Abscisic acid confers salinity tolerance in Chlamydomonas reinhardtii at different stages of its life cycle

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Abstract

The effect of abscisic acid (ABA) to increase the osmotolerance of the freshwater microalga Chlamydomonas reinhardtii at its different stages of life cycle was investigated. Exogenously added ABA enhanced the growth and photosynthesis of C. reinhardtii during the vegetative stage. The hormone also increased the tolerance of this alga to oxidative stress during gamete formation under nutrient depletion and high salinity, as it supported their survival for a longer period. We show that the level of reactive oxygen species (ROS) generated in the ABA-treated cells was significantly less than that in the untreated cells under inhibiting NaCl concentrations. Cell size examination showed that ABA prevents cells from forming palmella when exposed to high salinity. All together, these results suggest that ABA increases the tolerance of C. reinhardtii to salt stress conditions.

KEYWORDS

Abscisic acid, *Chlamydomonas* , Growth, Osmotolerance, Photosynthesis, ROS, Salinity

INTRODUCTION

Green algae are photoautotrophic eukaryotes, which are the ancestors to higher plants (Ligrone, 2019) and, therefore, they are identical to each other in their basic metabolism, including photosynthesis and photoprotection (Delaux et al., 2015). *Chlamydomonas reinhardtii*is a freshwater unicellular algal model species widely used for studying and revealing many biological aspects of microalgae relevant to higher plants such as evolution (Bell, 2005), biochemistry and physiology (Couso et al., 2018; Girolomoni et al., 2019; Juvale et al., 2016), mutant generation (Findinier *et al.* , 2017; Li et al., 2016;) and algae-based biofuel production (Scranton et al., 2015).*Chlamydomonas* cells are haploid, and under favorable growth conditions reproduce asexually during the dark phase of the light:dark cycle (Bruce & Bruce, 1981). However, environmental stresses, especially nitrogen limitation, promote sexual reproduction in*Chlamydomonas* , developing plus and minus gametes that mate and fuse to form a zygote, which undergoes meiosis to eventually release four new individual cells (Goodenough et al., 2007). It is well established that breeding, or outcrossing lines to generate hybrids in plants and animals can lead to segregants with new traits, a phenomenon known as heterosis (Shull, 1948), therefore sexual recombination in*Chlamydomonas* is a useful approach to optimize new complex traits.

Salinization is the accumulation of salts, mainly sodium and chloride salts, in the soil, which is considered one of the main environmental limiting factors to global crop productivity (Munns & Gilliham, 2015). Salinization also widely exists in aquatic environments that causes inhibition or induces cell death to many photosynthetic non-halophytic organisms including freshwater microalgae. High salt stress arrests cell division, where growth rate is immediately impacted, halts motility, reduces size, decreases photosynthesis in *Chlamydomonas* (Chen et al., 2016; Khona *et al.* , 2016).

Exposure to NaCl concentrations between 100 and 150 mM (equivalent to saline soils) and provokes formation

aggregates of smaller cells known as "palmelloids". Moreover, under sever salt stresses the levels of ROS increase in the chloroplasts as byproducts of photosynthesis (Asada et al., 1999; Rizhsky et al., 2003) resulting in oxidative damage to DNA, proteins and membranes, which eventually lead to cell death (Takahashi and Murata, 2008). To reduce the toxicity of ROS to improve cell stress tolerance, photosynthetic organisms have evolved several anti-oxidant and free-radical scavenging systems (Miller et al., 2010; Quesada and Fernández, 1994).

The plant hormone abscisic acid (ABA) plays significant roles in many biological pathways in the development and growth of plants including seed germination and bud dormancy (Han et al., 2018; Santner et al., 2009). ABA is also known in its vital involvement in plant tolerance to both biotic and abiotic stresses such as heavy metals, extreme temperature variations and high salinity (Suzuki et al., 2012; Vishwakarma et al., 2017), and in controlling stomata aperture to prevent water loss during drought (Mittler & Blumwald, 2015). Traditionally, plant hormones and synthetic metabolism regulators are used in research to elucidate phenotypical plant responses to external stimuli and their control mechanisms. It was reported that exogenous abscisic acid (ABA) modulates the abundance of mitochondrial reactive oxygen species (ROS) in plants via inhibition of cytochrome c oxidase (COX) or non-mitochondrial ROS signaling through inhibition of NADPH oxidases (Piantadosi, 2008), thus reducing ROS accumulation toxicity.

Although the effect of ABA in enhancing the growth of*Chlamydomonas* has been demonstrated (Chokshi et al., 2017; Yoshida et al., 2003 & 2004), the functional role of it in increasing microalgal osmotolerance and photosynthesis under salt stress remains unclear. Therefore, the objective of this study is to achieve a better understanding of how exogenous ABA regulates algal photosynthesis, tolerance and acclimation to NaCl stress, and to compare bioaccumulation of ROS in ABA-treated and ABA-untreated slat stressed cells. Moreover, this work examines the effects of ABA on gametes formation and surviving under inhibiting NaCl concentrations.

MATERIALS AND METHODS

Strain, Culture Conditions, and Growth Monitoring

Chlamydomonas reinhardtii strain CC-124 (mt- [137C]) was generously obtained from laboratory of Dr. Iftach Yacoby, Tel Aviv University, which was originally purchased from Chlamydomonas Resource Center, University of Minnesota. The seed cultures were grown at 23 °C on liquid Tris-acetate-phosphate (TAP) medium (Harris, 1989) in flasks capped with a silicone sponge, and were shaken at 100 rpm under continuous illumination of 150 μmol photons $m^{-2}s^{-1}$ at 16:8 h of light:dark period, and ambient CO_2 concentrations. Cell growth was determined by optical density measurements at 750 nm (OD_{750}) using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE). Cell count was performed with a BD FACSCalibur flow cytometer (Breda, Netherlands). Cultures were kept in the log phase with OD_{750} between 0.1 – 0.3 which is corresponding to a cell number of $3 - 9 \times 10^5$cells*mL^{-1} by dilution every 48 h to minimize self-shading.

Determination of salt stress

Exponential cultures of *C. reinhardtii* were inoculated onto TAP medium with elevated amounts of NaCl to final concentrations of 0, 100, 125, 175, 200, 250 and 300 mM, with initial OD_{750} of 0.1. The cultures were incubated at optimal conditions, in triplicates, and growth response was measured after 72 h of treatment.

Effect of ABA on *C. reinhardtii* in dense cultures

C. reinhardtii cells were harvested by centrifugation at 1500 g for 10 min and then inoculated onto 50 ml of fresh Tris-minimal medium (Gorman and Levine, 1965) to an OD_{750} of 1. For ABA experiments, stock of ABA solution was prepared by dissolving ABA powder (Duchefa Biochemie) in HPLC-grade ethanol to a final concentration of 100 mM. The stock solution was very concentrated to minimize the added volume to the algal cultures. To investigate the effect of ABA on the growth of *C. reinhardtii* under salt stress, two different concentrations of ABA were checked. Cultures were treated with 0 (control), 500 μM ethanol (mock), 50 and 500 μM ABA, and NaCl was added to the cultures to a final concentration of 125 mM

(this concentration was chosen based on the results of the previous section). The cultures were then further incubated for 48 h under continuous illumination of 100 µmol photons m^{-2} s^{-1} for 48 h without dark periods.

Cell viability

Cell number of exponential cultures was adjusted to ~ 2× 10^3 cells*mL^{-1} in fresh TAP medium containing 0 (control), 50 µM ethanol (mock) and 50 µM ABA then algal inoculates of 50 µl were spotted on TAP-1.5% Difco agar plates with and without 125 mM of NaCl (6 plates each treatment). The plates were then incubated for 6-8 days till no more colonies were developed. The survival rate of the cells incubated with NaCl was indicated as a percentage to the control colony forming units (CFUs) in which the cells were incubated without NaCl.

Monitoring of photosynthetic activity To enable measuring net photosynthetic O_2 production rates, a mini photobioreactor was equipped with a multi-sensor port, AlgaeGrowth-PAM, designed in the laboratory of Prof. Zvy Dubinsky at Bar Ilan University, and manufactured by Heinz Walz (Effeltrich, Germany) (Supplementary Fig. S1), controlled by custom WinControl-3 software (Fig. S4). Cultures were collected after 24 h of different treatments with continuous optimal illumination, then were loaded in the insolated metabolic chamber, dark-adapted for 10 min at 23 °C until the maximum quantum yield (Fv/Fm) was reached, then the cultures were illuminated with a stepwise increasing actinic light of 0, 110, 150, 230, 330, 480 and 1000 µmol photons m^{-2} s^{-1} for 2 min each step for a total period of 12 min. Oxygen production was monitored by an ultra-sensitive optical oxygen sensor (Pyroscience, Ltd) that uses a fluorescence signal (synthetic dye that changes fluorescence intensity with O_2 concentration) under vigorous stirring. Delta O_2 was calculated and exponential fitting curves were performed to extract Pmax values.

ROS generation experiment

Cells were harvested by centrifugation at 1500 g for 10 min the washed 3 times with sterile double distilled water before suspended in TAP medium. Cultures were then treated with 125 mM of NaCl and with or without ABA and were illuminated and shaken-incubated for 3 h, as described above in materials and methods. One-milliner samples were collected and stained with 20 µM of H_2DCFDA for 30 m (Colle'n, and Davison, 1997). The fluorescence of the dye was measured by a Fortessa flow cytometer (Breda, Netherlands). Cell vitality was carried out by inoculating of 50 µl drops of *Chlamydomonas*cultures after 24 h of treatment on TAP-1.5% Difco agar plates.

Microscope imaging

H_2DCFDA stained cells were visualized by a fluorescence microscope Leica SP8 confocal microscope equipped with Olympus DP10 digital camera, fitted with a 100 W High Pressure Mercury Burner with a lateral resolution down to 140 nm. Using CUDA based processing, differential interference contrast (DIC), chloroplast autofluorescence (excitation at 633 nm and emission at 670 nm) and DCF-DA epifluorescence (excitation: 488 nm, emission: 525 nm) images were obtained at 20× magnification.

Cell diameter and palmella formation

Cell diameter was determined by means of fluorescence activated cell sorter (FACS, BD Fortessa, USA) using side scatter pulse width (SSC-W) data from cells against size calibration bead standards of polystyrene (Spherotech, USA). Briefly, six different bead standards ranging between 3 µm and 16 µm were used to plot a calibration standard curve of amplifier gains where the pulse width was independent of peak height and peak area. The curve was then fitted with a linear curve according to Sharpless and Melamed (1976), and was used to transform the median SSC-W data from a microalgal cell population of 1 × 10^6 cells*mL^{-1} into a median cell diameter (Supplementary Fig. 2).

Effect of ABA on gametogenesis and gamete survival under salt stress, long term experiment

Cells were diluted to ~ 1 × 10^5 cells*mL^{-1} and were grown vegetatively to a concentration of ~ 1 × 10^6 cells*mL^{-1}, then were centrifuged at 1500 g for 10 min and washed twice with N-deficient (-N) TAP medium before resuspension in 10% N medium. Cultures were then treated with ABA or ethanol

before adding 125 mM NaCl as described above and were shaken-incubated at 200 rpm under continuous illumination of 150 µmol photons $m^{-2}s^{-1}$ without dark periods, and monitoring the optical density every 24 h for 72 h.

Statistical analysis

The experimental design included measurement of 6 cultures per treatment for the vegetative cells experiments (except photosynthesis measurements 2 cultures per treatment) and 3 cultures per treatment for the gametes' experiments. The effects of treatments and their interactions were analyzed using Student's t -test. Values represent means ± SD.

RESULTS

ABA enhances the growth and photosynthesis of *C. Reinhardtii* under salt stress

To investigate the sensitivity of *Chlamydomonas* to NaCl, cells were grown in TAP medium supplemented with increasing concentrations of NaCl from 100-300 mM. After 72 h of incubation the growth was reduced in which ˜ 60% of the cells survived at 125 mM NaCl, compared to the unstressed control (Supplementary Fig. S3). The negative effect of the salt on cell growth was more pronounced at higher concentrations such as 250 mM NaCl. However, *Chlamydomonas* is not fully arrested by NaCl at higher concentration, after longer incubation period we still detected slow growth of the cells (Supplementary Fig. S3). Therefore, the concentration of 125 mM NaCl was considered for salt stress treatments in this study.

Dimethylsulfoxide (DMSO) is widely used as a carrier solvent for small molecules in biological assays, with low toxicity at concentrations <10% (Szmant, 1975). In previous research DMSO was used in ABA experiments on *C. reinhardtii* (Yoshida *et al.* , 2003; Yoshida *et al.* , 2004), however its interfering influence on the grown was not evaluated. In this study, DMSO was used as a carrier solvent for ABA in low concentration of 50 µM. After 3 d of cultivation the growth increased 40% compared to control (Supplementary Fig. S4). Therefore, and instead, we decided to use methanol as a solvent for ABA for the experiments. To test whether ethanol has a positive impact on growth or not, we ran a mock treatment that contains only ethanol of a final concentration of 50 µM and *C. reinhardtii* cells, along with the other treatments.

We next set out to confirm whether ABA enhances the growth of *C. reinhardtii* under optimal and salt stress conditions or not. Fig. 1 shows that the addition of 50 µM and 500 µM ABA to dense cultures of*C. reinhardtii* increases about 10% of the growth without a lag phase compare to the untreated control, while adding ethanol as a mock treatment reduced the growth 20% after 48 h of incubation under favorable conditions (Fig. 1A). However, when ABA was added to cultures grown on TAP medium containing 125 mM NaCl, the growth increased 30%, with culture optical density 1.1 ± 0.1 and 1.3 ± 0.1, respectively, compared to untreated stressed cultures (0.8 ± 0.1), after 48 h (Fig. 1B). Since the alleviation of the negative impact of salinity was comparable between 50 µM and 500 µM ABA treatments, we used the lower concentration (50 µM) in the next experiments.

For a deeper investigation, it is critical to determine if the salt stress caused cellular death rather than cell inhibition, to understand the mechanisms involved. Using unstressed cultures as a reference, ˜ 60% of *C. reinhardtii* cells treated with salt survived and formed colonies. In comparison, the percent of cell vitality increased up to ˜90% when ABA was externally added (Fig. 1C). These results were in coherence with the optical density values.

The photosynthetic activities of the cultures were monitored by measuring O_2 production at different actinic light intensities in the P v E curve. *C. reinhardtii* cells grown under 125 mM NaCl displayed a lower light-saturated rate of photosynthesis ($P^E{}_{max}$) of 4.8 µmol· mmol Chl^{-1} s^{-1}compared to the unstressed control of 9.2 µmol · mmol Chl^{-1} s^{-1} (Fig. 3), indicating that a salinity-induced decline in the rate of photosynthesis is directly responsible for the decrease in cell growth. Surprisingly, when the cultures were incubated with 50 µM ABA the $P^E{}_{max}$ value increased to 20.1 µmol ·mmol Chl^{-1} s^{-1} and 12.3 µmol· mmol $Chl^{-1}s^{-1}$, with and without NaCl, respectively, which is ˜ 2-3 fold higher than the ABA-untreated controls. This result suggests that ABA does not only protect photosynthesis, but also enhance its rate.

ABA enhances salinity tolerance in *Chlamydomonas* by reducing ROS formation in cells

We used the fluorescent probe H_2DCFDA to estimate the cellular ROS-sensitive fluorescence in *Chlamydomonas* using confocal microscopy, where the green color indicates the presence of ROS and the red color indicates autofluorescence of chlorophyll (Fig. 4). ROS are natural by-products of cell metabolic processes (Mallick and Mohn, 2000), therefore non-stressed cells were also stained with H_2DCFDA to determine background levels of ROS for quantifying salt-stressed ROS induction compared to the non-stressed control. A very weak ROS green fluorescent signal was observed under control growth conditions. After salt treatment, strong ROS fluorescent signals were detected in the chloroplast and the mock sample, whereas slight ROS signal was observed in the ABA-treated cells (Fig. 4A). These results indicate that ABA decreased the ROS level in cells.

Intracellular ROS accumulation was measured in control and ABA-treated cells following exposure to 125 mM NaCl by using flow cytometry. All treatments with 125 mM NaCl significantly increased intracellular ROS after 3 h in comparison to controls (Fig. 4B). In ABA-treated cells there was a comparative significant reduction in ROS accumulation. Cells treated with 50 µM ABA induced ROS to ˜ 55% relative to stressed ABA-untreated cells.

ABA treated *Chlamydomonas* forms less palmelloid cells under NaCl stress

The formation of palmelloids in *Chlamydomonas* was studied using flow cytometry pulse width data as previously described by Chioccioli*et al.* , 2014, based on cell size. We found that after 12 h of stress with 125 mM NaCl, palmelloids constituted 30% of the total cell population which is ˜5-fold of the unstressed control (Fig. 5 & Supplementary Fig. 5). The remaining was freely motile cells. When assayed for extended time periods (24 h), the population distribution attained a plateau. However, the formation of palmelloids in ABA-treated cells under NaCl treatment did not significantly change from the unstressed control, which indicates that ABA prevents the cells from undergoing changes when exposed to NaCl stress.

ABA enhances gametogenesis and increases game tolerance to salinity

Once N depletion was initiated, all cultures continued to grow for 24, After that the growth, based on OD values, of N-depleted control and mock cells slowed till the end of the experiment, suggesting complete cessation of net cell division (Fig. 6). However, the rate of gamete formation in the ABA-treated *C. reinhardtii* cultures increased more than 2-fold (OD_{750} = 0.86) of that in the control cultures (OD_{750} = 0.39) after 48 h of induction under normal conditions, before they declined after 72 h.

In comparison, the rate of gamete formation was lower in all cultures under salt stress, with 24 h extended survival time in ABA-treated cells (Fig. 7).

Discussion

We examined the physiological phenotyping and chemical responses of*C. reinhardtii* treated with ABA and exposed to salt stresses in order to investigate whether ABA enhances salt tolerance in vegetative cells and gametes of this alga in a similar manner to that in higher plants or not.

The first and immediate indicator for increasing tolerance is the growth rate. *Chlamydomonas reinhardtii* cells are sensitive to high salt stress and therefore have lower growth rate under such conditions compared to unstressed cells (Hema et al., 2007; Mastrobuoni et al., 2012; Meijer et al., 2017). Yoshida et al. (2003) reported that exogenously added ABA enhances the growth of *C. reinhardtii* , however it was not clear whether this effect was due to the ABA or the interference of its carrier solvent. In this study, by using ethanol as a mock treatment we confirmed that ABA enhances the growth of microalgae and increases their tolerance to high-salinity stress, where ethanol showed no, or even slightly negative effect on algal growth (Figs. 1 & 2), unlike in plants where externally added ethanol was proven to increase salt stress tolerance (Nguyen et al., 2017).

It is known that salinity does not exert hostile effects on the stoichiometry and chlorophyll antenna size of the photosynthetic apparatus. However, salinity-stress increases the susceptibility of cells to photoinhibition

in *Chlamydomonas* (Neale & Melis, 1989). When *C. reinhardtii* was incubated with 125 mM NaCl for 24 h, as the alga photosynthetic activity recovers up by ˜30% of the original activity after 1 h of high salt exposure then it remains during the following 24 h (Vega et al., 2006), a decline in the light-saturated rate of photosynthesis (P^{E}_{max}) was observed (Fig. 3), indicating for PSII photoinhibition. However, ABA-treated cells showed up to ˜2 to 3-fold increase in the photosynthetic activity under salt stress compared to non-treated cells. These results suggest that ABA can participate in protecting PSII against photoinhibition in*C. reinhardtii* under high salinity, likely through protecting the cells from Na+ toxicity or less Na+ uptake (Gurmani *et al.* , 2007), as it has been evidenced that Na+ can irreversibly inactivate photosynthesis systems indirectly by promoting a secondary oxidative disruption, or directly by damaging the photosynthetic 32/34 kDa (D1/D2) proteins as in higher plants (Murata et al., 2007; Yang et al., 2014).

As almost all environmental stresses lead an increase in the production of ROS and thereby to oxidative stress in photosynthetic organisms (Mittler, 2002), the imposition of freshwater algae to salinity stress quickly leads to a rise in ROS in their cells (Mallick & Mohn, 2000). Indeed, based on ROS-sensitive fluorescence, ROS appeared to accumulate in the stressed ABA-untreated cells and in the stressed mock treatment, and ROS-sensitive fluorescence was especially prominent in the chloroplast (Figure 4A), where the chloroplasts look degraded. In contrast, ABA-treated cells showed weak ROS-sensitive fluorescence with no sign of chloroplasts degradation. The histograms in Fig. 4B clearly supports the microscope images that salinity stress significantly enhanced the intracellular ROS generation in the untreated cells. However, in ABA-treated cells ROS generation was significantly suppressed. These results suggest that ABA induces the elimination of high salinity ROS-reactions in *Chlamydomonas* cells.

Under different stress conditions such as phosphate limitation (Olsen et al., 1983), acidic environment (Visviki et al., 2000), the herbicide paraquat (Jamers et al., 2010) and high salinity (Khona *et al.* , 2016) *Chlamydomonas* cells have alternative ways to avoid chronic stress by forming stress-resistant life cycle stages undergo abnormal cell division with reduced individual cell size forming "palmelloids". In the current study, NaCl induced the formation of palmelloids in the stressed untreated-cells unlike in ABA-treated cells where the number of palmelloids was significantly reduced (Fig. 5). These results are evidence that ABA playing a crucial role in protecting*Chlamydomonas* cells from high salinity stress.

In this study, ABA supported the growth of gamete population, as it enhanced their tolerance to high salinity (Figs. 6 & 7). In*Chlamydomonas,* gametogenesis is triggered by N limitation. When N is depleted in the growth medium, the vegetative cells have programs (Beck & Haring, 1996; Goodenough, 1991). The first program is that the cells acclimate to N deprivation through metabolic changes, including synthesis of N-scavenging enzymes (Quesada & Fernández, 1994) and the renewal of ribosomes (Martin et al., 1976). The second program is that the vegetative cells underdo gametogenesis to produce Mt+ and Mt- cells for mating. While N starvation has been extensively studied in *Chlamydomonas* for producing alternative sources of energy by accumulation more TAG in the cells under N starvation (Montantes et al., 2018; Salas- Siaut et al., 2011; Yang et al., 2020), or as a new tool to create offspring with new traits (Kramer and Lucker, 2020), however, N-starved *Chlamydomonas* cells can also exhibit various biological pathways for managing photosynthesis to efficiently utilize the absorbed light energy (Saroussi et al., 2017). While underlying these pathways is still in its early stage, it could provide an important direction for developing a more comprehensive understanding of photosynthetic energetics and its control.

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Figure legends

Figure 1. The effects of ABA on growth of vegetative *C. reinhardtii* cultures under different salinity conditions. A). Time-course kinetics of optical density of cells grown under optimal conditions, and B). under salt stress of 125 mM NaCl for 48 h. C). Percent of colony forming units (CFU) of cultures grown under salt-stress conditions with and without 50 μM ABA and then inoculated on complete TAP agar plates for a 6-day recovery period. % is related to unstress conditions. Values are the mean of 6 biological replicates ± standard errors. Asterisks indicate significant difference compared to ABA-untreated cells and mock cells ($*P < 0.05$, $**P < 0.01$).

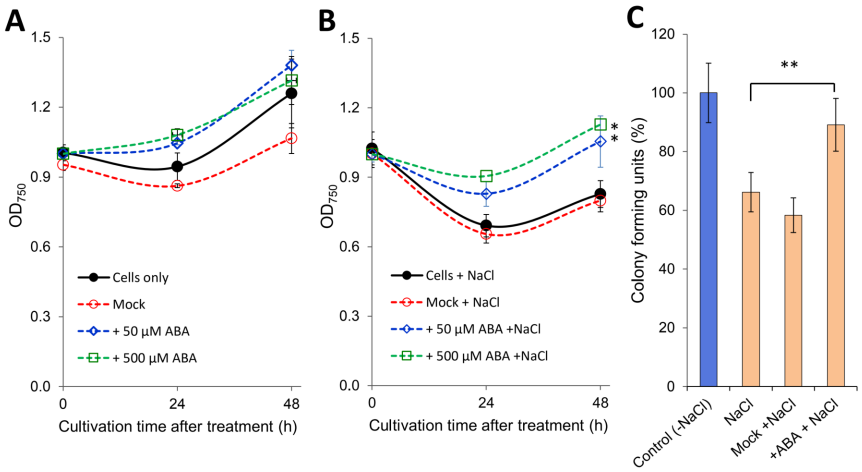
Figure 2. Changes in the light-saturated rate of photosynthesis (P^{E}_{max}) of *C. reinhardtii*subjected to stressing NaCl concentrations for 24 h in the presence and absence of ABA. Means of 2 replicates ± standard errors ($***P < 0.001$).

Figure 3. The effect of ABA on ROS generation in *C. reinhardtii* during incubation in the light for 3 h under NaCl stress conditions, then adding H2DCFDA stain as a fluorescent probe for ROS. A). Microscope images, green color indicates the presence of ROS; red color indicates autofluorescence of chlorophyll. Size bars 10 μm. B). Intracellular ROS level was determined by a flow cytometric analysis. Histograms show distribution of the fluorescent intensity of DCF among cell population of 1×10^3 cells*mL^{-1}. Values are the mean of 6 biological replicates ± standard errors. Asterisks indicate significant difference compared to ABA-untreated cells and mock cells by paired-sample Student's t test ($***P < 0.001$).

Figure 4. Palmella formation indicated by cell diameter per population. Fluorescence activated cell sorting (FACS) of *C. reinhardtii* cells was used to sort a mixture of cells and beads, according to SSC-W range. Cells and marker beads were photographed after sorting in the range SSC-W = 1×10^6 cells*mL^{-1} (cells grown in TAP).

Figure 5. The effects of ABA on kinetics of gamete formation and survival. A). Time-course kinetics of optical density of cells grown under optimal conditions, and B). under salt stress of 125 mM NaCl for 72 h ($*P < 0.05$, $***P < 0.001$).

Figure 6. Growth and survival of *Chlamydomonas* gametes after being subjected to salt stress conditions. Cells were serially diluted in water, and 50 μl-aliquots spotted on TAP-agar plates and incubated for 3 to 5 days, as indicated in A) and B), respectively, under continuous illumination.



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