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Prohexadione calcium regulates wheat tolerance to drought stress by maintaining water balance and promoting antioxidant metabolism and photosynthesis

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Abstract: This study explored whether and how prohexadione calcium (Pro-Ca) regulated wheat tolerance to drought stress (DS). Findings displayed that DS had significant influence on antioxidant metabolism, water balance and the photosynthesis. DS significantly improved the activity level of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX), the contents of non-enzymatic antioxidants ascorbic acid (AsA) and glutathione (GSH), electrolyte leakage (EL), malondialdehyde (MDA), and the contents of osmotic regulatory substances soluble protein (SP), soluble sugars (SS) and proline (Pro), compared with control. Whereas DS significantly reduced transpiration rate (T_r), stomatal conductance (g_s) and relative water content (RWC), photosynthetic pigments chlorophyll and carotenoid contents, net photosynthetic rate (P_n), maximum photochemical efficiency of PSII (F_v/F_m), plant height and biomass. Compared to DS, Pro-Ca plus DS significantly promoted the antioxidant metabolism by improving the activity level of SOD, CAT, POD and APX and increasing AsA and GSH contents, which in turn reduced MDA content and EL. In addition, Pro-Ca plus DS significantly maintained water balance by promoting the accumulation of osmolytes SP, SS and Pro, which in turn increased RWC, T_r and g_s. Pro-Ca plus DS also significantly promoted photosynthesis by increasing the contents of the above photosynthetic pigments, P_n and F_v/F_m, thereby promoting plant growth. These findings indicated that Pro-Ca was a potential agent to improve wheat tolerance under water deficit.

Keywords: water metabolism; plant growth regulator; antioxidant ability; dry period; gas exchange parameter

Crops often encounter water deficits throughout their entire growth and development. Water deficit induces drought stress (DS) in crops, which in turn breaks the antioxidant and water metabolism. Under DS, crops can activate the antioxidant defence system (ADS) to maintain antioxidant metabolism balance and enhance the water balance capacity (WBC) to keep water metabolism balance. The ADS includes antioxidant enzymes and antioxidants. The former mainly includes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), while the latter mainly includes ascorbic acid (AsA) and reduced glutathione (GSH). Increasing reports proved that exogenous chemicals could be used to help plants fight against DS by enhancing ADS. Lian et al. (2023) reported that sulphur reinforced the DS tolerance of mungbean (*Vigna radiata* (L.) R.Wilezek) *via* antioxidant enzymes. Urmi et al. (2023) found that salicylic acid (SA) and proline (Pro) improved rice (*Oryza sativa* L.) DS tolerance through antioxidant enzymes. It has also been reported that the WBC had close relationships with osmotic regulatory substances (ORS), soluble protein (SP), soluble sugars (SS) and Pro contents. As reported, exogenous chemicals could also help plants fight against DS by increasing ORS contents and improving WBC. Urmi et al. (2023) found that SA and Pro could improve rice WBC by enhancing the levels of Pro and SS under DS. Hafez et al.

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(2020) reported that biochar and chitosan increased barley WBC by enhancing the levels of Pro and SS under DS. DS also inhibited photosynthesis, which further inhibited plant growth. Previous reports also demonstrated that exogenous chemicals improved photosynthesis under DS. Urmi et al. (2023) found that SA and Pro enhanced rice photosynthesis by increasing photosynthetic pigments chlorophyll (*Chl*) and carotenoid (Car) contents and net photosynthetic rate (P_n) under DS. Luo et al. (2023) clarified that melatonin promoted the photosynthesis of *Chrysanthemum* seedlings by raising the values of P_n and *Chl* fluorescence parameters. These studies suggested that using exogenous chemicals under DS is a good method to regulate plant antioxidant capacity, water balance, and photosynthesis. Therefore, developing and utilising more useful exogenous chemicals to help wheat plants fight against DS will be meaningful.

Prohexadione calcium (Pro-Ca) is used as an important environmentally friendly plant growth regulator. Pro-Ca takes an important part in modulating plant growth and development, improving fruit rate, yield and quality, and alleviating the negative influence of chilling stress on tomato fruit and saline-alkali stress on soybean seedlings (Bizjak et al. 2013, Soleimani Aghdam 2013, Chang 2016, Feng et al. 2021, de Oliveira et al. 2023). Under stress, it is only documented that Pro-Ca regulated soybean tolerance to saline-alkali stress by improving antioxidant enzyme activities and antioxidant contents (Feng et al. 2021). Meanwhile, Pro-Ca also enhanced the water balance of soybean seedlings by modulating transpiration rate (T_r), stomatal conductance (g_s) and the contents of osmotic regulatory substances under saline-alkali stress (Feng et al. 2021). Moreover, Feng et al. (2021) uncovered that Pro-Ca enhanced soybean photosynthetic capacity under saline-alkali stress. Now, whether and how Pro-Ca regulates the DS tolerance is blank. Consequently, it is valuable to explore the effects of Pro-Ca on plant antioxidant capacity, water balance and photosynthesis under DS.

A current study was carried out to prove whether Pro-Ca protected wheat crops against DS by modulating antioxidant capacity, water balance and photosynthesis. For this aim, this study investigated the function of Pro-Ca in regulating malondialdehyde (MDA) content, electrolyte leakage (EL), the activity level of antioxidant enzymes, the contents of AsA, GSH, *Chl*, carotenoid and osmolytes, relative water content (RWC), gas exchange parameters, maximal photochemical efficiency of PSII (F_v/F_m), and growth parameters under DS. The current study will not only add more information on the role of Pro-Ca in fighting against DS but also supply the scientific basis for further application in wheat cultivation under DS.

MATERIAL AND METHODS

Plant material and treatments. Seeds of wheat cultivar Bainong 207, one important winter wheat cultivar in China, were supplied by the breeder Prof. Ou. Seeds with plump kernels and similar sizes were selected. After surface sterilisation with 10% sodium hypochlorite, seeds were soaked in a 25 °C incubator for 24 h. After the above treatment, seeds were transfered into Petri dishes with a diameter of 90 mm and germinated on moist filter paper. Then, all Petri dishes were transferred into an incubator to culture wheat seedlings. The growth conditions were as follows: 25 °C/15 °C day/night temperature, 500 μmol/m^2/s photosynthetic active radiation and a 10-h photoperiod. After the seedlings expanded the first leaves fully, the roots were submerged in half-strength Hoagland's solution contained in plastic boxes, and the roots were under dark conditions. Hoagland's nutrient solution was replaced every two days. To uncover the effect of DS, the seedlings (BBCH 13 stage) were chosen and transfered into beakers containing 200 mL 10% polyethylene glycol (PEG) solution under the same growth conditions as described above. Meanwhile, all roots were still under drak conditions during the whole period of the experiment.

To uncover the effects of different Pro-Ca concentrations, three groups of seedlings (BBCH 13 stage) were respectively treated with 50, 100 and 200 mg/L Pro-Ca for 10 h and then treated by DS or Hoagland's solution for 10 days under the same growth conditions as above. Hoagland's solution alone treated control seedlings (BBCH 13 stage) under the same growth conditions as above. For each treatment, there were three replications. Each replication per treatment includes 13 seedlings. After 5 days of treatment, the third leaves were sampled, frozen and then kept at –80 °C until the analysis for various indexes, including antioxidant enzymes, AsA and GSH contents, MDA content and EL, RWC, SP, SS and Pro contents, T_r, g_s, P_n, F_v/F_m, and *Chl* and Car contents. After 10 days of treatment, growth parameters were measured.

Assay of antioxidant enzymes. According to Shan and Zhao (2015), SOD, POD and CAT activities were

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assessed, and one enzyme activity unit of SOD was defined. Ascorbate peroxidase (APX) activity was assessed according to Zheng and Guo (2018). One enzyme activity unit of POD and APX was defined as the change of 0.01 in the absorbance value per minute. One enzyme activity unit of CAT was defined as the change of 0.001 per minute in the absorbance value. For SOD, POD, APX and CAT, the absorbance value was respectively recorded at 560, 470, 290 and 240 nm. All absorbance values were recorded by spectrophotometry. The final activity level of these antioxidant enzymes was represented as U/g fresh weight (FW).

Assay of AsA and GSH. AsA content was assessed according to the reduction of Fe^{3+} (Hodges et al. 1996). Samples were ground in 6% trichloroacetic acid and then centrifuged. The supernatant was added to the reaction mixture and incubated at 42 °C for 60 min. Then, the absorbance value at 525 nm was recorded by spectrophotometry. GSH content was assessed using GR and 2-vinylpyridine, according to Griffith (1980), and the absorbance value at 412 nm was recorded by spectrophotometry.

Assay of MDA and EL. MDA content was assessed using the thiobarbituric acid method by spectrophotometry (Hodges et al. 1999). EL were assessed according to Zhao et al. (2004). Samples were punched into small discs of uniform size. For each treatment, an equal amount of discs were immersed in the same volume of distilled water at room temperature for 3 h. Then, the above-distilled water containing leaf discs was boiled. The electrical conductivity was detected before and after boiling and recorded as EC_1 and EC_2, respectively. EL was represented as the percentage of the ratio between EC_1 and EC_2.

Assay of RWC and osmolytes. RWC was assessed using the following equation (Hou et al. 2018). RWC = [(fresh weight (FW) – dry weight (DW))/(saturated weight (SW) – DW)] × 100%. SP content was assessed using the coomassie blue staining according to Brafford (1976), and the absorbance value at 595 nm was recorded by spectrophotometry. SS content was assessed using the anthraquinone colourimetric method according to Smith et al. (1983), and the absorbance value at 620 nm was recorded by spectrophotometry. Pro content was assessed using the acid ninhydrin method reported by Bates et al. (1973), and the absorbance value at 520 nm was recorded by spectrophotometry.

Assay of T_r, g_s, Ls and WUE. Transpiration rate, stomatal conductance and stomatal limitation value (Ls) were measured by using the photosynthesis system (Licor-6400, Lincoln, USA) while measuring P_n. Water use efficiency (WUE) was calculated as the ratio of P_n to T_r.

Assay of *Chl*, Car, P_n and F_v/F_m. *Chl* and Car contents were assessed according to Song et al. (2016). P_n was measured by using the Licor-6400 photosynthesis system. F_v/F_m was measured after dark adaptation for 30 min through a PAM-2500 chlorophyll fluorometer (Effeltrich, Germany).

Assay of growth parameters. The natural vertical height of aboveground parts of wheat seedlings was measured by a ruler and recorded as wheat height. Plant biomass was assessed according to Zhao et al.

Table 1. Effects of different prohexadione calcium (Pro-Ca) concentrations on malondialdehyde (MDA) content, relative water content (RWC), photosynthetic rate (P_n) and plant height

Treatment	MDA (nmol/g FW)	RWC (%)	P_n (μmol/m^2/s)	Plant height (cm)
Control	6.90 ± 0.29^{e}	88.3 ± 1.86^{b}	17.5 ± 0.47^{d}	23.6 ± 0.46^{a}
DS	11.33 ± 0.66^{a}	78.0 ± 1.14	12.0 ± 0.31^{h}	17.3 ± 0.31^{d}
50 Pro-Ca	6.08 ± 0.27^{f}	90.2 ± 1.80^{ab}	18.6 ± 0.40^{c}	23.3 ± 0.52^{a}
100 Pro-Ca	5.10 ± 0.23^{g}	93.0 ± 2.10^{a}	20.9 ± 0.64^{a}	22.9 ± 0.44^{a}
200 Pro-Ca	5.74 ± 0.22^{f}	91.8 ± 1.73^{ab}	19.7 ± 0.50^{b}	22.7 ± 0.40^{a}
DS + 50 Pro-Ca	10.02 ± 0.48^{b}	80.7 ± 1.20^{d}	13.1 ± 0.37^{g}	18.2 ± 0.42^{c}
DS + 100 Pro-Ca	8.75 ± 0.41^{d}	84.9 ± 1.20^{c}	15.2 ± 0.40^{e}	20.0 ± 0.54^{b}
DS + 200 Pro-Ca	9.80 ± 0.49^{c}	82.5 ± 1.04^{d}	14.3 ± 0.39^{f}	19.3 ± 0.50^{bc}

Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; DS – 10% PEG (polyethylene glycol); 50 Pro-Ca – 50 mg/L Pro-Ca; 100 Pro-Ca – 100 mg/L Pro-Ca; 200 Pro-Ca – 200 mg/L Pro-Ca; DS + 50 Pro-Ca – 10% PEG + 50 mg/L Pro-Ca; DS + 100 Pro-Ca – 10% PEG + 100 mg/L Pro-Ca; DS + 200 Pro-Ca – 10% PEG + 200 mg/L Pro-Ca. Wheat plants were treated by Pro-Ca for 10 h and then treated with 10% PEG or Hoagland's solution for 5 days to measure MDA content, RWC and P_n and for 10 days to measure plant height

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Table 2. Effects of prohexadione calcium (Pro-Ca) on the activities of antioxidant enzymes

Treatment	SOD	POD	APX	CAT
	(U/g FW)			
Control	33.7 ± 1.10^{d}	13.2 ± 0.50^{d}	2.0 ± 0.12^{d}	3.3 ± 0.16^{d}
Pro-Ca	40.6 ± 1.40^{c}	16.4 ± 0.63^{c}	3.7 ± 0.20^{c}	4.5 ± 0.24^{c}
DS	45.4 ± 2.14^{b}	19.7 ± 0.74^{b}	4.6 ± 0.22^{b}	5.5 ± 0.22^{b}
DS + Pro-Ca	54.9 ± 2.10^{a}	25.9 ± 1.140^{a}	6.9 ± 0.38^{a}	7.8 ± 0.30^{a}

Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS – 10% PEG; DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days to measure these indicators; SOD – superoxide dismutase; POD – peroxidase; APX – ascorbate peroxidase; CAT – catalase; FW – fresh weight

(2023). Briefly, every seedling was harvested when the experiment was finished and dried to the constant weight in an 80 °C oven. Then, the dry mass was recorded as the biomass.

Statistical analysis. All data were the mean value of three replications. The values were compared by one-way analysis of variance (ANOVA) through version 22.0 of the statistical analysis software SPSS (IBM, Chicago, USA). The significance of the differences between treatments was determined using Duncan's multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Selection of optimal Pro-Ca concentration. DS significantly increased MDA content and decreased RWC, P_n and wheat height (Table 1). Compared to DS, different Pro-Ca concentrations significantly reduced MDA content and increased RWC, P_n and plant height under DS, especially for 100 mg/L Pro-Ca. Meanwhile, compared to control, different concentrations of Pro-Ca alone also decreased MDA content and increased RWC and P_n, especially for 100 mg/L Pro-Ca. Pro-Ca showed no obvious effect on plant height compared to the control. Therefore, 100 mg/L Pro-Ca was the optimal treatment concentration to explore the effect of Pro-Ca on wheat tolerance to DS.

Effects of Pro-Ca on antioxidant enzymes. Table 2 showed that DS increased SOD, POD, CAT and APX activities. Pro-Ca plus DS further reinforced these enzymes, compared to DS alone. In contrast with DS, Pro-Ca plus DS respectively increased SOD, POD, CAT and APX activities to 54.9, 25.9, 7.8 and 6.9 U/g FW. Compared to the control, Pro-Ca alone also enhanced the above antioxidant enzymes. Plant antioxidant capacity has a close relationship with the activities of antioxidant enzymes. Therefore, it is an effective measurement to enhance plant stress

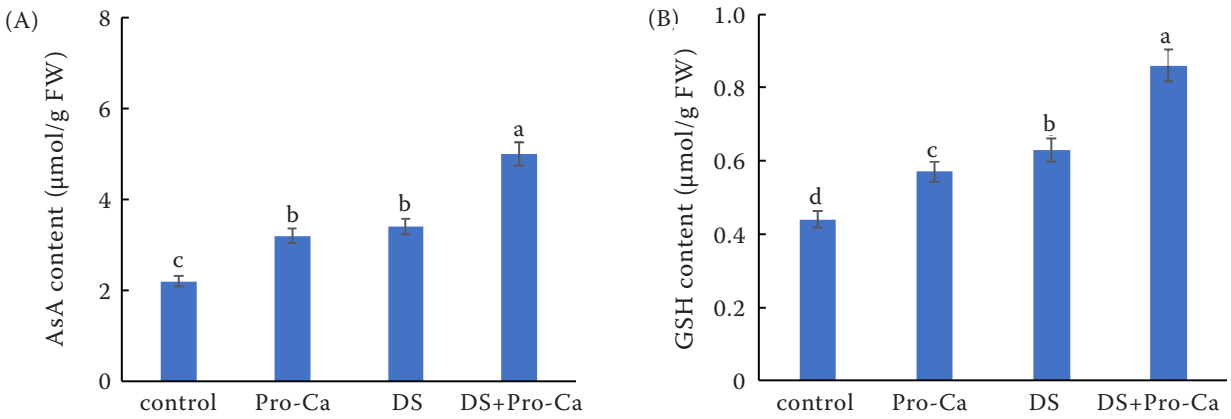

Figure 1. Effects of prohexadione calcium (Pro-Ca) on ascorbic acid (AsA) and reduced glutathione (GSH) contents. Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days to measure these indicators; FW – fresh weight

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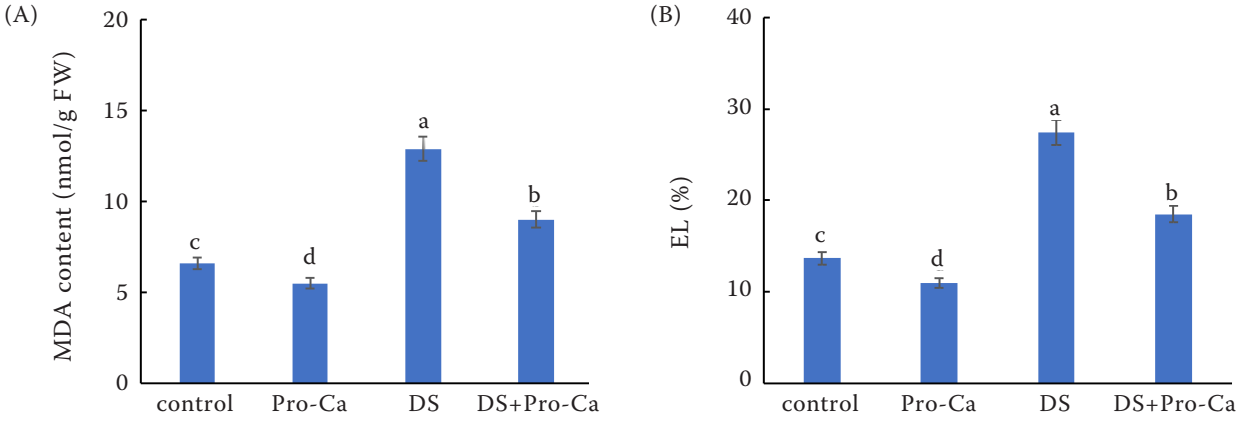

Figure 2. Effects of prohexadione calcium (Pro-Ca) on malondialdehyde (MDA) content and electrolyte leakage (EL). Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days to measure these indicators; FW – fresh weight

tolerance by modulating antioxidant enzymes. This study demonstrated that Pro-Ca enhanced SOD, POD, CAT and APX activities of wheat plants exposed to DS (Table 2). Current findings implied that Pro-Ca strengthened the antioxidant capacity of wheat plants by enhancing these antioxidant enzymes under DS.

Effects of Pro-Ca on AsA and GSH contents. DS enhanced AsA and GSH accumulation (Figure 1). Compared to DS, Pro-Ca further increased the accumulation of the above antioxidants under DS. In contrast with DS, Pro-Ca plus DS induced AsA and GSH contents up to 5.0 and 0.86 μmol/g FW, respectively. Compared to the control, Pro-Ca alone also increased above antioxidants. The above findings implied that Pro-Ca improved the antioxidant capacity by promoting antioxidants AsA and GSH accumulation under DS.

Plant antioxidant capacity is closely related to the contents of antioxidants. Therefore, it is also an effective measurement to enhance plant stress tolerance by modulating antioxidant contents. This study demonstrated that Pro-Ca improved AsA and GSH contents under DS (Figure 1). Current results implied that Pro-Ca strengthened the antioxidant capacity of wheat plants by increasing AsA and GSH contents under DS. In previous studies, it has been uncovered that AsA and GSH contents could be modulated by APX, GR, DHAR, MDHAR, GalLDH and γ-ECS. Whereas this study only explored the influence of Pro-Ca on APX activity. Thus, it is interesting to explore the influence of Pro-Ca on other enzymes responsible for AsA and GSH recycling and biosynthesis, which will add more new information for the physiological mechanism of Pro-Ca in modulating the antioxidant capacity under DS.

Effects of Pro-Ca on MDA and EL. DS raised MDA content and EL (Figure 2). In contrast with DS, Pro-Ca plus DS significantly decreased MDA

Table 3. Effects of prohexadione calcium (Pro-Ca) on relative water content (RWC) and osmolytes

Treatment	RWC (%)	SP (mg/g FW)	SS (mg/g FW)	Pro (μg/g FW)
Control	91.0 ± 1.60^{b}	150.2 ± 6.53^{d}	320.3 ± 13.90^{d}	40.6 ± 1.84^{d}
Pro-Ca	94.5 ± 1.44^{a}	202.7 ± 8.44^{c}	400.5 ± 17.74^{c}	60.5 ± 2.27^{c}
DS	81.0 ± 1.10^{d}	239.0 ± 9.27^{b}	465.0 ± 20.26^{b}	71.3 ± 3.15^{b}
DS + Pro-Ca	87.8 ± 1.25^{c}	295.4 ± 11.25^{a}	647.7 ± 28.14^{a}	109.8 ± 4.41^{a}

Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days; SP – soluble protein; SS – soluble sugars; Pro – proline; FW – fresh weight

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content and EL in wheat seedlings. In contrast with DS, Pro-Ca plus DS decreased MDA content and EL to 9.0 nmol/g FW and 18.5%, respectively. Compared with the control, Pro-Ca alone decreased the values of these indicators, too. The above results implied that Pro-Ca was vital in reinforcing wheat DS tolerance. As reported, Pro-Ca could mitigate the postharvest chilling injury of tomato fruit by reducing MDA content and EL (Soleimani Aghdam 2013). This study uncovered that Pro-Ca reduced MDA content and EL (Figure 2), which suggested that Pro-Ca may also mitigate DS-induced injury to wheat plants. The results of the current study and previous studies all indicated that Pro-Ca may mitigate stress-induced injury to plants.

Effects of Pro-Ca on RWC and osmolytes. In contrast with the control, DS significantly decreased RWC (Table 3). To fight against DS, wheat plants significantly enhanced the ability to regulate osmosis by increasing SP, SS, and Pro contents. In contrast with DS, Pro-Ca significantly promoted the accumulation of SP, SS and Pro under DS. Compared to DS, Pro-Ca plus DS increased SP, SS and Pro contents to 295.4 mg/g FW, 647.7 mg/g FW and 109.8 μg/g FW, which induced RWC up to 87.8%. Pro-Ca alone also improved SP, SS, Pro contents, and RWC more than the control. The present findings implied that Pro-Ca reinforced the ability to regulate osmosis by promoting the accumulation of osmolytes in wheat plants under DS.

Water balance is important for plants in fighting against DS. Plants can promote the accumulation of osmolytes, enhancing the ability to regulate osmosis and water balance. Under saline-alkali stress, Pro-Ca enhanced soybean water balance by improving osmotic regulatory substances SS and Pro contents (Feng et al. 2021). This study uncovered that Pro-Ca increased osmotic regulatory substances SS and Pro contents of wheat seedlings under DS (Table 3), which was consistent with the influence of Pro-Ca on SS and Pro in soybeans exposed to saline-alkali stress (Feng et al. 2021). I also discovered that Pro-Ca increased the osmotic regulatory substance SP content and RWC (Table 3). As saline-alkali stress and DS can all induce osmotic stress in plants, the above results of Feng et al. (2021) and mine indicated that Pro-Ca could enhance the osmotic regulation ability, maintaining the water balance indicated by RWC.

Effects of Pro-Ca on T_r, g_s, Ls and WUE. In contrast with control, DS significantly reduced T_r and g_s and increased Ls and WUE (Table 4). In contrast with DS, Pro-Ca plus DS significantly decreased Ls and increased T_r and g_s but did not obviously affect WUE. Compared to DS, Pro-Ca plus DS decreased Ls to 0.33 and respectively increased T_r and g_s to 3.48 mmol/m^2/s and 0.25 mol/m^2/s. In contrast with control, Pro-Ca alone also decreased Ls and increased T_r and g_s. These findings indicated that Pro-Ca was vital in maintaining the water metabolism under DS.

In plants, water balance is important to maintain the water metabolism. Under saline-alkali stress, Pro-Ca enhanced soybean water balance by improving ORS SS and Pro contents, raising T_r and g_s (Feng et al. 2021). This study uncovered that Pro-Ca enhanced wheat water balance by increasing ORS SS, SP and Pro contents under DS (Table 3) and increasing T_r and g_s (Table 4). As saline-alkali stress and DS can induce osmotic stress in plants, the above findings of Feng et al. (2021) and mine all implied that Pro-Ca enhanced water balance through osmotic regulation, which enhanced water metabolism. Additionally, current research showed that Pro-Ca decreased Ls but did not obviously affect WUE under DS (Table 4). Thus, my findings proved that Pro-Ca could main-

Table 4. Effects of prohexadione calcium (Pro-Ca) on transpiration rate (T_r), stomatal conductance (g_s), stomatal limitation value (Ls) and water use efficiency (WUE)

Treatment	T_r (mmol/m^2/s)	g_s (mol/m^2/s)	Ls	WUE (μmol/mmol)
Control	4.45 ± 0.18^b	0.30 ± 0.02^b	0.30 ± 0.01^c	4.04 ± 0.15^b
Pro-Ca	5.10 ± 0.17^a	0.36 ± 0.02^a	0.26 ± 0.01^d	4.07 ± 0.10^b
DS	2.80 ± 0.13^d	0.18 ± 0.01^d	0.39 ± 0.02^a	4.53 ± 0.17^a
DS + Pro-Ca	3.48 ± 0.16^c	0.25 ± 0.01^c	0.33 ± 0.01^b	4.59 ± 0.12^a

Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days

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Table 5. Effects of prohexadione calcium (Pro-Ca) on photosynthetic parameters

Treatment	*Chl* (mg/g FW)	Car (mg/g FW)	P_n (μmol/m^2/s)	F_v/F_m
Control	2.00 ± 0.09^b	0.48 ± 0.01^b	18.0 ± 0.84^b	0.77 ± 0.03^b
Pro-Ca	2.26 ± 0.10^a	0.60 ± 0.02^a	20.8 ± 0.80^a	0.85 ± 0.04^a
DS	1.40 ± 0.05^d	0.27 ± 0.01^d	12.7 ± 0.51^d	0.56 ± 0.02^d
DS + Pro-Ca	1.79 ± 0.08^c	0.40 ± 0.01^c	16.0 ± 0.66^c	0.68 ± 0.03^c

Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 5 days; *Chl* – chlorophyll; Car – carotenoid; P_n – photosynthetic rate; F_v/F_m – maximum photochemical efficiency of PSII; FW – fresh weight

tain the water metabolism by improving the osmotic regulation ability.

Effects of Pro-Ca on photosynthetic parameters. DS significantly decreased P_n, F_v/F_m, *Chl* and Car contents (Table 5). Compared to DS, Pro-Ca decreased above four indicators. Compared to DS, Pro-Ca plus DS increased P_n, F_v/F_m, *Chl* and Car contents to 16.0 μmol/m^2/s, 0.68, 1.79 mg/g FW and 0.40 mg/g FW, respectively. In contrast with the control, Pro-Ca raised these photosynthetic parameters, too. These findings further implied that Pro-Ca had vital roles in reinforcing the photosynthetic capacity of wheat plants under DS.

DS significantly inhibits plant photosynthetic performance. DS reduced *Chl* and Car contents and P_n regarding wheat (Ning et al. 2023, Jiang et al. 2024). Current research found that Pro-Ca increased *Chl* and Car contents under DS (Table 5). As *Chl* and Car play a major role in light absorption and utilisation, my results proved that Pro-Ca promoted light absorption and utilisation by increasing pigment contents, thereby increasing P_n (Table 5). Besides, F_v/F_m is another important indicator for evaluating plant photosynthetic capacity. F_v/F_m represents the maximum photochemical efficiency of PSII. This research proved that Pro-Ca raised F_v/F_m of seedlings under DS (Table 5), which suggested that Pro-Ca reinforced the photochemical efficiency of PSII under DS. Based on these results, the current study clearly proved that Pro-Ca reinforced wheat photosynthesis under DS.

Effects of Pro-Ca on wheat growth parameters. In contrast with the control, DS decreased the height and biomass (Figure 3). In contrast with DS, Pro-Ca plus DS improved these indicators. Compared with DS, Pro-Ca plus DS increased the height and biomass to 20.1 cm and 135.9 mg/plant, respectively. Compared to the control, Pro-Ca alone also increased plant biomass but had no obvious effect on plant height. The above results further indicated that Pro-Ca had vital roles in reinforcing wheat DS tolerance.

Plant growth is closely related to photosynthesis. The present study proved that Pro-Ca improved the

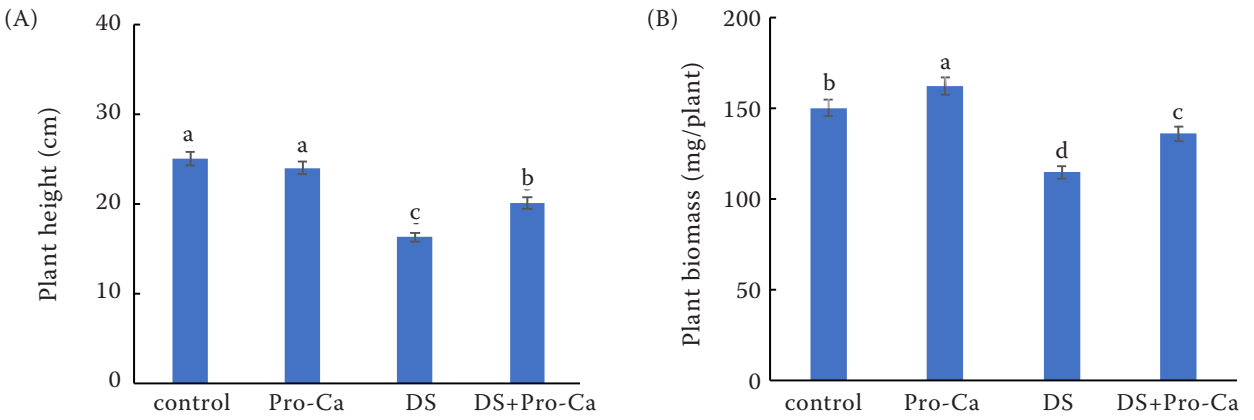

Figure 3. Effects of prohexadione calcium (Pro-Ca) on wheat growth parameters. Wheat plants were respectively subjected to the following treatment: Control – Hoagland's solution; Pro-Ca – 100 mg/L Pro-Ca; DS (drought stress) – 10% PEG (polyethylene glycol); DS + Pro-Ca – 10% PEG + 100 mg/L Pro-Ca. Wheat plants were treated with Pro-Ca for 10 h and then treated with DS or Hoagland's solution for 10 days

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photosynthetic capacity, increasing plant height and biomass under DS (Figure 3). However, current research showed that Pro-Ca significantly impacted plant height under DS but had no obvious influence on plant height under normal conditions (Figure 3). As reported, Pro-Ca had a major role in modulating endogenous hormones gibberellin (GA) and ABA levels in apple tree shoots (Wang 2021). Meanwhile, GA may promote plant shoot growth, and ABA can protect plants against various stresses. Thus, the difference in the effect of Pro-Ca on plant height under normal and DS was highly likely to be related to endogenous hormone levels of plants regulated by it. In further study, it is important to explore the influence of Pro-Ca on endogenous hormone levels of wheat plants under DS, which will add new insights into the regulatory mechanism of Pro-Ca in reinforcing DS tolerance.

In conclusion, the present research clearly indicated that Pro-Ca regulated wheat DS tolerance by enhancing antioxidant capacity, maintaining water balance and promoting photosynthesis. My findings added new insights into the function of Pro-Ca in modulating DS tolerance and supplied the foundation for Pro-Ca application in further actual wheat cultivation under water deficit.

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