



# Drought priming alleviated salinity stress and improved water use efficiency of wheat plants

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## Abstract

Global warming and salinization are inducing adverse effects on crop yield. Drought priming has been proved to improve drought tolerance of plants at later growth stages, however, whether and how drought priming at early growth stage alleviating salinity stress at later growth stage and improving water use efficiency (WUE) of plants remains unknown. Therefore, two wheat cultivars were subjected to drought priming at the 4th and 6th leaf stage and subsequent moderate salinity stress at 100 mmol NaCl applied at the later jointing growth stage. The growth, physiological responses, ABA signaling and WUE were investigated to unravel the regulating mechanisms of drought priming on subsequent salinity stress. The results showed that drought priming imposed at the early growth stage improved the leaf and root water potential while attenuated the ABA concentration in the leaves ( $[ABA]_{\text{leaf}}$ ) for the primed plants, which increased the stomatal conductance ( $g_s$ ) and photosynthesis ( $P_n$ ). Consequently, the biomass under the salinity stress was significantly increased due to earlier drought priming. Moreover, drought priming improved the specific leaf N content due to the facilitated root growth and morphology, and this could benefit high leaf photosynthetic capacity during the salinity stress period, improving the  $P_n$  and water uptake for the primed plants. Drought priming significantly improved plant level WUE ( $WUE_p$ ) due to considerably enhanced dry biomass compared with non-primed plants under subsequent salinity stress. The significantly increased leaf  $\delta^{13}C$  under drought priming further demonstrated that the improved leaf  $\delta^{13}C$  and  $WUE_p$  was mainly ascribed to the improvement of  $P_n$ . Drought primed plants significantly improved  $K^+$  concentration and maintained the  $K^+/Na^+$  ratio compared with non-primed plants under subsequent salinity stress, which could mitigate the adverse effects of excess  $Na^+$  and minimize salt-induced ionic toxicity by improving salt tolerance for primed plants. Therefore, drought priming at early growth stage could be considered as a promising strategy for salt-prone areas to optimize agricultural sustainability and food security under changing climatic conditions.

**Keywords** *Triticum aestivum* L. · Salinity tolerance · Hormones ·  $\delta^{13}C$  · ABA · Water stress

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## Introduction

Drought and salinity are the major causes for inhibited plant growth and development, which are becoming the major threats for wheat cultivation in many regions of the world (Farooq et al. 2014; Wang et al. 2018; Zhang et al. 2016; Isayenkov 2019). Wheat (*Triticum aestivum* L.) is moderately salinity tolerant, covering around 32% of the global region under cereal cultivation and providing food to over 35% of the worldwide population (FAO 2015). Soil salinity can reduce its grain yield by about 60% (El-Hendawy et al. 2017). Globally, more than 45 million hectares of irrigated lands have been interrupted, and every year around 1.5 million hectares are taken out from production due to high soil

salinity (Munns and Tester 2008; Robin et al. 2016). The coincidence of drought and salinity always exists in field condition, which can lead to multiple abiotic stresses for plants.

Drought stress always causes stomatal closing, which limits the diffusion of  $\text{CO}_2$  to the leaf, leading to a reduction in carbon assimilation and other photosynthetic processes (Guan et al. 2015; Voeselek and Sasidharan 2013). Abscisic acid (ABA) maintains stomatal closure under water deficit (Finkelstein et al. 2002), which plays a vital role in reducing abiotic stress oxidative disruption (Larkindale and Knight 2002). Besides, higher transpiration leads to more water loss and a decrease of leaf water potential ( $\psi_1$ ), and this could be prevented by closing of stomata, which prevents water vapor and  $\text{CO}_2$  transport and ultimately reduces photosynthesis and transpiration (Nishida et al. 2009). Salt stress influences physiological processes from germination to plant development, leading to less growth and yield due to decreased leaf area (Arif et al. 2019) and changed photosynthetic light phase (Qiu et al. 2003) by triggering ionic and osmotic stress (Ashraf 2004). Physiological changes under salinity stress include biochemical imbalance and low soil water capacity due to excess  $\text{Na}^+$  and  $\text{Cl}^-$  ions (Villalta et al. 2008). Soil salinity may restrict  $\text{K}^+$  uptake due to the major interaction with  $\text{Na}^+$  at the root surface. Plant's salt tolerance depends on their ability to maintain  $\text{K}^+/\text{Na}^+$  homeostasis, with low level of  $\text{Na}^+$  while high level of  $\text{K}^+$ .  $\text{K}^+$  acts as a major agent that can alleviate  $\text{Na}^+$  stress, thereby the ability of plants to survival salinity is heavily dependent on their  $\text{K}^+$  ion (Ahanger et al. 2019). Therefore, to lessen the adverse effects of  $\text{Na}^+$ , plants tend to decrease salt-induced ionic toxicity to improve salinity tolerance by lowering the level of  $\text{Na}^+$  in the photosynthesis system (James et al. 2006) and enhancing  $\text{K}^+$  levels, leading to increased  $\text{K}^+/\text{Na}^+$  ratio (Ahanger et al. 2019; Shabala and Cuin 2008). It has been proposed that a high cytosolic  $\text{K}^+/\text{Na}^+$  ratio in the cytoplasm is required for plant survival during salt stress. Roots are important in plant growth and grain yield, as they provide anchoring for plants and aid in nutrient and water uptake (White and Brown 2010). The acquisition of sufficient water and nutrient resources as a result of better root system results in a larger leaf area with improved photosynthesis, consequently increasing crop production (Farooq et al. 2012; Imran et al. 2013).

Multiple studies have reported that priming has significant implications in physiology of crop stress by triggering a quicker and stronger activation of defense mechanisms faced with subsequent stress occurrences (Abid et al. 2016, 2018; Backhaus et al. 2014; Banik et al. 2016; Bruce et al. 2007; Chen and Arora 2013; Pastor et al. 2013). Cui et al. (2019) noticed that drought primed plants at the tillering stage improved photosynthesis, leaf water potential, biomass and grain yield. Abid et al. (2016) reported that pre-drought priming during the vegetative growth stage showed

high potential to tolerate drought stress through improved leaf water potential, chlorophyll content, leaf gas exchange and enzymatic antioxidant system, leading to a less yield loss. Pre-drought primed plants sustained lower ABA levels during early grain filling stages, which led to a greater dry weight and grain yield compared to non-primed plants (Abid et al. 2017). Walter et al. (2011) found that drought priming enhanced biomass production and photoprotection in *Arrhenatherum elatius* under later drought stress.

Previous studies have demonstrated that drought priming at early growth stage could increase the drought tolerance of plants, but whether and how drought priming alleviating subsequent salinity stress at later growth stages remains unknown. It is hypothesized that drought priming can alleviate the salinity stress by improving leaf gas exchange and plant water status as well as modulation of plant hormones. Therefore, the objective of the present study was to assess whether and how drought priming at the early growth stage regulated plant growth, physiological responses, ABA signaling, plant water status and WUE at the later growth stages under subsequent salinity stress and unravel the underlying mechanisms for drought priming on subsequent salinity stress. The findings could be beneficial for understanding the physiological reactions of crops against multi-occurrence stresses that are essential for more accurate assessment of crop performance under climate changes.

## Materials and methods

### Experimental setup

The experiment was carried out in the glasshouse at Chinese Academy of Agricultural Sciences from April to August 2019. Two wheat cultivars (*Triticum aestivum* L. var. Heng0628 and XR4347) were cultivated in the pots with 22 cm height and 16 cm diameter. During the experimental period, the average day/night temperature was maintained at 27/22 °C and the density of photon flux ranged between 450 and 800  $\mu\text{molm}^{-2} \text{s}^{-1}$ . The seeds were sown on 15th April, 2019. The pots comprised 6.72 kg air-dried sandy loam soil with pH at 7.6, electrical conductivity (EC) at 128.1  $\mu\text{s cm}^{-1}$ , pot water holding capacity at 34.3%, permanent wilting point at 10.8% and soil bulk density at 1.2 g dry soil  $\text{cm}^{-3}$ . Sixteen surface-sterilized seeds were planted in free-draining pots and leaching was not observed during the experimental period. First thinning was carried out on 10 days after germination and six seedlings were removed per pot. After 7 days from first thinning, the second thinning was done and seven uniform seedlings were kept per pot. Fertilizer doses before seeding included 1.0 g N as the form of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 3.0 g P as mono-potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) and 3.0 g K as potassium

sulfate ( $K_2SO_4$ ) applied in each pot to avoid nutrient deficiency. Before the beginning of drought priming, the pots were watered to 85% of soil water holding capacity (SWHC).

## Experimental treatments

The experiment was a complete randomized design. The treatments consisted of two wheat cultivars, two drought priming levels (drought priming and non-drought priming) and two salinity levels (moderate and non-salinity). The plants were subjected to moderate drought stress at 60% SWHC for 6 days applied at the 4th and 6th leaf stage, respectively, as outlined in the experimental description (Table 1). After drought priming, the crops were instantly re-watered to 85% SWHC for recovery. The control pots continued to be irrigated at 85% SWHC throughout the growing period. Drought stress was maintained by weighing the pots and re-filling the water loss. Moderate salinity stress (100 mmol NaCl) was applied during the jointing growth stage after drought priming. During the experimental period, all the pots were rotated several times to minimize the impact of spatial exposures to the environment. To maintain soil water contents at the target levels, the experimental pots were evaluated daily.

## Sampling, measurement and analysis

Net photosynthetic rate ( $P_n$ ) and stomatal conductance ( $g_s$ ) of leaves were recorded weekly with photosynthetically active radiation at  $1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  and 400 ppm  $CO_2$  concentration using Li-6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE, USA) from 9:00 to 11:00 a.m. on upper completely expanded leaves. The intrinsic water use efficiency ( $WUE_i$ ) was computed as the ratio between net photosynthesis rate ( $P_n$ ) and stomatal conductance ( $g_s$ ).

**Table 1** The treatment design for the experiment. DP and NP indicate drought priming and non-drought priming. S0 and S1 denote non-salinity and salinity stress. WW represents well-watered condition

Growth stages	Tillering/4th leaf stage		Tillering/6th leaf stage		Jointing stage to harvest
	Priming	Recovery	Priming	Recovery	
Before priming					
WW	NP	WW	NP	WW	NPS0 NPS1
	DP		DP		DPS0 DPS1

The soil water content in the pots was controlled by weighing method. During the experimental period, the plant water use was estimated based on the amount of irrigation and soil water stored in the pots. The water used for irrigation was tap water with negligible quantities of nutrients. All the plants were harvested manually at the heading and flowering stage and the dry biomass was determined after oven drying at  $70^\circ\text{C}$  to constant weight. Plant water use efficiency ( $WUE_p$ ) was calculated as the ratio between the above ground dry biomass and plant water use during the experimental period.

The leaf area was measured using a leaf area meter (LI-3000 C, Li-Cor Inc., NE, USA). Root sections were tapped to remove adhering soil particles, briefly blotted with absorbent paper, and instantly put and secured within a plastic sample cup (< 10 s) for measurement of root water potential ( $\Psi_r$ ). For leaf water potential ( $\Psi_l$ ), the top fully extended leaf was separated from the plant in each pot. Then, it was instantly put inside a plastic cup and sealed. The time spent was less than 10 s from excision to sealing in the chamber. The  $\Psi_l$  and  $\Psi_r$  were determined by Dewpoint Potentiometer (WP4C, Meter Group Inc., WA, USA).

The dry leaves, stems and roots were ground and then digested in  $HNO_3$  by a Microwave Digestion System (CEM corporation, NC, USA). The  $Na^+$ ,  $K^+$  and  $Ca^{2+}$  concentrations were determined using the Atomic Absorption Spectrophotometer (AAS) (ICE3500, Thermo Fisher Scientific Inc., MA, USA).

At the end of the experiment, the upper-canopy fully expanded four leaves were sampled and frozen in liquid N immediately. In addition, a few of root segments (about 0.5 g) were excised from the root system in each pot, washed in tap water to remove adhering soil particles, blotted with absorbent paper, frozen in liquid N immediately. The leaf and root samples were stored at  $-80^\circ\text{C}$  before further analysis. The ABA concentration was determined by the indirect enzyme-linked immunosorbent assay (ELISA) (Yang 2001). Using an Elemental Analyser System (vario PYRO cube, Elementar Analysensysteme GmbH, Germany) combined with an Isotope Mass Spectrometer (Isoprime 100, Elementar Analysensysteme GmbH, Germany), the plant samples were determined for carbon isotopic composition ( $\delta^{13}\text{C}$ ). The carbon isotope composition was calculated as:

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample and the Pee Dee Belemnite standard, respectively.

All the roots in the pots were collected and washed with tap water to remove soil from root samples. Then the roots were preserved in 20% ethanol solution and scanned with a

root scanner (V800, Epson America Inc., CA, USA). The images were evaluated using WinRHIZO software (Regent Instruments Inc., Quebec, Canada).

## Statistical analysis

The data were statistically analyzed by SPSS 23.0 software (IBM corporation, New York, USA). The data during the drought priming period were compared using independent t-test between the drought priming and control treatments. The one-way analysis of variance (ANOVA) was performed to analyze the differences among the treatments at the significance level of 5%. Regression analyses were determined to assess the correlations between two parameters. Figures were made using OriginPro software (OriginLab Corporation, MA, USA) and Microsoft excel.

## Results

### Soil water dynamics

The daily average soil water content (SWC) for non-primed plants was kept around 23%, whereas the SWC for primed plants reduced from 23 to 14% for both cultivars for 6 days at the 4th and 6th leaf growth stage under drought priming (Fig. 1). The SWC was kept around 23% for all the plants during the water recovery period. The salinity stress was

applied at the jointing stage and the SWC of all the treatments was maintained at about 20%.

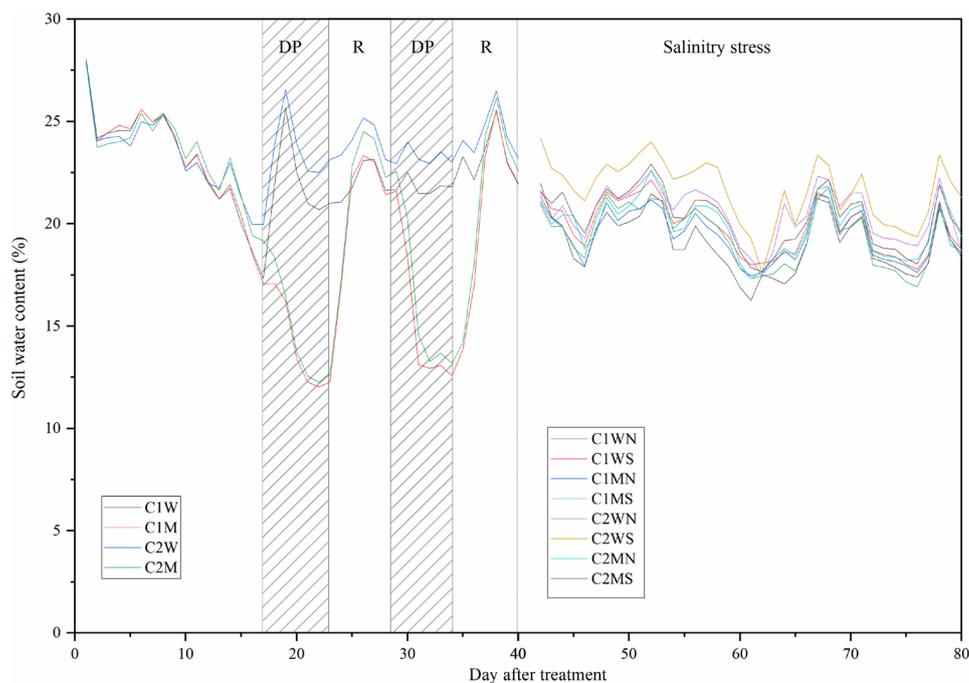
### Leaf gas exchange and intrinsic WUE

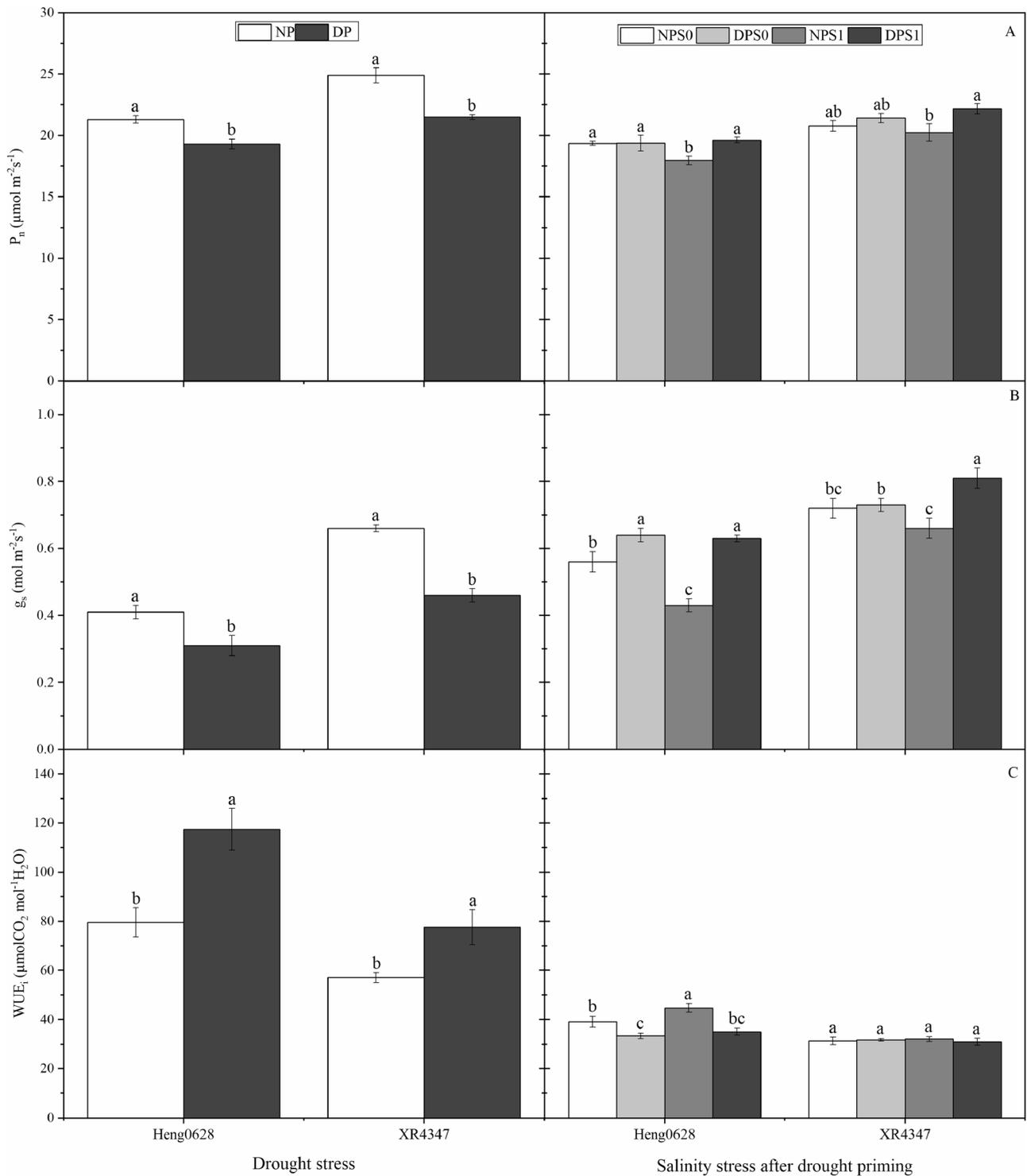
The moderate water stress during the drought priming period significantly decreased the  $P_n$  and  $g_s$  by about 9% and 26% in Heng0628 and 14% and 30% in XR4347 compared to the non-primed plants, respectively (Fig. 2). The  $WUE_i$  was significantly improved by 48% in Heng0628 and 36% in XR4347 cultivar under the drought stress. During the salinity stress period, significantly higher  $P_n$  and  $g_s$  were found for the primed plants than the non-primed plants. For the primed plants of Heng0628, the DPS1 increased the  $P_n$  and  $g_s$  by 9% and 47% while significantly reduced  $WUE_i$  by 14% compared with the NPS1 plants under the salinity stress. For XR4347, the DPS1 increased the  $P_n$  and  $g_s$  by about 10% and 23% while slightly decreased  $WUE_i$  by 4% compared to the NPS1 plants under the salinity stress.

### Leaf and root water potential

The drought primed plants under DPS1 significantly increased  $\Psi_1$  by 11% in Heng0628 and 13% in XR4347 compared with NPS1 plants under the salinity stress after drought priming (Fig. 3). Similarly, DPS1 treatment also

**Fig. 1** Changes of daily average soil water contents under drought priming (DP), recovery (R) and subsequent salinity stress period in two wheat cultivars. C1, C2, W, M, N and S denote Heng0628, XR4347, well-watered, moderate water stress, non-salinity and salinity stress. The shaded zones indicate the drought priming period



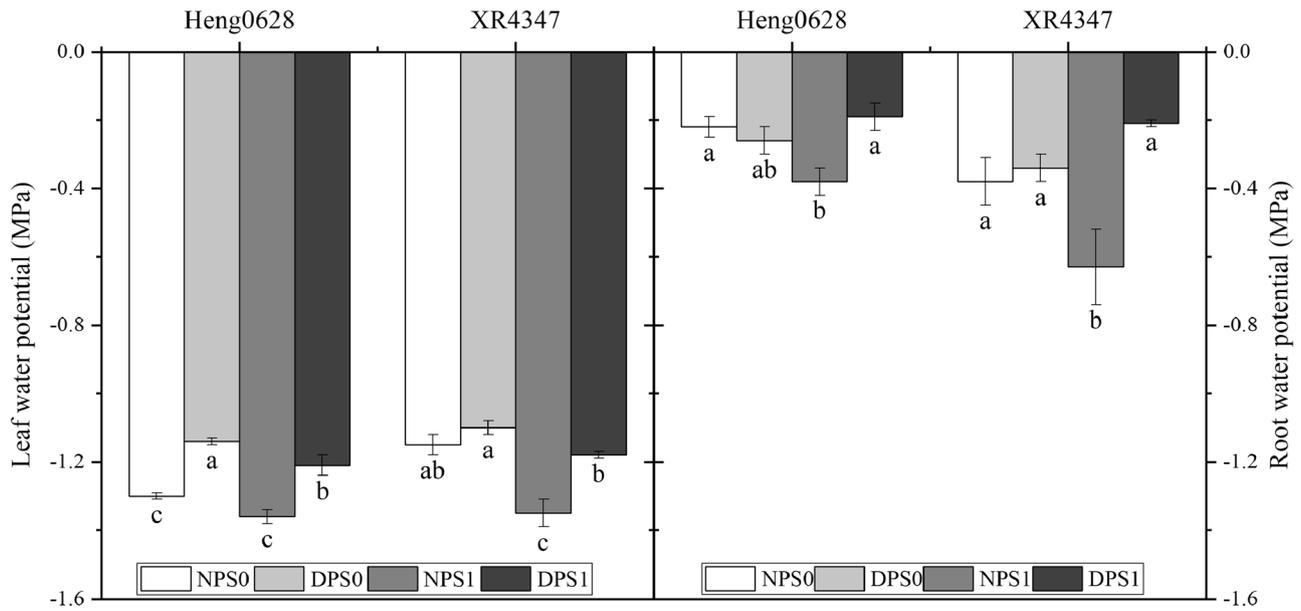


**Fig. 2** Effect of drought priming on **A** net photosynthetic rate ( $P_n$ ), **B** stomatal conductance ( $g_s$ ) and **C** intrinsic water use efficiency ( $WUE_i$ ) of wheat during drought priming and salinity stress. NP and

DP indicate non-priming and drought priming. S0 and S1 denote non-salinity and salinity stress. Difference letters above the columns indicate significant differences at  $P < 0.05$ . Error bars indicate SE ( $n = 4$ )

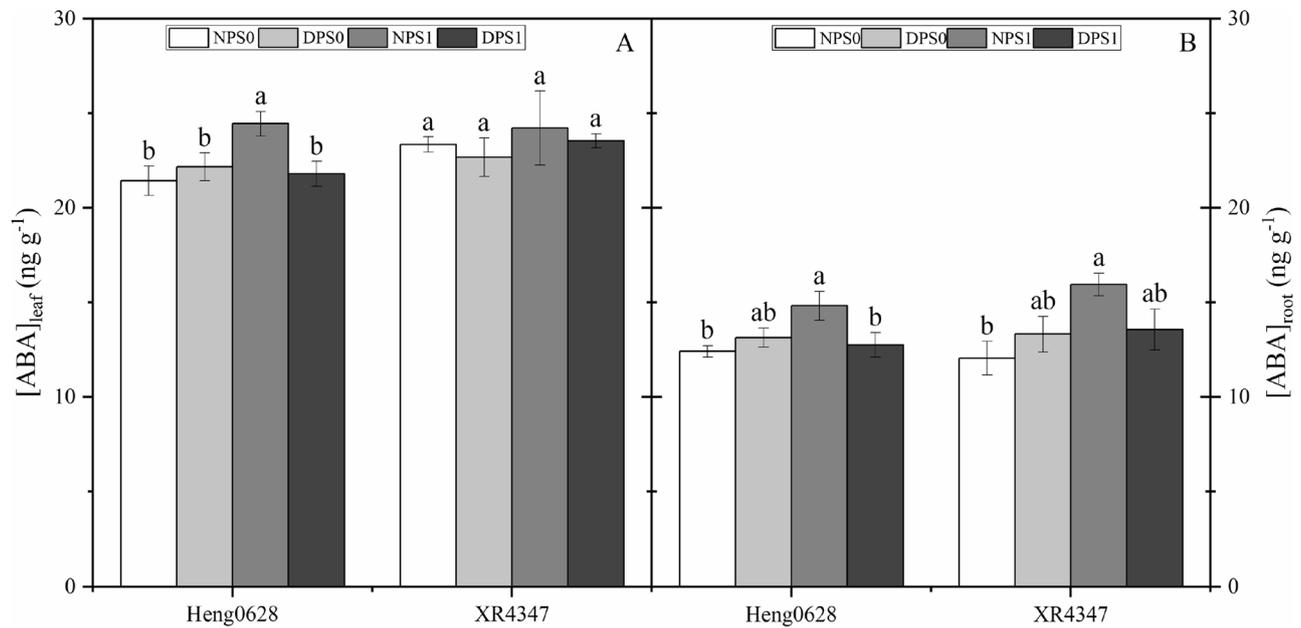
significantly improved the  $\Psi_r$  by 46% in Heng0628 and 66% in XR4347 cultivar compared with the NPS1 treatment. The  $\Psi_1$  under the DPS0 treatment slightly or significantly

increased, while the  $\Psi_r$  was similar compared with the NPS0 treatment.



**Fig. 3** Effect of drought priming on leaf and root water potential of wheat under subsequent salinity stress. NP and DP indicate non-priming and drought priming, respectively. S0 and S1 denote non-salinity

and salinity stress, respectively. Difference letters below the columns indicate significant differences at  $P < 0.05$ . Error bars indicate SE ( $n = 4$ )



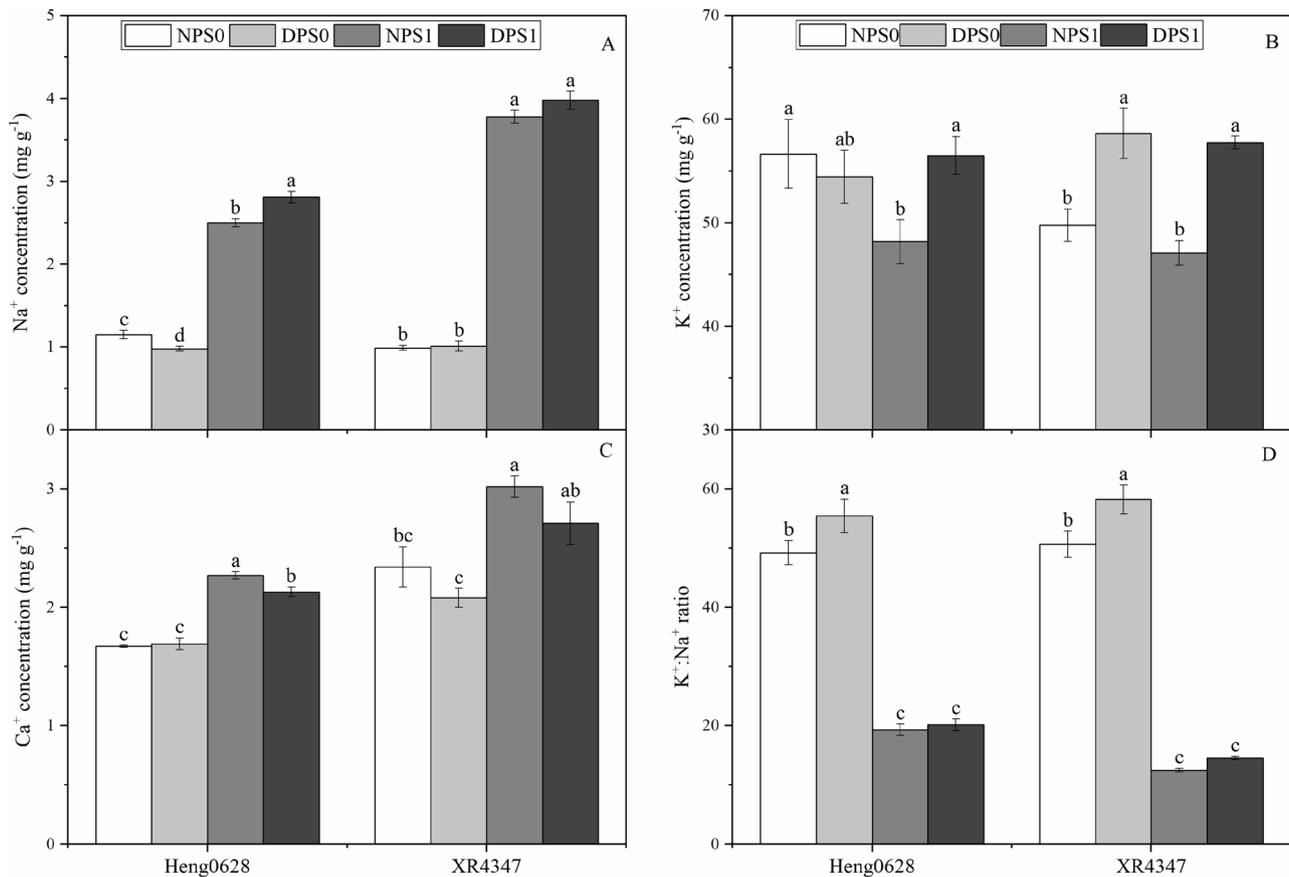
**Fig. 4** Effect of drought priming on **A**  $[ABA]_{leaf}$  and **B**  $[ABA]_{root}$  of wheat under subsequent salinity stress. NP and DP indicate non-priming and drought priming, respectively. S0 and S1 denote non-salinity

and salinity stress, respectively. Difference letters above the columns indicate significant differences at  $P < 0.05$ . Error bars indicate SE ( $n = 4$ )

**ABA concentrations in the leaf and root tissues**

The NPS1 treatment decreased the  $[ABA]_{leaf}$  and  $[ABA]_{root}$  compared with the NPS0 treatment under subsequent

salinity stress condition, and the differences were significant for Heng0628 (Fig. 4). However, the  $[ABA]_{leaf}$  and  $[ABA]_{root}$  were similar between the DPS0 and NPS0 treatment.



**Fig. 5** Effects of drought priming on **A** Na<sup>+</sup> concentration, **B** K<sup>+</sup> concentration **C** Ca<sup>2+</sup> concentration and **D** K<sup>+</sup>:Na<sup>+</sup> ratio of wheat under subsequent salinity stress. NP and DP indicate non-priming

and drought priming, respectively. S0 and S1 denote non-salinity and salinity stress, respectively. Difference letters above the columns indicate significant differences at  $P < 0.05$ . Error bars indicate SE ( $n = 4$ )

### Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> concentrations and K<sup>+</sup>:Na<sup>+</sup> ratio in the shoots

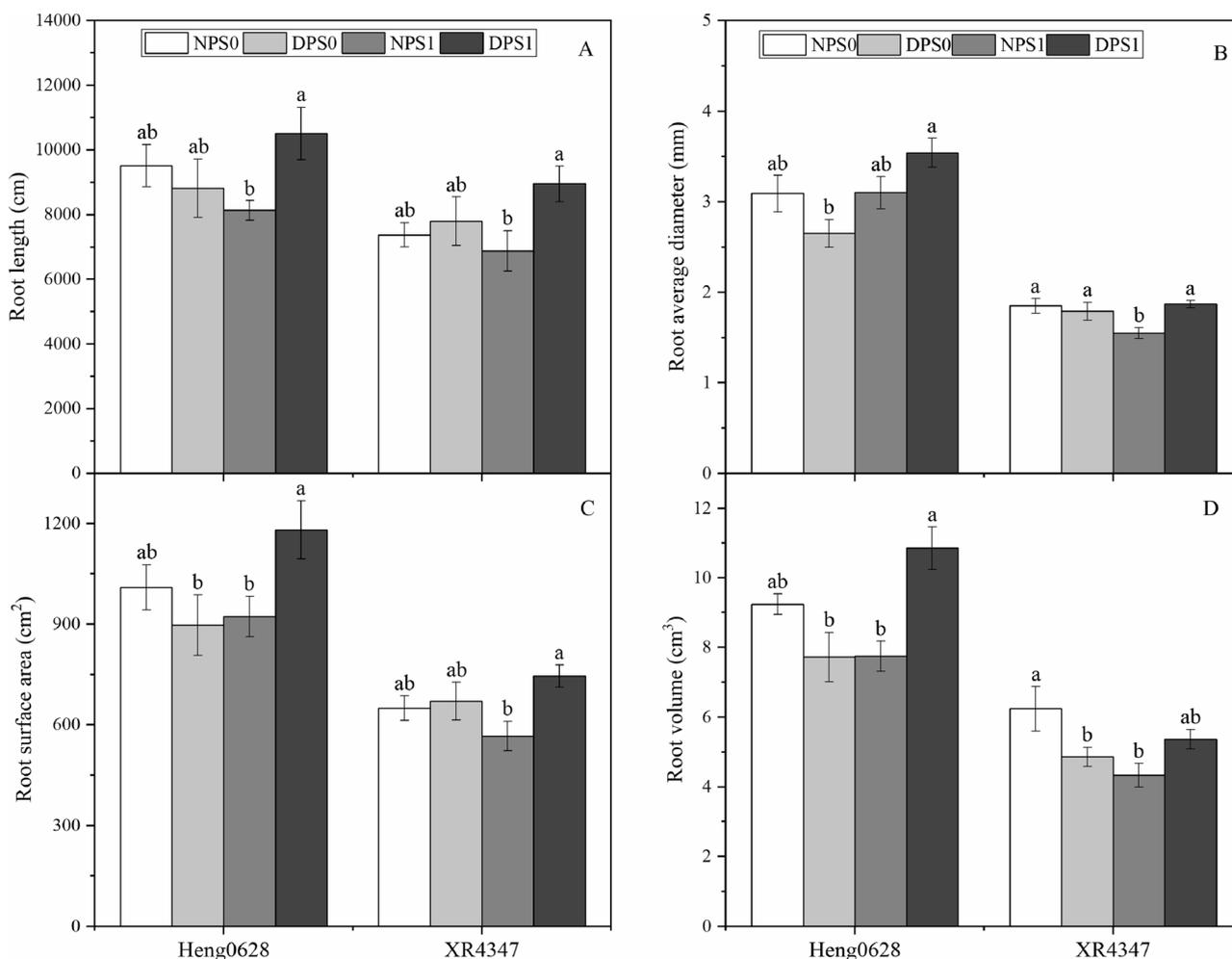
The Na<sup>+</sup> concentration in the NPS1 plants was significantly higher than the NPS0 plants (Fig. 5). The Na<sup>+</sup> concentration of the primed plants under the DPS1 treatment was significantly or slightly higher than that of the non-primed plants under the NPS1 treatment. The primed plants under the DPS1 treatments significantly increased K<sup>+</sup> concentration in both cultivars compared with the non-primed plants in the NPS1 treatment under the salinity stress. The Ca<sup>2+</sup> concentration in the primed plants under the DPS1 treatment decreased compared with the NPS1 plants under the salinity stress but increased compared to the NPS0 plants. The K<sup>+</sup>:Na<sup>+</sup> ratio of the primed plants under the DPS0 was significantly higher than that under the NPS0 treatment, whereas it was similar between the DPS1 and NPS1 treatment.

### Root morphology

The NPS1 treatment reduced the root length, diameter, area and volume compared with the NPS0 treatment (Fig. 6). The DPS1 treatment increased the root length, diameter, area and volume by 29, 14, 28 and 40%, respectively, in Heng0628, and elevated by 30, 21, 21 and 24%, respectively, in XR4347, compared with the non-primed NPS1 plants under the salinity stress.

### Leaf area, dry biomass, plant water use, WUE<sub>p</sub>, δ<sup>13</sup>C and specific leaf N content

The NPS1 treatment significantly decreased the leaf area, plant water use, δ<sup>13</sup>C and specific leaf N content compared with the NPS0 treatment (Table 2). For the primed plants, the DPS1 treatment improved leaf area by 2% in Heng0628 and 37% in XR4347 compared to the non-primed plants under the salinity stress. Similarly, the dry biomass in the DPS1 treatment was significantly improved compared to the NPS1 treatment under the salinity stress. Likewise,



**Fig. 6** Effects of drought priming on **A** root length, **B** root average diameter, **C** root surface area and **D** root volume of wheat under subsequent salinity stress. NP and DP indicate non-priming and drought

priming, respectively. S0 and S1 denote non-salinity and salinity stress, respectively. Difference letters above the columns indicate significant differences at  $P < 0.05$ . Error bars indicate SE (n = 4)

**Table 2** Effects of drought priming on dry biomass, plant water use, leaf area,  $WUE_p$ ,  $\delta^{13}C$  and specific leaf N content of wheat under subsequent salinity stress

Cultivar	Treatment	Leaf area (cm <sup>2</sup> pot <sup>-1</sup> )	Dry biomass (g pot <sup>-1</sup> )	Plant water use (L plant <sup>-1</sup> )	$WUE_p$ (g L <sup>-1</sup> )	$\delta^{13}C$ (‰)	Specific leaf N content (mg cm <sup>-2</sup> )
Heng0628	NPS0	1473.5a	67.7ab	55.94a	1.21b	- 32.03a	1.30a
	DPS0	1131.5b	65.8b	51.18ab	1.29b	- 32.28ab	1.29a
	NPS1	1220.0b	65.2b	50.42b	1.26b	- 32.51b	1.07b
	DPS1	1245.5ab	70.4a	52.40ab	1.35a	- 32.09a	1.26a
XR4347	NPS0	1264.8a	48.0a	49.39a	0.97ab	- 31.76a	1.53a
	DPS0	1051.3b	51.6a	48.13ab	1.07a	- 32.17bc	1.41ab
	NPS1	877.3c	36.1b	44.46c	0.81b	- 32.25c	1.31b
	DPS1	1203.0a	49.4a	46.59b	1.06a	- 31.91ab	1.61a

the DPS1 treatment improved the plant water use by 4% in Heng0628 and 5% in XR4347 cultivar compared with the NPS1 plants. The  $WUE_p$  significantly increased in the DPS1

treatment by 7% in Heng0628 and 31% in XR4347 compared to the NPS1 treatment. In addition, the drought primed plants under the DPS1 treatment significantly improved the

$\delta^{13}\text{C}$  and specific N content compared with the NPS1 treatment under the salinity stress.

## Discussion

Drought and salinity are the major environmental limitations on crop productivity, and they decrease plant water status, photosynthesis (Janda et al. 2016) and expansion of leaf area (Kozłowski 1997; Parida and Das 2005; Rahnesan et al. 2018). Previous studies have reported that priming can alleviate stress tolerance in wheat against post-anthesis waterlogging (Li et al. 2011), heat (Wang et al. 2014a, b), cold (Li et al. 2015), freeze (Wang et al. 2019) and drought stress (Wang et al. 2015). In the present study, whether drought priming can alleviate subsequent salinity stress and the underlying mechanisms was investigated. The results showed that drought primed plants achieved a stress imprint that minimized subsequent salinity stress as exemplified by the improvement of leaf gas exchange, plant water status and root growth as well as modulating plant hormones compared with the non-primed plants.

Photosynthesis ( $P_n$ ) can be greatly impaired by drought and/or salinity stress, resulting in a substantial loss of yield (Chaves et al. 2009; Downton et al. 1985; Farquhar et al. 1982). The reduction in photosynthetic rate under drought and salinity conditions is usually attributed to the suppression of mesophyll metabolism and stomatal closure at moderate and high pressure (Flexas et al. 2004; Chaves et al. 2009). In previous studies, improvement of leaf gas exchange was noticed in wheat after drought priming when plants suffered from drought stress at the later growth stage (Abid et al. 2017). Under subsequent salinity stress, it was noted that the primed plants improved the  $P_n$  and  $g_s$  of leaves when compared to the non-primed plants (Fig. 2). The leaf and root water potential of primed plants were significantly higher than the non-primed plants under subsequent salinity stress due to drought priming imposed at the early growth stage (Fig. 3). In addition, drought priming maintained lower  $[\text{ABA}]_{\text{leaf}}$  for the primed plants. Both the improved plant water status and reduced ABA signaling improved the opening of the stomatal aperture and significantly increased leaf photosynthesis, which preserved grain production throughout the salinity stress period. Thus, the biomass was significantly increased due to earlier drought priming under the salinity stress in the priming treatment (Table 2). Furthermore, drought priming improved the specific leaf N content due to the facilitated root growth and morphology (Fig. 6), and this could benefit high leaf photosynthetic capacity during the salinity stress period, improving the  $P_n$  for the primed plants. The improved specific leaf N content could also

directly impact the photosynthetic process by influencing the Rubisco content (Heckathorn et al. 1996).

The present study showed that drought priming significantly improved  $\text{WUE}_p$  due to considerably enhanced dry biomass compared with the non-primed plants under subsequent salinity stress, though the plant water use also increased for primed plants (Table 2). In order to further explore how drought priming regulated  $\text{WUE}$  in response to subsequent salinity stress, leaf  $\delta^{13}\text{C}$  was measured to demonstrate long-term utilization of water. It has been reported that drought and salinity stress increase leaf  $\delta^{13}\text{C}$  (Acosta-Motos et al. 2017; Flanagan and Farquhar 2014) when the stomata closure is a key factor in limiting photosynthesis. Consistently, drought primed plants increased leaf  $\delta^{13}\text{C}$ , indicating that the increased leaf  $\delta^{13}\text{C}$  and  $\text{WUE}_p$  for primed plants under subsequent salinity stress was due to the improved photosynthesis and/or decreased of  $g_s$ . Under this circumstance, the results clearly showed that the significantly higher leaf  $\delta^{13}\text{C}$  and  $\text{WUE}_p$  in the present study was mainly ascribed to the improvement of  $P_n$ .

Drought priming increased root length, diameter, area and volume compared with non-primed plants under the salinity stress (Fig. 6). The stimulated root growth and root morphological characteristics can increase water and nutrient absorption from the soil, allowing plants to cope with stressful conditions (Arif et al. 2019). It was found that drought priming increased water consumption for the primed plants compared with non-primed plants under subsequent salinity stress (Table 2), implying further the enhanced water uptake from the soil for the primed plants due to the stimulated root growth and morphology. The stimulated root growth and water uptake could contribute to the better plant water status, increased  $g_s$  and enhanced shoot growth of the primed plants. The significantly increased specific leaf N content (Table 2) demonstrated that the enhanced root growth after drought priming enhanced nutrient uptake from the soil.

Salinity stress lowers carbon fixation and biomass production in plants via reducing water availability,  $\text{Na}^+$  toxicity and ion imbalance (Ahanger et al. 2019). Maintaining cellular  $\text{K}^+/\text{Na}^+$  homeostasis is pivotal for plant survival in saline environments. El-Samad and Shaddad (1997) reported that plant reactions to salinity stress cause a drop in  $\text{K}^+$  content in plant tissues, and the replacement of  $\text{K}^+$  by  $\text{Na}^+$  might result in nutritional imbalances. In the current study, drought primed plants significantly improved  $\text{K}^+$  concentration than non-primed plants under subsequent salinity stress, and thus maintained the  $\text{K}^+/\text{Na}^+$  ratio, though the  $\text{Na}^+$  concentration was increased by drought priming under the subsequent salinity stress. The improvement of  $\text{K}^+$  level under salinity stress condition could mitigate the adverse effects of excess  $\text{Na}^+$  plants and minimize salt-induced ionic

toxicity by improving salt tolerance (Ahanger et al. 2019). The decreased  $\text{Ca}^{2+}$  concentration in the non-primed plants might compete with  $\text{Na}^+$  ion, resulting in reduced  $\text{Na}^+$  absorption and concentration in the treatment compared with the primed plants.

## Conclusions

Drought priming improved the plant water status while attenuated ABA signaling, which improved the stomatal opening and thus significantly increased leaf photosynthesis. Consequently, the biomass under the salinity stress was significantly increased for primed plants. Furthermore, drought priming facilitated the root growth and morphology, and thus improved the water and nutrient uptake. The increased specific leaf N content could benefit leaf photosynthetic capacity during the salinity stress period and increased the photosynthesis significantly for primed plants. Drought priming significantly improved plant level WUE ( $\text{WUE}_p$ ) due to considerably enhanced dry biomass compared with non-primed plants under subsequent salinity stress, and the significantly increased leaf  $\delta^{13}\text{C}$  under drought priming further demonstrated that the improved leaf  $\delta^{13}\text{C}$  and  $\text{WUE}_p$  was mainly ascribed to the improvement of photosynthesis. Drought primed plants significantly improved  $\text{K}^+$  concentration and maintained the  $\text{K}^+/\text{Na}^+$  ratio for the primed plants compared with non-primed plants under subsequent salinity stress, which could mitigate the adverse effects of excess  $\text{Na}^+$  and minimize salt-induced ionic toxicity by improving salt tolerance for primed plants. Therefore, drought priming at early growth stage could be considered as a promising strategy for salt-prone areas to optimize agricultural sustainability and food security through improving WUE of crops under changing climate. Based on the findings, hopefully, such innovative insight might provide directions in the research efforts aimed at improving synchronous drought and salinity tolerance in wheat.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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