Calcium amendment for improved germination, plant growth, and leaf photosynthetic electron transport in oat (Avena sativa) under NaCl stress

Xiaoshan Wang*, Qiyue Dingxuan, Mengmeng Shi

Department of Grassland Science, College of Animal Science and Technology, Yangzhou University, Yangzhou City, Jiangsu Province, The People's Republic of China

* xswang@yzu.edu.cn

Abstract

Calcium (Ca\(^{2+}\)) is an essential nutrient element for plants as it stabilizes the membrane system structure and controls enzyme activity. To investigate the effects of Ca\(^{2+}\) on plant growth and leaf photosynthetic electron transport in oat (Avena sativa) under NaCl stress, oat seeds and plants were cultivated in nutrient solutions with single NaCl treatment and NaCl treatment with CaCl\(_2\) amendment. By measuring the seed germination rate, plant growth, Na\(^{+}\) and Cl\(^{-}\) accumulation in leaves, ion leakage in seedlings and leaves, prompt chlorophyll a fluorescence (PF) transient (OJIP), delayed chlorophyll a fluorescence (DF), and modulated 820 nm reflection (MR) values of the leaves at different growth phases, we observed that Ca\(^{2+}\) alleviated the inhibition of germination and plant growth and decreased Na\(^{+}\) and Cl\(^{-}\) accumulation and ion leakage in the leaves under NaCl stress. NaCl stress changed the curves of the OJIP transient, induced PF intensity at P-step (F\(_{P}\)) decrease and PF intensity at J-step (F\(_{J}\)) increase, resulted in obvious K and L bands, and altered the performance index of absorption (PI\(_{ABS}\)), the absorption of antenna chlorophylls (ABS/RC), electron movement efficiency (ETo/TRo), and potential maximum photosynthetic capacity (F\(_{V}/F_{M}\)) values. With the time extension of NaCl stress, I\(_1\) and I\(_2\) in the DF curve showed a decreasing trend, the lowest values of MR/MR\(_{O}\) curve increased, and the highest points of the MR/MR\(_{O}\) curve decreased. Compared with NaCl treatment, the extent of change induced by NaCl in the values of OJIP, DF and MR was reduced in the NaCl treatment with CaCl\(_2\) amendment. These results revealed that Ca\(^{2+}\) might improve the photosynthetic efficiency and the growth of salt-stressed plants by maintaining the integrity of oxygen-evolving complexes and electron transporters on the side of the PSI receptor and enhancing the relationship between the functional units of the photosynthetic electron transport chain. The findings from this study could be used for improving crop productivity in saline alkali lands.
DF, delayed chlorophyll a fluorescence; ETO/TRO, probability that an electron moves further than QA-; F\textsubscript{i}, PF intensity at I-step; F\textsubscript{r}, PF intensity at J-step; F\textsubscript{o}, PF intensity at O-step; F\textsubscript{p}, PF intensity at P-step; F/V, the potential maximum photosynthetic capacity; M-PEA, Multi-functional Plant Efficiency Analyzer; MR, modulated 820nm reflection; OEC, oxygen evolving complex; OJIP, prompt chlorophyll a fluorescence transient; PC, plastocyanin; PF, prompt chlorophyll a fluorescence transient; PQ, plastocyanine; PSI, photosystem I; PSII, photosystem II; PSs, the photochemical efficiency of two photo systems; QA, the primary quinone electron acceptor of PSII; ROS, reactive oxygen species; QB, the secondary quinone electron acceptor of PSII; ROS, reactive oxygen species; VPSII-PSI, maximum slope decrease of MR/MRO; VPSII-PSI, maximum slope increase of MR/MRO; WOJ, the ratio of variable fluorescence F\textsubscript{v} - F\textsubscript{o} to the amplitude F\textsubscript{J} - F\textsubscript{o}; WOK, the ratio of variable fluorescence F\textsubscript{K} - F\textsubscript{o} to the amplitude F\textsubscript{K} - F\textsubscript{o}.

**Introduction**

Soil salinity can seriously affect crop growth at any stage of plant growth, and can ultimately reduce economic yield [1]. Sodium chloride (NaCl), one of the major salts in saline soils, usually causes Na\textsuperscript{+} and Cl\textsuperscript{-} toxicity and a low external osmotic potential, inducing the formation of reactive oxygen species (ROS), which could lead to oxidative damage in plants under salinity stress [2,3]. ROS, Na\textsuperscript{+} and Cl\textsuperscript{-}, can damage various cell components through oxidative stress and ion toxicity, and can interfere with the normal physiological processes of plant cells [2,3]. The photosynthetic activity of chloroplasts is usually inhibited by NaCl stress, which reduces the photochemical efficiency of photosystem I (PSI) and photosystem II (PSII) [4]. Thus, a better understanding of plant photosynthetic responses to NaCl stress is essential to improve the salt resistance of the plants in saline environments. In recent years, the development of a multi-functional plant efficiency analyzer (M-PEA) has allowed the rapid measurement of the forward and reverse electron transfer and redox states of reaction centers [5–8]. Some photosynthetic electron transfer signals, such as instantaneous fluorescence (PF), delayed fluorescence (DF), and 820 nm modulated reflection (MR), measured synchronously by M-PEA, can reflect the changes in the light energy absorption, transmission, and dissipation, revealing the changes in the photosynthetic processes in stressed plants [9–13].

As light energy is captured by the photochemical reaction center and is transferred through the electron transfer chain, the energy transfer is accompanied by energy dissipation in the form of heat and fluorescence. In the photoelectron transport chain, PF appears after light excitation and before the photochemical reaction uses the excitation energy. The prompt chlorophyll a fluorescence (PF) transient (OJIP) of the PF induction curve is determined by progressive changes in the electronic receptors Q\textsubscript{A}, Q\textsubscript{B}, and PQ of PSII [9]. DF is produced by electron reverse transfer in the photosynthetic chain. The DF decay curve can reflect multiple emission components in the redox state of PSII [11]. The maximum values of DF at 3 ms (I\textsubscript{1}) and DF at 100 ms (I\textsubscript{2}) are closely related to PSII and PSI after stress, respectively. The MR reflects the redox state of PSI under continuous illumination [5,9]. The absorbance value at 820 nm is directly proportional to the oxidized PSI and plastocyanin (PC) values [9].

Calcium (Ca\textsuperscript{2+}) is an essential nutrient element for plants. It stabilizes the membrane system structure and controls enzyme activity [14–16]. When the amount of Ca\textsuperscript{2+} required by plants under normal conditions was applied to salt-stressed plants, salt-stressed plants showed Ca\textsuperscript{2+} deficiency [17]. Adding an appropriate amount of exogenous Ca\textsuperscript{2+} could alleviate Ca\textsuperscript{2+} deficiency, increase the stability and function of the plasma membrane, and ensure the normal functioning of the Ca\textsuperscript{2+} signaling system under salt stress [17–19]. Ca\textsuperscript{2+} is also a necessary factor for photosynthetic oxygen release [16,20], and exogenous Ca\textsuperscript{2+} can significantly increase the chlorophyll content and net photosynthetic rate of some species under salt stress [17,18,21]. However, little information is available on the Ca\textsuperscript{2+}-induced changes in the reaction and mechanism of photosynthesis under salt stress.

Oat is a food and forage crop that has great tolerance and adaptability to saline environments, thus, oat has been considered an important crop for improving crop productivity in saline alkali lands. Many studies have been conducted on the gas exchange [22], ion absorption [22–24], and oxidative stress [25–27] of oat grown in saline soils. Under salt stress, the exogenous Ca\textsuperscript{2+} can reduce damage to plants by keeping the stability and functions of the plasma membrane in cells [17–19]. However, there have been few studies on oat salt stress and exogenous Ca\textsuperscript{2+} in photosynthesis, especially on the photosynthetic electron transport during photosynthesis. We hypothesized that salt stress could impair multiple sites in the photosynthetic electron transport chain of oat. Additionally, Ca\textsuperscript{2+} has been reported to play an important role in protecting the photosystems of the electron transport chain from damage caused by salt.
stress in oat [16–21]. In this study, we measured the seed germination, plant growth, and the signals of PF, DF, and MR in leaves of oat plants under NaCl stress and under NaCl stress with a CaCl$_2$ amendment. Our aim was to investigate the effect of NaCl stress on the forward electron flow, backward electron flow, and some electron carriers in the photosynthetic electron transport chain and to reveal the protective mechanism of Ca$^{2+}$ in the photosynthetic electron transport chain.

**Materials and methods**

**Oat seed treatment and germination test**

Oat seeds were disinfected with 6% NaClO solution for 6 min, washed with sterile water, and dried at room temperature (22–24˚C) for 2 h. For each treatment, 50 seeds were placed in each box (11 cm long, 11 cm wide, and 2.5 cm high) with a thin sponge and filter paper placed below to maintain moisture. There were four treatments in this test, including the control (distilled water), 10 mM CaCl$_2$, 150 mM NaCl, and 150 mM NaCl + 10 mM CaCl$_2$. Each treatment was replicated four times. The seeds were germinated in a light incubator with a photoperiod of 18 h/6 h (day/night) and a temperature regime of 30/25˚C (day/night). The water in each box was added every day to ensure consistency in the concentration. On day 7 after beginning the germination test, the germination rate was calculated ($\frac{\text{Number of germinated seeds}}{\text{number of tested seeds}} \times 100\%$) and the ion leakage of the seedlings was measured with an electric conductance meter (DDSJ-308A, Scientific Instruments Company, Shanghai, China).

**Controlled pot experiment**

Three disinfected oat seeds were planted in each pot (13 cm long, 13 cm wide, and 10 cm high) filled with 1 kg medium quartz sand (0.5 mm) and was watered with 300 mL standard nutrient solution every day. The standard nutrient solution was composed of KNO$_3$ (2.5 mM), Ca(NO$_3$)$_2$ (2.5 mM), (NH$_4$)$_2$HPO$_4$ (0.5 mM), MgSO$_4$ 7H$_2$O (1 mM), CuSO$_4$ 5H$_2$O (2×10$^{-4}$ mM), ZnSO$_4$ 7H$_2$O (1×10$^{-3}$ mM), EDTA.FeNa (0.1 mM), H$_3$BO$_3$ (2×10$^{-2}$ mM), (NH$_4$)$_6$Mo$_7$O$_{24}$$\cdot$4H$_2$O (5×10$^{-6}$ mM), and MnSO$_4$·H$_2$O (1×10$^{-3}$ mM). All the plants were placed in a greenhouse with a light (450 $\mu$mol m$^{-2}$ s$^{-1}$) for 14 h (7:00 am—21:00 pm) every day and with a temperature regime of 28/20˚C (light/dark). The nutrient solution was adjusted to pH = 7.0 using 0.01 mM KOH and/or H$_2$SO$_4$ before solution irrigation. When the plants had three leaves, the 10 mM CaCl$_2$, 300 mM NaCl, 300 mM NaCl + 10 mM CaCl$_2$, and control (standard nutrient solution with pH = 7) treatments were imposed, with four replications for each treatment.

**Measurements**

To determine the electrolytic leakage in the leaves and seedlings, 1.0 g of chopped leaves or seedlings was weighed and soaked in 25 mL deionized water for 24 h. Then, the electric conductivity of the leachates was measured using an electric conductivity meter (DDSJ-308A, Scientific Instruments Company, Shanghai, China).

The electrolytic leakage was calculated using the following formula [28]: electrolytic leakage (%) = \[\frac{\text{[electric conductivity of treatment} - \text{electric conductivity value of control}]\times 100}{\text{electric conductivity value of control}}\]

After 15 d of NaCl stress, the leaves of each treatment were dried at 105˚C for 0.5 h and at 65˚C for 48 h to a constant weight, then the leaves were crushed with a miniature crusher. Afterward, 50 mg of the dried sample from each treatment was placed into a 50 mL test tube filled with 20 mL of deionized water. The tubes were placed in boiling water for 1.5 h. After cooling, the extract was filtered into a 25 mL volumetric flask for the determination of Na$^+$ and
Cl− concentrations using a plasma emission spectrometer (ICP, PE3300DV, Perkin Elmer, Germany) [28].

On the day of NaCl imposition and days of 3, 6, 9, 12, and 15 hereinafter, the plants of each treatment were moved into a dark room. After 3 h in the dark, the 3rd fully expanded leaves of each treatment were used for the measurement of PF, DF, and MR by M-PEA (Hansatech, Norfolk, UK) [29].

The normalized curves in the PF transients were calculated using the following formulas [29]: \( V_t = \frac{(F_t - F_0)}{(F_P - F_0)} \) and \( V_t = \frac{(F_t - F_0)}{(F_P - F_0)} \).

The curves of the K-band in the PF transients were calculated using the following formulas [29]: \( W_{OJ} = \frac{(F_t - F_0)}{(F_J - F_0)} \) and \( \Delta W_{OJ} = W_{OJ}^{TR} - W_{OJ}^{td} \).

The curves of the L-band in the PF transients were calculated using the following formulas [29]: \( W_{OK} = \frac{(F_t - F_0)}{(F_K - F_0)} \) and \( \Delta W_{OK} = W_{OK}^{TR} - W_{OK}^{td} \).

The parameters, including the performance index of absorption (PI\(_{ABS}\)), absorption of antenna chlorophyll (ABS/RC), electron movement efficiency (ET\(_O\)/TR\(_O\)), and potential maximum photosynthetic capacity (F\(_V\)/F\(_M\)) were calculated from the OJIP transient [5].

The normalized curves from the DF values were calculated using the following formula [29]: \( (DF_t - D_0)/(DF_{I1} - D_0) \).

The experiment was a two factorial combination and three treatment: 10 mM CaCl\(_2\), 150 mM NaCl, and 150 mM NaCl + 10 mM CaCl\(_2\). All data were collated using Excel 2019 and were subjected to variance analysis using one-way ANOVA (SPSS v11.5). The means of each parameter were separated through Fisher’s least significant difference multiple comparison at the 0.05 probability level.

Results

The 150 mM NaCl stress significantly inhibited oat seed germination in both the NaCl treatment without CaCl\(_2\) amendment and the NaCl treatment with CaCl\(_2\) amendment (Fig 1A and Table 1). Nevertheless, the amendment of Ca\(^{2+}\) promoted germination and root extrusion under NaCl stress. Compared with the control, the germination rates decreased by 71% and 44% in the NaCl treatment without CaCl\(_2\) amendment and NaCl treatment with CaCl\(_2\) amendment, respectively, on day 7 after NaCl stress (Table 1). Under NaCl stress, the amendment of Ca\(^{2+}\) reduced ion leakage of the seedlings (Table 1), increased aboveground dry weight of the plant, and reduced ion leakage of the leaves (Fig 1B and Table 1).

On day 15 after NaCl stress, the leaf Na\(^+\) and Cl\(^-\) contents in the NaCl treatment were approximately 37-fold and 48-fold higher than those in the non-NaCl treatment, respectively, whereas the leaf Na\(^+\) and Cl\(^-\) contents in the NaCl treatment with CaCl\(_2\) amendment were approximately 28-fold and 41-fold higher than those in the non-NaCl treatment, respectively (Table 1). These indicated that Ca\(^{2+}\) significantly reduced the accumulation of Na\(^+\) and Cl\(^-\) in oat leaves under NaCl stress (Table 1).

In the control and CaCl\(_2\) treatments, leaf PF transients showed a similar shape to the typical polyphasic rise of a basic OJIP. In both NaCl treatments without CaCl\(_2\) amendment and NaCl treatment with CaCl\(_2\) amendment, the P value decreased gradually with increasing NaCl stress duration (Fig 2A and 2D). In the NaCl treatment without CaCl\(_2\) amendment, the P value decreased by 7.9%, 8.3%, 17.3%, 32.6%, and 42.0% on days 3, 6, 9, 12, and 15 of NaCl stress, respectively (Fig 2A). In the NaCl treatment with CaCl\(_2\) amendment, the P value decreased by 0.1%, 3.9%, 4.9%, 9.4%, and 18.6% on days 3, 6, 9, 12, and 15 of NaCl stress, respectively (Fig 2D).
For the results of transients normalized between O and P, the obvious increase in the J value of OJIP began on day 12 of the NaCl treatment, while in the NaCl treatment with CaCl$_2$, the obvious increase in the J value of OJIP began on day 15 (Fig 2B and 2E). In the J phase, the obvious peaks in the $\Delta V_t$ curves in the NaCl treatment appeared on day 12 after NaCl stress, while in the NaCl treatment with CaCl$_2$, obvious peaks in the $\Delta V_t$ curves appeared on day 15 (Fig 1).

Table 1. Germination rate, electrolytic leakages of oat seedlings in the control (water), CaCl$_2$ (10 mM), NaCl (150 mM), and NaCl (150 mM) + CaCl$_2$ (10 mM) treatments on day 7 after NaCl stress and aboveground dry weight, Na$^+$ and Cl$^-$ content, and electrolytic leakages of oat leaves in the control (nutrient solution), CaCl$_2$ (10 mM), NaCl (300 mM), and NaCl (300 mM) + CaCl$_2$ (10 mM) treatments on day 15 after NaCl stress.

| Treatment | Germination rates (%) | Electrolytic leakages in seedling (%) | Aboveground dry weight (g plant$^{-1}$) | Na$^+$ content (mg g$^{-1}$ DW) | Cl$^-$ content (mg g$^{-1}$ DW) | Electrolytic leakage in plants (%) |
|-----------|-----------------------|--------------------------------------|---------------------------------------|-------------------------------|-------------------------------|----------------------------------|
| Control   | 93±2a                 | 21.2±2.3c                            | 0.51±0.02a                            | 0.05±0.01c                    | 0.02±0.01c                    | 6.1±0.6c                          |
| CaCl$_2$  | 92±2a                 | 19.7±1.5c                            | 0.51±0.03a                            | 0.05±0.01c                    | 0.03±0.01c                    | 5.9±0.2c                          |
| NaCl      | 27±5c                 | 52.6±2.3a                            | 0.26±0.01c                            | 1.86±0.14a                    | 0.95±0.09a                    | 22.5±2.6a                         |
| NaCl + CaCl$_2$ | 52±6b | 40.3±2.0b                            | 0.35±0.03b                            | 1.40±0.16b                    | 0.81±0.02b                    | 18.1±0.2b                         |

Each value is the mean ± SE (n = 3). The data in the same column followed by different letters are statistically different at the 0.05 probability level.

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after NaCl stress (Fig 2B and 2E). For the I point, the obvious peaks in the NaCl treatment appeared on day 3 after NaCl stress, whereas in the NaCl treatment with CaCl$_2$ obvious peaks appeared on day 9 of NaCl stress (Fig 2C and 2F).

With the duration of NaCl stress, the K-band progressively increased in the NaCl treatment and in the NaCl treatment with CaCl$_2$ (Fig 3A and 3C). The obvious ascendant K-band in the NaCl treatment was found on day 6 of NaCl stress (Fig 3B). In the NaCl treatment with CaCl$_2$, an obvious ascendant K-band was found on day 12 of NaCl stress (Fig 3D). As early as day 3 of NaCl stress, an obvious ascendant L-band appeared in the NaCl treatment (Fig 3F), while in the NaCl treatment with CaCl$_2$ amendment, an obvious ascendant L-band appeared on day 12 of NaCl stress (Fig 3H).

For the plants, a DF induction curve was constructed for the signals measured at 20ms-delay per dark interval, which increased between the initial D$_0$ and I$_1$ in 3ms, then decreased to the platform D$_2$, and passed through the I$_2$ in 100ms. In both the NaCl treatment and the

![Fig 2. The curves of PF transients of oat leaves in the NaCl treatment and the NaCl treatment with CaCl$_2$ amendment on the day of NaCl stress and on days 3, 6, 9, 12, and 15 of NaCl stress. (A, B, C) PF intensity (rel.u.), normalized transients and ΔV$_{1}$ curves in single NaCl treatment. (D, E, F) PF intensity (rel.u.), normalized transients and ΔV$_{1}$ curves in the NaCl treatment with CaCl$_2$ amendment. The signals were plotted on a logarithmic time scale (JIP Time). Each value of the curves is the average of 3 replicates.](https://doi.org/10.1371/journal.pone.0256529.g002)
Fig 3. The curves of the K and L band in NaCl treatment and NaCl treatment with CaCl₂ amendment on the day of NaCl stress and on days 3, 6, 9, 12, and 15 of NaCl stress. (A, B, E, F) the curves of the K-band and L-band in NaCl treatment. (C, D, G, H) the curves of the K-band and L-band in NaCl treatment added CaCl₂. The signals were plotted on a logarithmic time scale (JIP Time). Each value of the curves is the average of 3 replicates.

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NaCl treatment with CaCl$_2$ amendment, the amplitude and shape of the DF induction curves were changed by NaCl stress (Fig 5A and 5B). NaCl stress led to a progressively decreased DF amplitude, and the reduction was more notable at the I$_1$ than at I$_2$ (Fig 5A and 5B). The DF amplitude in the NaCl treatment showed a much faster decrease than that in the NaCl treatment with CaCl$_2$ amendment (Fig 5A and 5B).

In the NaCl treatment, the I$_1$ maximum decreased by 12.4%, 21.2%, 44.1%, 62.0%, and 66.6% on days 3, 6, 9, 12, and 15 of NaCl stress, respectively (Fig 5A). In the NaCl treatment with CaCl$_2$ amendment, the I$_1$ maximum decreased by 3.5%, 17.2%, 21.0%, 42.2%, and 47.4% on days 3, 6, 9, 12, and 15 of NaCl stress, respectively (Fig 5B). In the normalization curves of both D$_0$ and I$_1$, the normalized I$_2$ values in the NaCl treatment were increased by NaCl stress (Fig 5C), while in the NaCl treatment with CaCl$_2$ amendment, there was no obvious change in the normalized I$_2$ values (Fig 5D). The I$_2$/I$_1$ ratio in the NaCl treatment increased, while in the NaCl treatment with CaCl$_2$ amendment, little change was observed (Fig 5C and 5D).

In the control and CaCl$_2$ treatments, the curves of the MR/MR$_O$ at 820 nm in the oat leaves showed a pattern of decline first, which then increased. In the NaCl treatment and the NaCl treatment with CaCl$_2$ amendment, the curve pattern of the MR/MR$_O$ changed significantly (Fig 6A and 6B). The NaCl stress induced an elevation in the descending phase of the MR/MR$_O$ curves and a reduction in the ascending phase of the MR/MR$_O$ curves in both the NaCl treatment and the NaCl treatment with CaCl$_2$ amendment. On day 9 of NaCl stress, the decrease then increase curve pattern of the MR/MR$_O$ was eliminated partially in the NaCl
treatment (Fig 6A), whereas in the NaCl treatment with CaCl$_2$, on day 12 of NaCl stress (Fig 6B).

**Discussion**

Salinity stress can cause damage to cellular membrane structure and can interfere with cellular functions at any stage of the plant growth [2,3,30]. In salt treatment, Ca$^{2+}$ amendment can alleviate damage to plants by keeping the stability and functions of the plasma membrane in cells [17,18]. In the present study, the growth of salt-stressed oat seedlings and plants was improved by the addition of Ca$^{2+}$. The lower ion leakage of oat seedlings and leaves in the NaCl treatment with CaCl$_2$ amendment than in NaCl treatment supported the idea that Ca$^{2+}$ could control membrane structure and function of cells by blocking Na$^+$ influx, binding with phospholipids, and stabilizing lipid bilayers [16], thus, alleviating the effects of NaCl stress on plants by controlling membrane permeability [17–19]. The components of the photosynthetic electron transfer chain are located on the thylakoid membrane in plant cells. Salt stress
damages the structure of the thylakoids in chloroplasts of leaves and decreases photosynthetic pigments contents and photosynthetic efficiency in some plant species [4,29,31–36]. Exploring the effects of Ca$^{2+}$ on the photosynthetic electron transfer under salt stress could further reveal the possible mechanisms of Ca$^{2+}$-enhanced salt tolerance of oats.

Changes in the OJIP transients of PF in oat leaves revealed that the normal photosynthetic electron transport is affected by salt stress in both NaCl treatment and NaCl treatment with CaCl$_2$ amendment. As NaCl stress continued, decreased I-P stage in OJIP curves, increased J-I stage in OJIP curves, and changed K-band and L-band were observed. Similar results have also been found in other plant studies [29,34]. The decreased I-P stage in OJIP under continuous NaCl stress revealed that salt stress reduced the redox capability of PSI [8,9]. The decreased P value was caused by degradation and denaturation of chlorophyll proteins at the PSI acceptor side and decreased numbers of closed PSII RCs [7,37]. The increased J-I stage in normalized transients of OJIP under continuous NaCl stress suggested that the active PQ molecular stock was reduced by salt stress in PSII [20]. At the later stage of NaCl stress, the higher P value and amplitude of the I-P phase in the OJIP curves in NaCl treatment with CaCl$_2$ amendment than in NaCl treatment indicated that Ca$^{2+}$ inhibited the degradation and denaturation of chlorophyll proteins at the PSI acceptor side and improved the redox rate of PSI under NaCl stress. The increased J point in normalized transients of OJIP suggested that the transfer rate of electrons from $P_{680}$ to QA was limited and the quantum yield of PF increased [37]. The changes in the K-band and L-band in the present study indicated that the oxygen-evolving complex was impaired by NaCl stress. Moreover, NaCl stress caused a reduction in electron transfer efficiency in the PSII donor side and inhibited the energy flow of PSII [12,20]. The obvious differences in the K-band and L-band between the NaCl treatment and NaCl treatment with CaCl$_2$ amendment suggested that Ca$^{2+}$ increased the electron transfer efficiency in the PSII donor side by protecting the oxygen-evolving complex under NaCl stress.

Several parameters, such as PI$_{ABS}$, ABS/RC, ETo/TRo and F$_{V}$/F$_{M}$ developed from the OJIP transient, can provide detailed information on the photosynthetic process [38,39]. In the present study, the increase in ABS/RC and the decrease in PI$_{ABS}$ indicated that NaCl stress reduced the number of active PSII reaction centers and decreased the overall PSII activity of
oat leaves in NaCl treatment and NaCl treatment with CaCl₂ amendment. The reduced number of active PSII reaction centers could lead to an increase in the necessary number of RC turnovers for full reduction of the PQ pool [20]. Compared with NaCl treatment, the changes in NaCl stress on ABS/RC and PIₐBS in oat leaves showed a lesser extent and appeared at a later stage in NaCl treatment with CaCl₂ amendment. The results indicated that Ca²⁺ could reduce the damage of NaCl stress on PSII reaction centers in the leaves of oat. The decrease in ETₕ/.TRₒ and Fᵥ/Fₘ in oat leaves suggested that NaCl stress decreased the capability of PSII to capture light energy for QA reduction in both NaCl treatments. Similarly, previous studies in maize have shown that salt stress decreases the open PSII energy capture efficiency [29]. The changes in NaCl stress on ETₒ/ TRₒ and Fᵥ/Fₘ in oat leaves were to a lesser extent and appeared at a later stage in the NaCl treatment with CaCl₂ amendment. The results indicated that Ca²⁺ improved the capability of PSII to capture light energy for QA reduction under NaCl stress.

NaCl stress decreased the intensity of DF and made the speed of reaching I₁ relatively faster in both the NaCl treatment and NaCl treatment with CaCl₂ amendment. The results indicated that NaCl stress decreased the activation of RCs, resulting in weakened backward electron transfer capacity and electron refilling of chlorophyll in the excited PSII antenna [5]. The I₁ value of the DF curves was affected by the transmembrane electrical gradient and Z⁺P₆80ₐQAₜQₐₐ accumulation in reaction centers, and the I₂ peak of DF was related to the reduction of PQ and the Z⁺P₆80QAₜQₐₐ increase in reaction centers [7,40]. The decreased I₁ and I₂ values suggested that the electron transfer capacity in both the donor and acceptor sides of PSII decreased due to NaCl stress [6,11]. At the later stage of NaCl stress, the higher amplitudes at I₁ and I₂ phases in the DF curves in the NaCl treatment with CaCl₂ amendment than that in NaCl treatment suggested that Ca²⁺ relieved the salt injuries to the donor and acceptor sides of PSII and improved the transmembrane electrical gradient and backward electron transfer capacity in the oat leaves under NaCl stress.

The lowest point of the decline phase of the MR/MRₒ transient significantly increased at the later stage of NaCl stress in both the NaCl and NaCl treatments with CaCl₂ amendment. This result showed that NaCl stress reduced the oxidation activity of PSI. In the NaCl treatment and NaCl treatment with CaCl₂ amendment, the signal weakening of the MR/MRₒ transient in the duration of NaCl stress showed that NaCl stress reduced the reduction activity of PSI. The reduction in the activity of PSI may be due to the decrease in the capacity of PSII to produce and transfer electrons, the electron transfer obstruction between PSII and PSI, and the inhibition of the receptor side of PSI [35]. Combining the results of the OJIP transients with their parameters and DF parameters, it can be deduced that NaCl stress damaged multiple sites of the photosynthetic electron transport chain [29]. Under the NaCl treatment, the lowest point of decline in the MR/MRₒ transient emerged earlier than that of the NaCl treatment with CaCl₂ amendment. These results showed that Ca²⁺ alleviated the damage on PSII and on some sites of the photosynthetic electron transport chain under NaCl stress.

Conclusions
In the present study, according to the increase in the ion leakage in the oat seedlings and leaves, we concluded that the 150 mM NaCl treatment damaged the cells in the oat seedlings, and the 300 mM NaCl treatment damaged the cells in the leaves of oat plants. The addition of 10 mM CaCl₂ improved seed germination and plant growth, decreased the accumulation of Na⁺ and Cl⁻ in the leaves of plants, and reduced the ion leakage of the oat seedlings and plant leaves. The changed curves of the PF, DF, and MR in the oat leaves at plant growth phases revealed that NaCl stress interfered with the oat photoreaction system in the NaCl treatment.
and NaCl treatment with CaCl₂ amendment. The oxygen-evolving complexes and electron transporters of the oat leaves were damaged by NaCl stress on the PSI receptor side. The NaCl treatment reduced the activity of the PSII reaction center and the interlinkage between functional units in the photosynthetic electron transport chain and decreased the rate of electron flow from Q₆ to Q₄ in the oat leaves. Compared with the single NaCl treatment, the degree of change in the photosynthetic electron transport induced by NaCl was decreased by the addition of Ca²⁺ in the NaCl treatment with CaCl₂ amendment. These results showed that Ca²⁺ could alleviate the damages caused by NaCl stress to the photosynthetic electron transporters and improve the salt resistance of plants. Further research on Ca²⁺ fertilizers of soil could improve crop yield in saline-alkali soil.

Supporting information
S1 File.

Author Contributions
Data curation: Xiaoshan Wang, Qiyue Dingxuan, Mengmeng Shi.
Formal analysis: Qiyue Dingxuan, Mengmeng Shi.
Funding acquisition: Xiaoshan Wang.
Methodology: Qiyue Dingxuan, Mengmeng Shi.
Writing – original draft: Xiaoshan Wang, Qiyue Dingxuan.
Writing – review & editing: Qiyue Dingxuan.

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