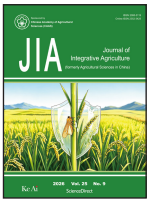
Journal of Integrative Agriculture 2026, 25(9): 3609–3618

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Drought priming enhances young spike development in wheat under drought stress during stem elongation

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Highlights

- Drought priming enhances photosynthesis and antioxidant defenses to improve drought tolerance.
- Through priming, spike development is stabilized to promote floret differentiation and fertility under drought.
- Optimized carbon metabolism and hormones support stress adaptation and yield improvement.

Abstract

Drought stress is a significant environmental stressor that can have detrimental effects on crop yields, especially during stem elongation. Drought priming has emerged as a promising technique for enhancing plant drought tolerance. However, the effects of drought priming on the spike differentiation process and its physiological basis in wheat are not clear. In this study, we investigated the effects of drought priming on spike development under drought stress by applying drought priming at the three-leaf stage and drought stress during stem elongation. This study demonstrated that drought priming significantly increased the photosynthetic rate of flag leaves by approximately 25.7% and improved leaf water potential by 17.4% during drought stress. Moreover, it mitigated oxidative damage by reducing the hydrogen peroxide and malondialdehyde levels by 30.6 and 11.1%, respectively, during stem elongation. Drought priming also markedly enhanced the activities of two key carbon metabolism enzymes, hexokinase and fructokinase, by 170.0 and 236.0%, respectively. This improved carbon metabolism and stabilized spike differentiation, leading to increased spikelet and floret primordia formation. Ultimately, drought priming achieved a 13.8% increase in kernel number per spike, demonstrating its potential for improving grain yield under drought conditions. This study innovatively revealed the “carbon homeostasis-spike development” coordination mechanism underlying drought priming-enhanced reproductive stress tolerance. The findings advance our understanding of stress memory as it relates to spatiotemporal regulation in crops and offer transformative solutions for stabilizing wheat production under climate change scenarios.

Keywords: wheat, drought priming, spike differentiation, carbon metabolism

1. Introduction

Wheat is a staple food crop that provides essential nutrients to more than one-third of the global population (Chen *et al.* 2024). As global warming intensifies, greenhouse gas emissions from human activities are expected to trigger significant water cycle changes on a global scale, which will pose serious threats to ecosystems, water resources and food security (Daryanto *et al.* 2016; Piedrahita *et al.* 2024). Studies have shown that wheat yields are significantly reduced by drought stress, and the crop phenological period is shorter with climate change (Hou *et al.* 2024). Although drought stress during critical stages such as stem elongation, anthesis, and grain filling can severely reduce grain yield, substantial yield losses occur when crops encounter water limitation during the stem elongation stage (Lan *et al.* 2022). Spikelets are one of the key components of wheat yield, and

Received 31 October 2024; Received in revised form 21 January 2025; Accepted 26 January 2025; Available online 19 February 2025
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doi: 10.1016/j.jia.2025.02.033

the final manifestation of the development, degradation, and setting of wheat florets. The number of fertile florets and grains per spike are positively correlated (Zhang *et al.* 2021). During the stem elongation stage, the wheat spike undergoes a series of developmental transitions, from the initiation of spikelet primordia to the determination of floret fertility. This process is very sensitive to environmental conditions, with water availability being a critical factor. Previous research has shown that drought stress during this stage disrupts cell elongation, shortens spike development, and reduces the number of fertile florets, ultimately reducing grain yield (Frantová *et al.* 2022). Therefore, developing strategies to enhance the resilience of wheat to drought stress during these crucial developmental stages is paramount for ensuring food security in the face of a changing climate.

Drought priming is a promising strategy for increasing the adaptive capacity of wheat plants to future drought stress (Wang *et al.* 2014). Several studies have demonstrated the effectiveness of drought priming in enhancing the drought tolerance of various species, including *Arabidopsis* (Ding *et al.* 2012), rice (Bahuguna *et al.* 2018) and wheat (Wang *et al.* 2014). Previous studies in wheat have shown that drought priming can reduce the yield loss of wheat under drought stress that occurs during the reproductive growth stage. Drought priming, applied either once or twice, alleviates the grain yield reduction caused by drought stress during grain filling (Wang *et al.* 2014). However, most studies on drought priming in wheat have focused on the seedling stage, while few have explored its effects on drought tolerance during later developmental stages, particularly the stem elongation stage.

In this study, we hypothesized that drought priming can stabilize spikelet development in wheat under drought stress during the stem elongation stage, leading to improved grain yield. We addressed this gap in the literature by investigating the effects of drought priming on spikelet development and drought tolerance during this critical phase.

The identification of key pathways involved in the drought priming response opens new avenues for the development of drought-resilient wheat varieties through targeted breeding and genetic engineering approaches. Moreover, our results highlight the potential of drought priming as a cost-effective and sustainable strategy for improving wheat productivity under water-limited conditions, thereby contributing to global food security in the face of climate change.

2. Materials and methods

2.1. Experimental design

Experiment I A pot experiment was conducted to analyze the effect of drought priming on drought tolerance in wheat at the stem elongation stage. Field-based pot experiments were conducted under shelters to block natural precipitation throughout the growth of wheat. Each pot contained 7.5 kg soil, 1.5 g urea, and 1 g potassium dihydrogen phosphate. Initially, 14 wheat seeds were planted in each pot, which were then narrowed down to seven seedlings once they reached the three-leaf stage.

Drought priming was applied at the three-leaf stage by controlling the water for 7 days to reach a relative soil water content at 60–65%, which was maintained for 2 days. Two treatments were established: control (C) and primed (P). Subsequently, during the stem elongation stage, drought stress was imposed by reducing the relative soil water content for 7 days to reach 40–45%, which was maintained for 2 days. These two steps established four treatments: non-primed plants under control (CC), primed plants under control (PC), non-primed plants under drought stress at stem elongation (CD), and primed plants under drought stress at stem elongation (PD). The net photosynthesis rate, chlorophyll fluorescence, antioxidant enzyme activities, malondialdehyde content, and carbon metabolizing enzyme activities of leaves were examined after drought priming at the three-leaf stage and drought stress at the stem elongation stage. Yield and tiller dynamics were examined after harvesting.

Experiment II Hydroponic experiments were conducted to further analyze the effect of drought priming on spikelet development under drought stress in wheat. Uniform seeds were selected and surface sterilized with 20% sodium hypochlorite for 10 min, followed by four rinses with sterile distilled water. Germination occurred in the dark at 22°C until the first leaf was fully extended. Subsequently, seedlings were transplanted into plastic containers filled with Hoagland's nutrient solution for hydroponic cultivation. Growth conditions were maintained at day/night temperatures of 22°C/15°C, with a photoperiod of 14 h at a light intensity of 350 μmol m^{-2} s^{-1}. The Hoagland culture solution was replaced every 3 d to ensure optimal nutrient availability for the growing seedlings.

As the plants reached the three-leaf stage, they were divided into two distinct groups. One group served as the control, receiving the standard hydroponic solution, whereas the other group underwent a drought priming treatment with 10% PEG6000 (–0.63 MPa) for a period of 48 h. During the stem elongation stage, the plants were subjected to drought stress by being exposed to 20% PEG (–0.94 MPa) for 48 h. After stress, the residual solution was carefully rinsed off, and the plants were then returned to a normal hydroponic solution for further cultivation. Dynamic observations of the spikelets and sampling were conducted throughout the experimental process, and the hormone levels in leaves and spikelets were examined after treatment.

2.2. Remobilization of dry matter and grain yield

Plants were harvested from experiment I at anthesis and physiological maturity. The grains in each pot were collected to determine grain yield. Each plant organ was promptly inactivated by exposure to 105°C for 30 min followed by drying at 75°C until the weight reached a constant value. Established formulas were used to calculate critical parameters such as the transport capacity of pre-anthesis storage substances (PTA), pre-anthesis storage material transport rate (PTR), contribution rate of pre-anthesis storage substances to grains (CTA), post-anthesis photosynthetic rate (PAA), contribution rate of photosynthetic compounds to grains after anthesis (CPA) (Fan *et al.* 2005).

PTA=Plant dry weight at anthesis–Nutritive organ dry weight at maturity

PTR (%)=TAA/Dry weight at anthesis×100

CTA (%)=TAA/Grain dry weight at maturity×100

PAA=Grain dry weight at maturity–TAA

CPA=PAA/Grain dry weight at maturity

2.3. Soil water potential and leaf water potential

After drought priming and drought stress, soil samples were collected at a depth of 10 cm from the pot barrel and placed in sample cups. A calibrated WP4C dew point microvolt meter (Wescor, Logan, UT, USA) was used for measuring soil water potential to ensure that the plants in CD and PD received the same stress (Appendix A). The last fully expanded leaves were cut into pieces and placed in sample cups, and a calibrated WP4C dew point microvolt meter was used for measuring leaf water potential (Wang *et al.* 2020).

2.4. Leaf net photosynthesis rate and chlorophyll fluorescence in wheat leaves

After drought priming and drought stress, the net photosynthesis rate was accurately measured using the IRGA Li-COR portable photosynthesis system (Li-6800 Inc.), as previously described by Wang (2014). The measurements were conducted on the last fully expanded leaf employing a photon flux density of 800 μmol photo m^{-2} s^{-1} and a CO_2 concentration of 400 μmol mol^{-1}, within the time frame of 9:00 a.m. to 12:00 p.m. The chlorophyll fluorescence was measured using a modulated fluorimeter (FluorPen FP 110) on the same leaves tested for net photosynthesis.

2.5. Antioxidant enzyme activities and malondialdehyde content

The last fully expanded leaves after drought priming and drought stress in experiment I were used to determine the activities of superoxide dismutase (SOD EC:1.15.1.1) and catalase (CAT EC:1.11.1.6), using the method described by Tan (2008). The reaction mixture was incubated under both light and dark conditions to monitor the photoreduction of nitro blue tetrazolium (NBT) at 560 nm, and the activity of SOD was expressed as U mg^{-1} protein. CAT activity was calculated by determining the change in OD_{240} min^{-1}.

The malondialdehyde (MDA) content was determined following the method of Tan (2008). Samples of 2 mL were mixed with 4 mL of TCA-TBA mixture solution. The mixture was kept in a boiling water bath for 20 min and centrifuged at 4,000×g for 10 min. The supernatant was collected and read using a visible light spectrophotometer at OD_{450}, OD_{532} and OD_{600} nm.

The hydrogen peroxide (H_2O_2) content was determined following the method of Velikova (2000). Samples of 0.5 g fresh leaf material were ground in 5 mL of 0.1% trichloroacetic acid solution. The extract was homogenized at 12,000 r min^{-1} for 15 min, and a 0.5 mL aliquot of the supernatant was mixed with 1 mL of 1 mol L^{-1} potassium iodide and 0.5 mL of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0). The absorbance of the mixture was noted at OD_{390} nm using a spectrophotometer.

2.6. Carbohydrate metabolism enzyme activities

Leaf samples (0.5 g) in experiment I were ground in liquid nitrogen, homogenized with 1 mL of extraction buffer (40 mmol L^{-1} Tris-HCl at pH 7.6, 3 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA, 0.1 mmol L^{-1} PMSF, 1 mmol L^{-1} benzamidine, 14 mmol L^{-1} β-mercaptoethanol, and 24 μmol L^{-1} NADP), and kept on ice for 30 min. The mixture was centrifuged at 13,200×g for 30 min at 4°C. The liquid supernatant was extracted to test the activities of hexokinase (HXK EC:2.7.1.1), fructose-1,6-bisphosphate aldolase (Ald EC:4.1.2.13), fructokinase (FK, EC:2.7.1.4), glucose-6-phosphate 1-dehydrogenase (G6PDH EC:1.1.1.49), phosphofructokinase (PFK EC:2.7.1.11), phosphoglucomutase (PGM EC 5.4.2.2), ADP-glucose pyrophosphorylase (AGPase EC:2.7.7.13), and UDP-glucose pyrophosphorylase (UGPase EC:2.7.7.9) according to the methods by Jammer (2015). The liquid supernatant was extracted and dialyzed overnight with 20 mmol L^{-1} potassium phosphate buffer, and then tested for the activity of Invertase (EC 3.2.1.26), including vacuolar invertase (VacInv), cell wall invertase (CwInv), and cytoplasmic invertase (CytInv), according to the method described by Jammer (2015).

2.7. Plant hormones

The flag leaf and spike of each main stem for each treatment from experiment II were collected at the end of drought stress and placed in liquid nitrogen. The contents of hormones in the leaves and spikes of wheat were determined according to the method of Pan (2010). Briefly, 0.2 g of wheat leaves or spikes were ground into powder, 1 mL of extraction solution (isopropanol/H_2O/HCl=2/1/0.002) was added, the mixture was sonicated for 30 min at 4°C and the sonication process was maintained for another 30 min with the addition of 2 mL dichloromethane. The samples were then centrifuged (13,000×g) at 4°C for 5 min. The lower liquid phase was transferred to a new centrifuge tube and the solution was concentrated to dryness with nitrogen, and 200 μL of pre-cooled 50% methanol solution was added to dissolve the sample residue. The solution was filtered through a 0.22 μm organic filtration membrane and transferred to a sample injector. The contents of abscisic acid (ABA), salicylic acid (SA), zeatin (ZT), and auxin (IAA) hormones in the sample solution were analyzed by LC-MS/MS (Triple Quad 6500+, AB Sciex, Tokyo, Japan).

2.8. Statistical analysis

The statistical analysis was performed using one-way ANOVA (Sigmaplot 11.0, Systat Software Inc., San Jose, CA, USA) to determine the differences between treatments, with significant differences considered at $P<0.05$ using Duncan's multiple range test.

3. Results

3.1. Effect of drought priming on dry matter accumulation and the distribution and grain yield of wheat under drought stress during stem elongation

Drought stress during the stem elongation stage significantly reduced the leaf water potential, in contrast to the primed plants that maintained higher leaf water

potential, with an increase of about 17.4% in the PD compared with the CD (Appendix A). Similarly, drought priming resulted in water loss from the leaves, but the damage to leaves was mild and could be recovered quickly after the water stress was alleviated. Therefore, although drought priming resulted in the loss of aboveground fresh weight, there was no significant difference between the CC and PC. In addition, drought stress resulted in a 49.6% reduction in leaf fresh weight and a 40.9% reduction in dry weight, whereas the biomass of plants in PD increased by around 42.5 and 33.8%, respectively, compared with plants in CD (Appendix A).

Grain yield was significantly reduced by drought stress at the stem elongation stage. Compared with plants in CC, the grain yields decreased by 24.9 and 10.9% in CD and PD, respectively. The kernels per spike decreased in CD and PD by 28.9 and 15.2%, respectively. Kernel weight decreased by 11.7 and 5.0%, respectively. Drought stress reduced the spike numbers in CD and PD, but with no significant difference between them. The yield and yield components showed no significant differences between PC and CC (Table 1).

Drought stress during stem elongation significantly reduced the post-anthesis assimilate accumulation (PAA) and contribution of post-anthesis assimilates to grain yield (CPA). Compared with CC, PAA and CPA in CD decreased by 18.1 and 14.8% respectively, while in PD they decreased by 9.8 and 4.7%. In contrast, drought stress significantly increased the transport capacity of pre-anthesis storage substances (PTA), the pre-anthesis storage material transport rate (PTR) and the contribution rate of pre-anthesis storage substances to grains (CTA), and they increased by 15.6, 16.8 and 23.8% in CD, respectively, compared with CC, while in PD they only increased by 2.6, 4.5 and 7.5%. The dry matter accumulation and distribution showed no significant differences between PC and CC (Table 2).

3.2. Effects of drought priming on photosynthesis and the leaf antioxidant system of wheat under drought stress during stem elongation

Drought priming significantly reduced the photosynthetic rate (P_n), transpiration rate (T_r) and stomatal conductance (g_s), but did not significantly influence the maximal photosynthetic efficiency (F_v/F_m) of the plants. The plants recovered rapidly when drought stress was alleviated, indicating that drought priming had a stimulating effect on photosynthesis in wheat. At the stem elongation stage, drought stress reduced P_n, g_s, T_r, and F_v/F_m by 40.2, 69.8, 61.3, and 11.0%, respectively, compared with plants in CC. However, plants in PD were able to maintain higher photosynthetic capacity, where the greatest effect by drought priming on the plants was reflected in the P_n, which increased by around 25.7% under drought stress (Fig. 1).

After drought priming, reactive oxygen species (ROS), including superoxide anion ($O_2^{-}\cdot$), H_2O_2, and MDA, accumulated in the plants and then gradually declined to their normal levels with the recovery of the external environment. In contrast, the primed plants accumulated less ROS and the

Table 1 Effect of drought priming on the grain yield of wheat under drought stress during stem elongation

Treatment[1)]	Spikes per pot	Kernels per spike	1,000-kernel mass (g)	Yield (g/plot)
CC	17.0 a	49.8 a	50.1 a	42.5 a
PC	17.5 a	50.1 a	50.5 a	43.2 a
CD	14.4 b	35.5 c	44.2 c	31.9 c
PD	14.9 b	42.3 b	47.6 b	37.8 b

[1)] CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation.
Letters mean significant differences among treatments (P<0.05).

Table 2 Effects of drought priming on dry matter accumulation and transport under drought stress during stem elongation[1)]

Treatment[2)]	PTA (mg/plant)	PTR (%)	CTA (%)	PAA (mg/plant)	CPA (%)
CC	714.5 c	23.4 bc	38.3 c	1,099.4 a	61.7 a
PC	708.3 c	22.7 c	38.7 c	1,146.4 a	61.3 a
CD	826.2 a	27.3 a	47.4 a	900.6 c	52.6 c
PD	733.4 b	24.4 b	41.2 b	991.2 b	58.8 b

[1)] PTA, transport capacity of pre-anthesis storage substances (g/plant); PTR, pre-anthesis storage material transport rate (%); CTA, contribution rate (%) of pre-anthesis storage substances to grains; PAA, post-anthesis photosynthetic rate (g/plant); CPA, contribution rate (%) of photosynthetic compounds to grains after anthesis.
[2)] CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation.
Letters mean significant differences among treatments (P<0.05).

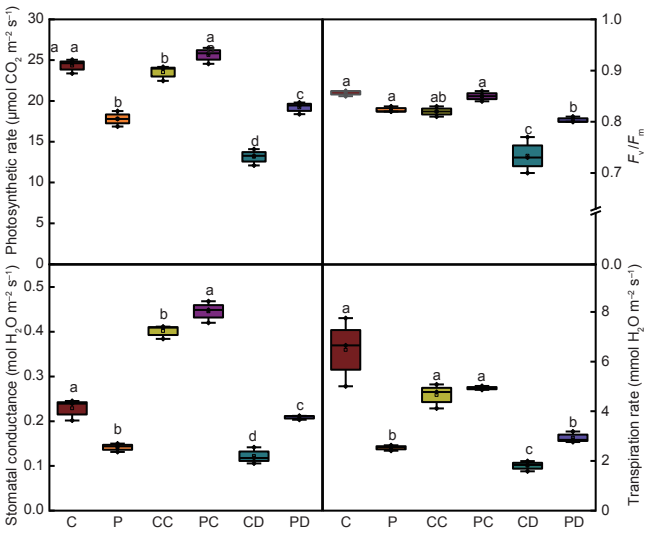

Fig. 1 Effect of drought priming on the photosynthesis of wheat under drought stress during stem elongation. C, control; P, primed; CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. F_v/F_m, the maximal photosynthetic efficiency. Box center horizontal lines represent the median value of each group; boxes represent standard error; the I-shaped whiskers show the maximum and minimum normal values of each group. Three biological replicates were analyzed. Different letters above the bars indicate significant differences at P<0.05.

contents of H_2O_2 and MDA were reduced by 30.6 and 11.1%, respectively, compared with CD under drought stress (Fig. 2). The reduction of ROS accumulation in the primed plants was attributed to the increases in antioxidant enzyme activities, so we tested the activities of SOD and CAT in the plants. The results showed that the activities of SOD and CAT increased by around 8.6 and 17.7%, respectively, in CD compared with CC under drought stress. Drought priming significantly enhanced the enzymatic activities under drought stress, and the activation of SOD by drought priming was more obvious, with its activity increasing by about 5% in PD compared with CD (Fig. 2).

3.3. Effect of drought priming on the development process of wheat young spikes at different stages

The development of wheat young spikes is divided into five periods: single ridge stage (SRS), double ridge stage (DRS), glume differentiation stage (GDS), floret differentiation stage (FDS), and pistil and stamen formation stage (PSFS). Drought stress led to a significant advancement in the development of young spikes. Compared with CC, the duration of young spikes development from SRS to PSFS was significantly reduced in CD, while the time of spike development in PD was prolonged under drought stress. The data on the development time of wheat spikes in each period showed that drought priming at the three-leaf stage mainly prolonged development from the DRS to PSFS stage but did not have a significant effect on the SRS to DRS stage (Table 3).

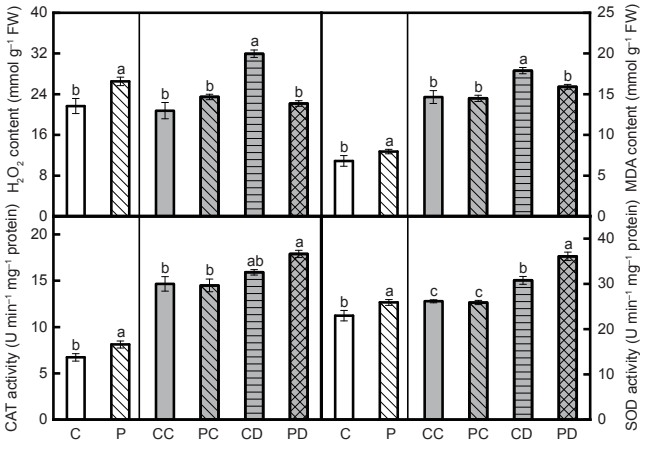

Fig. 2 Effect of drought priming on the antioxidant system in flag leaves under drought stress during stem elongation. H_2O_2, hydrogen peroxide; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase. C, control; P, primed; CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. White bars represent the drought priming stage; gray bars represent the drought stress stage. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

Table 3 Effects of drought priming on the development and differentiation times of wheat spikes at different stages[1)]

Treatment[2)]	SRS–DRS (d)	DRS–GDS (d)	GDS–FDS (d)	FDS–PSFS (d)	SRS–PSFS (d)
CC	28	7	5	8	48
PC	28	7	5	6	46
CD	26	2	6	9	43
PD	26	3	8	10	47

[1)] SRS, single ridge stage; DRS, double ridge stage; GDS, glume differentiation stage; FDS, floret differentiation stage; PSFS, pistil and stamen formation stage.
[2)] CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation.

3.4. Effect of drought priming on the activities of carbon metabolism enzymes in leaves under drought stress during the stem elongation stage

Wheat seedlings at the three-leaf stage were more sensitive to water deficit, so drought priming reduced the activities of three carbon metabolism enzymes in leaves, whereas drought stress during the stem elongation stage significantly elevated their activities. Of these three enzymes, only CytInv was influenced by drought priming under drought stress, and it was significantly elevated by 92.9 and 57.9% in CD and PD compared with CC, respectively. Drought priming significantly reduced the activity of CytInv under drought stress, thereby reducing sucrose catabolism (Fig. 3).

3.5. Effect of drought priming on the activities of glycolytic pathway enzymes in leaves under drought stress during the stem elongation stage

The glycolytic pathway is an important metabolic pathway that is affected by drought stress. Drought stress at the stem elongation stage significantly reduced the activities of hexokinase (HXK), glucose-6-phosphate-dehydrogenase (G6PDH), fructose-1,6-bisphosphate aldolase (Ald), and fructokinase (FK) in leaves compared with CC, which in turn affected the sugar metabolism under drought stress. In contrast, drought priming enhanced the activities of key enzymes under drought stress. For example, the activities of HXK and FK in primed plants were elevated by 170.0 and 236.0%, respectively, compared to the non-primed plants under drought stress. However, the activities of phosphoglucomutase (PGM) and Ald were not affected by drought priming. In addition, not all enzyme activities related to glycolytic pathways were inhibited by drought stress. For example, the activity of phosphofructokinase (PFK) was elevated by around 4.6-fold under drought stress, and drought priming had a further stimulatory effect (Fig. 4).

3.6. Effect of drought priming on the activities of enzymes related to nucleotide synthesis in leaves under drought stress during the stem elongation stage

Nucleotide synthesis is somewhat affected by drought stress, but two key enzymes in the pathway, ADP-glucose pyrophosphorylase (AGPase) and UDP-Glucose pyrophosphorylase (UGPase), did not respond to drought stress in the same way. The activity of AGPase was significantly reduced by 73.8% under drought stress compared with CC, while the activity of UGPase was less affected. Plants in PD were able to maintain a higher level of AGPase activity under drought stress, which was elevated

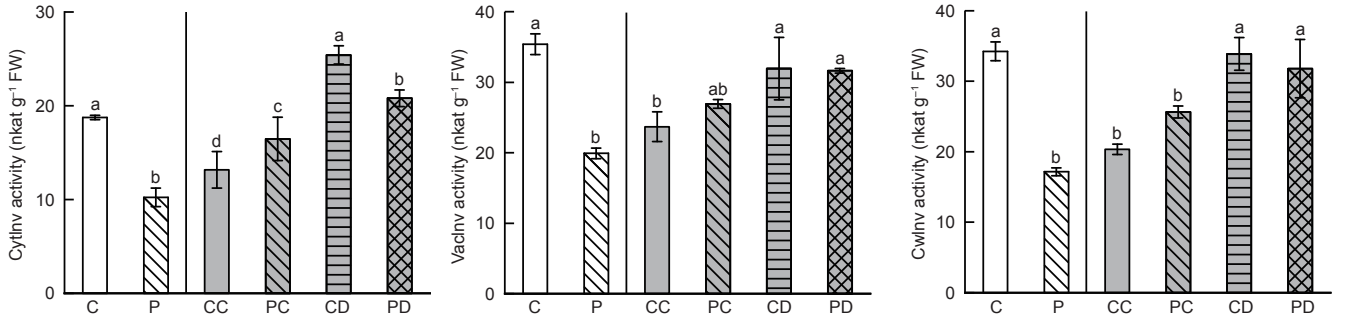

Fig. 3 Effect of drought priming on the activities of carbon metabolism enzymes under drought stress during stem elongation. VacInv, vacuolar invertase; CwInv, cell wall invertase; CytInv, cytoplasmic invertase. C, control; P, primed; CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. White bars represent the drought priming stage; gray bars represent the drought stress stage. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

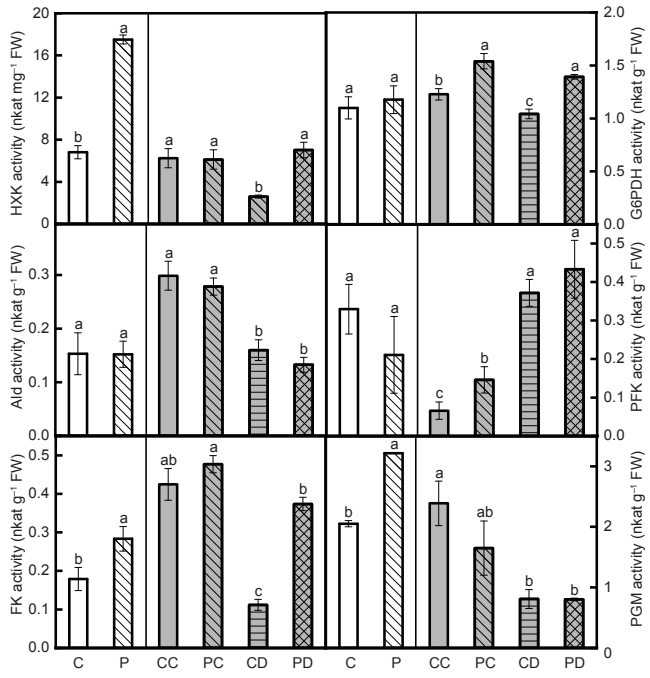

Fig. 4 Effect of drought priming on the activities of glycolytic pathway enzymes under drought stress during stem elongation. HXK, hexokinase; Ald, fructose-1,6-bisphosphate aldolase; FK, fructokinase; G6PDH, glucose-6-phosphate 1-dehydrogenase; PFK, phosphofructokinase; PGM, phosphoglucomutase. C, control; P, primed; CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. White bars represent the drought priming stage; gray bars represent the drought stress stage. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

around 2-fold compared with CD, which helped them to maintain a stable level of nucleotide metabolism (Fig. 5).

3.7. Effect of drought priming on the contents of hormones in spikelets and leaves under drought stress during the stem elongation stage

Drought stress affected the contents of various hormones in

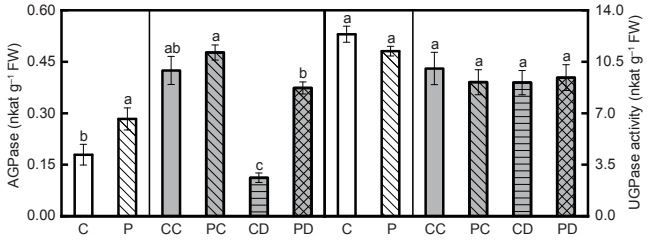

Fig. 5 Effect of drought priming on the activities of nucleotide synthesis enzymes under drought stress during stem elongation. AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase. C, control; P, primed; CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. White bars represent the drought priming stage; gray bars represent the drought stress stage. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

the leaves and spikes of wheat, and the contents of abscisic acid (ABA), salicylic acid (SA), zeatin (ZT), and auxin (IAA) were measured in the plants. Among them, ABA was the most affected by drought stress, and its contents in spikelets and leaves were elevated by about 5- and 3-fold after drought stress, whereas ABA accumulation levels in spikelets and leaves were elevated by 34.0 and 57.8%, respectively, in PD compared with CD. In addition, the accumulation of ZT in the leaves and spikelets of primed plants were elevated by 28.3 and 226.3%, respectively, compared to non-primed plants. In contrast, drought stress reduced the IAA contents in spikes and leaves, and plants in PD accumulated more IAA only in the leaves but not in the spikes (Fig. 6).

3.8. Effect of drought priming on the fertility of florets at different positions on the spikes in wheat

After 20 days of recovery, observations of the floret primordia in different parts of each group revealed great differences in the development of floret primordia in different parts of the spike. The fastest development was in the central section of floret primordia, followed by the apical floret primordia, while the basal floret primordia developed the slowest. Drought

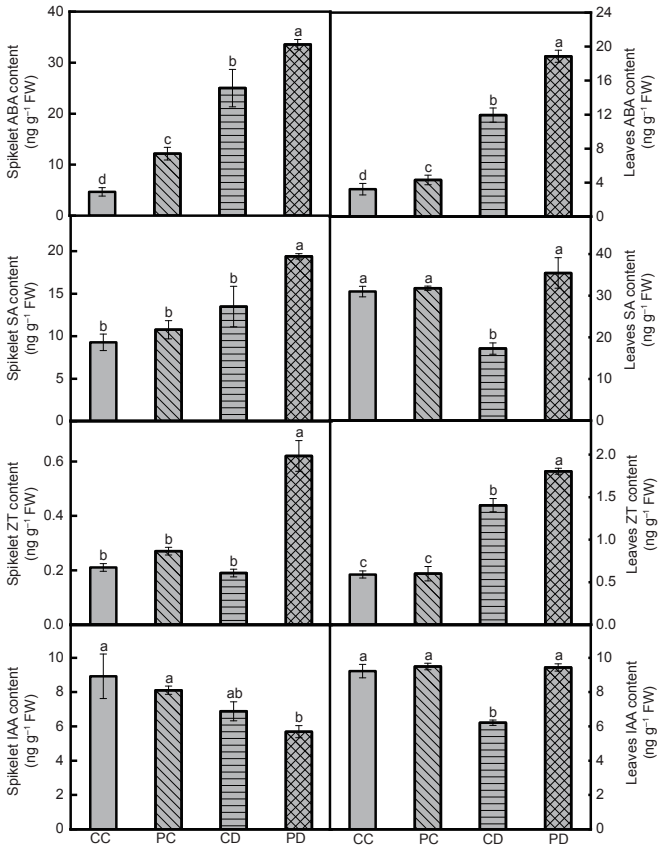

Fig. 6 Effect of drought priming on the hormone contents in spikelets and leaves under drought stress during stem elongation. ABA, abscisic acid; SA, salicylic acid; ZT, zeatin; IAA, auxin. CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

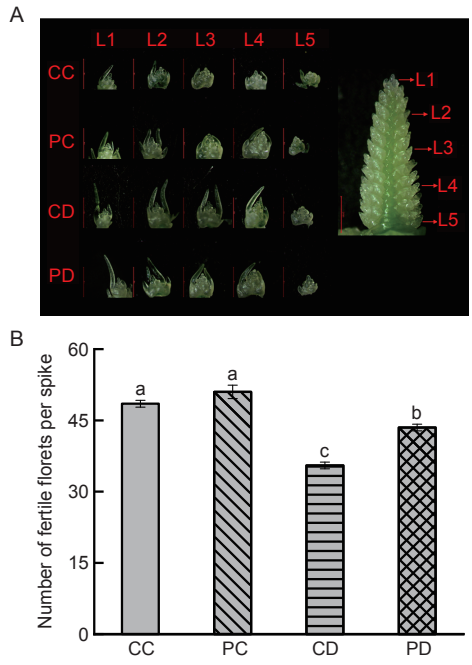

Fig. 7 Effect of drought priming on the fertility of florets at different positions along young spikes in wheat. A, effect of drought priming on floret primordia at different positions on young spikelets in wheat. The young spikes were divided into five parts from top to bottom, namely L1, L2, L3, L4 and L5. B, effect of drought priming on the number of fertile florets. CC, non-primed plants under control; PC, primed plants under control; CD, non-primed plants under drought stress at stem elongation; PD, primed plants under drought stress at stem elongation. Bars mean SD (n=3). Different letters above the bars indicate significant differences at P<0.05.

stress significantly enhanced the development of the floret primordia, resulting in faster development and longer awn primordia lengths. This led to the development of young spikes in CD entering the connective formation stage more quickly than in CC and shortened the developmental process of the young spikes. Under the same drought conditions, the development of floret primordia of wheat spikes in PD was similar to PC, but slightly faster than CC, which stabilized and prolonged the development of young spikes to a certain extent (Fig. 7-A). Drought stress greatly affected the number of fertile florets per spike in wheat, as it was significantly reduced in both CD and PD, but drought priming enhanced the fertility of florets to some extent. Counting the fertile florets revealed that their number increased by about 22.5% in PD plants compared to CD (Fig. 7-B).

4. Discussion

The stem elongation phase represents a pivotal period for wheat yield formation, marking the transition from vegetative to reproductive growth. During this phase, the apical meristem shifts its focus from producing leaves to producing spikes. Drought stress poses a significant threat to wheat across all growth stages. However, its impact is particularly severe during stem elongation, when it leads to substantial yield losses due to disruptions in photosynthesis that affect the grain weight (Ahmad *et al.* 2018), and it also leads to the abortion of pollen and a reduction in grain number (Ji *et al.* 2010). Previous studies have demonstrated that drought priming during the vegetative growth stage enhances the tolerance to drought stress during grain filling. However, the effect of drought priming on drought stress during stem elongation is far from clear. In this study, we found significant reductions in grain yield, spike number, and thousand-kernel weight due to drought stress during the stem elongation stage. However, compared to non-primed plants, drought-primed plants exhibited higher grain numbers per spike and increased grain weight. Therefore, our analysis primarily focused on elucidating the physiological mechanisms underlying the impact of drought priming on grain weight and kernel yield under drought stress during stem elongation.

4.1. Drought priming enhances grain weight by modulating leaf photosynthesis and biomass translocation during drought stress at stem elongation

Photosynthesis plays a crucial role in determining crop yield, with water deficiency adversely affecting its rate by inducing stomatal closure to regulate CO_2 diffusion, and ABA is involved in stomatal regulation (Mittler *et al.* 2004). In addition, the

Calvin cycle adapts to the reduced NADPH demand during stomatal closure by reducing photosynthesis, leading to an over-reduction of the photosynthetic electron transport chain. This excess excitation energy can be transferred to molecular oxygen, forming ROS, thereby causing ROS accumulation and exacerbating membrane peroxidation (Xing *et al.* 2016). Previous research has indicated that drought priming can enhance photosynthesis stability and activate antioxidant capacity during grain filling, thereby enhancing drought tolerance (Wang *et al.* 2015). Our findings demonstrated that primed plants maintained a higher leaf water potential and mitigated the damage to flag leaf photosystems under drought stress during stem elongation, thereby preserving greater photosynthetic capacity. Furthermore, primed plants exhibited a more robust antioxidant system, with elevated SOD and CAT activities, to facilitate rapid ROS removal and reduce cellular oxidative damage.

Apart from photosynthesis, nutrient substance translocation from vegetative organs to the grain is another crucial factor contributing to grain weight formation (Wu *et al*. 2022). Drought stress increased the proportion of pre-anthesis assimilates mobilized to grain, which may be related to the fact that drought stress during stem elongation reduces leaf photosynthesis, accelerates plant senescence and thus facilitates the remobilization of pre-anthesis assimilates (Han *et al.* 2023). Primed plants demonstrated greater developmental stability under drought stress while maintaining higher levels of photosynthesis and a lower plant senescence rate, thus increasing post-anthesis assimilate allocation to grains and providing more substrates for grain filling, ultimately contributing to increased yields under drought stress during stem elongation. The enhancement of grain weight by drought priming was mainly due to higher post-anthesis photosynthate assimilation and an increased translocation ratio to the grain under drought stress during stem elongation.

4.2. Drought priming stabilizes spike development under drought stress at stem elongation

The differentiation of spikes plays a crucial role in determining panicle number and kernels per spike (Pradhan *et al.* 2012). Drought stress accelerates the development of spikes by shortening the duration of stem elongation, which ultimately reduces the number of fertilized florets and impacts grain yield (González *et al.* 2002). By subdividing spike development into five periods and comparing the duration of each period between treatments, we found that drought stress significantly shortened the duration from the double ridge to glume differentiation stage, which may be an important reason for the effect of drought stress on grain yield (Frank *et al.* 1987). However, drought priming stabilized the time of wheat spike development from the double ridge to glume differentiation stage and then prolonged the duration time from the glume differentiation to pistil and stamen formation stage, which allowed the spikes to obtain adequate development time.

In addition to the spikes, the number of grains is also influenced by the number of fertile florets. Studies have shown that a prolonged duration of stem elongation increases the number of fertile florets due to extended spike growth and enhanced dry matter partitioning to the spike. This, in turn, increases the number of grains per spike and subsequently enhances the yield during this critical period of active juvenile spikelet growth. Drought stress during stem elongation tends to induce floral organ immaturity, leading to higher spikelet abortion rates, thereby affecting grain number per spike (Prasad *et al.* 2006). Drought stress induces pollen sterility, resulting in poor pollination and reduced kernel numbers, along with higher rates of blighted grains in maize (Shen *et al.* 2020). While these findings highlighted the differential effects of drought stress on cob pollination in maize, similar studies in wheat are limited.

Therefore, we counted the number of fertile florets in each spike and found that drought stress primarily reduced the number of fertile florets by increasing floret failure, whereas the number of fertile florets in primed plants was less affected by drought stress. This suggests that drought priming may confer a protective effect against the detrimental impacts of drought stress on wheat spikelet fertility, potentially contributing to an improved grain yield under challenging environmental conditions.

4.3. Drought priming modulates substrate supply and hormone levels in wheat spikes under drought stress at stem elongation

The potential number of grains per spike is intricately linked to the extent of floret primordia death, which is inversely correlated with the availability of resources for spike growth (Whitechurch *et al.* 2007). AGPase, an allosteric enzyme catalyzing the initial step in starch biosynthesis, has been reported to exhibit significantly reduced activity under drought stress, which directly impacts grain yield and starch content (Kaur *et al.* 2017). Our study demonstrated that primed plants exhibit elevated AGPase activity, indicating more carbohydrate metabolism crucial for enhancing plant tolerance to stress by regulating osmotic potential and energy supply.

Notably, the sucrose metabolism and drought tolerance were correlated with lower CytInv activity in dehydration-tolerant wheat seedlings, which is consistent with previous studies in barley (Ye *et al.* 2022). The lower CytInv activity in primed plants suggests advantages in sucrose conservation and possibly ATP preservation in primed plants. HXK, which catalyzes the irreversible reaction of glucose phosphorylation, is important for regulating plant metabolism and sugar signaling, and FK can phosphorylate fructose, thereby enhancing respiration rates and the transformation of fructose to sucrose, which is the more stable form of carbohydrate (Ahmad *et al.* 2024). Our findings revealed that primed plants maintained higher activities of the FK and HXK enzymes under severe drought stress conditions, which facilitates precursor substance availability for glucose-6-phosphate and fructose-6-phosphate synthesis. This, in turn, enhances sucrose biosynthesis and improves cell osmotic potential, ultimately contributing to enhanced drought tolerance in primed wheat plants.

Phytohormones play pivotal roles in abiotic stress responses and spike developmental regulation (Jiang and Asami 2018). ABA, which is critical for drought adaptation, usually increases in leaves and spikes with drought stress. On the one hand, ABA regulates photosynthesis under drought stress by modulating stomata, but on the other hand, the elevated ABA content in the spikelet was accompanied by floret abortion and yield reduction (Ji *et al.* 2011). In addition, interactions between ABA and SA also have implications for crop yield under drought (Shokat *et al.* 2021). Exogenous phytohormone SA analogs can significantly improve drought tolerance in plants, but the molecular mechanisms and their interactions with plant drought tolerance remain unclear (Sohag *et al.* 2020). We found that drought priming promotes the accumulation of ABA and SA in spikelets and leaves, so it enhances wheat tolerance by regulating photosynthesis, maintaining osmotic stability, and ultimately increasing the yield under drought stress. ZT is particularly important for floral bud development, which is severely retarded in the presence of ZT deficiency (Kiba *et al.* 2013). In this study, we found a significant elevation of ZT in the spikelets of primed plants, which helped to stabilize the development of florets, thereby enhancing the wheat yield under drought stress.

5. Conclusion

This study demonstrates that drought priming at the three-leaf stage can effectively reduce the yield loss under drought stress during stem elongation. Drought priming enhances the activities of carbon metabolism enzymes in flag leaves, improving photosynthetic efficiency and carbon transport capacity. It also stabilizes spike differentiation, resulting in increased spikelet and floret primordia formation and ultimately boosting kernel count per spike. Notably, drought priming attenuates the adverse impact of drought stress on floret fertility (Fig. 8). These findings underscore the potential of drought priming as a strategic approach for enhancing wheat productivity under challenging environmental conditions.

Acknowledgements

This study was supported by projects of the National Natural Science Foundation of China (32272213 and 31771693), the National Key Research and Development Program of China (2024YFD2301305), the Jiangsu Innovation Support Program for International Science and Technology Cooperation Project, China (BZ2023049), the China Agriculture Research System (CARS-03), and the Jiangsu Collaborative Innovation Center for Modern Crop Production, China (JCIC-MCP).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendix associated with this paper is available at https://doi.org/10.1016/j.jia.2025.02.033

References

Ahmad K S, Shehzad M A, Javid H, Mehmood A, Akhtar G, Zafar S, Mahroof S, Mahmoud E A, Elansary H O, Ulfat A, Abid H. 2024. Transgenerational seed exposure to elevated CO involves stress memory regulation at metabolic levels to confer drought resistance in wheat. *Acs Omega*, **9**, 20042–20055.

Ahmad Z, Waraich E A, Akhtar S, Anjum S, Ahmad T, Mahboob W, Hafeez O B, Tapera T, Labuschagne M, Rizwan M. 2018. Physiological responses of wheat to drought stress and its mitigation approaches. *Acta Physiologiae Plantarum*, **40**, 80.

Bahuguna R N, Tamilselvan A, Muthurajan R, Solis C A, Jagadish S V K. 2018. Mild preflowering drought priming improves stress defences, assimilation and sink strength in rice under severe terminal drought. *Functional Plant Biology*, **45**, 827–839.

Chen Z D, Wang J F, Dong D Q, Lou C, Zhang Y, Wang Y X, Yu B, Wang P F, Kang G Z. 2024. Comparative analysis of TaPHT1;9 function using CRISPR-edited mutants, ectopic transgenic plants and their wild types under soil conditions. *Plant and Soil*, **1**, 1–12.

Daryanto S, Wang L, Jacinthe P A. 2016. Global synthesis of drought

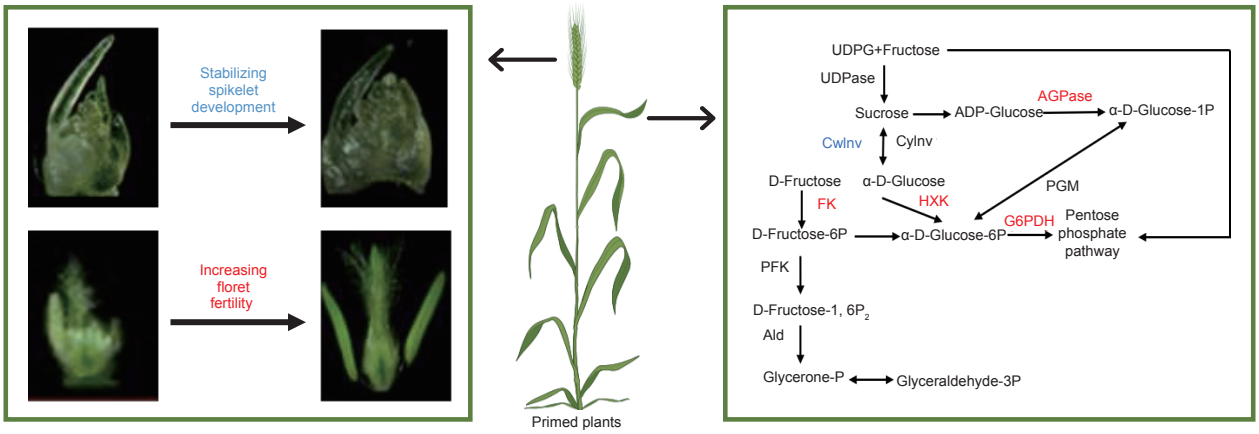

Fig. 8 Drought priming regulates the activities of carbon metabolism enzymes and spikelet development, and it plays a role in drought stress tolerance. HXK, hexokinase; Ald, fructose-1,6-bisphosphate aldolase; FK, fructokinase; G6PDH, glucose-6-phosphate 1-dehydrogenase; PFK, phosphofructokinase; PGM, phosphoglucomutase. AGPase, ADP-glucose pyrophosphorylase; UGPase, UDP-glucose pyrophosphorylase. CwInv, cell wall invertase; CytInv, cytoplasmic invertase. Red and blue colors indicate higher and lower activities in primed plants under drought stress at stem elongation (PD) compared with non-primed plants under drought stress at stem elongation (CD), and the black color indicates no difference between PD and CD.

effects on maize and wheat production. *PLoS ONE*, **11**, e0156362.
Ding Y, Fromm M, Avramova Z. 2012. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. *Nature Communications*, **3**, 740.
Fan X, Jiang D, Dai T, Jing Q, Cao W. 2005. Effects of nitrogen supply on flag leaf photosynthesis and grain starch accumulation of wheat from its anthesis to maturity under drought or waterlogging. *Chinese Journal of Applied Ecology*, **16**, 1883–1888.
Frank A B, Bauer A, Black A L. 1987. Effects of air-temperature and water-stress on apex development in spring wheat. *Crop Science*, **27**, 113–116.
Frantová N, Rábek M, Elzner P, Streda T, Jovanovic I, Holková L, Martinek P, Smutná P, Prásil I T. 2022. Different drought tolerance strategy of wheat varieties in spike architecture. *Agronomy-Basel*, **12**, 2328.
González F G, Slafer G A, Miralles D J. 2002. Vernalization and photoperiod responses in wheat pre-flowering reproductive phases. *Field Crops Research*, **74**, 183–195.
Han Y J, Wang Y C, Zhang D M, Gao H, Sun Y, Tao B, Zhang F Y, Ma H, Liu X M, Ren H L. 2023. Planting models and deficit irrigation strategies to improve radiation use efficiency, dry matter translocation and winter wheat productivity under semi-arid regions. *Journal of Plant Physiology*, **280**, 153864.
Hou M L, Li Y, Biswas A, Chen X G, Xie L L, Liu D L, Li L C, Feng H, Wu S F, Satoh Y, Pulatov A, Siddique K H M. 2024. Concurrent drought threaten wheat and maize production and widen crop yield gaps in the future. *Agricultural Systems*, **220**, 104056.
Jammer A, Gasperl A, Luschin-Ebengreuth N, Heyneke E, Chu H, Cantero-Navarro E, Grosskinsky D K, Albacete A A, Stabentheiner E, Franzaring J, Fangmeier A, van der Graaff E, Roitsch T. 2015. Simple and robust determination of the activity signature of key carbohydrate metabolism enzymes for physiological phenotyping in model and crop plants. *Journal of Experimental Botany*, **66**, 5531–5542.
Ji X M, Dong B D, Shiran B, Talbot M J, Edlington J E, Hughes T, White R G, Gubler F, Dolferus R. 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. *Plant Physiology*, **156**, 647–662.
Ji X M, Shiran B, Wan J L, Lewis D C, Jenkins C L D, Condon A G, Richards R A, Dolferus R. 2010. Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat. *Plant Cell and Environment*, **33**, 926–942.
Jiang K, Asami T. 2018. Chemical regulators of plant hormones and their applications in basic research and agriculture. *Bioscience Biotechnology and Biochemistry*, **82**, 1265–1300.
Kaur V, Madaan S, Behl R K. 2017. ADP-glucose pyrophosphorylase activity in relation to yield potential of wheat: Response to independent and combined high temperature and drought stress. *Cereal Research Communications*, **45**, 181–191.
Kiba T, Takei K, Kojima M, Sakakibara H. 2013. Side-chain modification of cytokinins controls shoot growth in *Arabidopsis*. *Developmental Cell*, **27**, 452–461.
Lan Y Z, Chawade A, Kuktaite R, Johansson E. 2022. Climate change impact on wheat performance — Effects on vigour, plant traits and yield from early and late drought stress in diverse lines. *International Journal of Molecular Sciences*, **23**, 3333.
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. Reactive oxygen gene network of plants. *Trends in Plant Science*, **9**, 490–498.
Pan X, Welti R, Wang X. 2010. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. *Nature Protocols*, **5**, 986–992.
Piedrahita V A, Roberts A P, Rohling E J, Heslop D, Zhao X, Galeotti S, Florindo F, Grant K M, Hu P X, Li J H. 2024. Dry hydroclimates in the late palaeocene-early eocene hothouse world. *Nature Communications*, **15**, 7042.
Pradhan G P, Prasad P V V, Fritz A K, Kirkham M B, Gill B S. 2012. Effects of drought and high temperature stress on synthetic hexaploid wheat. *Functional Plant Biology*, **39**, 190–198.
Prasad P V V, Boote K J, Allen L H, Sheehy J E, Thomas J M G. 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, **95**, 398–411.
Shen S, Liang X G, Zhang L, Zhao X, Liu Y P, Lin S, Gao Z, Wang P, Wang Z M, Zhou S L. 2020. Intervening in sibling competition for assimilates by controlled pollination prevents seed abortion under postpollination drought in maize. *Plant Cell Environment*, **43**, 903–919.
Shokat S, Grosskinsky D K, Liu F L. 2021. Impact of elevated CO on two contrasting wheat genotypes exposed to intermediate drought stress at anthesis. *Journal of Agronomy and Crop Science*, **207**, 20–33.
Sohag A A, Tahjib-Ul-Arif M, Brestic M, Afrin S, Sakil M A, Hossain M T, Hossain M A, Hossain M A. 2020. Exogenous salicylic acid and hydrogen peroxide attenuate drought stress in rice. *Plant Soil and Environment*, **66**, 7–13.
Tan W, Liu J, Dai T, Jing Q, Cao W, Jiang D. 2008. Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. *Photosynthetica*, **46**, 21–27.
Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants — Protective role of exogenous polyamines. *Plant Science*, **151**, 59–66.
Wang X, Vignjevic M, Jiang D, Jacobsen S, Wollenweber B. 2014. Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (*Triticum aestivum* L.) var. Vinjett. *Journal of Experimental Botany*, **65**, 6441–6456.
Wang X, Vignjevic M, Liu F L, Jacobsen S, Jiang D, Wollenweber B. 2015. Drought priming at vegetative growth stages improves tolerance to drought and heat stresses occurring during grain filling in spring wheat. *Plant Growth Regulation*, **75**, 677–687.
Wang X, Zhang J, Song J, Huang M, Cai J, Zhou Q, Dai T, Jiang D. 2020. Abscisic acid and hydrogen peroxide are involved in drought priming-induced drought tolerance in wheat (*Triticum aestivum* L.). *Plant Biology*, **22**, 1113–1122.
Whitechurch E, Slafer G, Miralles D. 2007. Variability in the duration of stem elongation in wheat and barley genotypes. *Journal of Agronomy and Crop Science*, **193**, 138–145.
Wu X L, Liu M, Li C S, Mchugh A D, Li M, Xiong T, Liu Y B, Tang Y L. 2022. Source–sink relations and responses to sink–source manipulations during grain filling in wheat. *Journal of Integrative Agriculture*, **21**, 1593–1605.
Xing X H, Fang C W, Li L, Jiang H Q, Zhou Q, Jiang H D, Wang S H. 2016. Improved drought tolerance by α-naphthaleneacetic acid-induced ROS accumulation in two soybean cultivars. *Journal of Integrative Agriculture*, **15**, 1770–1784.
Ye F, Jiang M, Zhang P, Liu L, Liu S Q, Zhao C S, Li X N. 2022. Exogenous melatonin reprograms the rhizosphere microbial community to modulate the responses of barley to drought stress. *International Journal of Molecular Sciences*, **23**, 9665.
Zhang Z, Li J, Hu N Y, Li W, Qin W L, Li J P, Gao Y M, Liu Y, Sun Z C, Yu K, Wang Z M, Zhang Y H. 2021. Spike growth affects spike fertility through the number of florets with green anthers before floret abortion in wheat. *Field Crops Research*, **260**, 108007.