The Crop Journal xxx (xxxx) xxx

Contents lists available at ScienceDirect

The Crop Journal

journal homepage: www.keaipublishing.com/en/journals/the-crop-journal/

Spermidine alleviates drought-induced wheat floret degeneration by mitigating oxidative damage and maintaining energy homeostasis

Juan Li [a,b], Gege Li [a], Zhiyuan Li [a], Jiayu Li [a], Jianke Xiao [a], Vinay Nangia [c], Yang Liu [a,*]

[a] College of Agronomy, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] Institute of Grain Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang, China
[c] International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 6299-10112, Rabat, Morocco

ARTICLE INFO

Article history:
Received 30 January 2024
Revised 10 July 2024
Accepted 22 July 2024
Available online xxxx

Keywords:
Drought stress
Polyamines
Grain number
Amino acid
Carbohydrate metabolism

ABSTRACT

Drought stress at the booting stage causes severe floret degeneration and a decrease in grain number. Polyamines are involved in wheat floret development under drought stress, but the underlying physiological mechanisms are unclear. This study showed that drought-induced accumulation of reactive oxygen species led to wheat spikelet cell apoptosis and floret degeneration. Drought induced stomatal closure to reduce photosynthesis, then inhibited the activities of sucrose phosphate synthase, sucrose synthetase (cleavage direction) and ADP-glucose pyrophosphorylase in spikes and leaves, and soluble vacuolar invertase and cell wall invertase in spikes, thus providing a poor nutrient base for floret development. Exogenous spermidine application increased antioxidant enzyme activities and polyamine metabolism, promoted starch and sucrose metabolism, amino acid utilization and increased the levels of glycolytic and tricarboxylic acid cycle intermediates to mitigate oxidative damage and maintain energy homeostasis in the spike, thereby reducing floret degeneration and increasing grain number.

1. Introduction

Wheat is one of the world's most important food crops, and more than 60% of China's wheat crops are grown in arid and semi-arid areas [1]. It is urgent to study wheat breeding and cultivation practices for maintaining yield and drought tolerance. The grain number per spike in wheat is one of the key traits of yield components and is ultimately determined by floret differentiation, degeneration, and fruiting processes [2]. Drought stress at meiosis during wheat floret degeneration can severely reduce grain number [3,4]. Therefore, it is desirable to investigate drought-induced floret degeneration at the pre-reproductive stage and identify ways of mitigating this stress to ensure yield under water scarcity.

Drought causes extensive physiological damage to plant development through complex physiological activities such as reactive oxygen species (ROS) metabolism [5], hormone signaling [6], and nutrient allocation and utilization [7,8]. Redox homeostasis is a key physiological activity for maintaining the normal growth and development of plant cells [9]. Enzymatic and nonenzymatic antioxidant defense systems are generated in plants to resist adverse conditions [10]. In wheat under drought stress, the activities of antioxidant enzymes (such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX)) first increase and then decrease to scavenge excess ROS [11]. Ascorbic acid (AsA) content increases to reduce the toxicity of hydrogen peroxide (H_2O_2) under drought stress [10]. When plants are subjected to severe drought stress, the antioxidant mechanism is impaired, and the balance between ROS production and their scavenging is disrupted, leading to excessive ROS production [6,12,13]. Subsequently, cells are in a state of oxidative stress, which leads to lipid peroxidation, protein oxidation, nucleic acid damage and enzyme inactivation [14]. The overaccumulation of H_2O_2 leads to programmed cell death (apoptosis), which is the major cause of apoptosis in most spikelet degeneration mutants [13]. Under normal growth conditions, the rice spikelet degeneration mutant *tutou1* showed degeneration of the apical spikelet and high levels of ROS in the spike tissue compared with that in the wild type [15]. Alleviating drought-induced oxidative cellular damage and preventing excessive stimulation of programmed cell death are necessary for mitigating floret degeneration.

Energy is required to develop reproductive organs and to resist adversity (clean up excess ROS) [16,17]. Under severe drought stress, energy expenditure increases dramatically, energy

* Corresponding author.
E-mail address: liuyang0328@126.com (Y. Liu).

https://doi.org/10.1016/j.cj.2024.07.017

production is inhibited, and when energy levels fall below a certain threshold, growth stops, damage accumulates, and the organism eventually dies [18,19]. Sucrose is the carbon skeleton and energy supply for floret development, and sucrose catabolism plays a key role in maintaining energy balance [20]. Sucrose is split into glucose and fructose by invertase [21], and glucose enters the glycolytic and tricarboxylic acid (TCA) cycle to produce ATP; in this process, amino acids can be used as alternative substrates for organic matter under adverse conditions [16]. The catabolism of valine and branched-chain amino acids (BCAAs) provide alternative energy sources during seed development or under prolonged dark conditions [22], and this pathway has also been identified as an essential factor for drought tolerance in *Arabidopsis* [23]. Sucrose synthesizes starch under the action of sucrose synthase (SuS), ADP-glucose pyrophosphorylase (AGPase) (which can provide the direct precursor adenosine diphosphate glucose for starch synthesis), soluble starch synthase (SSS) and granule-bound amylase [24], and as starch is hydrolyzed into monosaccharides, the monosaccharides are metabolically broken down to produce ATP, which in turn provides energy for organisms [25]. Drought-mediated inhibition of invertase activity reduces the glucose and fructose contents of pistils and anthers, which leads to sucrose starvation of anthers and reduced fertility [7,26,27]. Hu et al. [18] showed that drought stress reduces the content of pyruvate and acetyl coenzyme A, thereby increasing the difficulty of generating sufficient ATP in the TCA cycle in cotton pollen tubes. Genes encoding AGPase and amylopectin synthase are downregulated, and insufficient starch accumulation is a common feature of abnormal pollen grain development under drought stress [28,29]. Sucrose feeding can partially reverse the effect of water deficiency on infertility [30]. However, balancing the utilization of sucrose and amino acids under drought conditions to stabilize the energy supply and maintain metabolic homeostasis is a major challenge for the development of florets under drought stress.

Polyamines (PAs), with arginine and ornithine serving as synthetic precursors, function as exogenous substances in the regulation of plant cell division, floral organ differentiation and homeostatic regulation induced by adaptation to various abiotic stresses [31,32]. Spermidine (Spd) and putrescine (as a diamine; Put) are common PA in higher plants [33,34]. PAs are involved in oxidative damage and/or carbon and nitrogen metabolism in cells under stresses such as drought, salt, low and high temperatures [11,33–35]. ROS production is closely linked to the process of PA catabolism given that H_2O_2 is produced by the action of diamine oxidase and/or PA oxidase (PAO) [36]. Exogenous Spd and overexpression of the PA biosynthesis gene *SlSPDS2* increase POD and CAT enzyme activities while decreasing H_2O_2 and malondialdehyde (MDA) contents in the leaves of plants under drought and low-temperature stress to alleviate cellular oxidative damage [34,35]. Exogenous Spd also increased the photosynthetic rate of the flag leaf to increase carbohydrate accumulation in the plant and enhanced the activities of SuS and acid invertase involved in sucrose unloading in the sink organ to facilitate the transport of sucrose from the stem to the sink organ [36–38]. Both the endogenous Spd and Put (the starting point of the PAs pathway) contents were positively correlated with the number of fertile florets under drought stress, and the balance of ethylene (ETH) and PAs under drought treatment was associated with the abortive process [8,39]. Exogenous Spd alleviated floret degeneration and increasing spike grain number under drought stress [39,40]. However, the mechanism through which Spd mitigates floret degeneration by modulating oxidative damage and energy homeostasis in young spike cells is unclear.

The objective of the present study was to elucidate the physiological mechanisms by which PAs mediate drought-induced floret degeneration and grain number formation in the wheat plant, beginning with two hypotheses: (1) Spd reduces drought-induced oxidative damage in spikes by activating the ROS scavenging system, and (2) Spd mitigates floret degeneration by balancing the utilization of sugars and amino acids to maintain energy homeostasis.

2. Materials and methods

2.1. Field experiment

2.1.1. Plant materials and cultivation

The field experiment was conducted at the Yangling Smart Agriculture Demonstration Park, Shaanxi province, China (34°15′N, 108°02′E), during the 2020–2022 growing seasons. The composition of the surface soil layer (0–20 cm) at the beginning of the experiment and the daily air temperature during the growth period of winter wheat were obtained as described by Li et al. [39]. The soil was a sandy loam with an electrical conductivity (EC) of 1.02 dS m^{-1}. Two dryland winter wheat cultivars (Changhan 58 (CH58, relatively strongly drought tolerant), Luohan 6 (LH6, relatively weakly drought tolerant)) were used in this experiment. CH58 was developed from Changwu112/PH82–2 at the Changwu County Agricultural Technology Extension Center in Shaanxi province and LH6 from Yumai 9/Shannong 45 at the Luoyang Agricultural Science Research Institute. The two cultivars showed similar yield performance in production. Seeds were sown on October 25, 2020 and October 20, 2021 under a rain shelter at a rate of 16.5 g (about 370–390 seeds) m^{-2} by strip sowing in rows 25 cm apart. The rain shelter was 78 × 18 m, with removable plastic films on the sides (always open) and on the top (closed only during rain). Compound fertilizers were applied during both growing seasons, with amounts equivalent to N 150 kg ha^{-1}, P_2O_5 90 kg ha^{-1} and K_2O 30 kg ha^{-1}.

2.1.2. Soil moisture and chemical application treatment

Experiment I: in the 2020–2021 growing season, three water treatments—a severely dry soil (DS) treatment (soil relative humidity 50%–55%), a moderately dry soil (MD) treatment (soil relative humidity 60%–65%) and a normal soil water (CK) treatment (soil relative humidity 70%–75%) from the jointing to heading period—were used. The average soil bulk density and field water capacity were 1.65 g cm^{-3} and 23.0%, respectively. The soil relative humidity for severe drought stress was set after a pre-experiment to simulate the natural drying process of the soil, and the settings for control and moderate drought stress were taken from Liu et al. [41]. The soil water content was monitored with an AZS-100 TDR meter (Beijing Aozuo Ecological Instruments Co., Ltd.). The irrigation amounts for CK and MD were calculated based on the difference between the soil moisture content detected by TDR (three replications) and the target soil moisture content. The plants in the DS treatment were not irrigated during the soil moisture treatment period. The experiment used a randomized complete block design with three replicates. The area of each plot was 6 m^2 (2 × 3 m), and the plots were separated by intervals of 2 m.

Experiment II: in the 2021–2022 growing season, after Experiment I was repeated, DS (soil relative humidity 50%–55%) and CK (soil relative humidity 70%–75%) treatments were established to conduct an exogenous treatment. Exogenous Spd and the PA inhibitor propamidine hydrazone (MGBG, an inhibitor of S-adenosylmethionine decarboxylase (SAMDC)) and distilled water were used for the DS treatment, and the treatment combinations used were DS-Spd, DS-MGBG and DS. CK treatment with exogenous distilled water only. Water was used for solution preparation and all spray solutions contained 0.01% Tween-20. Each plot was 6 m^2. Exogenous MGBG was applied to the 1 m^2 micro-area of

the plot. The spray volume was 225 mL m^{-2}. Based on the previous research [42,43], water (as control), 1 mmol L^{-1} Spd and 5 mmol L^{-1} MGBG were applied by spraying from 16:00–18:00 (wind spend <1.8 m s^{-1}) for 2 consecutive days, and the application started 5 d before the booting stage (flag leaf pillow pulled out 4–6 cm) (158 d after sowing) and heading stage (the spikes were pulled out from 4–6 cm) (166 d after sowing). The air temperatures during the treatment were maintained at 15.8–18.0 and 17.5–22.0 °C. The light intensities were maintained at 800 and 900 μmol m^{-2} s^{-1}.

2.2. Pot experiment

During the 2022–2023 growing season, a pot experiment was conducted at Northwest Agriculture and Forestry University, Yangling, Shaanxi province, China (34°17′N, 108°05′E) to verify the effects of exogenous Spd and MGBG on the drought-induced inhibition of floret development. Each pot (31 cm in diameter and 20 cm in height) was filled with 8 kg of soil and vermiculite (soil: vermiculite = 3:1). The soil was taken from the wheat field. The mixed soil contained 17.1 g kg^{-1} organic matter, 1.42 g kg^{-1} total N, 19.5 mg kg^{-1} available P, and 120.4 mg kg^{-1} available K. The EC of pot soil was 0.94 dS m^{-1}. A total of 1.88 g of urea (containing 46% N) and 1.48 g of diammonium phosphate (containing 18% N and 46% P_2O_5) were dissolved in water and applied to each pot at the seedling stage (application rates corresponding to 150 kg ha^{-1}N and 90 kg ha^{-1} P_2O_5 in the field). Each pot contained 20 wheat seedlings. The soil moisture treatments were the same as those in experiment I. Among them, two gradients, CK (70%–75%) and DS (50%–55%), were applied exogenously. Water control started 12 d before the booting stage (135 d after sowing), and water was resumed at the end of the heading stage (141 d after sowing). Water, Spd and MGBG solutions were sprayed as described in Experiment II at a rate of 500 μL $plant^{-1}$. Young spikes and leaves were sampled at the booting stage for terminal deoxynucleotidyl transferase dUTP neck-end labeling (TUNEL) method and determination of energy metabolism in young spikes.

2.3. Terminal deoxynucleotidyl transferase dUTP neck-end labeling (TUNEL) assay

The spikelets at the booting stage (flag leaf pillow pulled out 4–6 cm from the meiosis stage) were collected and fixed in formaldehyde alcohol acetic acid fixation solution (containing an 18:1:1 [v/v] mixture of 70% ethanol, formalin, and acetic acid) and stored overnight at 4 °C. After dehydration, the materials were embedded in conventional paraffin sections, and the samples were continuously sectioned at a thickness of 10 μmol L^{-1} The TUNEL assay was performed with a TUNEL Cell Apoptosis Detection Kit (CF488; Servicebio, Wuhan, Hubei, China). The sections were subjected to microscopic detection, and images were collected by fluorescence microscopy and imaging systems. The green fluorescence of the FITC fluorescent signal was measured at 465–495 nm (excitation) and 515–555 nm (detection). The blue fluorescence of the DAPI fluorescent signal was measured at 330–380 nm (excitation) and 420 nm (detection) [44]. Three biological replicates were performed for each treatment.

2.4. Record of dynamic development of florets

Spike differentiation was observed and recorded by an XTS2022 electron optical microscope (Beijing Tech). At the jointing stage (the flag leaf tip 2–4 cm) and heading stage (the spike of wheat emerged 4–6 cm), 10 main stems with consistent development were sampled to record the maximum floret differentiation number and the number of fertile florets (florets with complete green anthers and feathery stigmas were considered fertile florets). The floret degeneration rate (DR) was calculated as

$$DR = \frac{MF_0 - FN_0}{MF_0} \quad (1)$$

where MF_0 is the maximum number of floret differentiation events and FN_0 is the number of fertile florets.

At maturity, 10 spikes were sampled to record grain number per spike. Three replicates were performed for each treatment. A 1 m^2 area of each plot from the CK, DS and DS-Spd treatments was sampled to record spike number, thousand grain weight, and yield.

2.5. Observation of stomatal morphology

At the booting stage, fresh flag leaves were collected, cleaned with sterile water and stored in 4% glutaraldehyde for 24 h at 4 °C. The samples were then dehydrated with increasing concentrations of ethanol. The dried samples were sputtered with a 60:40 gold/palladium ratio, and the stoma morphology was observed under a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan) as described by Ma et al. [45]. Three biological replicates were performed for each treatment.

2.6. Monitoring of gas exchange, relative water and chlorophyll contents of leaves

At the booting stage, six flag leaves were selected from each plot to determine the relative water content (RWC, %) following Hunt et al. [46], with slight modifications. RWC was calculated as RWC (%) = (fresh weight (g) − dry weight (g))/(turgor weight (g) − dry weight (g)) × 100. Net photosynthetic rate (P_n), stomatal conductance (G_s) and transpiration rate (T_r) were monitored for the six flag leaves in each treatment between 9:00 and 11:30 on a sunny day (wind speed 2.1 m s^{-1}) using a Li-6400XT system (Li-COR, USA). The relative humidity of the air was maintained at 35%–42%. The light intensity was maintained at 1100 μmol m^{-2} s^{-1}. The instantaneous water use efficiency (WUEi) of the leaves was calculated as

$$WUEi = P_n/T_r \quad (2)$$

The relative chlorophyll content (SPAD) was determined for the six flag leaves in each treatment using a SPAD 502 Plus system (Konica Minolta, Japan).

2.7. Oxidative damage, soluble protein content, enzymatic and non-enzymatic antioxidant activity assays

The superoxide anion radical (O_2^-) and H_2O_2 contents of young spikes and leaves at the booting stage were measured as described by Chen et al. [47]. The soluble protein content was determined by the Coomassie brilliant blue method according to Kielkopf et al. [48]. The MDA content was measured according to Hodges et al. [49]. The activities of SOD and CAT were assayed as described by Jiang and Zhang [50]. The POD activity was determined following the methods of Maehly and Chance [51]. The method of Sharma and Dubey [52] was used to determine ascorbate peroxidase (APX) activity. The reduced glutathione (GSH) and AsA contents in young spike and flag leaves were measured by GSH and AsA assay kits (Geruisi Biotechnology) following the manufacturer's instructions. Glutathione reductase (GR) activity was detected with a GR activity assay kit (Geruisi Biotechnology). All measurements were performed with three biological replicates.

2.8. Endogenous spermidine content, putrescine content, enzyme activity, and rate of ethylene evolution

At the booting stage, Spd and Put were extracted from 0.5 g fresh young spike and flag leaf samples with 3 mL precooled 5% (v/v) $HClO_4$. The peaks corresponding to the contents of Spd and Put were detected by HPLC (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) at an absorbance of 254 nm. The activities of PAO, arginine decarboxylase (ADC), ornithine decarboxylase (ODC), SAMDC, and Spd synthase (SPDS) were quantified by enzyme-linked immunosorbent assay. Following Yang et al. [53] with slight modifications, two fresh young spikes were collected, weighed and immediately placed in a 30 mL airtight sample container. The containers were capped and incubated at room temperature (25 °C) for 8 h in the dark, after which 1 mL of gas was injected with a syringe into a gas chromatograph (Agilent 7890B, USA) to measure the concentration of ETH. Each treatment was replicated three times.

2.9. Total nitrogen, nitrate and ammonium concentrations and key nitrogen metabolism enzyme activities

Total nitrogen of wheat spikes and leaves was measured as described by Li et al. [39]. The nitrate (NO_3^-) and ammonium (NH_4^+) concentrations of wheat spikes and leaves were extracted at 30%. (v/v) trichloroacetic acid and determined by an AA3 continuous flow analyzer (SEAL Analytical, Germany). The activities of nitrate reductase (NR), glutamate synthase (GOGAT), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) levels of young spikes and leaves from the booting stage were determined by assay kits following the manufacturer's instructions. The assay kits were purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. (Nanjing, Jiangsu, China). All analyses were performed with three biological replicates.

2.10. Sugar and starch content and key enzymes in their metabolism

Sugars were extracted according to Wardlaw and Willenbrink [54] with slight modifications. Dried spike and leaf samples (0.1 g) from the booting stage were extracted in 5 mL of alcohol (90%, v/v) in a 80 °C water bath for 10 min, and the extraction was repeated twice. The supernatant was used to measure glucose, fructose, and sucrose concentrations by HPLC. The HPLC column ZORBAX carbohydrate column (4.6 × 150 mm, 5 μm) was used, the mobile phase system was an acetonitrile: water mixture (80:20, v/v), and the flow rate was 1 mL min^{-1}. The residues were extracted in perchloric acid for starch determination via the anthrone method [55]. All analyses were performed with three biological replicates.

The extraction and determination of SPS, SS-I, AGPase, INV and CWINV of fresh spikes and leaves at the booting stage were performed using assay kits from Solarbio Biotechnology Co., Ltd. (Beijing, China). Briefly, SPS activity was measured by recording the change in the characteristic absorbance peak of the reaction between sucrose and resorcinol at 480 nm. SS-I activity was measured by recording the change in the maximum absorption peak of the brownish-red substance produced by the reaction of fructose with 3,5-dinitrosalicylic acid at 480 nm. AGPase activity was assayed by recording the rate of decrease in NADH absorbance at 340 nm. INV and CWINV activities were determined by changes in the absorption peaks of brown–red substances resulting from the reaction of reducing sugars with 3,5-dinitrosalicylic acid were recorded. All analyses were performed with three biological replicates.

2.11. Detection of targeted metabolites

After the sample (young spike from the booting stage) was thawed and ground, 0.05 g of the young spike sample was mixed with 500 μL of 70% methanol/water. After centrifugation, 200 μL of the supernatant was transferred through a protein precipitation plate for further liquid chromatography-mass spectrometry (LC–MS) separation. All metabolites were detected by MetWare (https://www.metware.cn/) based on the AB Sciex QTRAP 6500 LC–MS/MS System. HPLC column used was an ACQUITY UPLC BEH Amide (i.d. 2.1 × 100 mm, 1.7 μm), the flow rate was 0.4 mL min^{-1}, and the column temperature was 40 °C. The electrospray ionization source operation parameters were as follows: ion source, ESI+/-; source temperature, 550 °C; ion spray voltage (IS), 5500 V (positive), –4500 V (negative); and curtain gas (CUR), 35 psi. Tryptophan and its metabolites were measured by scheduled multiple reaction monitoring (MRM). Data acquisition was performed using Analyst 1.6.3 software (Sciex). All metabolites were quantified using Multiquant 3.0.3 software (Sciex). Three biological replicates were performed for each treatment.

2.12. Transcriptome sequencing

Total RNA was extracted from young spikes at the booting stage using an E.Z.N.A. plant RNA kit (Omega Bio-Tek, USA) following the manufacturer's instructions. Three biological replicates were performed. After assessing the concentration and purity of the RNA using Nanodrop, high quality RNA samples (OD260/OD280 1.9–2.0) were used for library construction. Sequencing libraries were constructed with an Illumina NEBNext Ultra II RNA Library Prep Kit (NEB, Ipswich, USA). The libraries were sequenced on an Illumina HiSeq 2500 platform (BioMarker Technologies, Beijing, China). Sequence alignment results between the sample sequencing data and selected reference genomes as shown in Table S1 and Table S2. For comparisons among samples, the expression of each gene was normalized to fragments per kilobase of transcript per million reads (FPKM) values. Differential gene expression between DS and CK was estimated using DESeq2. Genes with a P value ≤ 0.01 and a $\log_2$fold change > 1 were defined as DEGs. Functional enrichment analyses of DEGs were conducted using gene ontology (GO) and KEGG pathway database enrichment analysis.

2.13. Gene expression analysis

Total RNA of young spikes at the booting stage was extracted using an EZNA. plant RNA kit (Omega Bio-Tek, USA) and reverse transcribed to cDNA using a PrimeScript RT Reagent Kit (Takara). Quantitative real-time polymerase chain reaction (qRT–PCR) was performed using SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, China) was used on a QuantStudio 7 Flex System (Thermo Fisher Scientific, USA). Three biological replicates were performed. Relative expression of genes (Table S3) was quantified with *TaActin2* and *TaGAPDH* as internal reference genes.

2.14. Statistical analysis

Differences were identified by a least significant difference (LSD) test at a P value < 0.05. Two-way ANOVA with cultivar and treatment as fixed factors was fitted to determine the significance of the treatment effects. Linear and nonlinear fitting was used to describe the relationships between the number of fertile florets and the sucrose and starch contents of young spikes.

3. Results

3.1. Spermidine alleviates drought stress-induced floret degeneration and cell death in spikelet

Compared to those in the CK treatment, the number of fertile florets in the DS treatment decreased by 13.6% (CH58) and 16.7% (LH6), and the number of grains per spike at maturity decreased by 16.5% (CH58) and 23.1% (LH6) (Fig. 1A). Compared to DS, DS-Spd effectively alleviated floret degeneration, increased the number of fertile florets, and thus reduced the drought-induced decrease in the number of grains per spike (with an amplitude of 12.0%–22.0%) (Fig. 1A). However, compared to DS, DS-MGBG (a PA inhibitor) further exacerbated the rate of floret degeneration, leading to an increase in the negative impact of drought stress on grain number formation (Fig. 1A). A strong green fluorescence

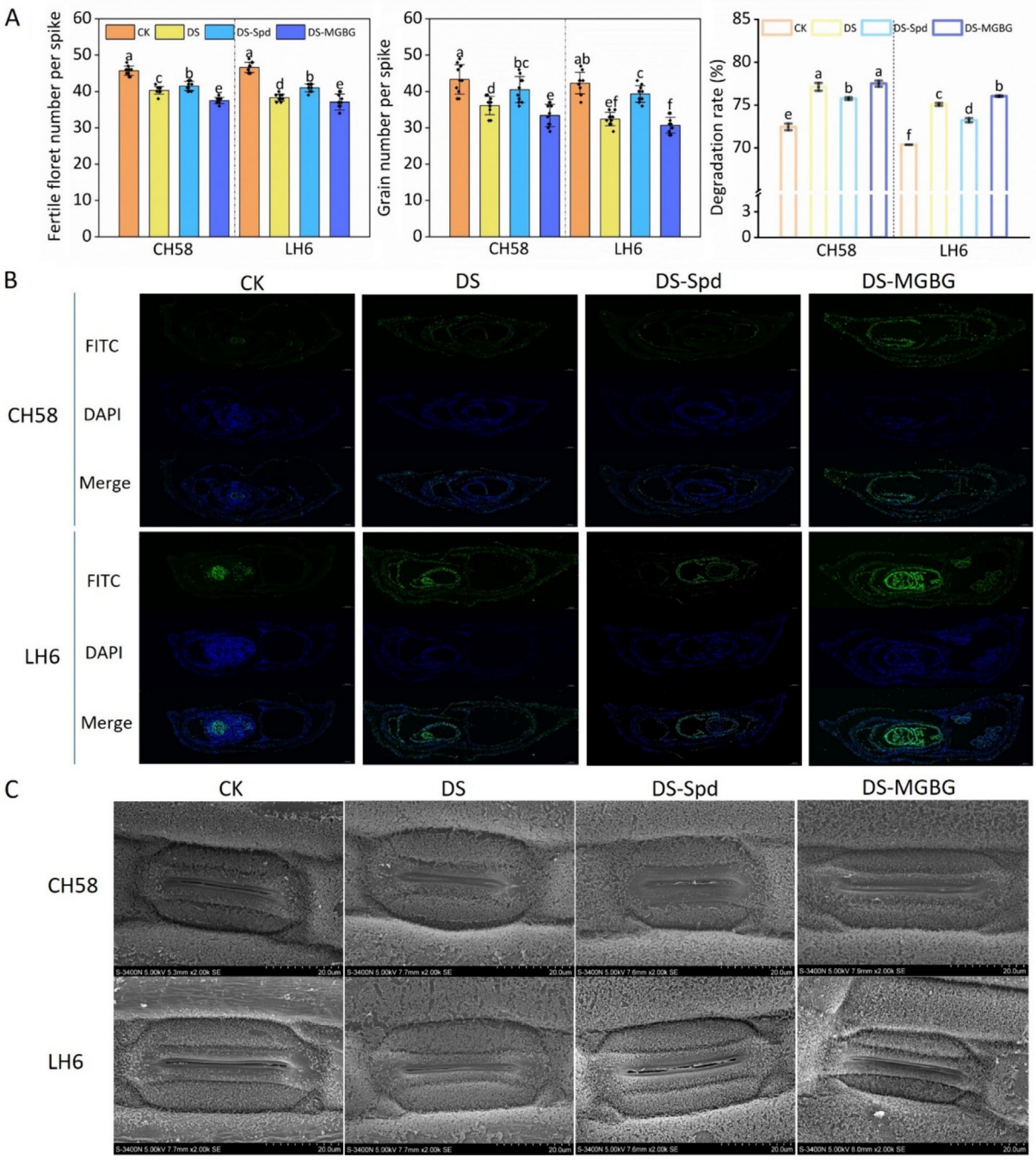


Fig. 1. Characteristics of floret development and phenotypes of young spikelets and flag leaves under drought and chemical treatments. (A) Number of fertile florets and grains per spike and the floret degeneration rate of wheat cultivars CH58 and LH6 in the field trial, the values are the means ± SD (n = 10). CK and DS represent well-watered conditions and drought stress, respectively. DS-Spd and DS-MGBG represent exogenous spermidine and the polyamine inhibitor propamidine hydrazone, respectively. The data were obtained from the 2022–2023 growing season of field experiment II. Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test, $P < 0.05$). (B) TUNEL assay of spikelets cells of CH58 and LH6 of subjected to various soil water and exogenous treatments in the pot trial. DAPI-stained nuclei are blue, while FITC-fluorescein-labeled positive apoptotic nuclei are green. Scale bar, 200 μm. The data were obtained from the 2022–2023 growing season of pot experiment. (C) Leaf stomatal morphology of the wheat cultivars CH58 and LH6 under various soil water and exogenous treatments in the field trial. Scale bar, 20 μm. The data were obtained from the 2021–2022 growing season of field experiment II. CH58, Changhan 58; LH6, Luohan 6.

signal (indicating the degree of cell apoptosis) was observed in DS and DS-MGBG, while fewer green signals were observed in the spikelet shell cells of DS-Spd (Fig. 1B). These findings indicated that DS-Spd resulted in less cell death than DS [44]. Yield and spike number were lower in the DS than in the CK treatment (Table S3). Compared to DS, DS-Spd increased yield, whereas there were no effects of exogenous chemical treatments on spike number (Table S4). Overall, Spd alleviated the degeneration of florets and reducing the number of grains per spike after exposure to drought stress.

3.2. Exogenous spermidine affects stomata and promotes photosynthesis in leaves under drought stress

Soil water and exogenous chemical treatments affected the RWC, SPAD and photosynthetic parameters of the flag leaves (Table S5). Compared to those of the CK treatment, the DS treatment reduced the RWC and SPAD of the flag leaves (Table S5). However, the DS-Spd treatment increased the RWC and SPAD of the two cultivars on average compared with those in the DS treatment, with values of 3.6% and 5.7%, respectively. Flag leaf stomata were closed after drought stress but tended to open slowly under the DS-Spd treatment (Fig. 1C). The photosynthetic parameters P_n, T_r, and G_s decreased under the DS treatment. Compared to DS, DS-Spd significantly improved the P_n of the two cultivars and the T_r of LH6 (Table S5). Therefore, exogenous Spd can effectively improve the water content, chlorophyll content and stomatal conditions of flag leaves, thereby enhancing photosynthesis [56].

3.3. Spermidine increases the antioxidant capacity and balances polyamine and ethylene metabolism in wheat leaves and young spikes

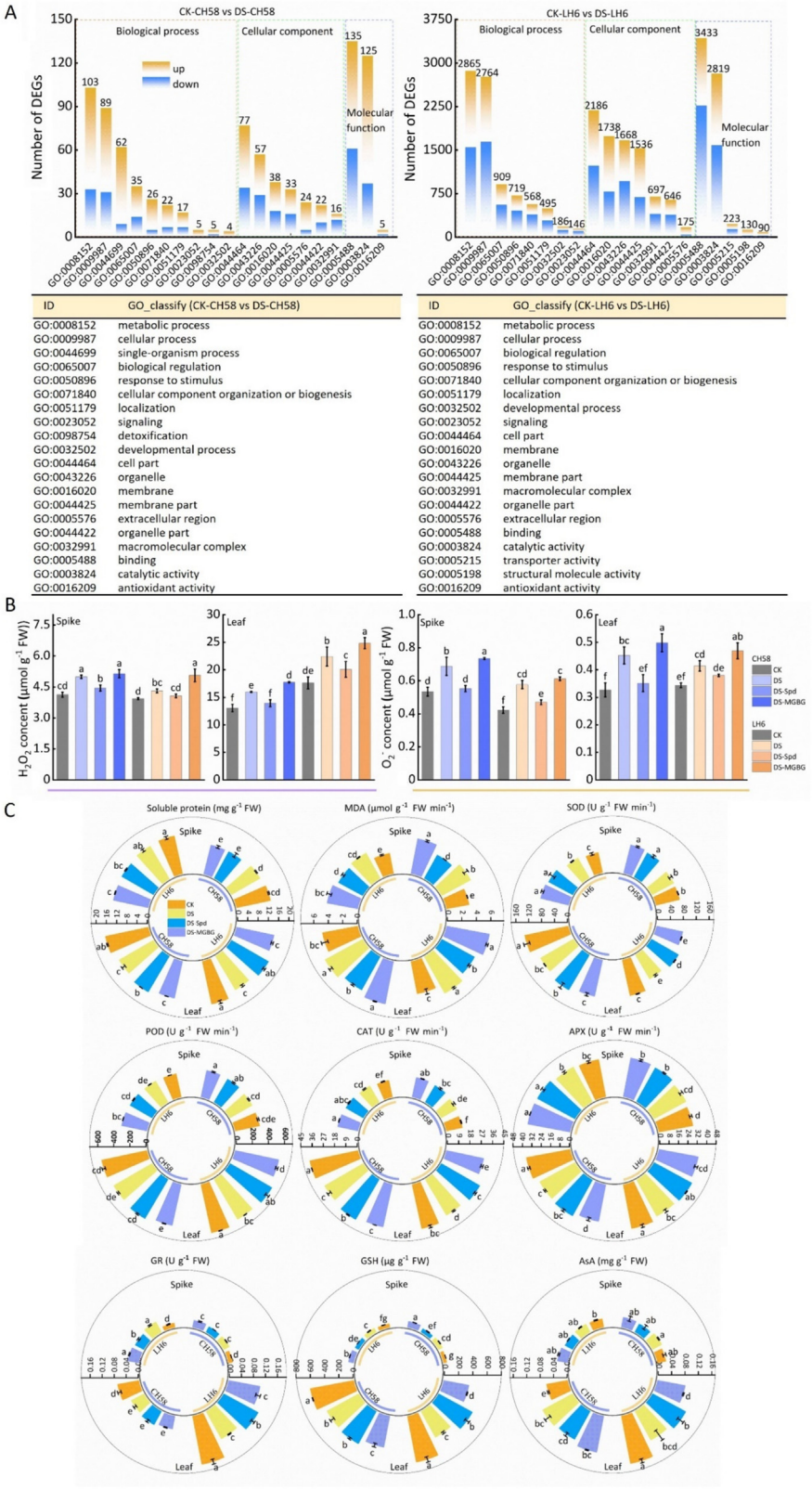
More DEGs were up- or downregulated in the young spikes of LH6 than in those of CH58 (Table S6). Nine genes relevant to this study were analyzed for quantification relative to the RNA-Seq results, and they showed similar expression patterns in qRT–PCR (Fig. S1). GO functional enrichment of the DEGs revealed distinct enrichment in metabolic processes, biological regulation, stimulus response, signaling and antioxidant enzyme activity (Fig. 2A). Severe drought upregulated the expression of genes encoding SOD, CAT, POD and APX (with LH6 being more pronounced) but downregulated the expression of genes encoding the GR enzyme (Fig. S2). The O_2^- and H_2O_2 contents of young spikes increased significantly in CH58 and LH6 plants under drought stress during the booting stage (Figs. S3, 2B). Compared to DS, SOD enzyme activity in the young spikes of both cultivars and POD, CAT and APX enzyme activities in the young spikes of LH6 increased under DS-Spd. However, the AsA content in the young spikes of both cultivars and the GSH content and GR activity in the young spikes of LH6 were reduced under DS-Spd treatment. In the leaves, exogenous Spd increased the activities of the CAT and GR enzymes as well as the GSH content to a greater extent than did DS. The level of H_2O_2 was reduced in leaves after exogenous Spd application (Fig. 2B), and cellular peroxidation was attenuated [56,57]. The soluble protein content in young spikes and leaves decreased after drought treatment, whereas the MDA content in young spikes and leaves increased in the two cultivars (Fig. 2C). Compared to those in the CK treatment, the activities of SOD, CAT and APX in the leaves in the DS treatment were lower, while the activities of POD and CAT were greater in the young spikes, especially in the LH6 treatment. Thus, exogenous Spd application further alleviated peroxidative damage in young spikes and leaves by enhancing the ROS scavenging system under drought stress.

These DEGs were enriched in the glutathione metabolism, arginine metabolism and proline metabolism pathways (Fig. S4). Expression of the gene encoding PAO was up-regulated, but the expression of the SPDS and SAMDC genes was down-regulated in young spikes under DS conditions (Fig. 3A), resulting in a decrease in endogenous Spd and Put contents but an increase in the rate of ETH release in DS than in CK (Fig. 3B), especially for CH58. The enzyme activities of PA synthesis and decomposition in spikes and leaves were affected by drought and exogenous treatment, but not by their interaction (Table S7). Compared to DS, SPDS activity was significantly increased but PAO activity was significantly inhibited under DS-Spd treatment (Fig. 3C), which induced a significant increase in the endogenous Spd content (36.1%–47.0%) and Put content (7.7%–12.0%) and a decrease in the ETH release rate (12.9%–33.0%) in young spikes under drought stress (Fig. 3B). Compared to DS, DS-MGBG increased PAO enzyme activity but decreased ADC activity in the spikes and flag leaves (Figs. 3C, S5), which caused a significant decrease in the endogenous Spd content but increased the ETH release rate in spikes and leaves of both cultivars (Figs. 3B, S5). The levels of endogenous Spd and Put, as well as ADC and SPDS enzyme activities in young spikes and leaves, were positively correlated with the number of fertile florets (Table S8). The ETH release rate and PAO enzyme activity in young spikes strongly and positively correlated with the rate of floret degeneration (Table S8). Compared to DS, the endogenous Spd content of young spikes was reduced by 5.1%–15.1%, the Put content was increased by 8.5%–9.1% (Fig. S6A), and the number of grains in the middle and top of the spike was significantly reduced when exogenously applied ethene (10 mmol L^{-1}) at the booting stage under DS treatment (Fig. S6B). Taken together, these results suggested that exogenous Spd application contributes to maintain endogenous PAs and ETH homeostasis in young spikes and leaves [58,59].

3.4. Spermidine promotes starch and sucrose metabolism pathways in young spikes and leaves under drought stress

The accumulation of dry matter in leaves also changes with decreasing or increasing photosynthesis (Fig. S7). Compared to CK, the dry matter weight of CH58 and LH6 leaves in the DS was reduced during the booting stage. However, the dry matter weight of LH6 leaves treated with DS-Spd was increased compared to DS until the heading stage (Fig. S7). The sucrose and starch contents of young spikes were strongly positively correlated with the number of fertile florets (Fig. 4A). Compared to CK, the fructose, glucose, sucrose and starch contents in young spikes of both cultivars were reduced in the DS treatment (Fig. 4B). Compared to those in DS, the fructose, glucose, sucrose and starch contents in LH6 spikes and the glucose content in CH58 spikes were increased in the DS-Spd treatment. Conversely, the glucose and starch contents in young CH58 spikes were significantly inhibited by DS-MGBG treatment. In leaves, the sucrose content in leaves of both cultivars was significantly lower in DS-Spd than in DS, but the starch content was increased, with the effect being more pronounced in LH6.

Compared with those in CK, the activities of the SPS and AGPase enzymes in both cultivars, as well as the INV and SS-I enzymes in LH6, were inhibited in young spikes under drought, while the activity of the SS-1 enzyme in CH58 increased (Fig. 4C). Compared with those in DS, the SPS activity in young spikes of both cultivars and the SS-I, AGPase, CWINV and INV activities in young spikes of LH6 were effectively increased under DS-Spd conditions. The SPS, SS-I and AGPase activities in flag leaves were significantly lower in the DS treatment than in the CK treatment. SS-I and AGPase activities in flag leaves were significantly greater in DS-Spd than in DS. However, the SPS, SS-I and AGPase enzyme activities in LH6 flag leaves were significantly reduced by DS-MGBG, whereas the SS-I enzyme activity in CH58 flag leaves increased. Therefore, Spd increased the synthesis and degeneration of sucrose and starch



ID	GO_classify (CK-CH58 vs DS-CH58)
GO:0008152	metabolic process
GO:0009987	cellular process
GO:0044699	single-organism process
GO:0065007	biological regulation
GO:0050896	response to stimulus
GO:0071840	cellular component organization or biogenesis
GO:0051179	localization
GO:0023052	signaling
GO:0098754	detoxification
GO:0032502	developmental process
GO:0044464	cell part
GO:0043226	organelle
GO:0016020	membrane
GO:0044425	membrane part
GO:0005576	extracellular region
GO:0044422	organelle part
GO:0032991	macromolecular complex
GO:0005488	binding
GO:0003824	catalytic activity
GO:0016209	antioxidant activity

ID	GO_classify (CK-LH6 vs DS-LH6)
GO:0008152	metabolic process
GO:0009987	cellular process
GO:0065007	biological regulation
GO:0050896	response to stimulus
GO:0071840	cellular component organization or biogenesis
GO:0051179	localization
GO:0032502	developmental process
GO:0023052	signaling
GO:0044464	cell part
GO:0016020	membrane
GO:0043226	organelle
GO:0044425	membrane part
GO:0032991	macromolecular complex
GO:0044422	organelle part
GO:0005576	extracellular region
GO:0005488	binding
GO:0003824	catalytic activity
GO:0005215	transporter activity
GO:0005198	structural molecule activity
GO:0016209	antioxidant activity

Fig. 2. RNA-seq analysis of spikes and ROS metabolism of spikes and leaves. (A) GO enrichment of young spikes at the booting stage under soil water treatment. CK-CH58, CH58 in the normal soil moisture treatment; DS-CH58, CH58 in the severe drought treatment; CK-LH6, LH6 in the normal soil moisture treatment; DS-LH6, LH6 in the severe drought treatment. The data were obtained from the 2020–2021 growing season of field experiment I. (B) O_2^- and H_2O_2 contents in the spikes and leaves of wheat cultivars CH58 and LH6 under various soil water and exogenous treatments in the field trial. The data were obtained from the 2021–2022 growing season of field experiment II. (C) Antioxidant enzyme and nonenzymatic antioxidant contents in the spikes and leaves of wheat cultivars CH58 and LH6 under various soil water and exogenous treatments in the field trial. The data were obtained from the 2021–2022 growing season of field experiment II. The values are means ± SD (n = 3). Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test, $P < 0.05$). CH58, Changhan 58; LH6, Luohan 6.

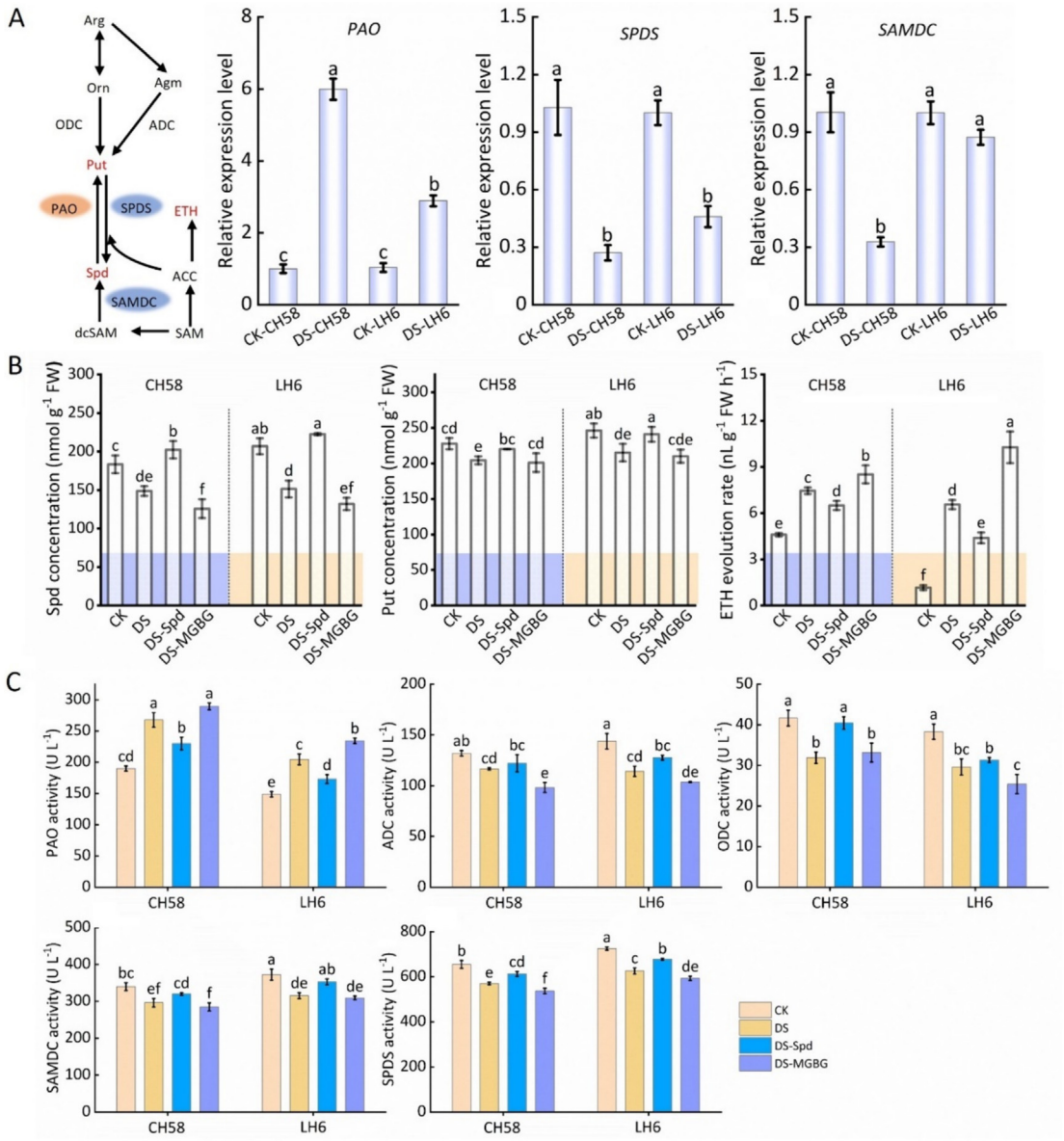


Fig. 3. Expression of genes encoding key enzymes involved in polyamine synthesis and decomposition by qRT-PCR (A), endogenous polyamine content and ethylene release rates (B), and activity of key enzymes involved in polyamine metabolism (C) in the spikes of wheat cultivars CH58 and LH6. CK and DS represent normal water conditions and drought stress, respectively. DS-Spd and DS-MGBG represent exogenous spermidine and the polyamine inhibitor propamidine hydrazone, respectively. The pathway diagram in (A) was from Li et al. [39] with permission from Oxford University Press. The data for (A) were obtained from the 2020–2021 growing season of field experiment I, and the data for (B) and (C) were obtained from the 2021–2022 growing season of field experiment II. Values are means ± SD (n = 3). **, $P < 0.01$ level. Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test, $P < 0.05$). Arg, arginine; Orn, ornithine; Agm, agmatine; SAM, S-adenosylmethionine; dcSAM, decarboxylated S-adnosylmethionine; Put, putrescine; Spd, spermidine; ETH, ethylene; ACC, 1-aminocyclopropane-1-carboxylic acid; ODC, ornithine decarboxylase; ADC, arginine decarboxylase; PAO, polyamine oxidase; SPDS, spermidine synthase; SAMDC, S-adenosylmethionine decarboxylase. CH58, Changhan 58; LH6, Luohan 6.

in young spikes and flag leaves under drought stress [38], and its effect on LH6 was more pronounced.

3.5. Spermidine promotes the synthesis and utilization of amino acids in young spikes under drought stress

Drought stress significantly reduced the total nitrogen content of wheat spikes and leaves at the booting and heading stages than those of CK (Fig. S8). Compared to DS, total nitrogen contents in spikes of CH58 at the booting stage and in spikes and leaves of two varieties at the heading stage were significantly increased in the DS-Spd treatment. To investigate the effects of drought stress and exogenous Spd on amino acid synthesis and catabolism, the results showed that the activities of NR, GS, GOGAT and GDH enzymes in young spikes were significantly higher under DS-Spd conditions compared to those in DS treatment (Fig. 5A). As amino acid synthesis and catabolism increased [39,60], NO_3^- levels in young spikes gradually decreased, and NH_4^+ levels gradually increased in DS-Spd than in DS, while glutamate and glutamine levels in young spikes in DS-Spd was gradually decreased to CK levels (Fig. 5B, C). For leaves, compared to DS, GOGAT and GDH activities of both cultivars as well as NR and GS enzyme activities of LH6 were significantly increased under DS-Spd treatment (Fig. S9A), and the NH_4^+ content in the leaves of both cultivars increased (Fig. S9B). The contents of serine, lysine, tyrosine, asparagine, threonine and arginine were significantly greater in the DS

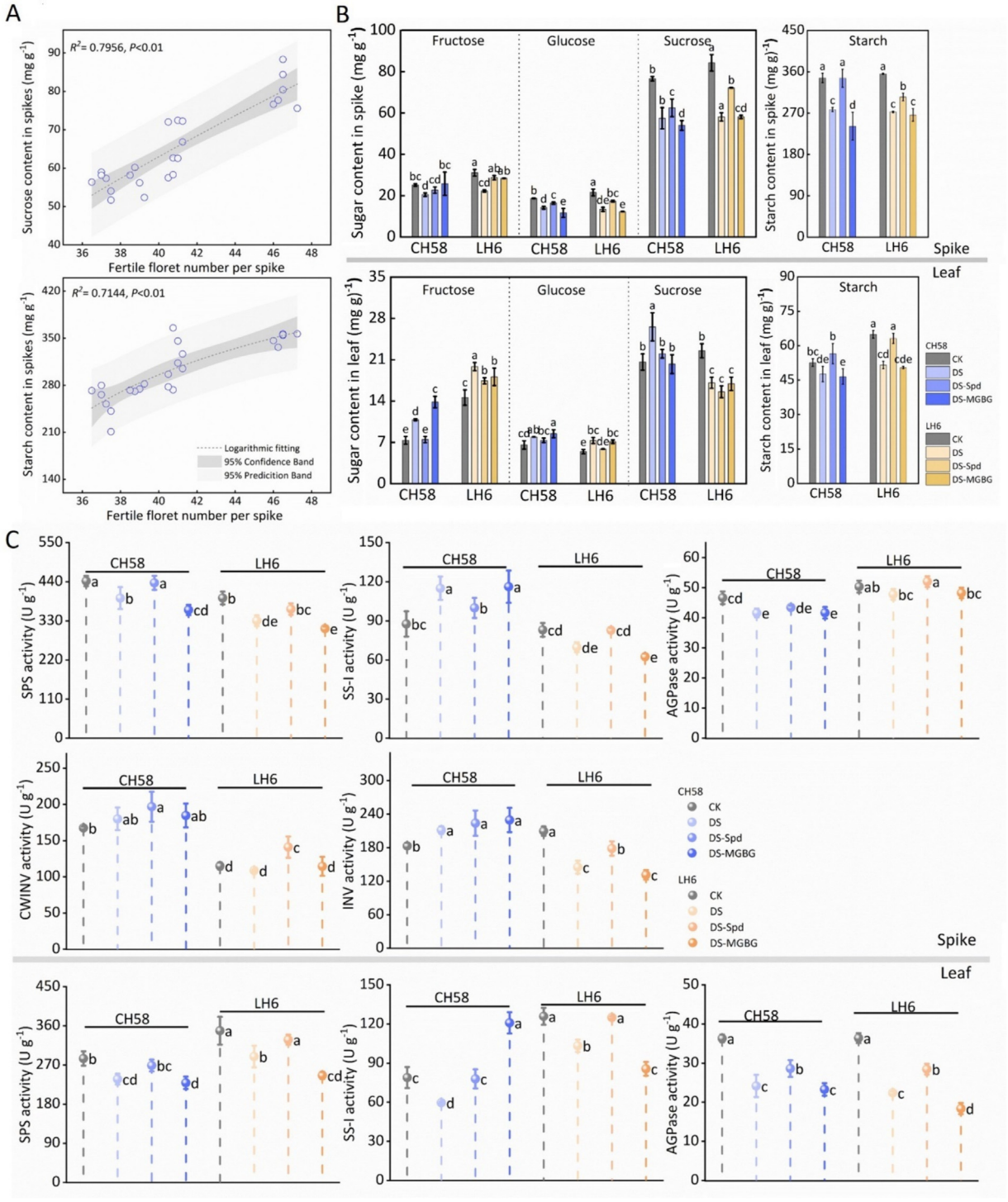


Fig. 4. The relationships between sugar and starch and between the number of fertile florets (A), the contents of sugar and starch (B), and the activities of key enzymes involved in sucrose and starch metabolism (C) in the spikes and leaves of wheat cultivars CH58 and LH6. CK and DS represent well-water conditions and drought stress, respectively. DS-Spd and DS-MGBG represent exogenous spermidine and the polyamine inhibitor propamidine hydrazone, respectively. The data were obtained from the 2021–2022 growing season of Field Experiment II. The values are the means ± SD (n = 3). Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test; $P < 0.05$). SPS, sucrose phosphate synthase, SS-I, sucrose synthetase (cleavage direction), AGPase, ADP-glucose pyrophosphorylase; INV, soluble vacuolar invertase; CWINV, cell wall invertase; CH58, Changhan 58; LH6, Luohan 6.

treatment than in the CK treatment, but the contents of these amino acids were significantly lower in the DS-Spd treatment than in the DS treatment (Figs. 5D, S10). Compared to DS, serine, lysine, asparagine, proline and arginine levels were significantly increased, and ornithine levels were significantly decreased under DS-MGBG treatment (Figs. 5D, S10). As a result, it speculated that exogenous Spd may facilitate the utilization of amino acids in young spikes under drought stress [61,62].

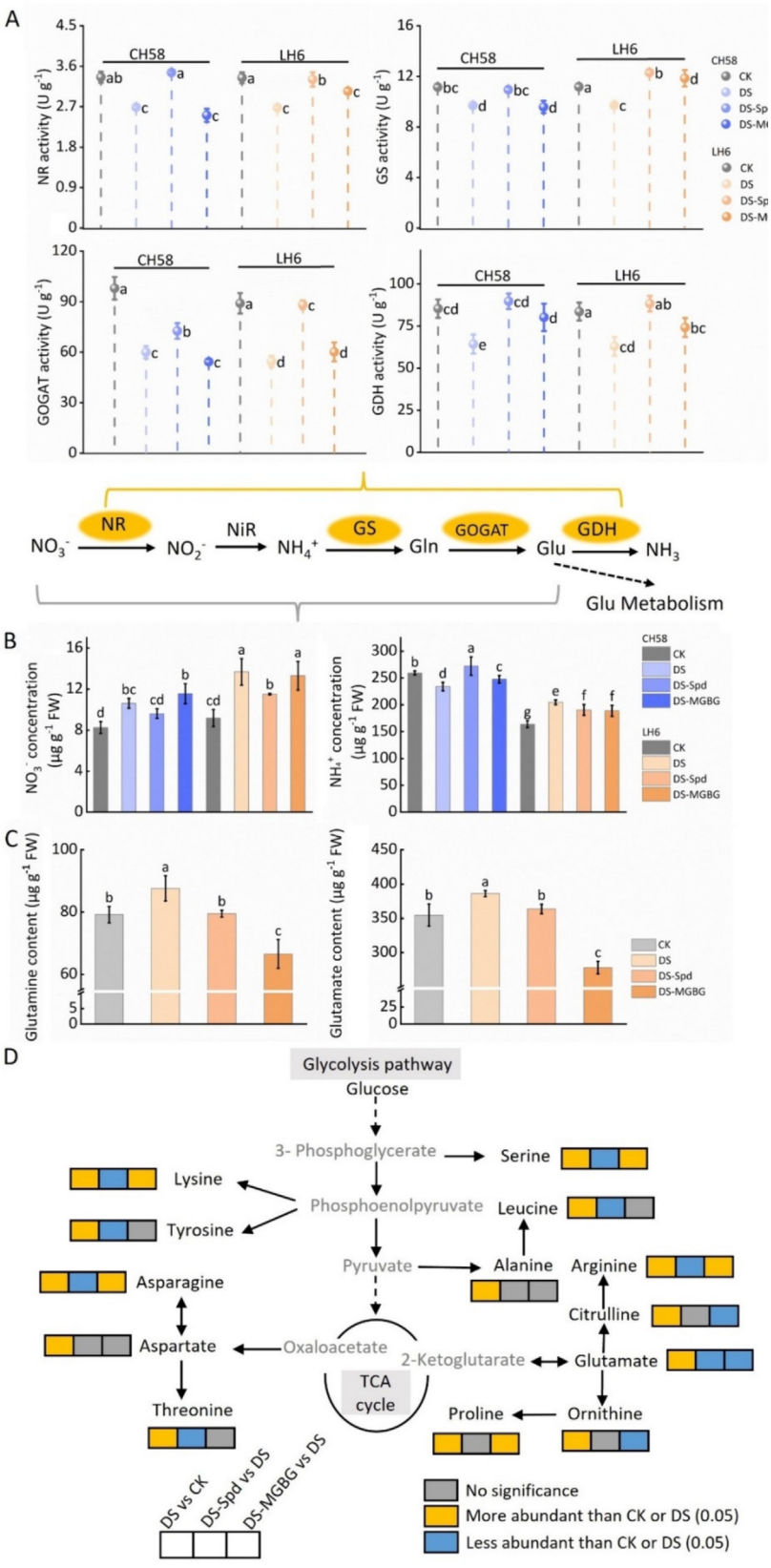


Fig. 5. Key enzyme activities involved in nitrogen metabolism (A); nitrate (NO_3^-) and ammonium (NH_4^+) nitrogen (B) contents; glutamic acid and glutamine (C) levels; and other amino acid content in the spikes of the wheat cultivar LH6 (D). CK and DS represent well-water conditions and drought stress, respectively. DS-Spd and DS-MGBG represent exogenous spermidine and the polyamine inhibitor propamidine hydrazone, respectively. The pathway diagram in (A) was from Li et al. [39] with permission from Oxford University Press. The pathway diagram in (D) was constructed according to Liu et al. [75] with slight modifications. The data for (A, B) were obtained from the 2021–2022 growing season of Field Experiment II. The data for (C, D) were obtained from the 2022–2023 growing season of Pot Experiment. Values are means ± SD (n = 3). Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test, $P < 0.05$). NR, nitrate reductase; GS, glutamine synthetase; GOGAT, glutamate synthetase; GDH, glutamate dehydrogenase; NO_3^-, nitrate; NO_2^-, nitrite; NH_4^+, ammonium; Glu, glutamate; Gln, glutamine; NH_3, ammonia. CH58; Changhan 58; LH6; Luohan 6.

3.6. Spermidine modulates the TCA cycle and glycolytic pathways in young spikes to maintain energy homeostasis under drought stress

The effects of PAs on glycolysis and the TCA cycle under drought conditions were further explored, and the results showed that the contents of fructose-1,6-bisphosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, and pyruvate were significantly lower in response to drought and that exogenous Spd significantly increased the levels of these intermediates of phosphoglycolysis in young spikes compared to those in DS plants (Fig. 6A). However, the glucose-6-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate levels were more significantly suppressed by DS-MGBG than by DS. Moreover, compared with that in the DS treatment, the glyceraldehyde-3-phosphate content in young spikes increased in response to drought, and the content was further reduced by DS-Spd. Thus, exogenous Spd provided a sufficient substrate for the synthesis of lysine, tyrosine and serine [23]. Citrate, *cis*-aconitate, isocitrate, and L-malate contents were significantly increased by drought, and the oxaloacetate, fumarase, and succinate contents were significantly decreased in young spikes (Fig. 6B). Compared to those in DS, the L-malate and *cis*-aconitate contents in DS-Spd further increased, but the succinate content decreased, while the oxaloacetate, fumarase, succinate and 2-ketoglutarate contents significantly decreased in the DS-MGBG treatment. In general, exogenous PA inhibitors further exacerbate the disruption of glycolysis and the TCA cycle under drought stress.

Glycolysis and the TCA cycle proceed through the synthesis and consumption of energy [17–19]. Compared to CK, ATP and NAD contents significantly increased under DS conditions, while the energy charge and NADPH content significantly decreased (Fig. 6C). Compared with DS, DS-Spd significantly increased the ATP (39.0%) and NADPH (50.2%) contents, whereas DS-MGBG had no significant positive effect on the energy content or energy charge. Consequently, exogenous Spd promoted energy production in young spikes [63].

4. Discussion

Drought intensifies floret degeneration, resulting in a significant reduction in the number of fertile florets and in the number of grains in the spikes at maturity [3,4,8]. The degeneration of florets is largely due to cell apoptosis [2,13]. In this study, the cell death signals associated with apoptosis by TUNEL staining were more pronounced in spikelets under DS than in CK. ROS have been implicated as signaling molecules during plant spikes, pollen development and programmed cell death in the tapetum, and the ROS scavenging status of young spikes under adverse conditions is associated with the fate of floret development [64]. Previous studies have shown that drought damages the ROS scavenging system, disrupting the balance between ROS production and scavenging, which results in a burst of ROS in tissues [5,12]. In the present study, O_2^- and H_2O_2 still over-accumulated in the young spikes of CH58 and LH6 despite the upregulation of the expression of genes encoding SOD, CAT, POD, and APX in response to severe drought stress. ROS bursts under adverse conditions by driving reprogramming of the transcriptome, metabolism and proteome for plant adaptation and survival [12]. RNA-Seq revealed that in addition to exhibiting antioxidant enzyme activity, a large number of DEGs were enriched in young spikes in response to stimuli, biological regulation and metabolic processes. KEGG functional enrichment showed that both the CH58 and LH6 DEGs were enriched in the glutathione metabolism, arginine metabolism and proline metabolism pathways. Arginine is a key synthetic precursor of PAs, and DS treatment decreased endogenous levels of Spd and Put, but increased the rate of release of ETH. ETH and PA compete for the precursor S-adenosylmethionine [31,40]. This was also the result of drought-induced upregulation of *PAO* gene expression in young spikes, as well as repression of the expression of *SPDS* and *SAMDC* genes. Similar results were obtained for wheat seedlings, rice and *Sophora acacia* [32,34]. The endogenous Spd and Put contents and the activities of ADC and SPDS in young spikes and leaves were significantly and positively correlated with the number of fertile florets, while the PAO enzyme activities in young spikes and flag leaves were highly significantly and positively correlated with the rate of floret degeneration. These findings indicated that PA synthesis has a positive effect on floret development. Therefore, drought-induced ROS bursts occur in young spikes, and floret degeneration intensifies; this process may be related to PA synthesis and catabolism [59].

Exogenous Spd effectively alleviated floret degeneration and increased the number of fertile florets, which in turn reduced the decrease in grain number caused by drought. However, exogenous PA inhibitors (e.g., O-phen and MGBG) decrease tissue antioxidant enzymes and antioxidants, causing an increase in cellular ROS bursts [33,65]. The dynamic equilibrium state of ROS in plants is maintained by their own efficient antioxidant enzyme components and nonenzymatic systems [34,56,57,66,67]. In this study, exogenous Spd promoted the enzymatic activities of SOD, POD, CAT and APX (more significantly in LH6) more than did the nonenzymatic substances AsA and GSH, especially in young spikes. Moreover, a lower H_2O_2 content was detected in young spikes and leaves treated with exogenous Spd under drought stress, and the degree of cellular peroxidation was partially alleviated. In this study, exogenous MGBG further exacerbated floret degeneration, leading to an increase in the negative effect of drought on floret development. These findings also indicate that the regulation of ROS metabolism by exogenous Spd in young spikes and flag leaves at the booting stage influences the induction of floret degeneration by drought. Exogenous PAs or the overexpression of PA biosynthesis genes to increase endogenous PA levels results in increased drought and salt tolerance in plants [32,56,59]. In the present study, exogenous Spd significantly increased the endogenous Spd and Put contents and decreased the ETH release rate in young spikes under drought stress. This was because exogenous Spd increased SPDS and SAMDC enzyme activities but inhibited PAO enzyme activity. Exogenous application of ethephon at the booting stage resulted in a significant decrease in endogenous Spd content, a significant increase in Put content in young spikes and a significant decrease in the number of grains in the middle and top of the spikes. Thus, the restoration of PA and ETH homeostasis further contributed to the alleviation of drought-induced floret degeneration by Spd. Moreover, photosynthesis is the primary pathway for accessing plant assimilates. One of the major physiological effects of drought stress is low CO_2 availability due to a decrease in stomatal conductance and activity of the key photosynthesis-related enzyme ribulose-1,5-bisphosphate carboxylase and consequent inhibition of photosynthesis [68,69,70]. PAs improve the gas exchange characteristics of plants suffering from drought and salt stress [56]. Exogenous Spd alleviated drought stress-induced changes in RWC and SPAD in both cultivars and further increased the P_n of the flag leaves of the two cultivars and the T_r of LH6. The rates of photosynthesis (i.e., CO_2 uptake) and transpiration (i.e., H_2O) are regulated by the stomatal behavior of plants [71]. The slow opening of stomata by exogenous Spd also supported this view.

Metabolic reprogramming is vital for maintaining plant energy homeostasis, survival and development under stress [17]. Sucrose and starch metabolism are known as the main sources of stored energy for the development of reproductive organs in plants [8,60]. The sucrose content in the leaves was affected differently

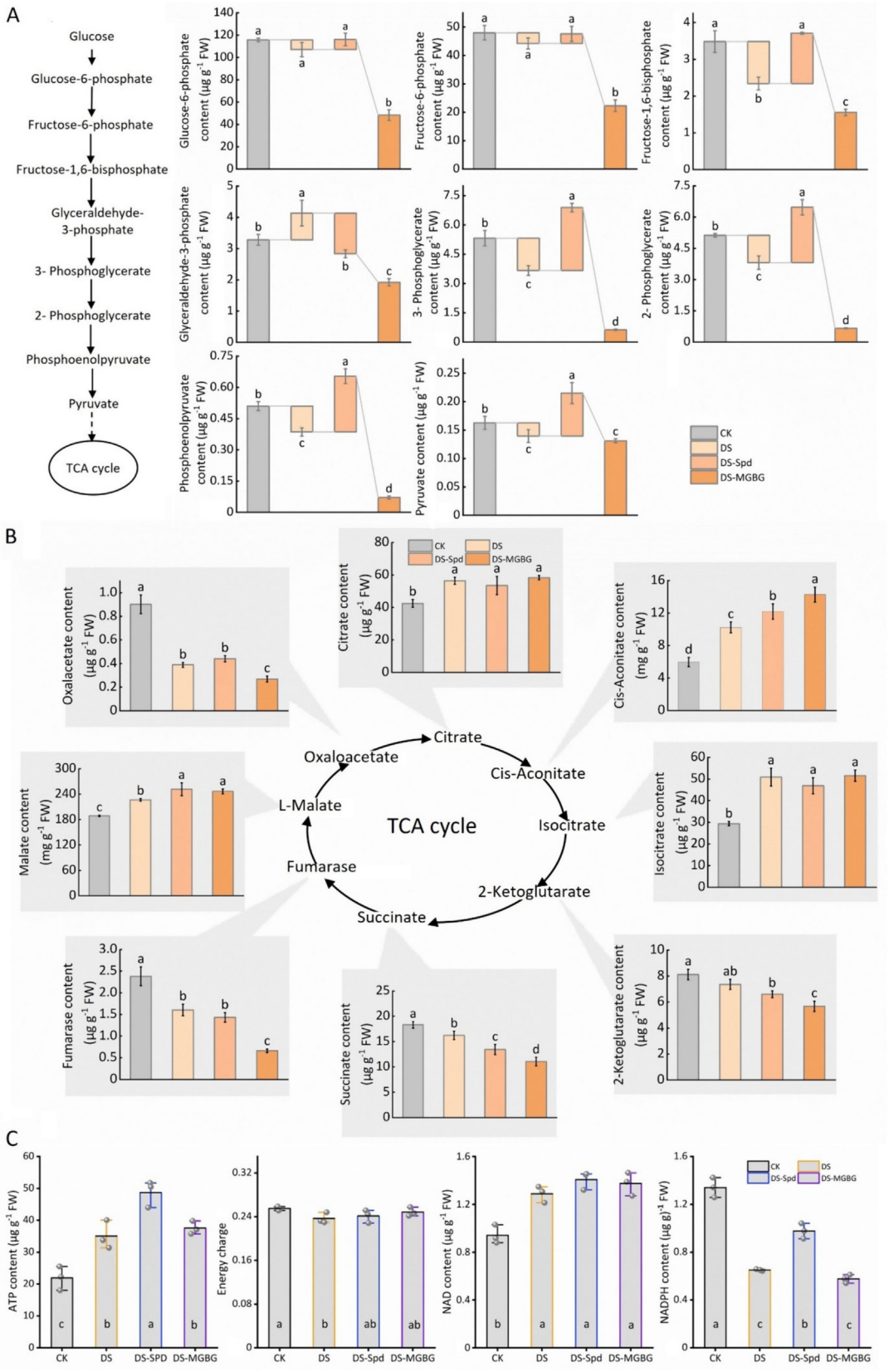


Fig. 6. Intermediacy of glycolysis (A) and the TCA cycle (B), and contents of adenosine triphosphate (ATP), energy charge, nicotinamide adenine dinucleotide (NAD) and dihydronicotinamide adenine dinucleotide phosphate (NADPH) in the spikes of the wheat cultivar LH6 (C).CK and DS represent well-water conditions and drought stress, respectively. DS-Spd and DS-MGBG represent exogenous spermidine and the polyamine inhibitor propamidine hydrazone, respectively. The pathway diagram in (A) and (B) were from Liu et al. [75] and Gao et al. [76]. The data were obtained from the 2022–2023 growing season of pot experiment. Values are means ± SD (n = 3). Different lowercase letters denote significant differences (ANOVA, Fisher's least significant difference test, $P < 0.05$). LH6, Luohan 6.

by drought due to the differential drought resistance of the cultivars; however, the fructose and glucose contents in the leaves of both cultivars increased under drought treatment, and the starch content decreased in both cultivars. These findings indicated that hexose utilization and starch synthesis in the leaves were inhibited by drought. Exogenous PAs play a positive role in regulating sucrose and starch synthases, thereby increasing sugar and starch contents in tissues to enhance plant stress tolerance [38]. Both the sucrose and starch contents of young spikes at the booting stage were strongly significantly and positively correlated with the number of fertile florets. In the present study, DS-Spd effectively alleviated the drought-induced inhibition of sucrose catabolic enzymes (SS-I, CWINV and INV) and AGPase activity in young spikes of LH6, which promoted the synthesis of starch and increased the glucose content in young spikes under drought stress. Glucose undergoes glycolysis to produce pyruvate, which further produces acetyl-CoA and enters the TCA cycle to produce ATP, which is directly used by plants [17,18,19]. Hu et al. [18] reported that drought reduces pyruvate content, leading to a reduction in energy consumption, which subsequently leads to a failure in the development of reproductive organs. Similarly, exogenous Spd effectively increased the contents of fructose-1,6-bisphosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and pyruvate in young spikes. Most glycolytic metabolites are used in the biosynthesis of amino acids (e.g., alanine, leucine and lysine) [72]. Thus, the effects of drought and Spd on glycolytic intermediates further regulate amino acid synthesis. Pires et al. [23] demonstrated that drought-induced metabolic reprogramming led to the accumulation of TCA cycle intermediates, thus improving plant drought tolerance. Drought induced an increase in the levels of citrate, *cis*-aconitate, isocitrate and L-malate in young spikes. Interestingly, exogenous Spd further increased the L-malate and *cis*-aconitate contents. Malate is essential for normal spike development, and injection of malate into spikes of the spikelet apical abortion *paab1-1* mutant alleviated the spikelet degeneration phenotype [44]. However, 2-ketoglutarate levels were significantly reduced in young spikes under DS-MGBG conditions. Based on the knowledge that 2-ketoglutarate can be converted by transaminases to glutamic acid, it may also be the reason for the decrease in glutamic acid content in young spikes.

Amino acids are precursors for the synthesis of secondary metabolites, and their accumulation under stress may enhance ROS scavenging and provide an additional pathway for energy supply [17,72]. Catabolic metabolites of BCAAs (e.g., isoleucine, leucine and valine) provide substrates for the TCA cycle to produce more ATP for adverse resistance and organism development [22,23]. There are interconversion pathways between BCAAs and glutamate: glutamate produces alanine and leucine via alanine aminotransferase, and in the presence of BCAA aminotransferases, BCAAs are degraded to branched chain alpha-keto acids, which further produce glutamate [23]. The contents of serine, lysine, tyrosine, asparagine, threonine and arginine significantly increased under DS treatment. However, DS-Spd significantly increased the enzyme activities of NR, GS, GOGAT and GDH in young spikes, and the contents of glutamate, glutamine, serine, lysine and tyrosine were still reduced. Thus, Spd replenishes the energy supply of plants by activating the respiratory pathway, which uses amino

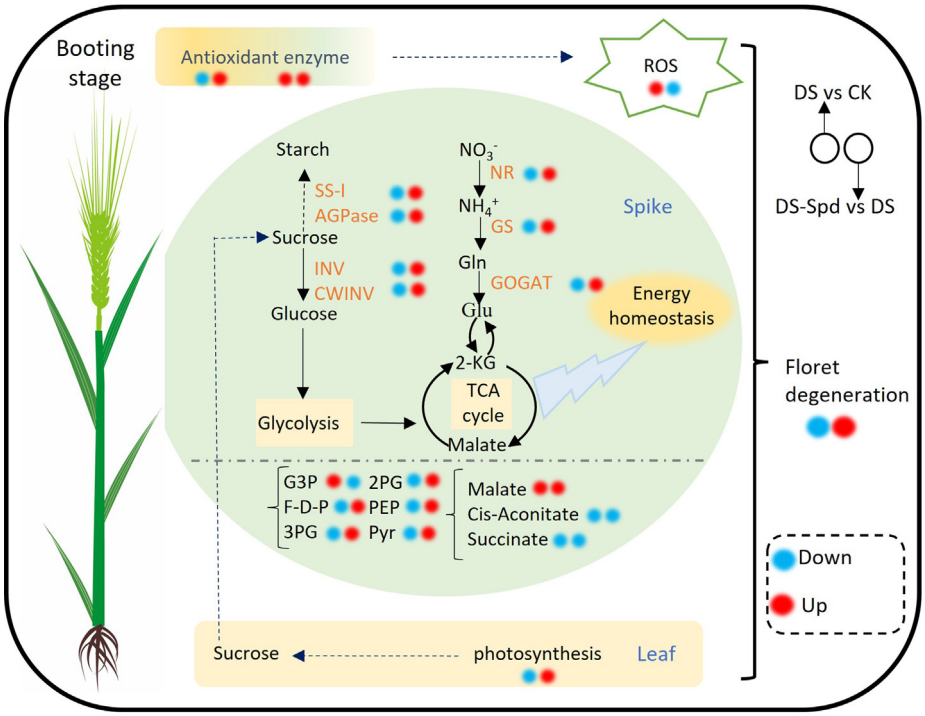


Fig. 7. Physiological mechanism of the effect of spermidine on floret degeneration under drought stress. Under drought stress, the excessive accumulation of cellular ROS and impaired sugar and amino acid metabolism in young spikes induced severe floret degeneration. Exogenous spermidine helped to increase leaf photosynthesis and alleviate drought-induced peroxidation in spikes by increasing antioxidant enzyme activities. Moreover, exogenous Spd promoted starch and sucrose metabolism and amino acid utilization by increasing the activities of invertase and key enzymes involved in nitrogen metabolism. Further improvements in glycolysis and the TCA cycle provided energy homeostasis for the floret development process, which consequently reduced floret degeneration. CK, well-water conditions; DS, drought stress; DS-Spd, exogenous spermidine under DS conditions. DS vs. CK, significant changes in indicators in DS treatment compared to CK treatment; DS-Spd vs. DS, significant changes in indicators in DS-Spd treatment compared to DS treatment; ROS, reactive oxygen species; SS-I, sucrose synthetase (cleavage direction); AGPase, ADP-glucose pyrophosphorylase; INV, soluble vacuolar invertase; CWINV, cell wall invertase; NR, nitrate reductase; GS, glutamine synthetase; GOGAT, glutamate synthetase; NO_3^-, nitrate; NH_4^+, ammonium; Glu, glutamate; Gln, glutamine; 2-KG, 2-ketoglutarate; G3P, glyceraldehyde-3phosphate; F-D-P, fructose-1,6-bisphosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate, Pyr, pyruvate.

acids as alternative substrates. According to Hu et al. [18] and Yu et al. [73], drought caused a significant decrease in the ATP content in cotton pistils and anthers, which made pollination difficult. However, the results of the present study are inconsistent with previous results. In this study, the energy charge and NAPDH content tended to decrease significantly under DS, while the ATP content increased significantly, and the ATP energy gradually recovered to the CK level after exogenous Spd treatment. This difference may be due to drought inhibiting the physiological activities of the florets, leading to a decrease in ATP consumption [74]. Overall, Spd promoted energy homeostasis for floret development and grain number formation under drought stress by alleviating sucrose and starch metabolism in young spikes and leaves and promoting amino acid utilization, glycolysis and the TCA cycle in young spikes.

5. Conclusions

Drought induces oxidative damage and excessive accumulation of cellular ROS in spikes, which leads to increased apoptosis and disturbed energy metabolism, thereby causing severe floret degeneration. Exogenous Spd contributes to increasing leaf photosynthesis and alleviating drought-induced peroxidation in the spikes by increasing antioxidant enzyme activity and PA metabolism. Moreover, exogenous Spd improved starch and sucrose metabolism; promoted amino acid utilization, glycolysis and the TCA cycle; and promoted energy homeostasis for floret development. Overall, Spd facilitated the formation of a network of multiple metabolic homeostasis pathways under drought stress, which alleviated oxidative damage in young spikes, thereby reducing floret degeneration and providing sufficient energy for grain number formation (Fig. 7).

CRediT authorship contribution statement

Juan Li: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Gege Li:** Methodology, Writing – review & editing. **Zhiyuan Li:** Data curation, Formal analysis. **Jiayu Li:** Data curation, Formal analysis. **Jianke Xiao:** Investigation, Supervision. **Vinay Nangia:** Investigation, Supervision. **Yang Liu:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Maomao Wang and Hongxia Li from the Crop Biology Innovation Center for their help in the determination of polyamines and sugar components by high-performance liquid chromatography. This work was supported by the National Natural Science Foundation of China (31871567) and the Key Research and Development Program of Shaanxi (2021NY-083).

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2024.07.017.

References

[1] Z. Wang, Y. Zhang, Z. Kang, H. Mao, Improvement of wheat drought tolerance through editing of TaATX4 by CRISPR/Cas9, J. Genet. Genomics 50 (2023) 913–916.
[2] A. Ferrante, R. Savin, G.A. Slafer, Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability, J. Exp. Bot. 64 (2013) 169–184.
[3] X. Ji, B. Shiran, J. Wan, D.C. Lewis, C.L. Jenkins, A.G. Condon, R.A. Richards, R. Dolferus, Importance of pre-anthesis anther sink strength for maintenance of grain number during reproductive stage water stress in wheat, Plant Cell Environ. 33 (2010) 926–942.
[4] Z. Zhang, J. Huang, Y. Gao, Y. Liu, J. Li, X. Zhou, C. Yao, Z. Wang, Z. Sun, Y. Zhang, Suppressed ABA signal transduction in the spike promotes sucrose use in the stem and reduces grain number in wheat under water stress, J. Exp. Bot. 71 (2020) 7241–7256.
[5] A. Fabian, E. Safran, G. Szabo-Eitel, B. Barnabas, K. Jager, Stigma functionality and fertility are reduced by heat and drought costress in wheat, Front. Plant Sci. 10 (2019) 244.
[6] A. Gupta, A. Rico-Medina, A.I. Caño-Delgado, The physiology of plant responses to drought, Science 368 (2020) 266–269.
[7] X. Li, L.M. Lawas, R. Malo, U. Glaubitz, A. Erban, R. Mauleon, S. Heuer, E. Zuther, J. Kopka, D.K. Hincha, K.S. Jagadish, Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress, Plant Cell Environ. 38 (2015) 2171–2192.
[8] S. Shen, X.G. Liang, L. Zhang, X. Zhao, Y.P. Liu, S. Lin, Z. Gao, P. Wang, Z.M. Wang, S.L. Zhou, Intervening in sibling competition for assimilates by controlled pollination prevents seed abortion under postpollination drought in maize, Plant Cell Environ. 43 (2020) 903–919.
[9] W. He, W. Li, X. Luo, Y. Tang, L. Wang, F. Yu, Q. Lin, Rice FERONIA-LIKE RECEPTOR 3 and 14 affect grain quality by regulating redox homeostasis during endosperm development, J. Exp. Bot. 74 (2023) 3003–3018.
[10] P.I. Kerchev, F. van Breusegem, Improving oxidative stress resilience in plants, Plant J. 109 (2022) 359–372.
[11] H. Wang, N. Li, H. Li, S. Zhang, X. Zhang, X. Yan, Z. Wang, Y. Yang, S. Zhang, Overexpression of NtGCN2 improves drought tolerance in tobacco by regulating proline accumulation, ROS scavenging ability, and stomatal closure, Plant Physiol. Biochem. 198 (2023) 107665.
[12] A.R. Devireddy, S.I. Zandalinas, Y. Fichman, R. Mittler, Integration of reactive oxygen species and hormone signaling during abiotic stress, Plant J. 105 (2021) 459–476.
[13] Q. Gan, F. Song, C. Zhang, Z. Han, B. Teng, C. Lin, D. Gu, J. Wang, H. Pei, J. Wu, J. Fang, D. Ni, Ca^{2+} deficiency triggers panicle degeneration in rice mediated by Ca^{2+}/H^{+} exchanger OsCAX1a, Plant Cell Environ. 46 (2023) 1610–1628.
[14] C.H. Hu, Q.D. Zeng, L. Tai, B.B. Li, P.P. Zhang, X.M. Nie, P.Q. Wang, W.T. Liu, W.Q. Li, Z.S. Kang, D.J. Han, K.M. Chen, Interaction between TaNOX7 and TaCDPK13 contributes to plant fertility and drought tolerance by regulating ROS production, J. Agric. Food Chem. 68 (2020) 7333–7347.
[15] J. Bai, X. Zhu, Q. Wang, J. Zhang, H. Chen, G. Dong, L. Zhu, H. Zheng, Q. Xie, J. Nian, F. Chen, Y. Fu, Q. Qian, J. Zuo, Rice *TUTOU1* encodes a suppressor of cAMP receptor-like protein that is important for actin organization and panicle development, Plant Physiol. 169 (2015) 1179–1191.
[16] J. Tang, D.C. Bassham, Autophagy in crop plants: what's new beyond Arabidopsis, Open Biol. 8 (2018) 180162.
[17] B. Heinemann, T.M. Hildebrandt, The role of amino acid metabolism in signaling and metabolic adaptation to stress-induced energy deficiency in plants, J. Exp. Bot. 72 (2021) 4634–4645.
[18] W. Hu, Y. Liu, D.A. Loka, R. Zahoor, S. Wang, Z. Zhou, Drought limits pollen tube growth rate by altering carbohydrate metabolism in cotton (*Gossypium hirsutum*) pistils, Plant Sci. 286 (2019) 108117.
[19] B. Feng, Y. Xu, W. Fu, H. Li, G. Li, J. Li, W. Wang, L. Tao, T. Chen, G. Fu, RGA1 negatively regulates thermo-tolerance by affecting carbohydrate metabolism and the energy supply in rice, Rice 16 (2023) 32.
[20] Y. Du, Q. Zhao, L. Chen, X. Yao, W. Zhang, B. Zhang, F. Xie, Effect of drought stress on sugar metabolism in leaves and roots of soybean seedlings, Plant Physiol. Biochem. 146 (2020) 1–12.
[21] X. Guan, Y. Zhang, L. Zhou, M.A.U. Asad, Q. Zhao, G. Pan, F. Cheng, Disruptions of sugar utilization and carbohydrate metabolism in rice developing anthers aggravated heat stress-induced pollen abortion, Plant Physiol. Biochem. 202 (2023) 107991.
[22] W.L. Araujo, K. Ishizaki, A. Nunes-Nesi, T.R. Larson, T. Tohge, I. Krahnert, S. Witt, T. Obata, N. Schauer, I.A. Graham, C.J. Leaver, A.R. Fernie, Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of Arabidopsis mitochondria, Plant Cell 22 (2010) 1549–1563.
[23] M.V. Pires, A.A. Pereira Junior, D.B. Medeiros, D.M. Daloso, P.A. Pham, K.A. Barros, M.K. Engqvist, A. Florian, I. Krahnert, V.G. Maurino, W.L. Araujo, A.R. Fernie, The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in Arabidopsis, Plant Cell Environ. 39 (2016) 1304–1319.
[24] Y. Liu, J. Hou, X. Wang, T. Li, U. Majeed, C. Hao, X. Zhang, The NAC transcription factor NAC019-A1 is a negative regulator of starch synthesis in wheat developing endosperm, J. Exp. Bot. 71 (2020) 5794–5807.
[25] H.M. Wu, D.J. Xie, P.F. Jia, Z.S. Tang, D.Q. Shi, G.H. Shui, G.D. Wang, W.C. Yang, Homeostasis of flavonoids and triterpenoids most likely modulates starch

metabolism for pollen tube penetration in rice, Plant Biotechnol. J. 21 (2023) 1757–1772.
[26] O. Turc, F. Tardieu, Drought affects abortion of reproductive organs by exacerbating developmentally driven processes via expansive growth and hydraulics, J. Exp. Bot. 69 (2018) 3245–3254.
[27] Y. Li, P. Zhang, Z. Wang, Y. Zhang, F. Zhu, Y.H. Liu, A. Jones, L. Wu, Y. Song, Coordinated regulation of sucrose and lignin metabolism for arrested silk elongation under drought stress in maize, Environ. Exp. Bot. 214 (2023) 105482.
[28] H. Zhang, W. Liang, X. Yang, X. Luo, N. Jiang, H. Ma, D. Zhang, Carbon starved anther encodes a MYB domain protein that regulates sugar partitioning required for rice pollen development, Plant Cell 22 (2010) 672–689.
[29] Y. Jin, H. Yang, Z. Wei, H. Ma, X. Ge, Rice male development under drought stress: phenotypic changes and stage-dependent transcriptomic reprogramming, Mol. Plant 6 (2013) 1630–1645.
[30] J.E. McLaughlin, J.S. Boyer, Sugar-responsive gene expression, invertase activity, and senescence in aborting maize ovaries at low water potentials, Ann. Bot. 94 (2004) 675–689.
[31] R. Alcazar, T. Altabella, F. Marco, C. Bortolotti, M. Reymond, C. Koncz, P. Carrasco, A.F. Tiburcio, Polyamines: molecules with regulatory functions in plant abiotic stress tolerance, Planta 231 (2010) 1237–1249.
[32] H.T. Ebeed, N.M. Hassan, A.M. Aljarani, Exogenous applications of Polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes, Plant Physiol. Biochem. 118 (2017) 438–448.
[33] A. Korbas, J. Kubiś, M. Rybus-Zając, T. Chadzinikolau, Spermidine modify antioxidant activity in cucumber exposed to salinity stress, Agronomy 12 (2022) 1554.
[34] X. Sun, A. Lv, D. Chen, Z. Zhang, X. Wang, A. Zhou, X. Xu, Q. Shao, Y. Zheng, Exogenous spermidine enhanced the water deficit tolerance of *Anoectochilus roxburghii* by modulating plant antioxidant enzymes and polyamine metabolism, Agric. Water Manage. 289 (2023) 108538.
[35] J. Yang, P. Wang, X. Li, D. Zhou, X. Cai, X. Hu, S. Hu, Regulation of cold resistance by the polyamine biosynthetic gene *SlSPDS2* via modulating the antioxidant enzyme system and osmotic regulatory substances in *Solanum lycopersicum*, Environ. Exp. Bot. 216 (2023) 105531.
[36] C.W. Qiu, Y. Ma, Q.Q. Wang, M.M. Fu, C. Li, Y. Wang, F. Wu, Barley HOMOCYSTEINE METHYLTRANSFERASE 2 confers drought tolerance by improving polyamine metabolism, Plant Physiol. 193 (2023) 389–409.
[37] E. Verma, B. Sharma, H.R. Singal, R. Munjal, Purification of sucrose synthase from thermotolerant wheat grains and its characterization, J. Environ. Biol. 39 (2018) 459–466.
[38] J. Luo, B. Wei, J. Han, Y. Liao, Y. Liu, Spermidine increases the sucrose content in inferior grain of wheat and thereby promotes its grain filling, Front. Plant Sci. 10 (2019) 1309.
[39] J. Li, Q. Li, N. Guo, Q. Xian, B. Lan, N. Vinay, F. Mo, Y. Liu, Polyamines mediate the inhibitory effect of drought stress on nitrogen reallocation and utilization to regulate the grain number in wheat, J. Exp. Bot. 75 (2024) 1016–1035.
[40] W. Zhang, Y. Chen, Z. Wang, J. Yang, Polyamines and ethylene in rice young panicles in response to soil drought during panicle differentiation, Plant Growth Regul. 82 (2017) 491–503.
[41] E.K. Liu, X.R. Mei, C.R. Yan, D.Z. Gong, Y.Q. Zhang, Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes, Agric. Water Manage. 167 (2016) 75–85.
[42] X.K. Lyu, X.X. Wen, Y.C. Liao, Y. Liu, Effect of exogenous polyamines on mechanism of floret degeneration in wheat, Acta Agron. Sin. 42 (2016) 1391–1401.
[43] Q. Chen, The mechanism of exogenous polyamine in the regulation of wheat grain filling under drought stress, Master Thesis, Northwest A&F University, Xianyang, Shaanxi, China, 2017 (in Chinese with English abstract).
[44] Y. Heng, C. Wu, Y. Long, S. Luo, J. Ma, J. Chen, J. Liu, H. Zhang, Y. Ren, M. Wang, J. Tan, S. Zhu, J. Wang, C. Lei, X. Zhang, X. Guo, H. Wang, Z. Cheng, J. Wan, OsALMT7 maintains panicle size and grain yield in rice by mediating malate transport, Plant Cell 30 (2018) 889–906.
[45] Q. Ma, E. Wu, H. Wang, Y. Yuan, Y. Feng, J. Liu, L. Zhao, B. Feng, Exogenous 24-epibrassinolide boosts plant growth under alkaline stress from physiological and transcriptomic perspectives: the case of broomcorn millet (*Panicum miliaceum* L.), Ecotoxicol. Environ. Saf. 248 (2022) 114298.
[46] E.R. Jr, B.N. Hunt, P.S.N. Rock, Measurement of leaf relative water content by infrared reflectance, Remote Sens. Environ. 22 (1987) 429–435.
[47] Y.E. Chen, J.M. Cui, G.X. Li, M. Yuan, Z.W. Zhang, S. Yuan, H.Y. Zhang, Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings, Biol. Plant. 60 (2016) 139–147.
[48] C.L. Kielkopf, W. Bauer, I.L. Urbatsch, Bradford assay for determining protein concentration, CSH Protoc. 2020 (2020) 102269.
[49] D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Improving the thiobarbituric acid-reactivesubstances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, Planta 207 (1999) 604–611.
[50] M. Jiang, Zhang J, Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves, J. Exp. Bot. 53 (2002) 2401–2410.
[51] A.C. Maehly, B. Chance, The assay of catalases and peroxidases, Method Enzymol. 2 (1955) 764–775.
[52] P. Sharma, R.S. Dubey, Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective roles of osmolytes, Plant Sci. 167 (2004) 541–550.
[53] J. Yang, J. Zhang, Z. Wang, K. Liu, P. Wang, Post-anthesis development of inferior and superior spikelets in rice in relation to abscisic acid and ethylene, J. Exp. Bot. 57 (2006) 149–160.
[54] I.F. Wardlaw, J. Willenbrink, Carbohydrate storage and mobilization by the culm of wheat between heading and grain maturity: the relation to sucrose synthase and sucrose-phosphate synthase, Aust. J. Plant Physiol. 21 (1994) 255–271.
[55] J.M. Hanft, R.J. Jones, Kernel abortion in maize: I. Carbohydrate concentration patterns and acid invertase activity of maize kernels induced to abort in vitro, Plant Physiol. 81 (1986) 503–510.
[56] M.A. Ahanger, C. Qin, Q. Maodong, X.X. Dong, P. Ahmad, E.F. Abd Allah, L. Zhang, Spermine application alleviates salinity induced growth and photosynthetic inhibition in *Solanum lycopersicum* by modulating osmolyte and secondary metabolite accumulation and differentially regulating antioxidant metabolism, Plant Physiol. Biochem. 144 (2019) 1–13.
[57] K. Nahar, M. Hasanuzzaman, M.M. Alam, A. Rahman, T. Suzuki, M. Fujita, Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems, Ecotoxicol. Environ. Saf. 126 (2016) 245–255.
[58] N. Napieraj, M. Janicka, M. Reda, Interactions of polyamines and phytohormones in plant response to abiotic stress, Plants 12 (2023) 1159.
[59] M.A. Blazquez, Polyamines: their role in plant development and stress, Annu. Rev. Plant Biol. 75 (2024) 95–117.
[60] A. Kiran, P.N. Sharma, R. Awasthi, H. Nayyar, R. Seth, S.S. Chandel, K.H.M. Siddique, G. Zinta, K.D. Sharma, Disruption of carbohydrate and proline metabolism in anthers under low temperature causes pollen sterility in chickpea, Environ. Exp. Bot. 188 (2021) 104500.
[61] C. Liu, Z. Xue, D. Tang, Y. Shen, W. Shi, L. Ren, G. Du, Y. Li, Z. Cheng, Ornithine delta-aminotransferase is critical for floret development and seed setting through mediating nitrogen reutilization in rice, Plant J. 96 (2018) 842–854.
[62] Y. Xu, S. Tang, C. Jian, Y. Liu, K. Li, K. Zhu, W. Zhang, W. Wang, Z. Wang, J. Yang, Polyamines and ethylene interact in mediating the effect of nitrogen rates on synthesis of amino acids in rice grains, Food Energy Secur. 11 (2022) e408.
[63] Y. Huang, G. Mei, D. Cao, Y. Qin, L. Yang, X. Ruan, Spermidine enhances heat tolerance of rice seeds during mid-filling stage and promote subsequent seed germination, Front. Plant Sci. 14 (2023) 1230331.
[64] Q. Zhao, X. Guan, L. Zhou, M.A. Asad, Y. Xu, G. Pan, F. Cheng, ABA-triggered ROS burst in rice developing anthers is critical for tapetal programmed cell death induction and heat stress-induced pollen abortion, Plant Cell Environ. 46 (2023) 1453–1471.
[65] M. Zhong, R. Song, Y. Wang, S. Shu, J. Sun, S. Guo, TGase regulates salt stress tolerance through enhancing bound polyamines-mediated antioxidant enzymes activity in tomato, Environ. Exp. Bot. 179 (2020) 104191.
[66] C.H. Foyer, A.V. Ruban, G. Noctor, Viewing oxidative stress through the lens of oxidative signalling rather than damage, Biochem. J. 474 (2017) 877–883.
[67] P. Cao, J. Yang, L. Xia, Z. Zhang, Z. Wu, Y. Hao, P. Liu, C. Wang, C. Li, J. Yang, J. Lai, X. Li, M. Deng, S. Wang, Two gene clusters and their positive regulator SlMYB13 that have undergone domestication-associated negative selection control phenolamide accumulation and drought tolerance in tomato, Mol. Plant 17 (2024) 579–597.
[68] X. Huang, G. Zhou, W. Yang, A. Wang, Z. Hu, C. Lin, X. Chen, Drought-inhibited ribulose-1,5bisphosphate carboxylase activity is mediated through increased release of ethylene and changes in the ratio of polyamines in pakchoi, J. Plant Physiol. 171 (2014) 1392–1400.
[69] Y. Salmon, A. Lintunen, A. Dayet, T. Chan, R. Dewar, T. Vesala, T. Holtta, Leaf carbon and water status control stomatal and nonstomatal limitations of photosynthesis in trees, New Phytol. 226 (2020) 690–703.
[70] Q. Zhang, W. Tang, Z. Xiong, S. Peng, Y. Li, Stomatal conductance in rice leaves and panicles responds differently to abscisic acid and soil drought, J. Exp. Bot. 74 (2023) 1551–1563.
[71] S. Karwa, J. Taunk, S. Maurya, A. Das, G.K. Krishna, S.S. Arya, A. Kumar, S. Kumar, P. Kumar, V. Chinnusamy, M. Pal, Spermidine exogenous application mollifies reproductive stage heat stress ramifications in rice, Front. Plant Sci. 13 (2022) 1027662.
[72] W. Batista-Silva, B. Heinemann, N. Rugen, A. Nunes-Nesi, W.L. Araujo, H.P. Braun, T.M. Hildebrandt, The role of amino acid metabolism during abiotic stress release, Plant Cell Environ. 42 (2019) 1630–1644.
[73] H. Yu, Y. Cao, Z. Wang, J. Zhang, L. Yang, Z. Zhao, S. Wang, Z. Zhou, D.A. Loka, W. Hu, Identification of the most sensitive stage of cotton microspore development to water deficit and analysis of carbohydrate metabolism related to pollen viability, Environ. Exp. Bot. 206 (2023) 105168.
[74] P. Yu, N. Jiang, W. Fu, G. Zheng, G. Li, B. Feng, T. Chen, J. Ma, H. Li, L. Tao, G. Fu, ATP hydrolysis determines cold tolerance by regulating available energy for glutathione synthesis in rice seedling plants, Rice 13 (2020) 23.
[75] H. Liu, Y. Su, Y. Fan, D. Zuo, J. Xu, Y. Liu, X. Mei, H. Huang, M. Yang, S. Zhu, Exogenous leucine alleviates heat stress and improves saponin synthesis in *Panax notoginseng* by improving antioxidant capacity and maintaining metabolic homeostasis, Front. Plant Sci. 14 (2023) 1175878.
[76] J.J. Gao, Z.J. Li, B. Zhu, L.J. Wang, J. Xu, B. Wang, X.Y. Fu, H.J. Han, W.H. Zhang, Y. D. Deng, Y. Wang, Z.H. Zuo, R.H. Peng, Y.S. Tian, Q.H. Yao, Creation of environmentally friendly super "dinitrotoluene scavenger" plants, Adv. Sci. 10 (2023) e2303785.