the three-leaf stage, the pots were divided into two equal groups, a salt-treated group and a control group. Each group contained 20 pots of maize plants. For the salt-treated group, 100 ml of 200 mM NaCl was added to the pots, and this saline solution was applied every day for the remainder of the experiment. For the control group, the pots received 100 ml of water every day. The treatments lasted for 16 d.

2.2. Determination of Na^+

Na^+ concentrations were measured as described previously (Stepien and Johnson, 2009), with minor modifications. At 0 and 16 d of treatment, the mature upper second leaves of nine plants from three pots in both the salt-treated and control groups were harvested and washed with deionized water. The three leaves collected from the same pot were mixed into one leaf sample. Leaf samples were dried at 65 °C for 3 d, and the dried leaves were then milled to powder for mineral nutrient analyses. Powdered samples (0.5 g) were extracted using 10 ml