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Exogenous Calcium Improves Photosynthetic Capacity of *Pinus sylvestris* var. *mongolica* under Drought

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Abstract: Calcium (Ca), a secondary messenger, plays an essential role in improving drought resistance. We used the Fast Chlorophyll Fluorescence Induction Dynamics technique to investigate the effects of exogenous calcium on electron transport and energy fluxes in an 8-year-old Mongolian pine to investigate the mechanism of action of Ca in regulating drought adaptation in *Pinus sylvestris* var. *mongolica*. We found water stress significantly decreased Pn and Gs, but exogenous calcium significantly improved photosynthesis under water stress. The chlorophyll a fluorescence transient (OJIP) analysis revealed that water stress increased Fo and decreased Fm, inactivating reaction centers. Water stress reduced V_I and V_J while increasing Mo, destroying the electron transport chain. Exogenous calcium increased Sm while decreasing V_I and Mo under water stress, enhancing electron transport from QA to QB. Furthermore, 5 mM Ca^{2+} increased I-P phase and ψPo, δRo, and φRo, decreasing the drought-induced reduction in electron accepters of PSI. The increase in ABS/RC, TRo/RC, ETo/RC, and DIo/RC caused by 5 mM Ca^{2+} demonstrated that calcium can regulate photoprotection to promote photosynthetic activity. Thus, exogenous calcium alleviated drought-induced reductions in photosynthetic activity by regulating photoprotection and boosting the electron transport efficiency at the acceptor side of PSII and PSI.

Keywords: chlorophyll a fluorescence; photosynthesis; drought; calcium

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1. Introduction

Mongolian pine (*Pinus sylvestris* var. *mongolica*) is the most important afforestation tree species in Three-North (northwest, north, and northeast) China, particularly in sandy areas [1–3]. However, Mongolian pine plantations have experienced serious dieback and decline since the 1990s [4]. Drought is a major abiotic factors influencings forest production and the global carbon balance. Drought mainly affects plant development and metabolism via altering photosynthesis [5]. Drought influences photosynthesis by lowering assimilate carbon, as plants adapt to drought by rapidly reducing stomatal openings [6]. Furthermore, drought reduces photosystem II (PSII) activity, enhances oxidation efficiency, limits electron transport, and damages the oxygen-evolving complex [7–9]. Photosynthesis is an essential basic metabolic process in plants that provides energy and material for plant growth. Previous studies have demonstrated that drought reduced the efficiency of chloroplasts in capturing energy and, eventually, inhibited electron transport [10]. Furthermore, drought decreased the activity of the oxygen-evolving complex and produced photoinhibition of PSII [11]. To avoid photoinhibition of the photosystem caused by drought, plants can regulate cyclic electron flow and non-photochemical quenching [12–14]. Drought, on the other hand, can restrict plant regulatory mechanisms. Thus, there were lots of measures to improve drought-induced photosynthesis inhibition, such as fertilization, growth regulators, and other exogenous applications.

Calcium (Ca) is thought to play an important role in tree acclimation to drought stress, not only by stabilizing the cell wall and cell membrane as an essential macronutrient, but

also by regulating stomatal closure, transducing drought signals, and regulating a series of enzyme activities as a second messenger [15–18]. In addition to influencing photosynthesis through stomatal regulation, calcium is also known to be a cofactor in the formation of the structure of PSII [19,20]. Ca is also involved in linear and cyclic electron flow, as well as regulation of numerous photosynthetic proteins [21,22], which involves the regulation of photosynthetic function in response to other surroundings [23]. Ca, different from other essential plant nutrients such as nitrogen, phosphorus, and potassium, is primarily acquired mainly by the apoplastic pathway and transported upward via the xylem with rare redistribution via the phloem [24,25]. These characteristics make it vital, but challenging, for trees to obtain a continuous Ca supply under drought conditions. Despite the fact that exogenous Ca has been shown to improve tree drought resistance, investigations on the influence of calcium on photosynthetic activity have been limited [26–28].

Chlorophyll fluorescence is a useful indicator for the assay and analysis of photosynthetic performance and has been utilized extensively in drought physiological processes given its sensitivity to the identification of changes in the photosynthetic system [29–31]. When photosynthetic plants are moved to high light intensity after long durations of darkness, chlorophyll a fluorescence, which is divided into two phases: fast phase and slow phase, is triggered. The fast phase is called OJIP, where O is the origin, or the minimum fluorescence (Fo), J and P are the middle phases, and P is the peak, or the maximum fluorescence (Fm). In addition to reflecting the PSII photochemical processes and information about electron transport under stress, OJIP is sensitive to various environmental stresses [32–34]. The JIP test, which quantifies the activity of PSII and estimates energy fluxes, is an analysis of the OJIP [35,36]. The OJIP parameters, such as absorbance (ABS) of photons, intermediate trapping flux (TR), electron transport (ET), and reduction in the end-electron at PSI electron acceptor side (RE), were utilized to better analyze the absorption, conversion, and dissipation of light energy in plants. Chl absorbs the photon absorption flux (ABS), which are then captured by the reaction center (TR), transferred by electron transport (ET), and stored by RE to fix CO_2 or other processes [37–40]. This study aimed to investigate the effect of different exogenous calcium concentrations on the photosynthetic electron transport process of *Pinus sylvestris* var. *mongolica* under drought stress and to clarify the mechanism of calcium on drought-tolerant photosynthetic organs. We subjected 8-year-old Mongolian pine to drought and applied different calcium concentrations to investigate the impacts of exogenous calcium on electron transport and energy conversion by chlorophyll a fluorescence.

2. Materials and Methods

2.1. Experimental Design and Plant Growth Conditions

We used 8-year-old Mongolian pine trees (height 1.5–1.7 m) from the Institute of Sand Fixation and Silviculture of Zhanggutai, Zhangwu Country, Liaoning province, which is the first place that Mongolian pine was successfully planted and spread to other parts of China. In April 2020, these trees were transplanted into pots filled with local sandy soil. All trees were acclimated for 2 months in the greenhouse belonging to the College of Forestry, Shenyang Agricultural University, and watered at a normal moisture level (70% pot capacity) with sterile water. In June 2020, we divided the trees into six treatments of 5 individuals arranged in a randomized plot: (1) control (CK, 70% pot capacity, zero $CaCl_2$); (2) drought (DC, 30% pot capacity, zero $CaCl_2$); (3) 5 mM Ca^{2+} (Ca5, 70% pot capacity, 5 mM $CaCl_2$); (4) drought with 5 mM Ca^{2+} (DC-Ca5, 30% pot capacity, 5 mM $CaCl_2$); (5) 10 mM Ca^{2+} (Ca10, 70% pot capacity, 10 mM $CaCl_2$); (6) and drought with 10 mM Ca^{2+} (DC-Ca10, 30% pot capacity, 10 mM $CaCl_2$).

The pots exposed to the water-limited development conditions were no longer watered after the addition of $CaCl_2$ aqueous solution until the end of experiment, while the pots that had been adequately watered were watered at a normal moisture level. During the experiment period, trees were growth in the greenhouse, receiving natural light with photosynthetic photon flux density averaging 1000 $\pm$ 200 μmol m^{-2} s^{-1}, ambient temperatures

ranging from 21 ± 2 °C for night to 28 ± 2 °C for day and relative humidity of 60 ± 5%. After two weeks, photosynthetic and fluorescence were measured, and after a month we harvested the newest fully developed mature leaves and measured the growth parameters (dry weight per leaf and leaf area per leaf) and calculated the specific leaf area.

2.2. Gas Exchange

Photosynthetic rates (Pn, μmol m^{-2} s^{-1}) and stomatal conductance (Gs, mol m^{-2} s^{-1}) were determined from 9:00 to 11:00 on a sunny day with a portable photosynthesis system (Li-6400, Li-Cor Inc., Lincoln, NE, USA). The most recent fully elongated sunlight leaves were randomly selected for measurement. Photosynthetic photon flux density was set at 1000 μmol m^{-2} s^{-1}.

2.3. Chlorophyll (Chl) a Fluorescence Measurement

The Chl a fluorescence transients were measured with a portable pulse-modulated chlorophyll fluorescence analyzer (OS5p+, USA). Before measurement, leaves were adapted in the dark for 20 min to ensure complete oxidation of the reaction center. Three Chl a measurements were carried out on each pot. Leaves were exposed to saturated pulsed light (3000 μmol m^{-2} s^{-1}), and the fluorescence transients were recorded from 10 μs to 1 s. The fluorescence intensity at 20 μs was determined to be Fo and maximum fluorescence was defined as Fm. The different steps of the multi-phase fluorescence transient are marked in alphabetical order: when all reaction centers are open, there is minimal fluorescence intensity (the O step); the photochemical phase, or the fluorescence intensity at 2 ms (the J step), is the most noticeable stage and provides information about antenna size and the connection between PSII reaction centers [41]. When all RCs of PS II are closed, the greatest fluorescence intensity is known as P (the P step), the fluorescence intensity at 0.3 ms and 30 ms is referred as the K and I steps.

The JIP test, which has been proposed for the characterization and quantification of OJIP, has shown to be a useful tool for learning about the structural and functional aspects as well as the photosynthetic behavior of samples. The JIP test was conducted based on the calculation of Strasser and Stirbet [36,42]. The quantum yields and efficiencies were calculated according to Gururani [33]. The photosynthetic performance indexes (PI_{ABS} and PI_{total}) were calculated [43].

2.4. Statistical Analysis

Statistical analysis was carried out by SPSS 19. Analysis of variance (ANOVA) was carried out on the data (ANOVA) and Duncan's multiple range test was used to find out significant differences among group means. Before ANOVA, the data were tested by the Kolmogorov–Smirnov test. When the data were not normally distributed, logarithmic or square root transformations were applied to meet the criteria of normal distribution. If transformation did not meet the criteria, a nonparametric test was used. The significance of effects of water and calcium, and their interaction were tested by a two-way ANOVA.

3. Results

The dry weight (DW) and leaf area (LA) per leaf were significantly influenced by water and calcium ($p < 0.01$, Figure 1). Moreover, calcium significantly affected special leaf area (SLA). Under drought stress, the dry weight (DW) and leaf area (LA) per leaf were significantly reduced in three Ca concentrations ($p < 0.05$, Figure 1). Both DW and LA per leaf were significantly increased by 5 mM Ca^{2+} in well-watered seedlings. Moreover, exogenous calcium increased the DW per leaf in water-stress seedlings compared with 0 mM Ca^{2+}, which was significant in 5 mM Ca^{2+}. However, the LA per leaf was not obviously different among the three Ca concentrations under water stress. The SLA is a useful parameter that measures the photosynthetic performance of leaves. Though no significant effects on SLA were observed between the water treatments. Under 5 mM and 10 mM Ca^{2+} conditions, water stress decreased SLA by 5.10% and 6.31%, respectively.

Additionally, under 0 mM Ca^{2+} conditions, water stress increased SLA by 4.28%. There was no difference among the three Ca concentrations in well-watered seedlings. While both 5 mM and 10 mM Ca^{2+} significantly decreased SLA compared with 0 mM Ca^{2+} under water stress.

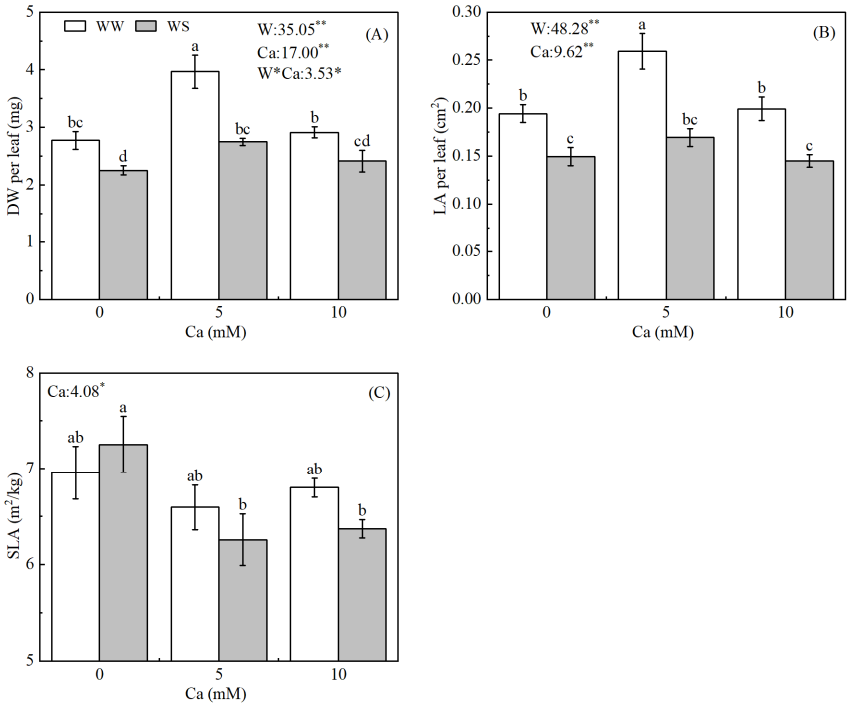

Figure 1. Leaf dry weight (DW, **A**), leaf area per leaf (LA, **B**), and special leaf area (SLA, **C**) of *Pinus sylvestris* var. *mongolica* exposed to well water (WW), water stress (WS) and different calcium (Ca) concentration. Different letters indicate significant differences at $p < 0.05$ determined by Duncan's multi-range test. F values are provided for the significant effects of the main variables calcium (Ca), water (W) and their interactions, and the single * in the upper right corner indicated a significant effect at 0.05 level, and the double * indicated a significant effect at 0.001 level. F is not provided when Ca, W, and their interactions are not significant.

Water, calcium, and their interaction considerably impacted Pn and Gs (Figure 2). both Pn and Gs were significantly decreased by water stress (p <0.05, Figure 2). Pn and Gs were decreased by 77.36% and 88.84%, respectively; for 0 mM Ca^{2+}, by 35.58% and 38.56%, respectively; for 5 mM Ca^{2+} and by 56.09% and 73.40%, respectively; for 10 mM Ca^{2+}. Compared with 5 mM Ca^{2+} and 10 mM Ca^{2+} treatments, this decrease was more prominent under 0 mM Ca^{2+} treatments. Under well-watered treatments, the application of 10 mM Ca^{2+} induced a significant decrease in Pn and Gs compared with 0 mM Ca^{2+} and 5 mM Ca^{2+}. However, the lowest Pn and Gs were observed in 0 mM Ca^{2+} under water stress. Moreover, Pn and Gs were significantly increased by 5 mM Ca^{2+} under droughted treatments compared with 0 mM Ca^{2+} and 10 mM Ca^{2+}.

Fluorescence transient curves for all samples displayed feature sequences for OJIP steps, and K was visible in all treatments in the OJIP transient. The O-K and K-J phases of the fluorescence transient were no significant differences among all treatments. While the variable fluorescence amplitude of J-I and I-P phases showed obvious differences. Regardless of the Ca concentrations, water stress led to a considerable decrease in variable fluorescence amplitude of J-I and I-P phases (Figure 3A). Under well-watered treatments, the curve for 10 mM Ca^{2+} treatment was at the highest and there were no differences between 0 mM Ca^{2+} and 5 mM Ca^{2+}, with the exception of P-step (Fm). Under water stress treatments, the J-I and I-P phase curves decreased with rising Ca concentrations. The first normalization of chlorophyll a fluorescence (Ft/Fo) was performed at Fo (Figure 3B) and

O, K, J, I, and P steps were also present. Water stress decreased the variable fluorescence amplitude of J-I and I-P phases, and exogenous calcium caused a decrease under water stress compared with 0 mM Ca^{2+}.

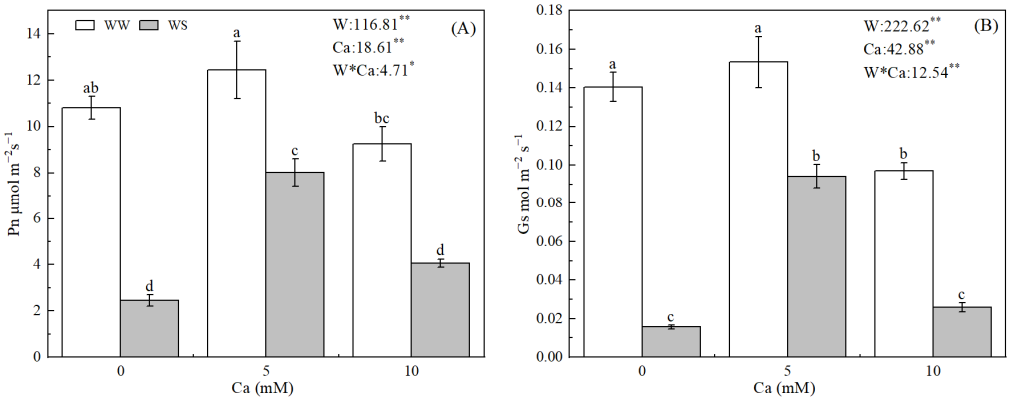

Figure 2. Photosynthetic rate (Pn, **A**) and stomatal conductance (Gs, **B**) of *Pinus sylvestris* var. *mongolica* exposed to well water (WW), water stress (WS), and different calcium (Ca) concentrations.

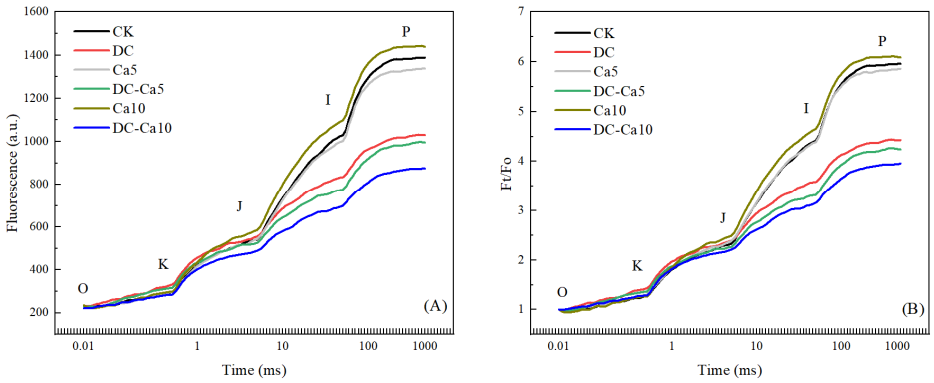

Figure 3. Chlorophyll a fluorescence OJIP transient curves of *Pinus sylvestris* var. *mongolica* exposed to well water, water stress, and different calcium concentrations, descripting the fluorescence rise from Fo (O) to Fm (P). Chl a fluorescence transient curves exhibiting fluorescence intensity (a.u.: arbitrary unit); (**A**) normalization at Fo (Ft/Fo); (**B**) CK: Control; DC: drought; Ca5: 5 mM Ca^{2+}; DC-Ca5: combined drought and 5 mM Ca^{2+}; Ca10: 10 mM Ca^{2+}; DC-Ca10: combined drought and 10 mM Ca^{2+}.

To compare visually and better assess the information about the OJIP curve reflected in the O-J, J-I, and I-P phases, the fluorescence data were double normalized (between Fo and Fm) and expressed as relative variable fluorescence (Vt). A significant increase in Vt of O-K, K-J, and J-I phase was observed when seedlings were subjected to water stress (Figure 4), and V_J was increased by 62.21% for 0 mM Ca^{2+}, 48.43% for 5 mM Ca^{2+} and 45.19% for 10 mM Ca^{2+}, which was much more increase in 0 mM Ca^{2+} due to water stress (Table 1). No considerable differences were detected in V_J among the three Ca concentrations in water stress. Moreover, under water stress, V_I was significantly larger in 0 mM Ca^{2+} than in 5 mM Ca^{2+} and 10 mM Ca^{2+} (Table 1). Water had a significant influence on V_J and V_I, and the interaction of water and calcium had a considerable impact on V_I.

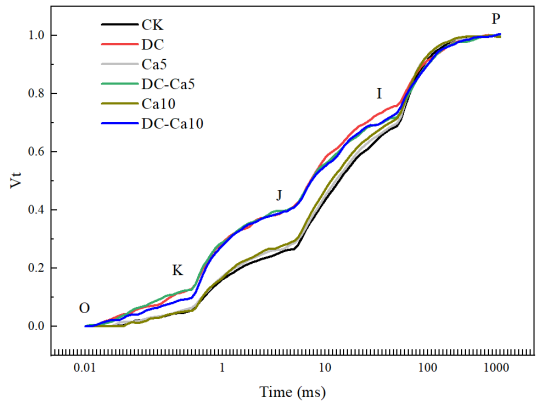

Figure 4. Relative variable fluorescence of *Pinus sylvestris* var. *mongolica* exposed to well water (WW), water stress (WS), and different calcium (Ca) concentrations. The relative variable fluorescence was a double normalization of the Chl a fluorescence transient at Fo and Fm and was calculated as Vt = (Ft − Fo)/(Fm − Fo).

Table 1. The parameters derived from the chlorophyll fluorescence curve. Sm = Area/F_V, the normalized total complementary area above the O-J-I-P transit; V_J and V_I are relative variable fluorescence at the J-step and I-step, respectively; Mo = 4 (F300 μs − Fo)/(Fm − Fo), the approximated initial slope of the fluorescence transient.

	Fo	**Fm**	**Sm**	**V_J**	**V_I**	**Mo**
CK	233.00 ± 5.57 b	1390.67 ± 45.32 a	27.13 ± 0.63 a	0.23 ± 0.00 b	0.71 ± 0.01 b	0.27 ± 0.02 c
DC	259.50 ± 3.18 a	1030.33 ± 35.33 b	21.62 ± 1.02 b	0.37 ± 0.04 a	0.77 ± 0.02 a	0.54 ± 0.03 a
Ca5	228.33 ± 9.84 b	1338.33 ± 91.55 a	27.13 ± 0.96 a	0.24 ± 0.01 b	0.72 ± 0.02 b	0.30 ± 0.01 c
DC-Ca5	232.00 ± 9.87 b	996.00 ± 6.11 b	26.74 ± 1.46 a	0.36 ± 0.05 a	0.74 ± 0.02 b	0.46 ± 0.01 b
Ca10	235.33 ± 9.96 b	1442.67 ± 64.24 a	24.07 ± 1.44 ab	0.25 ± 0.02 b	0.74 ± 0.00 b	0.29 ± 0.01 c
DC-Ca10	221.67 ± 2.19 b	874.33 ± 39.82 c	25.90 ± 0.49 a	0.37 ± 0.00 a	0.75 ± 0.01 b	0.43 ± 0.02 b

	Fo		**Fm**		**Sm**		**V_J**		**V_I**		**Mo**	
	F	P	F	P	F	P	F	P	F	P	F	P
W	0.80	0.39	154.83	**0.00**	8.08	**0.02**	34.30	**0.00**	19.81	**0.00**	371.08	**0.00**
Ca	3.40	0.07	0.89	0.60	10.45	**0.00**	0.07	0.93	2.03	0.17	7.99	**0.01**
W*Ca	3.60	0.06	4.54	**0.03**	20.71	**0.00**	0.17	0.84	4.61	**0.03**	16.12	**0.00**

Different letters within a single column indicate significant differences at $p < 0.05$ determined by Duncan's multirange test. F and P values are provided for the effects of the main variables water (W), calcium (Ca), and their interactions (W*Ca). $p < 0.05$ are shown by bold letters.

O-steps did not appear different on the OJIP curve among the treatments (Figures 3 and 4). In fact, Fo was larger in 0 mM Ca^{2+} than in 5 mM Ca^{2+} and 10 mM Ca^{2+} under water stress (Table 1). A considerable decrease in Fm was observed under water stress. Compared with DC, 10 mM Ca^{2+} significantly decreased Fm under water stress ($p < 0.05$). Sm and Mo reflected the change in acceptor of PSII. DC significantly decreased Sm, but Sm was unchangeable when the presence of exogenous calcium. Water stress significantly increased Mo, but Mo was significantly reduced by exogenous calcium, and Ca concentrations. Water and the interaction of water and calcium had a significant influence on Fm, Sm, and Mo, but not Fo (Table 1). Sm and Mo were significantly affected by calcium.

The I-P phase was assessed by double standardization of the fluorescence transient between F_I and F_P, denoted as V_{IP} = (Ft − F_I)/(F_P − F_I). V_{IP} was almost similar in all treatments, while V_{IP} was significantly decreased in water stress compared with well water, regardless of the Ca concentrations (Figure 5C). In addition, V_{OI} was also used to assess I-P phase. The same as V_{IP}, exogenous calcium did not affect the V_{OI} (<1) in either well-watered or water stress (Figure 5A). Whereas contrary to V_{IP}, water stress increased the V_{OI}

(<1). The V_{OI} (≥1) was obviously different among treatments (Figure 5B). Compared with well water, water stress significantly decreased the V_{OI} (≥1). This decline had become more pronounced without exogenous calcium. The V_{OI} (≥1) was decreased with the increasing in Ca concentrations. Under water stress, 10 mM Ca^{2+} induced a lower V_{OI} (≥1) than 5 mM Ca^{2+}.

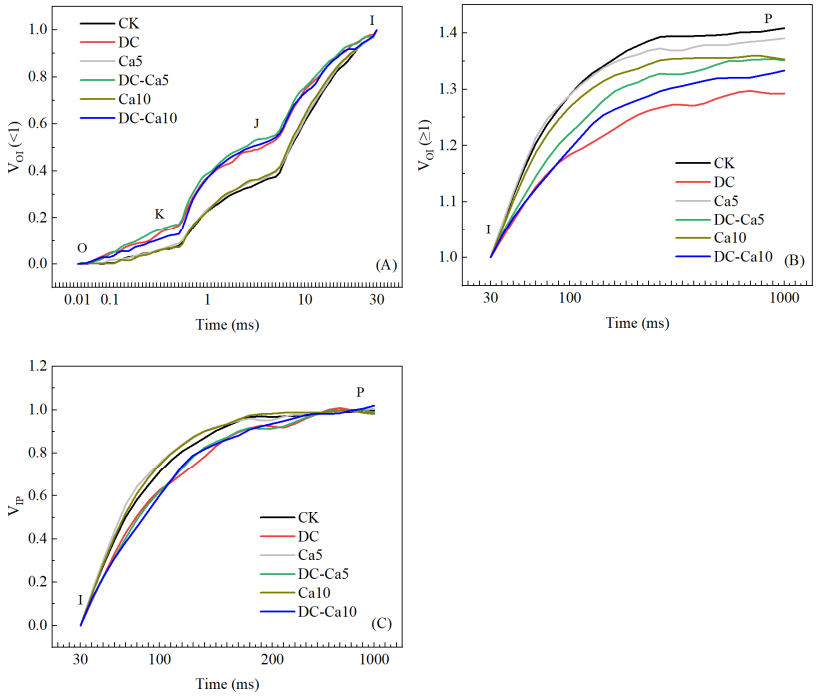

Figure 5. Variable fluorescence transients double normalized at Fo and F_I: $Vo_I = (Ft - Fo)/(F_I - Fo)$ (**A**,**B**) and at F_I and F_P phases: $V_{IP} = (Ft - F_I)/(F_P - F_I)$ (**C**).

Water and the interaction of water and calcium significantly influenced $\triangle V_{IP}$ (Figure 6). Water stress decreased $\triangle V_{IP}$ by 21.58%, 4,99%, and 7.25% in the three Ca concentrations, which were significant in 0 mM Ca^{2+} and 10 mM Ca^{2+} (Figure 6). The $\triangle V_{IP}$ was decreased with the increasing in Ca concentrations under well-watered treatments, and a significant decrease was observed in 10 mM Ca^{2+} compared with 0 mM Ca^{2+}. In contrast, exogenous calcium significantly increased $\triangle V_{IP}$ by 16.85% for 5 mM Ca^{2+} and by 8.48% for 10 mM Ca^{2+} under water stress, which was thought to be a tolerance to drought.

Except for DIo/CS, water stress significantly reduced the other phenomenological energy fluxes (ETo/CS, ABS/CS, and TRo/CS, Figure 7). Compared with 0 mM Ca^{2+}, ETo/CS, ABS/CS, and TRo/CS were decreased by 10 mM Ca^{2+} in water stress. Water significantly affected phenomenological energy fluxes (ETo/CS, ABS/CS, TRo/CS, and DIo/CS). Additionally, the interaction of water and calcium significantly influenced ABS/CS, TRo/CS, and DIo/CS. A significant decrease in RC/CS was observed in water stress compared with well water. The ABS/RC, TRo/RC, and DIo/RC were increased in water stress. The specific energy fluxes showed almost no differences among Ca concentrations under well-watered treatments. Ca significantly influenced specific energy fluxes (ABS/RC, TRo/RC, ETo/RC, and DIo/RC). Moreover, the interaction of water and calcium significantly influenced TRo/RC, ETo/RC, and DIo/RC. ABS/RC, TRo/RC, and ETo/RC showed a similar trend under water stress, and 10 mM Ca^{2+} decreased ABS/RC, TRo/RC, and ETo/RC under water stress compared with 0 mM Ca^{2+} and 5 mM Ca^{2+}. The quantum yields and efficiency were significantly decreased by water stress. Moreover, δRo was significantly affected by the interaction of water and calcium. The ψPo, δRo, and φRo were reduced with the increasing in Ca concentration in well-watered treatments. In contrast to well water, the application of exogenous calcium increased the ψPo, δRo, and φRo under water stress, which was much more pronounced in 5 mM Ca^{2+}. Exogenous calcium, however, had no effect on

ψEo, φPo, and φEo in both water conditions. Water significantly affected PI_{ABS} and PI_{total}, and obviously reduced PI_{ABS} and PI_{total} ($p < 0.05$, Figure 8). Although exogenous calcium did not significantly affect PI_{ABS} water conditions (Figure 8A), 5 mM Ca^{2+} significantly increased PI_{total} under water stress compared with 0 mM Ca^{2+} and 10 mM Ca^{2+} (Figure 8B). Calcium and the interaction of water and calcium significantly influenced PI_{total}.

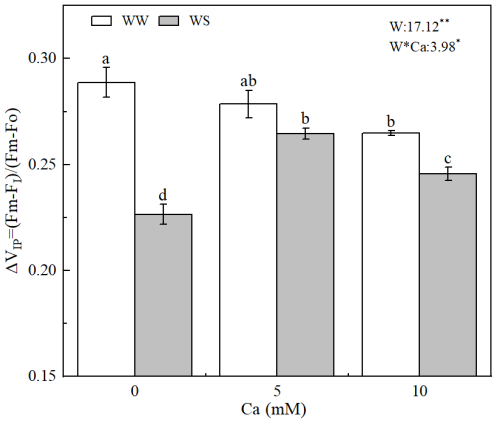

Figure 6. The effects of well water (WW), water stress (WS) and different calcium (Ca) concentrations on relative amplitude of the I–P phase, $\triangle V_{IP} = (Fm - F_I)/(Fm - Fo)$.

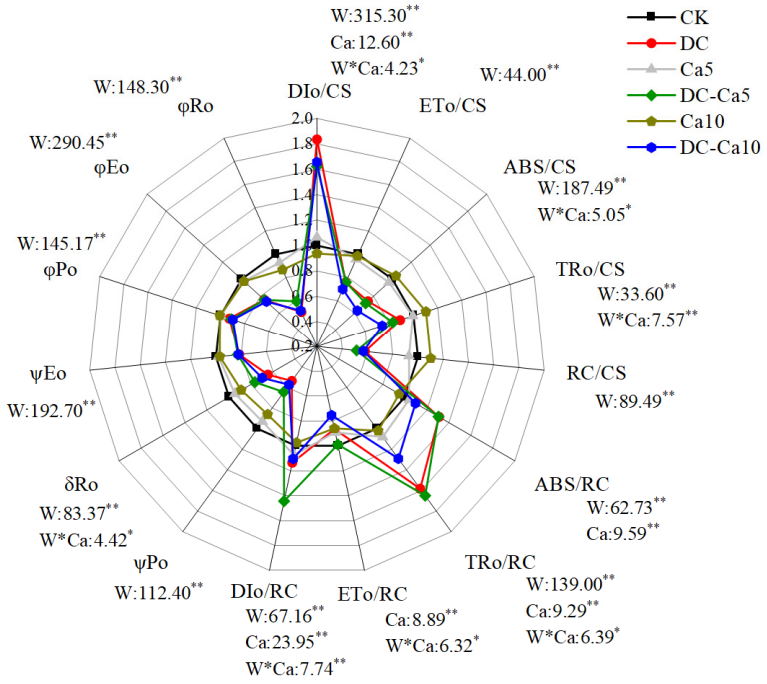

Figure 7. A 'spider plot' of well water (WW), water stress (WS), and different calcium (Ca) concentrations on specific energy fluxes (ABS/RC, TRo/RC, ETo/RC, and DIo/RC), phenomenological energy fluxes (ETo/CS, ABS/CS, TRo/CS, and DIo/CS) and the quantum yields and efficiency (ψEo, ψPo, δRo, φPo, φEo, and φRo). All data are normalized to the control and all variables are normalized to provide a value of 1. The single * in the upper right corner indicated a significant effect at 0.05 level, and the double * indicated a significant effect at 0.001 level. F is not provided when Ca, W, and their interactions are not significant. $\psi Eo = ETo/TRo = (1 - V_J)$, $\psi Po = REo/TRo = (1 - V_I)$, $\delta Ro = REo/ETo = (1 - V_I)/(1 - V_J)$, $\varphi Po = TRo/ABS = Fv/Fm$, $\varphi Eo = ETo/ABS = [1 - (Fo/Fm)]$ ψEo, φRo = REo/ABS = (1 − VJ) φPo. ABS/RC = Mo $(1/V_J)$ (1/φPo), TRo/RC = Mo $(1/V_J)$, ETo/RC = Mo $(1/V_J)$ ψEo, DIo/RC = (ABS/RC) − (TRo/RC). ABS/CS = Fm, TRo/CS = φPo (ABS/CS), DIo/CS = (ABS/CS) − (TRo/CS), ETo/CS = φEo (ABS/CS).

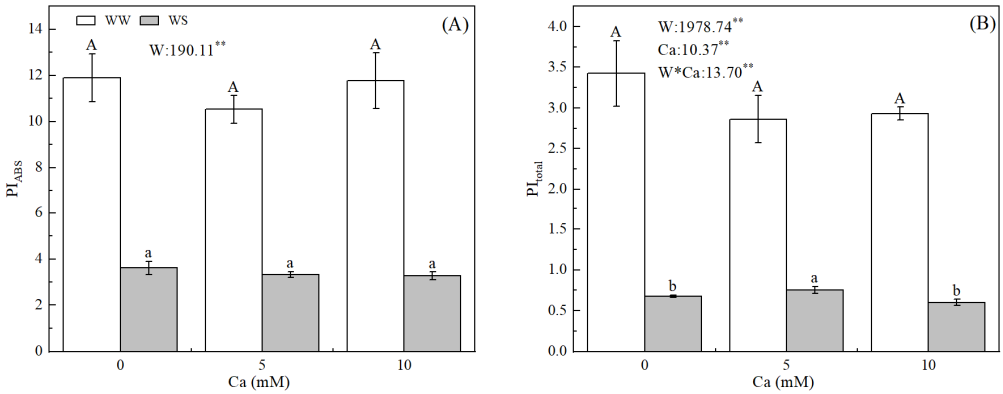

Figure 8. Effect of well water (WW), water stress (WS), and different calcium (Ca) on PI_{ABS} (performance index, **A**) and PI_{total} (total performance index, **B**). The capital letters indicated a significant difference at 0.05 among well water treatments. Moreover, the little letters indicated a significant difference at 0.05 among water stress treatments. The single * in the upper right corner indicated a significant effect at 0.05 level, and the double * indicated a significant effect at 0.001 level. F is not provided when Ca, W, and their interactions are not significant. PI_{ABS} = (RC/ABS) (φPo/(1 − φPo)) − (ψo/(1 − ψo)), PI_{total} = (RC/CS) (φPo/(1 − φPo)) − (ψo/(1 − ψo)).

4. Discussion and Conclusions

Water stress significantly decreased the DW and LA per leaf (Figure 1A,B), and Pn and Gs were also significantly reduced (Figure 2A). The inhibition of photosynthesis by drought is the main reason of the reduced growth. Plants adapt to drought by changing the stomatal aperture to balance water, transpiration, and photosynthesis, preventing water loss and reducing drought damage [44]. However, this change limits CO_2 uptake and reduces photosynthesis [45]. In this study, exogenous calcium was found to boost Pn and Gs under water stress, suggesting that calcium can maintain growth by improving photosynthesis. However, excess soil Ca might be counteracted any beneficial impacts, as the DW and LA did not show any differences between 0 mM Ca^{2+} and 10 mM Ca^{2+} under water stress (Figure 1A, B). This can be related to changes in Ca-induced soil water potential under drought. Compared with 0 mM Ca^{2+}, exogenous calcium significantly decreased SLA under water stress, and there was no considerable difference between 5 mM Ca^{2+} and 10 mM Ca^{2+}, suggesting that 10 mM Ca^{2+} might be offset partial positive impacts but not be a Ca salt. Severe drought damaged chloroplasts, reduced PSII activity, and blocked electron transport, resulting in lower photosynthetic rates. It is well-known that there is a calcium pool in the chloroplast that regulates photosynthesis [21]. This study clarifies how calcium alters photosynthesis in terms of electron transport and energy conversion between PSI and PSII to promote drought resistance.

In this study, water stress destroyed the electron transport chain and blocked electron transport. However, exogenous calcium lessened this effect. Water stress led to an increase in V_J and a decrease in ψEo and φEo indicating the accumulation of QA^-, blocked electron transfer from QA to QB on the PSII receptor side, and reduced electron transport capacity of PSII [46]. The increased V_I suggested water stress inhibited PQ reoxidation and reduced the electron-accepting capacity of the PQ pool. Meanwhile, the reduction in Sm also suggested the reduction in PQ pool, which was the reason for weakened electron transport capacity from QA to QB [47,48]. In addition, elevated Mo due to water stress reflected a faster rate of QA decline, indicating water stress decreased the activity of reaction centers. The increase in Fo and the decrease in Fm due to water stress further illustrated the disruption of the PSII reaction centers. Abiotic stresses increase ROS accumulation, inhibit D1 protein reorganization, and reduce photosynthetic rates [49]. Previous studies discovered Ca-binding sites on the D1 protein and demonstrated exogenous calcium could increase D1 protein content, balance ROS, protect photosystem from ROS damage and maintain electron transport [50–52]. In this study, exogenous calcium significantly decreased Fo under water

stress and alleviated drought damage on PSII reaction centers. Moreover, the reduction in V_I and Mo and the increase in Sm indicated exogenous calcium improved the electron transportation chain from QA to QB. In comparison with 10 mM Ca^{2+}, 5 mM Ca^{2+} had a stronger mitigating effect on the reaction centers.

PI_{ABS} and PI_{total} quantified the effect of drought on PSII activity. Drought significantly decreased PI_{ABS} and PI_{total} (Figure 8). Comparing the two parameters, we found that PI_{total} was more sensitive to perceived drought. Exogenous calcium had no impact on PI_{ABS} under water stress, but 5 mM Ca^{2+} caused a higher PI_{total} value than 0 mM Ca^{2+} and 10 mM Ca^{2+}, indicating that calcium improved drought resistance. Water stress decreased ETo/CS, ABS/CS, TRo/CS, and RC/CS, possibly due to reaction centers degradation or inactivation, which would increase burden on the remaining active reaction centers [53]. The increase in specific energy fluxes (ABS/RC, TRo/RC, ETo/RC) also demonstrated partial inactivation of reaction centers. However, an increase in DIo/CS and DIo/RC suggested that the reaction centers have triggered the appropriate defense mechanism, i.e., dissipating the excess excitation energy as heat, reducing the damage to the plant from the excess light energy. Under water stress, 5 mM Ca^{2+} obviously increased DIo/CS compared with 0 mM Ca^{2+}, indicating calcium can enhance the self-protection mechanism of plants under water stress. Different from 5 mM Ca^{2+}, 10 mM Ca^{2+} directly inhibited ABS uptake, which may be related to the Chl size. Even though this avoids damage to the reaction centers caused by excess light energy, ETo/RC was reduced.

Water stress significantly decreased the quantum yields and efficiency. However, exogenous calcium had a negligible impact on quantum efficiency of the continuous process at the receptor side of PSII, such as φPo, ψEo, and φEo. It was worth noting that exogenous calcium had an important effect on quantum yields and efficiency at the PSI acceptor side, such as ψPo, δRo, and φRo. Simultaneously, the I-P phase is related to the reduction in the acceptor side of PSI, reflecting the reversion of plastocyanin and $P700^+$ in PSI [54,55]. Exogenous calcium significantly increased V_{OI} ($\geq$1) and $\triangle V_{IP}$ under water stress (Figure 5), indicating the application of calcium improved the final electron acceptor of PSI [56]. Furthermore, the increases in ψPo, δRo, and φRo also reflected enhanced electron transport efficiency to the PSI receptor side (Figure 7). Based on the changes in quantum yields and efficiency (ψPo, δRo, and φRo) and I-P phase, we found 5 mM Ca^{2+} was more effective than 10 mM Ca^{2+} in improving the drought tolerance.

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