

Thirty years of genetic progress combined with seed ethylene priming improved drought resistance of wheat through long-term drought stress memory

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Abstract

Knowledge of short-term physiological adaptation of wheat to soil water deficit is well understood, but little is known about seed ethylene priming effect on long-term drought stress memory of dryland winter wheat. The 42 leading and new cultivars released between 1992 to 2017 were subjected to drought (45% Field water-holding capacity), and well-watered (75% Field water-holding capacity) conditions aim to screen cultivars with contrasting drought resistance and grain yield. Seeds primed with ethylene were subjected to both PEG-8000 and pure water to uncover ethylene-induced stress memory at both physiological and organ levels. Results showed that the soil water deficit (45%FC) that occurred at the tillering stage resulted in 3.2% to 67.4% yield loss for 42 cultivars, which was determined mainly by the decrease in the fertile spike. Seed ethylene priming maintained leaf water by reducing root volume and dry weight, which played a crucial role in drought avoidance. Seed ethylene priming decreased malondialdehyde content by regulating auxin and abscisic acid signaling, reactive oxygen species scavenging capability, and osmotic regulation, which plays a crucial role in drought tolerance. Seed ethylene priming improved drought tolerance of the wheat through metabolic modification of carbon metabolism, glutathione metabolism, and phenylpropanoid biosynthesis. The seed ethylene priming induced long-term stress memory that improved tillering capacity and reduced wheat spikelets abortion, which provided extra 0.3 t ha⁻¹ of grain yield. These results suggested that seed ethylene priming allowed the recall of long-lasting stress defensive memory, increasing grain yield by both drought avoidance and drought tolerance.

1. Introduction

The monitoring of global climate changes indicated that extreme climate events occurred more frequently and in more severity (IPCC 2016). Globally, a series of studies published from 1980 to 2015 have shown that the drought leads to 21–40% yield reduction in wheat, and the severity and duration of the drought determine the extent of yield loss (Daryanto et al. 2017; Fahad et al. 2017). Though all wheat stages could be affected by drought stress, the tillering and grain-filling are more susceptible than other wheat-growing stages, and the soil water deficit that occurred at tillering will cause an unrecoverable reduction in ear-bearing tillers and grain yield (Farooq et al. 2014). The direct grain yield loss by soil water deficit is up to 48.9 million tons as the soil water deficit will affect the soil water and root relation at all levels from molecular, cellular metabolic events and organs to the whole plant (Muscolo et al. 2015). Preventing such high yield loss is particularly crucial to address the challenges of climate change.

Drought avoidance and drought tolerance are the dominant approaches for drought resistance conferred by crops than drought escape, and the drought resistance is associated with physiology and biochemical responses at molecular, cellular, tissue, organ, and whole plant levels (Fang and Xiong 2015; Li et al. 2021). Plants adapt to soil water deficit at the whole plant level by generating a more in-depth root system, smaller leaves, and rolling of leaves for reducing leaf water loss (Liu et al. 2006). To survive under extreme soil water deficit when the plant cannot avoid it, the plants have evolved sophisticated mechanisms to alleviate organelle damage when they perceive the signal of environmental deterioration. At the cellular level, the soil water deficit impairs plant growth mediated by overproduction of reactive oxygen species (ROS) resulted in peroxidation of the cell membrane, thereby caused leakage of cell metabolites and degradation of enzyme proteins (Li and Liu 2016; Miller et al. 2010). Consequently, at the organic level, the drought stress led to leaf water loss (Egea et al. 2018), decreased plant photosynthesis, plant biomass, and final grain yield (Pinheiro and Chaves 2011). The drought tolerance has been reported to rely mainly on reactive oxygen species (ROS) scavenging enzymes, osmotic adjustments, and stomatal regulation. The ROS scavenging mediated by both enzymatic and non-enzymatic antioxidant defense systems ensures that the production and removal of free radicals in plants are balanced (Sairam and Saxena 2000; Abid et al. 2017). The osmotic adjustments reduce organelle damage by maintaining leaf water content under soil water deficit conditions (Ashraf 2010; Wang et al. 2019). The root to leaf ABA signal is the crucial factor for stomatal regulation that ensures the stomatal CO₂ exchanges and leaf water loss in a balanced state (Tombesi et al. 2015). At the molecular level, these defense mechanisms have been reportedly modulated by the transcription factors and phytohormone signal transduction pathways, which further regulate root osmolyte levels by triggering sugar catabolism, regulating stomatal closure by transducing root to leaf ABA

signaling, increasing cell antioxidants level by activating the ROS defense system (Wang et al. 2021; Harb et al. 2020; Li et al. 2019). Although the short-term adaption of plants to drought stress has been clarified, the knowledge of long-term drought stress memory is more valuable for avoiding final grain yield loss by soil water deficit.

Previous exposure to abiotic stress can affect subsequent physiological responses and eventually prepare plants to respond more quickly and actively to future stress pressures (Wang et al. 2019; Abid et al. 2018). Seed hormonal priming is pre-sowing partial hydration of seeds with hormones without allowing radicle emergence, which has proved to be an effective method in imparting stress tolerance to plants (Jisha et al. 2013; Abid et al. 2018). Seed priming induces stress memory that was mediated by a coordinated reaction at organismal, cellular, and genome levels (Virilouvet and Fromm 2015). This memory is always accompanied by plant growth, even inherited by the next generation (Abid et al. 2018; Kinoshita and Seki 2014). Ethylene (Eth) is a phytohormone directly associated with plant growth and abiotic stress responses due to its role in activating defense responses and reducing the damaging effect of various abiotic stress (Kolbert et al. 2019). It is widely acknowledged that the Eth regulates many developmental and physiological processes such as cell division, root growth, anthocyanin accumulation, and embryogenesis (Divte et al. 2019). The endogenous Eth level was enhanced in many species when plants received environmental deterioration, such as cold stress, soil water deficit, and increased salinity (Kemal 2015). However, the seed ethylene priming effect on long-term stress memory and final grain yield remains unclear.

Our previous results have clarified the genetic yield gain in a historical set of cultivars released between 1965 to 2017 in Southwest China. In this study, the 42 cultivars were subjected to drought and well-watered conditions to identify the critical drought avoidance traits for enhancing drought resistance in the future. The seeds ethylene priming was used to evaluate short-term physiological adaption and long-term drought stress memory of wheat plants to enhance drought tolerance for sustainable yield production in an increasingly changing environment. Comparative root hair transcriptome analysis is conducted to understand the underlying mechanism of seed ethylene-mediated drought tolerance. The current study was conducted based on the hypothesis that seed ethylene priming allowed recalling the long-term drought stress memory that improves drought resistance of the wheat by multi-phytohormone cross-talk, ROS scavenging capability, and metabolic alterations, thereby reducing leaf water loss and increasing final grain yield.

2. Materials And Methods

2.1 Plant material and experimental design

The field trials were carried out at Wenjiang Experimental Station (29° 51'N, 104° 12'E) of Sichuan Agriculture University. The experimental site's soil is a typical purple lithomorph soil as per FAO soil taxonomy (Shi et al. 2006). A consecutive 22 years (1997-2019) climate data showed that the accumulative rainfall from wheat seed sowing to the time of harvest at the experimental site ranged from 123mm to 203mm, and spread unevenly over the years. The dry climate frequently occurred in wheat tillering, and the extreme droughts are becoming more severe in recent decades (Yang et al. 2020). Thus, the experiments with cultivars were carried out with contrasting drought resistance and seed priming treatments under both well-watered and drought conditions.

During the 2016-2017 and 2017-2018 cropping seasons, 42 leading and new cultivars released between 1965 and 2018 were collected from 9 breeding institutions, and it was selected to represent the 51 years of past genetic gain in grain yield and drought tolerance in Southwestern Wheat Production Region of China (supplemental file 1)(Zhou et al. 2007). The 42 leading and new cultivars were subjected to the water-deficit ($45\pm 5\%$ field water holding capacity) and well-watered conditions ($75\%\pm 5\%$ FC), respectively, to investigate how newly released cultivars adapted to drought stress in an increasingly variable climate. After screening the breakthrough cultivars with high yield and drought resistance, the seeds of drought-sensitive cultivar were primed with ethylene to enhance the wheat's drought resistance. The ethylene was applied for the treatments because the seeds primed with 200 mg kg^{-1} ethylene increased both the grain yield and drought resistance significantly more than other treatments (Table 2). In short, the

wheat seeds were sterilized with 200mg kg⁻¹ ethylene for 10 h and then rinsed three times with distilled water prepared for seed ethylene priming, and the seeds presoaked with distilled water were taken as the control. After priming, the seeds for both treatments were washed with distilled water and then re-dried near to their original weight with forced air at 27 ± 3 °C under shade. The seeds were sown in the soil maintained at 75 % FC (well-watered) and 45% of FC (drought) until the end of wheat jointing using an equitensiometer and automatic irrigation system to control the soil water content. Finally, four treatments were formed: unprimed seedlings grown at water sufficient condition (E0P0), unprimed seedlings grown at water deficit condition (E0P1), primed seedlings grown at water sufficient condition (E1P0), and primed seedling grown at water deficit condition (E1P1), respectively. To reduce the random effect caused by the soil environment when investigating different expressed genes response to soil water deficit, the PEG-8000 applicated because it is better than low molecular weight PEG for simulating soil drought (Kumar et al. 2019). Therefore, both primed and unprimed seedlings (2 weeks old) were subjected to 20% (v/v) PEG8000 in the growth cabinet at 25 °C for two weeks with 13 h of light and 11 h of darkness in 24 h period. Each sample's root hairs were collected at 1 h and 12 after treatment and then stored at -80°C prepared for RNA-sequencing (RNA-seq) analysis to clarify the short-term physiological mechanism of primed and unprimed plants adapt to drought (45% FC). Consecutive 15 wheat plants were collected at 24h and 9 days after imposing 20% (v/v) PEG8000, prepared for the observations of plant hormones, antioxygen enzymes, and osmotic adjustment substance. The yield-associated traits were determined at maturation to clarify the long-term stress memory effect on the final yield loss by drought.

2.2 Sampling and Lab analyses

2.2.1 Yield loss by soil water deficit

The fertile spikes, the number of grains per panicle, and the 1000-kernel weight were determined for calculating grain yield for each treatment at 13.5% moisture content. The number of the spikes in a representative plot of 1 m² were counted, and their grains were collected, air-dried, and then threshed for seed moisture determination using a digital moisture tester (DMC-700, Seedburo, Chicago, IL, USA). The grain number per ear was measure by counting the grains of consecutive 30 wheat plants. The air-dried grains were used to measure 1000-kernel weight. The yield loss by soil water deficit was calculated as follows: $Y_{loss}=(Y_c-Y_d)\times 100/Y_c$, where the Y_c and Y_d represent the yield of control and drought stress treatments, respectively.

2.2.2 Root morphology traits, plant biomass, and flag Leaf water loss

Fifteen consecutive wheat plants were randomly collected using the soil core method (Bohm 1979). The collected root samples were soaked in 1 M NaCl for 16 h to remove soil aggregates and then washed with a hydropneumatic washing machine. After the remaining mineral particles and organic debris were removed, the root samples were rinsed and then placed in the distilled water. The root morphology characteristics, including total root length, root surface area, root diagram, root volume, and the number of root tips, were recorded digitally using a flatbed scanner (Epson Expression 1600XL-Pro), and these parameters were estimated using the WinRHIZO root analysis software (WinRHIZO, Regent Instruments, Canada).

According to a previous protocol, the root system vigor of collected hairy roots was stained at room temperature for 24 h in 1mol/L phosphate buffer solution containing 1% triphenyl tetrazolium chloride (Li et al. 2016). After the sample was washed in distilled water, it was homogenized in ethyl acetate and then centrifuged at 5000×g for 10 min at 4°C. The supernatant homogenate was assayed immediately at 485 nm, and the overall root system vigor was expressed as mg benzophenone hydrazine (TTCH) generated from each gram of fresh soil per hour (mg TTCH g⁻¹ FW h⁻¹).

A consecutive 15 plants were collected at the wheat tillering in each plot and then separated into leaf, stem, and root. The aboveground plant biomass was measured by the oven-drying method. The relative leaf water content was calculated as the decrease

percentage of leaf water in soil water deficit treatments compared with well-watered condition (Wang et al. 2019) using the following

equation:
$$RWC(\%) = \frac{FW - DW}{TM - DM} \times 100$$
, in which FW refers to fresh weight for those plants primed with Ethylene, TW refers to dry weight for those plants without priming, and the DM refers to the dry weight of oven-dried leaves at 75°C till constant weight.

2.2.3 Endogenous hormones of root hairs

The root hairs were collected and then frozen in liquid nitrogen immediately and stored at -80°C until analysis. The collected root hairs (1g) were homogenized in 5ml of cold 80% aqueous methanol overnight at 4 °C and then centrifuged at 10,000 × g for 20 min at 4 °C. After the crude extract liquid was purified by passing a C18 Sep-Pak Catridge, a 400ul of extract solution was condensed using an N₂ and then re-dissolved in 400ul phosphate buffer (0.01 mol/L, pH 7.4) prepared for phytohormones analysis. The indole-3-acetic acid (IAA) content was determined as following Tian et al. (2008). The gibberellin (GA₁ and GA₃) content was carried out using a previously described method (Castillo and Martinez 1997). Extraction and assay of ABA were conducted as described previously (Lang et al. 2018).

2.2.4 Membrane lipids peroxidation, antioxidation enzymes, and osmotic adjustment substance assays

The oxidative damage to the membrane lipids was estimated by malondialdehyde (MDA), a major thiobarbituric acid, as an indicator for membrane peroxidation (Wei et al. 2016). The fully expanded leaves (0.2 g) were ground in 5 mL of 95% (v/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 15,000 × g for 10 min at 4 °C. An equal volume of supernatant and 0.6% (w/v) thiobarbituric acid was added to the TCA (20%), followed by heating the solution at 96 °C for 30min. The MDA concentration was measured by recording 450nm, 532nm, and 600 nm using the formula: $C = 6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}$, and the MDA content was expressed as mol g⁻¹ FW.

The enzyme activities of SOD, POD, and CAT were extracted and assayed as per the previous protocol (Wang et al. 2019) with minor modifications. The frozen leaf tissue (0.3 g) was ground in 5 mL of 0.1 M Tris-HCl buffer (pH=7.8) containing 1 mM DTT, 1 mM EDTA and 1% PVP. The homogenate was centrifuged at 20,000 × g for 20 min at 4 °C. The supernatant was collected for measuring enzyme activities. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium. Catalase (CAT, EC 1.11.3.6) activity was assayed by potassium permanganate titration and expressed as M H₂O₂ g⁻¹ FW min⁻¹. Peroxidase (POD, EC 1.11.1.7) activity was estimated as the guaiacol oxidation rate by measuring the rate of change in absorbance at 436 nm.

The amount of free proline was extracted from fresh flag leaves of the wheat, and it was measured by using the ninhydrin reagent as following the method of Wang et al (2019) with minor modification. After the leaves were ground in 5 mL of 3% (w/v) sulfosalicylic acid, the homogenate was centrifuged at 15,000 × g for 10 min at 4 °C. The collected supernatant was re-extracted with 2ml toluene-20, and the absorbance was recorded at 520nm. The free proline was expressed as mmol proline g⁻¹ FW.

The total water-soluble carbohydrates (WSC) were determinate by the anthrone method (Yang et al. 2017). In brief, 0.1 g dry leaf powder was extracted three times, with 80% ethanol, in a water bath at 80°C for 30 min. The non-structural carbohydrates were diluted to a final volume of 25 mL, and the non-structural carbohydrates were measured spectrophotometrically at 620 nm using an anthrone reagent.

2.3.5 Transcriptome profiling of wheat root hair cell

RNA extraction, cDNA library construction, and RNA sequencing: In each plot, the total RNA from root hairs was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and purified using Total RNA Kit (Qiagen, Valencia, CA) according to the instructions of the manufacturer. The RNA concentration and absorption curves were checked using a NanoDrop 2000 spectrophotometer (ND-2000, Thermo Fisher Scientific, Inc., USA). The RNA integrity number values were assessed on a Bioanalyzer 2100 (Alignment, Santa Clara, CA), and integrity number values were more significant than 9 for all total RNA samples. Sequencing libraries with an average insert size of 200 bp were prepared with TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, USA) and sequenced on HiSeq2000 (Illumina, San Diego, USA) according to the manufacturer instructions (Illumina, San Diego, CA). In short, the Poly-A-containing mRNA was purified twice from the total RNA using poly-T oligo-attached magnetic beads. First-strand cDNA was generated using reverse transcriptase and random primers. Following the second strand cDNA synthesis and adaptor ligation, 200-bp cDNA fragments were isolated using gel electrophoresis and amplified by 18 cycles. The products were loaded onto an Illumina HiSeq2000 instrument and subjected to 100 cycles of paired-end (2×100 bp) sequencing. The processing of fluorescent images into sequences, base-calling, and quality value calculations were performed using the Illumina data processing pipeline (version 1.8).

Gene ontology and pathway enrichment of DEGs: The high-quality reads were obtained by removing low-quality reads ($Q < 20$), and then mapped onto the international wheat genome sequencing consortium (IWGSC) using Bowtie2 (v2.2.9). The differential expression level was estimated using R software with edgeR package (R version: 4.0, edgeR version: 2.3.52). A strict algorithm was developed to identify differentially expressed genes (DEGs) between samples, and false positive and false negative errors are performed using Benjamini and Yekutieli hypergeometric test (FDR) method (Wei et al. 2020). Expression levels that exhibited an absolute \log_2 (FC) ≥ 1 and an absolute $-\log_{10}$ (P) ≥ 1.41 as the threshold to be significantly differentially expressed genes (DEGs). Two-fold up-or down-regulation compared to the corresponding control condition were taken only into consideration for further analysis. Gene Ontology (GO) enrichment was based on AgriGO software with a hypergeometric statistical test and Hochberg (FDR). Pathway enrichment analysis of DEGs was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg/>). This analysis identifies enriched signals transduction pathways and metabolic pathways in DEGs compared with the whole genome background.

Validation of differentiated expressed genes by qRT-PCR: Total RNA for each sample was pretreated with DNase I, and the first-strand cDNA was generated using a RevertAid First Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania) with oligo (dT) 20 primer following manufacturer instructions. The SYBR-based qRT-PCR reactions (SYBR Green I, Osaka, Japan) were performed in a 10 μ l reaction volume on a Light Cycler 480 system (Roche, Basel, Switzerland) using the following reaction conditions: 95°C for 1 min followed by 40 cycles of 95°C for 10 s and 60°C for 15 s. The detection threshold cycle for each reaction was normalized against the housekeeping gene expression level with primer sequences 5'-GACCGTATGAGCAAGGAGAT-3' and 5'-CAATCGCTGGACCTGACTC-3'). All qRT-PCR reactions were replicated three times, and the results were analyzed based on the $\Delta\Delta$ CT method (Roche), where $\Delta\Delta$ CT represents Δ CT condition of interest - Δ CT control. The results obtained were transformed to a \log_2 scale.

2.3 Statistical analysis

Two-way ANOVA was applied to analyze the difference between treatments performed using SAS version 9.1.3 (Statistical Analysis System, Institute INC, Box 8000, Cary, North Carolina 27511, USA). A significant level was identified at the 0.05 probability level by the LSD test. Graphs were plotted by using the Origin software, version 9.0.

3. Results

3.1 Drought sensitivity analysis of 42 leading and new cultivars released between 1965 to 2017

The soil water deficit (75% FC) resulted in 42 cultivars' grain yields decreased by between 3.2% and 67.4%, compared with those under well-watered conditions. The higher grain yield observed under well-watered conditions indicated a more significant yield loss rate by soil water deficit (Fig 1A). The first two principles explained the 26.7% and 15.3% of the yield variations, where cultivars with PC1 scores >0 identified as high-yielding cultivars; PC2 scores >0 identified as higher drought resistance cultivars. The PCA1 mainly relates to root length, chlorophyll content, and leaf area, while PCA2 mainly relates to root length, chlorophyll content, leaf water loss, and yield loss. The loading scores combined with the relative contribution to the sum of squares revealed that the yield loss attributed mainly to fertile spikes and panicle weight (Fig1, Table 1). Cultivars derived the negative relationship between grain yield and yield loss rate to identify drought avoidance traits. The drought-tolerant group includes five cultivars that showed both high yield and drought resistance. The drought sensitivity group consists of seven cultivars with a low yield and higher yield loss rate when the wheat is grown in 45% FC compared with those grown under well-watered conditions (75% FC). The drought sensitivity (CM-16), intermediate (CM-51), and tolerance (CM) cultivars with similar genetic backgrounds were screening from PCA biplot (Fig 1B). There was a dramatic decrease in plant biomass by soil water deficit (45% FC) for the drought-sensitive cultivar (CM-104), while the drought-tolerant cultivars (CM-16) did not show a significant difference in the plant biomass compared with those well-watered plots (75% FC). The yield components confirmed that the yield loss by soil water deficit could be attributed mainly to the decrease in fertile spikes and less by grains number per spike (Fig 1 C).

3.2 Seed ethylene priming effect on yield associated wheat phenotypes

Seed ethylene priming increased grain yield mainly by fertile spikes ($P<0.05$, Table 2), and there were no significant differences in plant height, grain number per panicle, and 1000-kernel weight ($P>0.05$). The seed ethylene priming decreased the degraded spikelets by 24.2% compared with unprimed plants, and there was no significant difference for fertile spikelets. Seed ethylene priming increased tillering capacity by 21.5%, and the tiller survival rate increased by 14.7%, compared with those without seed ethylene priming. These results suggest that seed ethylene priming increased grain yield mainly by the fertile spike that was resulted from the increased tillering capacity and tiller survival rate.

3.3 Seed ethylene priming effect on root morphology traits and shoot biomass under soil water deficit

The soil water deficit resulted in root length, surface area, and root volume decreased by averages of 39.3%, 30.0%, and 22.5%, respectively, compared with those under well-watered conditions, whereas the root diameter increased 16.4% (Table 3). As a result of decreased root surface area, the soil drought (45% FC) leads to the plant height, shoot dry weight, and leaf area per plant decreased by 31.4%, 37.6%, and 62.7%, respectively. In contrast, the seeds ethylene priming decreased leaf area reductions, shoot dry weight, and root dry weight, but not the plant height. Significant drought and priming interaction was observed in seedling heights and root to shoot ratio, but not the root surface area and shoot dry weight. These results suggested that seed ethylene priming improved plant resistance to soil water deficit by reduced root dry weight and LAI losses.

3.4 Comparative phytohormones of root hairs and signal transduction pathways

The unprimed plants grow on soil water deficit condition (45%FC) lead to the root hair IAA, CTK, and GA₁₊₃ contents decreased by an average of 8.5%, 17.9%, and 15.4%, respectively, as compared to the plant grown under well-watered condition (Fig 2). In contrast, the primed plant is grown at water deficit condition (45%FC) lead to the IAA, CTK, and GA₃ contents decreased by an average of 18.1%, 26.6%, and 29.4%, respectively, as compared to the well-watered treatments. The primed plants grown at the soil water deficit condition have greater ABA and ethylene contents than the unprimed treatments. These results suggested that plants primed with ethylene increased drought tolerance by increasing IAA, CTK, and GA₃ contents in early responses.

The differently expressed genes related to drought stress response and ethylene mediated tolerance response revealed that the ABA and CTK signals were upregulated, including *PP2C*, *SnRK2*, *B-ARR*, which further regulate stomatal closure and cell division. In contrast, the IAA signal including *AUX1*, *AUX/IAA*, *GH3*, and *SAUR* increased under soil drought, which further suppressed plant growth. Ethylene mediated drought tolerance associated with plant hormone signal was associated with Ethylene, IAA, and ABA signal pathways because the jasmonate ZIM domain (JAZ), ethylene response factor 1 (ERF-1) were down-regulated, and SAUR were upregulated response to enhanced drought tolerance. The above findings demonstrate that the ethylene-mediated drought tolerance relies on diverse signal pathways, including upregulated IAA signal transduction and the down-regulated ABA, JA, and Ethylene mediated stress responses.

3.4 Root hair antioxidative enzymes, osmotic adjustment substance, and leaf water loss

ROS-scavenging enzymes' activities were further examined to clarify the underlying ethylene-mediated short-term physiological adaption to soil water deficit (Table 4). As expected, drought stress (45%FC) leads to overproduction of the ROS, which caused lipid oxidation as shown in the MDA content, and there were significant increases in the activities of SOD, POD, and CAT in both primed and unprimed treatments as compared to the well-watered treatment (75%FC). Compared with unprimed control, the primed plants showed lower CAT activity and soluble sugar content, whereas increasing the activity of SOD. There was a five-fold increase in both SOD activity and soluble sugars content between well-watered and soil drought stress treatments, whereas primed plants decreased soluble sugar by 12.7% compared with unprimed plants. The proline in primed plants increased by 3.2% compared with unprimed plants. The plants grown under soil water deficit conditions (45% FC) led to 7.6%~6.9% of leaf water loss and 1.2~2.0% of root water loss than those grown under well-watered conditions (75% FC). The seed ethylene priming did not affect root moisture but reduced leaf water loss and leaf water content compared with those unprimed plants. These results suggested that seed ethylene priming reduced leaf water loss under soil water deficit by modifying ROS scavenging enzymes and osmotic adjustment substances.

3.5 Global gene expression of root hairs by transcriptome sequencing

Both primed and unprimed plants were subjected to 8% PEG-8000 to simulate drought stress for 1h and 12h to evaluate the similarities and differences in both drought-induced and Ethylene-induced transcriptomes responsive effects. The experiment performed deep RNA sequencing of 1-week old wheat seedling leaves using the Illumina sequencing platform. After removing low-quality reads, the eight materials yielded 79.3 million to 148.5 million clean reads (see [Methods](#), supplemental file 2). The high-quality reads accounted for more than 99.3% of the raw reads, and the Q30 for each sample was above 90.86%. Clean reads of each sample were aligned with the reference genome, and the alignment efficiency ranged from 61.94% to 65.20%. Fold Change (> 2) and FDR < 0.001 were used as screening criteria for differentially expressed genes (DEGs). The controlled and drought-stressed groups were separated by the first principle component representing 56.7% of the total variation and the second principle representing 19.4% of the total variance (Fig3 A). Drought stress-induced 3 197 DEGs at 1 hour after subjected to drought stress compared with controlled, of which 1953 genes were upregulated, and 1244 genes were down-regulated (Fig 3B). Seeds Ethylene priming induced 2586 DEGs, of which 1698 genes were upregulated and 888 genes were down-regulated. Both droughts induced DEGs and Ethylene induced DEGs at 12h after treatments were significantly higher than that of at 1h subjected to drought stress, of which 1744 and 2272 DEGs were upregulated, and 2743 and 2711 DEGs were down-regulated. The Venn diagram plot showed that there were 642 and 1779 genes associated with drought tolerance in both 1h and 24h after subjected to drought stress, in which 730 unique genes were expressed after subjected to drought stress (Fig 3C). All the DEGs were analyzed for time-scale variation in their expression and were classified into 9 clusters (Fig 3D). The clustering analysis also allowed us to sort genes responses according to their overall behavior. This analysis has demonstrated that rapid gene response could be classified into up to 9 clusters. We identified 1479 genes that were consistently upregulated (cluster 1), and 1352 genes (cluster 9) were always down-regulated reposed to drought stress. Seeds Ethylene priming induced 22 genes that consistently upregulated in root hairs. In contrast, the 19 genes that consistently down-regulated in root hairs. The chromosome-wise gene expression pattern revealed nearly the same expression pattern in 1h and 24 h subjected to drought stress. DEGs associated with drought stress were mainly distributed on Chr3 BL (5%), Chr5

BL (5%), Chr5 DL (4%), and Chr2 BL (4%). In contrast, DEGs associated with Ethylene mediated drought tolerance were mainly distributed on Chr4 DS (6%), Chr5 BL (6%), and Chr3 B (5%).

3.6 Go and KEGG analysis of the differently expressed genes of root hairs

Go enrichment analysis and were placed into three major categories: "biological process", "cellular components", and "molecular function"(Fig 4). The transcripts of E0P0 vs. E0P1 related to drought stress response in the biological process category mainly involved metabolite process, cellular process, single organism process, response to the stimulus. The drought stress-induced DEGs in the cellular components category mainly expressed in the cell part, organelle, and membrane. The E0P1vs E1P1 related to Ethylene mediated drought tolerance that associated with increased gene expression in the cell wall (9.67% vs. 11.76%), chloroplast (13.44% vs. 18.62%), and cytosol (8.60% vs. 10.78%), and decreased gene expression in Golgi apparatus (16.12% vs. 7.84%). The most enriched GO term under molecular function was catalytic activity, which was 21.02% and 17.89% in the E0P0 vs. E0P1 and E0P1 vs. E1P1 comparisons. The other GO terms under molecular function were binding, electron carrier activity, transporter activity, and nucleic acid binding transcription factor activity.

The pathway-based analysis was performed using the Kyoto Encyclopedia of Genes and genomes pathway database (Fig 5). The results showed that the drought stress-induced 3197 DEGs (1h) and 4487 DEGs (12h) involves 20 metabolites pathway and 17 functional pathways (Q-value < 1). Most of the DEGs associated with drought stress-related to plant hormone signals, glucosinolate biosynthesis, taurine, and hypotaurine metabolism, zeatin glutathione metabolism, flavone, and flavonol biosynthesis. In the environmental information procession module, forty-six DEGs were enriched in the plant hormone signal transduction pathway, including 12 down-regulated genes related to auxin signal transduction, one upregulated gene related to zeatin auxin signal transduction, and two down-regulated genes connected to the ABA signal transduction pathway. The acid receptor PYR/PYL family were down-regulated; 13 genes encoding protein phosphatase 2C were upregulated; 1 gene encoding serine/threonine-protein kinase SRK2 was upregulated; 2 genes encoding ethylene response transcription factor were upregulated; 8 genes related to jasmonic acid signal transduction pathway were upregulated; and 8 genes encoding salicylic acid were upregulated. It was found that 100 differentially expressed genes were enriched in the glutathione metabolic pathway, in which three genes encoding glutamate-cysteine ligase were upregulated, six genes encoding glutathione reductase (NADPH) were upregulated, three genes encoding glutathione peroxidase were upregulated, and 85 genes encoding glutathione S-transferase were upregulated. Drought stress also induced 105 DEGs enriched in the phenylpropanoid biosynthesis pathways and 81 DEGs enriched in the phenylalanine metabolism pathways. A Total of 902 transcription factors were identified response to drought stress, and the top 5 leading transcription factors were RLK-Pelle_DLSV, MYB, RLK-Pelle_WAK, RLK-Pelle_LRR-I-1, and C₂H₂.

Carbon metabolism, carbon fixation, and phenylpropanoid biosynthesis were the top 3 crucial pathways associated with Ethylene mediated drought tolerance. The DEGs encode ribulose-bisphosphate carboxylase, glyceraldehyde-3-phosphate dehydrogenase (NADP+) glyceraldehyde-3-phosphate dehydrogenase were upregulated response to Ethylene mediated drought tolerance. The DEGs encoding peroxidase enriched in phenylpropanoid biosynthesis were a down-regulated response to Ethylene mediated drought tolerance. A total of 42 transcription factors were identified, which were associated with ethylene-mediated drought tolerance response, and the 3 top transcription factors were the RLK-Pelle_DLSV, NAC, and AP2/ERF-EPF.

3.7 Validation of DEGs using qRT-PCR

The qRT-PCR validation of the DEGs was performed using cDNA isolated from treatments. The selection of high up- and down-regulated genes was based primarily on regulating various pathways related to drought stress and ethylene mediated drought tolerance (Fig 6). Because the number of down-regulated genes was greater than that of upregulated genes in 12h after subjected to soil drought, the experiment chose more downregulated genes for validation through qRT-PCR. The expression level of each selected DEG for both priming and drought stress treatment compared to the expression level indicated by its transcriptome sequencing data. The differential gene expression detected by qRT-PCR was consistent with the

up-and down-regulation trends of gene expression obtained from transcriptome sequencing ($R^2=0.89$; $n=24$). The results were indicating that the analytical results of transcriptome sequencing were reliable.

4. Discussion

4.1 Genetic modification of drought avoidance traits combined with seed ethylene priming provided an extra 0.3t hm^{-2} yield

The natural and artificial selection of cultivars with high yield and drought resistance processes numerous physiological and molecular mechanisms adapt to drought stress while realizing the potential yield (Liu and Qin 2021). Identifying and utilizing traits associated with drought tolerance and drought avoidance is the most effective measure for reducing leaf water loss and stress injury (Kamphorst et al. 2019; Pouri et al. 2019; Katuwal et al. 2020; Liu and Qin 2021). Notably, the higher the grain yield under well-watered conditions (75 % FC), the more significant yield loss by soil water deficit (45% FC). It is possible that cultivars with increasing drought resistance indicating higher metabolic cost while the yield potential realization. The intrinsic properties of 42 leading and new cultivars acclimate to soil water deficit provided a prospect for drought tolerance improvement while yield realization. There is no doubt that wheat cultivars selected from well-watered conditions have little contribution to improving drought tolerance even through genetic gain in different wheat production regions. The breakthrough cultivars released in recent years have both yield and drought resistance provided insights for future improvements. The yield loss by soil water deficit was determined mainly by the decrease in fertile spike, and the drought persistence primarily relates to root length, root volume, leaf chlorophyll content, and leaf water loss rate, which plays a significant role in drought avoidance. Plants that avoid drought stress rely on maximizing root surface area, minimizing leaf area, and decreasing leaf water loss (Fang et al. 2017; Sadok and Schoppach 2019; Pace et al. 2015). High leaf water content has been associated with higher leaf photosynthesis levels, cell membrane stability, ROS-savaging enzymes, and grain yield (Wang et al. 2021; Larkunthod et al. 2018). Although deeper root exploration may enhance water absorption in drought-stressed conditions, the metabolic cost cannot compensate for the loss of aboveground plant growth. Similarly, small leaf and prolonged stomatal closure increased the capability of drought avoidance, but in the long run, it may lead to a significant reduction in grain yield (Li et al. 2021). Therefore, a further increase in root length is not a wise approach for both grain yield and drought resistance because it will cause a decrease in carbon economy at the whole-plant scale. A novel finding from the present study is that the seed Ethylene priming enhanced both root volume and shoot dry weight without further metabolic cost on the deeper root. The drought resistance conferred by crops with increased root volume that improved tillering capability and spikelet survival rate and provided extra 0.3 t hm^{-2} grain yield under soil water deficit conditions (45% FC).

4.2 Seed ethylene priming recalling long-lasting stress memory through an altering signaling pathway

Drought tolerance involves mechanisms operating at temporal scales, from short-term physiological feedback process, plants maintain leaf water, and long-term feedback strategies have participated in the trade-off between carbon accumulation and risks of deleterious soil water depletion. For most short-term physiological processes, the short-term (1h, 12h) response is involved in changes in stomatal control, hydraulic tissue conductance, osmotic adjustment, root-to-leaf growth response optimization of carbon metabolism (Tardieu et al. 2018). As expected, the ethylene-responsive factors 1 and 2 were down-regulated at both 1h and 12h subject to drought stress, and the EIN 3 was upregulated compared with those grown under well-watered conditions. These results can be attributed to the ethylene-responsive factor (ERF) function involved in diverse plant reactions to biotic or abiotic stresses (Seo et al. 2010). *ETR*, *EIN2*, and *EIN3* in soybean (Arraes et al. 2015), *PYL* in cotton (Liang et al. 2017), and *JAR1* and *MYC2* in soybean (Takagi et al. 2016) were reported to be positively correlated with drought tolerance. Seed ethylene priming allowed the recall of long-lasting stress defensive memory, which decreased the Ethylene perception signal's gene expression. The Ethylene perception signal and root to shoot ABA secondary signal involve a dialogue between ABA, IAA, and SA, and the ABA is the dominant hormone regarding its role in plant drought tolerance (Wang et al. 2017b; Zhu et al. 2019; Harb et al. 2020). The production of ABA in the root hair and then mobilized to the leaves will maintain plant water status through stromal regulation (Zhang et al. 2018), and then postpone the development of water deficit in the aboveground (Liu et al. 2005). PP2C, PYL, and SnRK2 are the three critical components of the ABA signaling pathways; PP2C acts as a negative regulator, while SnRK2 acts as a positive regulator (Khan et al. 2019; Liang et al. 2018). In

the present study, the decreased expression of ABA receptor genes *PYR/PYL* combined with increased expression of *PPC2* and *SnRK2* is crucial for maintaining leaf water under drought-stressed conditions due to their function in stomatal regulation. Notably, it was reported that salicylic acid (SA) production also affects stomatal opening, photorespiration, and antioxidant defenses before any detectable change in the relative water content (Sanchez-Martin et al. 2015). The Ethylene mediated drought stress memory was associated with increased SA signal transduction gene *TGA* expression in 1h after subjected to drought stress but not 12h, which confirmed the crucial role of SA in the early stage of drought response. As a result of decreased ABA and Ethylene contents treated with seed Ethylene priming, the IAA responsive gene *SAUR* was significantly increased, whereas the Jasmonate ZIM-domain and *ERF1/2* were significantly decreased. The identified phytohormone signals cross-talk opened promising avenues to maintain leaf water under soil water deficit.

4.3 Transcription factor and phytohormones co-mediated for ethylene mediated drought tolerance

The transcription factor played a central role in regulating gene responses to abiotic stress in plants (Gollack et al. 2014). The MYB, C2H2, NAC, AP2/ERF-ERF, and bHLH were identified and coupled with the soil water deficit in this study. These identified transcription factor families are well known in plants' stress tolerance (Zhang et al. 2015; Zhu et al. 2019). The present study's significant contribution is the Ethylene mediated drought tolerance transcription factors, including the NAC, AP2/ERF-ERF, bHLH, SNF2, WRKY, and C₂H₂. Recently, many transcription factors have been identified as critical players for drought tolerance. The NAC transcription factor gene *TaNAC29*, *SNAC3*, *OsNAC52*, and *PtrNAC72* have been reported to enhance dehydration stress tolerance by modifying the ABA signal pathway, putrescine biosynthesis, and improving ROS scavenging capability under drought (Huang et al. 2015; Fang et al. 2015; Wu et al. 2016). NAC-coding genes' overexpression could improve drought tolerance and be more effective when under the control of stress-responsive or tissue-specific promoters (Nakashima et al. 2014; Brasileiro et al. 2015). The transcription factor ERF has been reported to enhance osmotic tolerance by modulating the increase in stress-responsive gene expression, including MYB transcription factor, ABA synthesis gene, and photosynthesis-related genes (2010), thereby regulating shoot branching, lateral root, leaf senescence even the leaf wax accumulation (Nie et al. 2018; Ranjan et al. 2012). The bHLH transcription factor reported enhanced drought tolerance not only by regulating stomatal density, transpiration, photosynthesis, and flavonoid biosynthesis (Zhai et al. 2016; Dong et al. 2014; Wang et al. 2016), but also associating with ABA biosynthesis, JA module component, and root development (Seo et al. 2011; Li et al. 2019). The loss of function of the SWI2/SNF2 chromatin remodeling ATPase BRAHMA (BRM) causes ABA hypersensitivity during post-germination growth arrest, demonstrating the crucial role of SNF2 on drought sensitivity of plants (Han et al. 2012). In rice plants, *ALT1*, an Snf2 family chromatin remodeling ATPase, reported alkaline tolerance negatively through enhanced defense against oxidative stress (Guo et al. 2014). The WRKY transcription factor's biological function in abiotic stress has been reported enhancing drought tolerance by ABA synthesis, AsA accumulation, and MAPK signal (Rushton et al. 2010; Ren et al. 2010). *GmWRKY13*, *GmWRKY21*, and *GmWRKY54* have been reported to play crucial roles in regulating both drought and salt tolerance of soybean (Zhou et al. 2008). In barley, the overexpression of the C₂H₂ type zinc finger member *TaZFP1B* resulted in an increase in drought tolerance (Cheuk et al. 2020), which can be mediated via a phytosulfokine receptor since overexpression *AtPSKR1* improves growth in Arabidopsis.

4.4 Antioxidant enzyme and osmotic adjustment co-mediated ROS scavenging involved in Ethylene mediated drought tolerance

Drought signals enable plants to slow down leaf water loss, stimulate the production of osmosis-regulating metabolites, activates the internal drought defense system to prevent the lipid oxidation of cell membrane (Singh et al. 2017; Gupta et al. 2020). The MDA content has been suggested as a marker of lipid peroxidation of the cell membrane in response to abiotic stress (Moller et al. 2007). The seed Ethylene priming reduced root hair MDA content, indicating the cell membrane's stress-induced injury was alleviated compared to the cultivars without seed ethylene priming. Lower MDA content can be mainly explained by increasing ROS scavenging enzymes, osmotic adjustment, and metabolic alternation (Wang et al. 2017a; Hameed et al. 2011; Simova-Stoilova et al. 2009). The seed Ethylene priming increased SOD and CAT activities but not POD activities under water deficit. This is clear that seed Ethylene priming reduced oxidative stress by increasing ROS scavenging enzymes triggered to detoxify cells. The differently expressed genes associated with glutathione metabolism, phenylpropanoid biosynthesis, and flavonoid biosynthesis confirmed the Ethylene mediated drought tolerance associated with the internal

drought defense system. The osmolytes included the soluble sugar and proline contents, which have been reported to stabilize membranes and maintain protein structure at stressed conditions, which appears to be consistent with yield and yield stability (Singh et al. 2017; Blum 2017). A novel finding from the present study is that the seed Ethylene priming increased Proline content but significantly decreased soluble sugar content. This helps reduce the consumption of carbohydrates assimilates while maintaining osmotic balance, thereby partitioning more carbohydrates for leaf growth that would increase aboveground plant growth. Previous results reported that plant growth and photosynthesis remain affected by water deficit even though leaf cell turgor is maintained via osmotic adjustment (An-Ching and Boyer ; Bouchabk et al. 2006). Seed Ethylene priming increased root hair Proline level, but not soluble sugar content explains why the root length did not increase significantly in seed ethylene priming treatment. Seed ethylene priming increases root volume that enables more N to accumulate in the leaves, thereby affecting light capture and biomass accumulation. Seed Ethylene priming increased root hair proline level that enhances drought tolerance by maintaining the osmotic balance of cells and ultimately helps plant recovery from drought stress. These short-term physiological changes also have a profound long-term consequence for plants' carbon budget at plant scale. The long-lasting stress memory reduced plant biomass loss by maintaining the leaf area and chlorophyll content, which further increased fertile tillers and grain number, thereby reduced yield loss under drought stress.

4.5 DEGs involved in metabolites pathways and their expression pattern in response to drought stress

The root is the first to encounter low soil moisture in the soil, and the transcriptome profiling of root hairs provide a good target for evaluating genes and key process underling plant adaption and tolerance to soil water deficit. Some antioxidants and osmotic regulation substances such as ascorbic acid (AsA), glutathione, glutathione, and flavonoid can be formed to effectively eliminate ROS and improve drought defense. Glutathione transferases are involved in protection under various stress conditions by detoxifying toxins, increasing oxidative stress (Singh et al. 2017). Glutathione S-transferases play a significant role under drought conditions by conjugating glutathione with electrophilic substrates to increase their solubility and facilitate further metabolic processing (Yilmaz and Iscan 2014). Expression levels of glutathione S-transferase and glutathione peroxidase activity under drought in flag leaves were found correlated with high yield stability (Galle et al. 2009). Genes encoding glutathione-disulfide reductase and glutathione peroxidase were upregulated under drought stress. In contrast, genes encoding glutathione transferase were down-regulated for both 1h and 12h subjected to soil drought, indicating that upregulated gene expression involved in glutathione biosynthesis was responsible for drought tolerance in plants. The antioxidant enzymes constitute the "first line of defense" against ROS-induced cell membrane peroxidation during drought stress, and the phenylpropanoids constitute a secondary antioxidant system. The phenylpropanoid biosynthesis was activated due to the depletion of primary antioxidant defenses and control cellular H₂O₂ within a sublethal concentration range (Sun et al. 2011) explained genes expression involved in phenylalanine ammonia-lyase, cinnamoyl-CoA reductase, and trans-cinnamate 4-monooxygenase were increased under drought. The catalase-peroxidase and cinnamyl-alcohol dehydrogenase were significantly decreased, which explains the decreased plant growth under drought stress. Both these findings indicate a potential role in drought stress responses of dryland winter wheat.

Seed ethylene priming induces physiological changes that affect the metabolism through the up-or down-regulation of genes involved in the phytohormone signal transduction, phenylpropanoid biosynthesis, carbon fixation, carbon metabolism, galactose metabolism. Seed ethylene priming upregulated genes related with caffeate O-methyltransferase and peroxidase, which must have played a significant role in the seed Ethylene mediated drought stress memory. The catalase and non-specific peroxidase activities can directly inhibit the production of H₂O₂ (Katuwal et al. 2020; Abid et al. 2018). It was speculated that seed Ethylene priming improved drought resistance related to its ability to scavenge ROS. The upregulated DEGs related to peroxidase prepared the plant response more quickly and actively to drought. It was reported that drought stress resulted in the reduction of leaf elongation by less lignin biosynthesis, which was highly corrected with the accumulation of caffeate O-methyltransferase (Vincent et al. 2005). The caffeate O-methyltransferase occupies a pivotal position in the phenylpropanoid pathway, which catalyzes the O-methylation of caffeic acid to ferulic acid, a precursor of lignin polymer and a crucial component of the cell wall structure (Vincent et al. 2005; Parizotto et al. 2020). Therefore, seed

ethylene priming increased root volume and leaf area resulted from the increased gene expression mainly involved in phenylpropanoids biosynthesis.

4.6 Prospect for future improvement

The short-term physiological changes do not necessarily indicate they affect the final yield (Sadras and Richards). The seed Ethylene priming avoids yield loss of 363 kg hm⁻² under 45% field water capacity but does not necessarily indicate they can avoid yield loss under more severe drought stress. In this investigation, the extent of yield loss by seed Ethylene priming depended on soil water availability. A significant contribution from the present study is to deepen the current knowledge on ethylene-mediated drought tolerance memory. The root-level determinations of hormone signals were made based on root hairs, while the increased water retention capability was determined on leaf levels. It is speculated that the Ethylene mediated drought tolerance was mediated by root-to-leaf cross talk. Secondly, the drought stress memory is always accompanied by wheat growth, and as approved by the present study, the final fertile spikes were significantly reduced by ethylene priming. Previous results indicated that the drought stress memory was inherited to the next generation by epigenetic modification. However, how many generations can remember this precious memory remains unknown. Thirdly, seed Ethylene priming activates genes associated with Eth sensing signals and triggers multi-hormone signals, indicating a cross-tolerance to multiple stresses condition. It is crucial to reduce the adverse effects of global climate change on crop yield. Over the past decades, researchers have made great efforts to elucidate the complex genetic architectures and a regulatory mechanism involving drought tolerance in an increasingly changing variable environment. The identified agronomy traits and genes that exert their effects on drought tolerance capability remain mostly unknown. It is of great importance to integrate multi-omics into association mapping to bridge the knowledge gap. The integration of multi-omics data will provide integrative information that increases the current knowledge of drought stress memory and multi-stress adaption. Undoubtedly, the drought tolerance improvement by using genetic approaches or seed priming, the understanding of the complex regulatory network associated with drought tolerance will help fine-tune the adaptability of wheat to withstand the challenges in an increasing changeable environment. In any case, further studies are required to shed light on (1) the exact mechanisms controlling root-to-leaf signals transduction; (2) the exact generations of the existence of the drought stress memory; (3) multi hormone signals commination adapt to cross stresses tolerance.

5. Conclusions

The results obtained from a combination of controlled lab experiments with observations under field drought-stressed conditions revealed that seed ethylene priming helped alleviate drought stress-induced growth inhibition by enhancing both short-term drought adaptation and long-term drought memory. Seed ethylene priming maintained leaf water by increasing root hair antioxidant enzymes and osmolytes regulation, resulting in lower membrane lipid damage than non-primed seedlings. The increased proline content and reduced soluble sugar content provided an economical approach for enhancing osmolytes regulation while realizing potential yield. The maintaining of root hair homeostasis endogenous IAA and ABA levels participates in recalling drought stress memory by affecting carbon metabolism, carbon fixation, and phenylpropanoid biosynthesis. Due to these beneficial effects, the long-lasting stress memory increased tillering capacity and florets efficiency, which provided an additional 0.3t yield in the dryland farming system. Future studies on Ethylene priming-induced epigenetic modifications, transgenerational stress memory, and cross stress memory are still required to fully elucidate the role of Ethylene priming on long-term stress memory in an increasingly changing environment.

Declarations

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Tables

Table 1 Relative contribution to the total sum of squares (SS) and the level of significance of source of variance for 13 traits of 42 wheat genotypes grown at soil with 45% FC compared with those well-watered conditions.

Trait	Unit	Drought		Reduction by soil water deficit	Contribution to the total SS		
		75%FC	45%FC		G	D	G×D
traits at tillering stage							
Plant height	cm	28.7	28.72	0.1	56.0**	0.06	19.1**
Plant dry weight	g plant ⁻¹	0.12	0.09	26.3	2.8**	73.2**	1.1 ^{ns}
		0.18	0.16	13.2	3.4**	28.3**	1.5*
traits at tillering stage							
Leaf area	-	1.12	0.93	17.0	34.8**	803.4**	10.6**
Leaf water loss rate	%	10.5	10.1	3.7	1.7**	0.6	1.0 ^{ns}
Chlorophyll content	mg g ⁻¹	14.2	13.7	3.7	7.0**	37.2**	2.8**
Plant biomass	g plant	0.79	0.66	16.2	3.3**	59.3**	1.0 ^{ns}
traits at maturation stage							
Plant height	cm	89.3	88.0	1.5	69.1**	26.4**	1.8**
Stem length	cm	10.9	10.4	4.6	28.5**	70.9**	2.0**
Stem sheath	no	18.54	17.5	5.5	13.6**	136.3**	2.2**
Stem tillers per plant	no	2.96	2.49	16.0	16.7**	480.0**	10.8**
Stem weight	g	2.57	1.94	24.6	248.2**	11072.9**	130.5**
Stem yield per plant	g	7.63	4.81	36.9	40.2**	3395.1**	29.4**

Note: * and ** Significant at the 0.05 and 0.01 probability levels, respectively. G, cultivars; D, drought stress; FC, field water holding capacity.

Table 2 Seed priming effects on yield associated wheat phenotypes under soil water deficit.

	Concentration (mg kg ⁻¹)	Plant height (cm)	Tillering capacity	Tiller survival rate (%)	Fertile spikes	Fertile spikelet	Degraded spikelet	Grain number	1000- kernel weight (g)	Grain yield (kg hm ⁻²)
	200	83abc	2.43a	61.6a	18.5ab	16.4cd	2.38b	45.0bc	49.9ab	6064a
	3000	82bcd	1.81d	61.3ab	20.0a	16.7bc	2.50b	47.5a	50.2ab	5950ab
de	100	84ab	2.21ab	56.3bc	17.2bc	16.9ab	2.10c	46.2b	47.6b	5829bc
	0	82cd	2.00bcd	53.7c	15.8c	16.1d	3.14a	44.8c	49.9ab	5701cd
	3	81d	2.14bc	53.1c	20.1a	16.3cd	1.33d	45.5bc	49.0ab	5656d
ic	10	85a	1.79d	57.9abc	20.2a	16.3d	2.40b	45.7bc	50.3ab	5489e
	20	84ab	2.00bcd	60.8ab	18.3ab	17.3a	2.52b	47.6a	52.6a	5344ef
	10	81cd	1.93cd	54.7c	18.7ab	15.5e	1.39d	43.1d	52.4a	5268f
		83.0	2.04	57.4	18.6	16.4	2.22	45.7	50.2	5662
		5.29**	8.55**	5.08**	5.03**	12.70**	80.87**	14.15**	1.87ns	31.74**

Table 3. Seed ethylene priming effects on above- and below-ground phenotypes of wheat grown under both well-watered (75% FC) and drought (45% FC) conditions.

Treatment	Drought Stress (S)	Seedling height (cm)	Shoot dry weight (mg plant ⁻¹)	Root dry weight (mg plant ⁻¹)	Leaf area (cm ²)	Root shoot ratio	Root Length (cm)	Root Surface Area (cm ²)	Root Diameter (mm)	Root Volume (cm ³)
Unpriming	75%FC	27.7a	37.7b	9.2b	12.5b	0.25c	176.0	17.7b	0.33b	0.15b
	45%FC	18.0c	22.9d	9.0b	4.7c	0.38a	112.1	12.9c	0.38a	0.12c
Seed ethylene priming	Avg.	22.8	30.3	9.1	8.6	0.31	144.0	15.3	0.35	0.13
	75%FC	26.6b	40.4a	10.5a	13.5a	0.26c	173.8a	18.8a	0.34b	0.16a
	45%FC	18.5c	25.9c	9.2b	5.1c	0.36b	100.2b	12.6c	0.40a	0.12c
	Avg.	22.6	33.2	9.9	9.3	0.31	137.0	15.7	0.37	0.14
F-Value	P	1.2 ^{ns}	19.8**	10.2*	24.7**	0.8	1.9 ^{ns}	1.2 ^{ns}	2.3 ^{ns}	9.0*
	S	1773.0**	513.3**	11.0*	2921.5**	458.3**	176.6**	279.5**	27.8**	69.4**
	P×S	14.0**	0.05 ^{ns}	0.04*	4.8 ^{ns}	11.0*	0.9 ^{ns}	4.2 ^{ns}	0.3 ^{ns}	2.8 ^{ns}

Note: P, S, and FC represent the seed priming, soil drought, and field water holding capacity. Different letters indicate significance at the 5% probability level.

Table 4 The root hair anti-oxidative enzymes, MDA, and soluble sugar contents and plant water loss in drought compared with those under well-watered condition.

Primed (P)	Stress (S)	Root water Content (%)	Leaf water Content (%)	Root system vigor (%)	MDA (μmol g ⁻¹ DW)	SOD (Ug ⁻¹ FW)	POD (g ⁻¹ FW)	CAT (g ⁻¹ FW)	Pro (mg g ⁻¹ DW)	Soluble sugars (mol g ⁻¹ DW)
Unprimed	75%FC	95.1a	89.7a	426.2b	0.22c	65.48c	152.2d	57.7c	2.30d	0.48c
	45%FC	93.1b	82.2c	488.6ab	0.46a	200.40b	541.0a	81.4a	9.59b	2.68a
	Avg.	94.1	85.95	457.4a	0.34	132.94	346.6	69.6	5.94	1.58
Primed	75%FC	94.5a	89.9a	434.2b	0.18d	50.59c	277.8c	47.8d	2.33c	0.50c
	45%FC	93.2b	83.0b	558.1a	0.36b	251.43a	412.2b	67.2b	9.93a	2.27b
	Avg.	93.8	86.5	496.2a	0.27	151.01	345.0	57.5	6.13	1.38
F-Value	P	1.83 ^{NS}	9.57*	1.6 ^{NS}	22.3**	6.99*	0.02 ^{NS}	27.5**	1055**	13.9**
	S	58.8**	1824.4**	9.4**	180.2**	603**	646.1**	87.7**	1642570**	1486.5**
	P×S	3.6 ^{NS}	3.68 ^{NS}	1.0 ^{NS}	2.95	23.2**	152.8**	0.9 ^{NS}	700**	17.5**

Note: P, S, and FC represent the seed priming, soil drought, field water holding capacity. Different letters indicate significance at the 5% probability level.

Figures

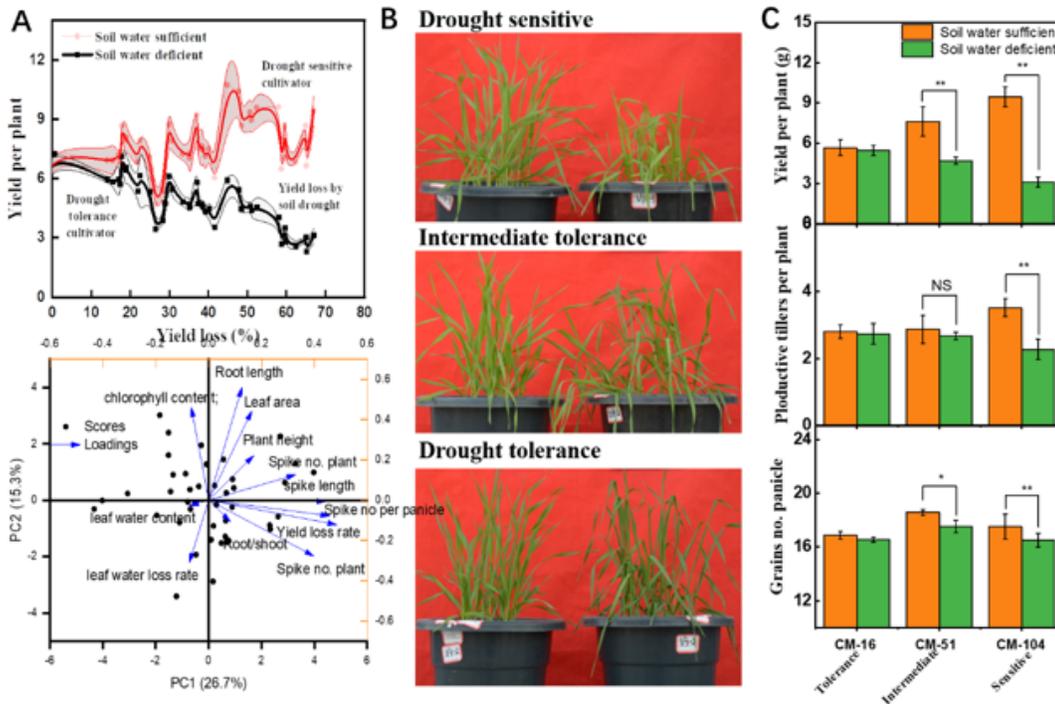


Figure 1

Phenotypes of drought resistance of 42 leading and new wheat cultivars released between 1965 and 2017 grown under both well-watered (75%FC) and drought (45%FC) conditions. (A) Screening of promising cultivars and their drought avoidance traits in both well water and drought conditions. (B) Comparisons of 3 cultivars with contrasting drought resistance grown under well-watered (left) and drought conditions (right). (C) Grain yield and yield components of drought sensitivity, tolerance, and intermediate cultivars were grown under drought-stressed (45% FC) and well-watered conditions (75% FC). Each column represents mean \pm S.D. (four replicates).

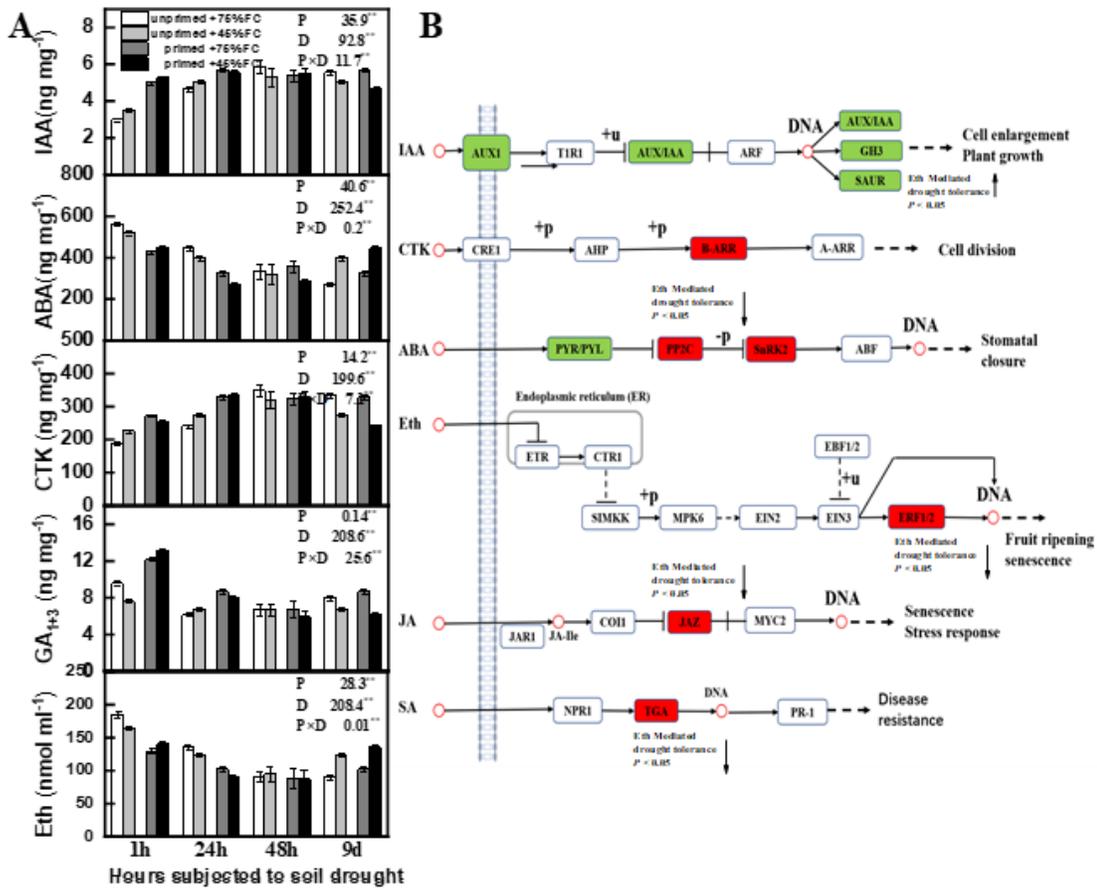


Figure 2

The changes in root hair hormone contents (A) and hormone signal transduction pathways (B) of seedlings primed with ethylene grown under well-watered (75%FC) and drought stress (45%FC) conditions.

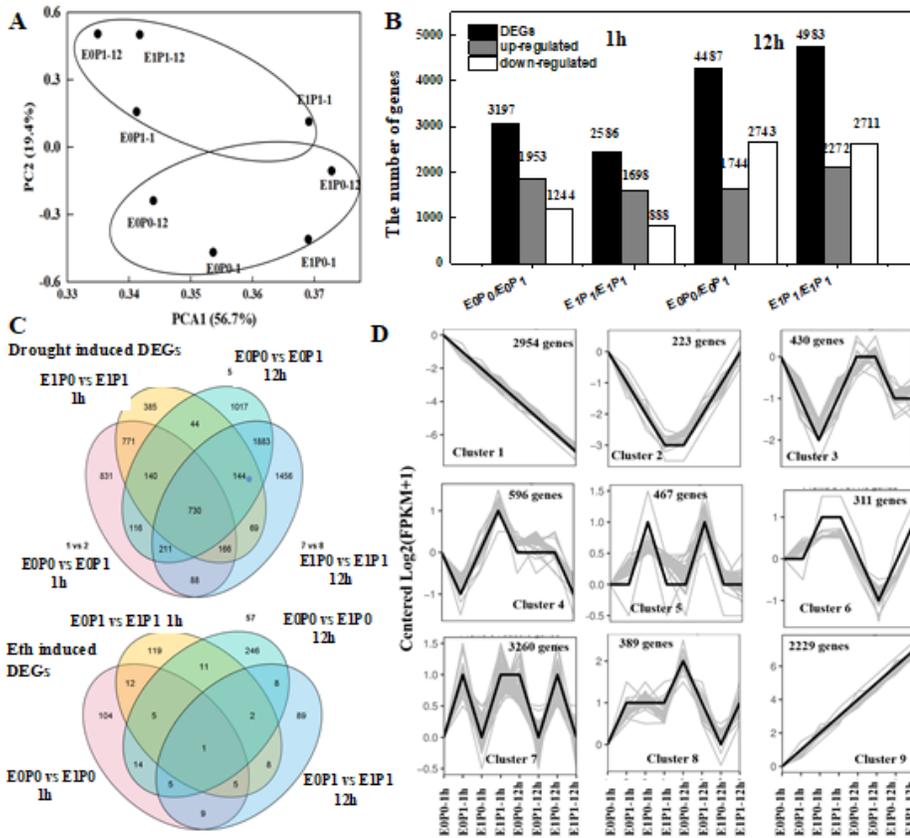


Figure 3

The differently expressed genes (DEGs), satisfying the criteria of $p \leq 0.01$, identified from drought response and ethylene mediated drought response. The ≥ 2 -fold change transcripts were used for the time series analysis

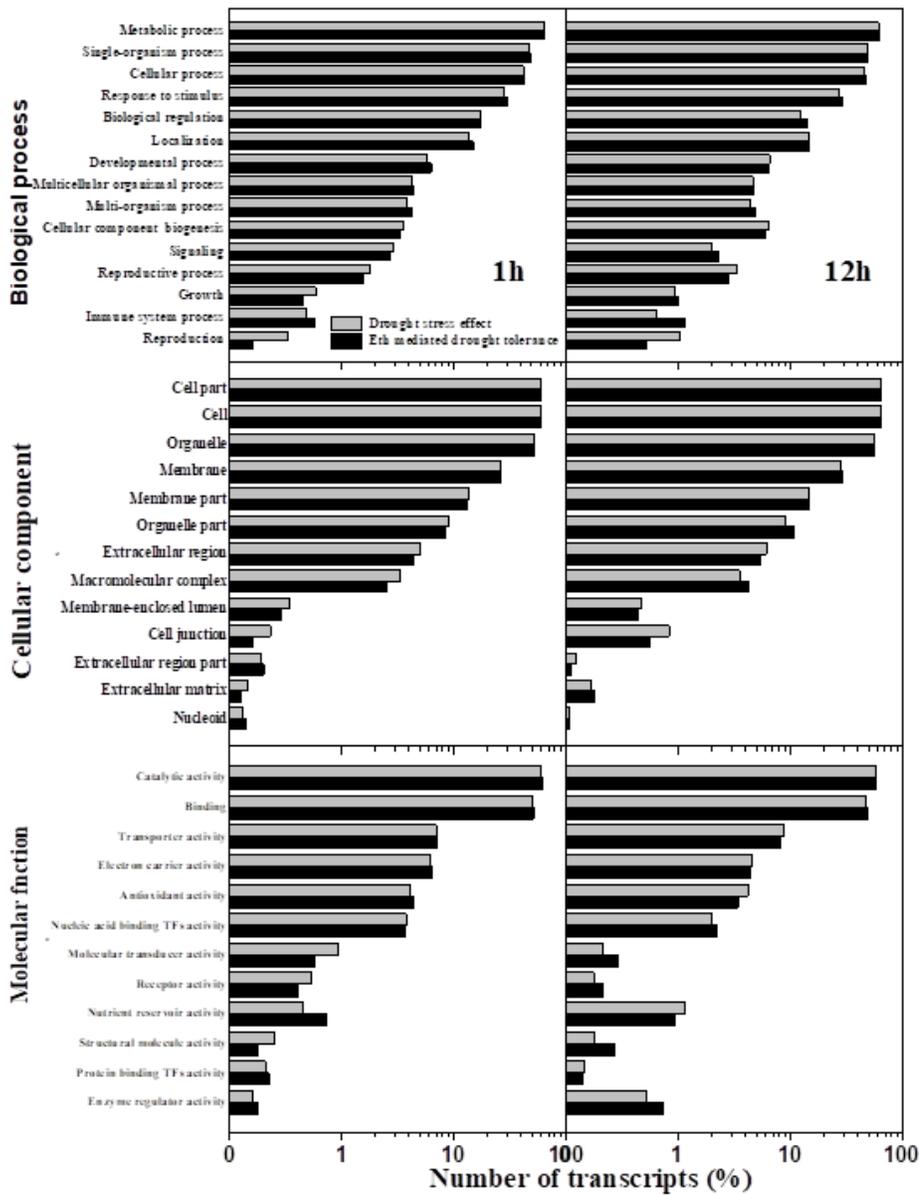


Figure 4

Gene ontology (GO) term assignment to the transcripts of primed and control plants exposed to 12h of drought stress in different categories of biological process, cellular component, and molecular function in the E0P0 vs. E0P1 and E0P1 vs. E1P1 samples. The X-axis shows the percentage of transcripts associated with the enriched GO terms.

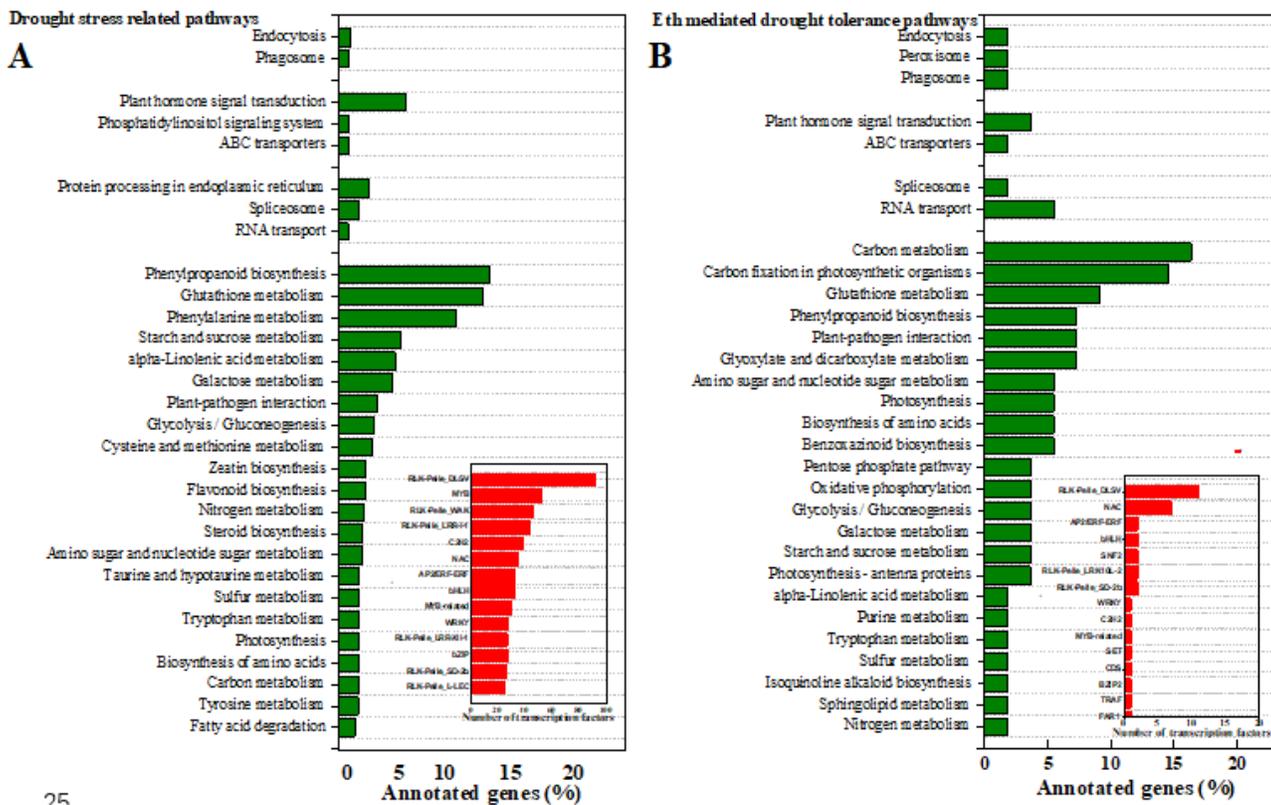


Figure 5

Top pathway entries of the DEGs related to drought response (A), and seed ethylene (Eth) priming mediated drought response (B).

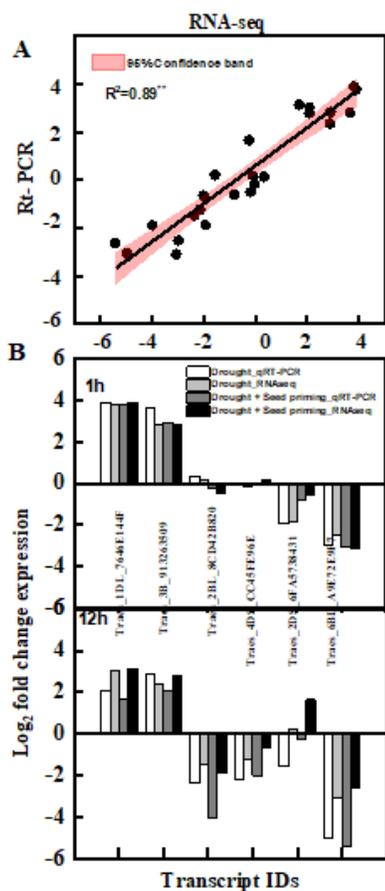


Figure 6

Validation of RNA-Seq data using quantitative real-time polymerase chain reaction (qPCR). Bar graphs depict selected transcripts' expression patterns for both primed and CK plants under well water and drought stress conditions. All the data points are represented by the log₂ fold change in the values.

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