The different root apex zones contribute to drought priming induced tolerance to a reoccurring drought stress in wheat
Wang, Xiao; Chen, Jing; Ge, Jiajun; Huang, Mei; Cai, Jian; Zhou, Qin; Dai, Tingbo; Mur, Luis Alejandro Jose; Jiang, Dong

Published in:
Crop Journal
DOI:
10.1016/j.cj.2020.11.008
Publication date:
2021

Citation for published version (APA):

Document License
CC BY-NC-ND

General rights
Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400
e-mail: is@aber.ac.uk
The different root apex zones contribute to drought priming induced tolerance to a reoccurring drought stress in wheat

Xiao Wang¹, Jing Chen¹, Jiakun Ge¹, Mei Huang¹, Jian Cai¹, Qin Zhou¹, Tingbo Dai¹, Luis Alejandro Jose Murb, Dong Jianga,³

¹National Technique Innovation Center for Regional Wheat Production/Key Laboratory of Crop Ecophysiology, Ministry of Agriculture/Nanjing Agricultural University, Nanjing 210095, Jiangsu, China
²Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth SY23 3DA, UK

Abstract

Drought priming is a promising approach to improve tolerance to further drought in wheat. The root apex plays important roles in drought however, its contribution to drought priming remains unknown. To provide mechanistic insights into this process, the transcriptomes and proteomes at three different zones along the root axis under drought stress were analyzed. Physiological assessment of root growth indicated that priming augmented roots growth in response to drought and also the levels of protective protein and glycine betaine. Scanning across the proximal to the distal zones of the root apex indicated increases the transcription of genes involved in primary and secondary metabolism. Conversely, genes related to translation, transcription, folding, sorting and degradation, replication and repair were increased in the apex compared to the proximal zone. A single drought episode suppressed their expression but prior drought priming served to maintain expression with recurrent drought stress. The differentially primed responses genes were mainly involved in the pathways related to plant hormone signaling, stress defense and cell wall modification. The prediction of regulatory hubs using Cytoscape revealed that priming augmented roots growth in response to drought. Based on our integrative transcriptomic-proteomic assessments we present a model for drought priming protected plant hormone signaling transduction pathways to drive the cell cycle and cell wall loosening to confer beneficial effects on roots to counter the effects of drought. This model provides a theoretical basis for improvement of drought tolerance in wheat, via an increased understanding of drought priming induced drought tolerance.

1. Introduction

Drought stress is one of the major challenges for crop production around the world, and can cause crop yield losses of over 30% [1]. Moreover, both the frequency and severity of drought events are predicted to increase in most crop producing regions in the world as a consequence of the global climate change [2,3]. Wheat is very sensitive to drought stresses and those occurring during
critical growth stages such as heading and grain filling, could result in a yield losses of 58%–92% [4,5]. This drives the need to explore means of conferring drought tolerance.

Considering the organs that could be most important in drought, roots are usually the earliest organ to perceive the drought stress and then communicate this to shoots and leaves [6]. Drought stress usually inhibits shoot growth but stimulates root growth to accelerate the remobilization of photo-assimilates from shoots to roots to cope with drought stress [7]. Root growth in response to drought stress has reported in many plant species [7,8]. For instance, the DEEPER ROOTING 1 gene increases yield under moderate and severe drought stress in rice [9]. In wheat, drought tolerant varieties can have fewer roots in the surface soil but have a dense root architecture in the subsoil layer. This allows penetration into deep soil layers to absorb water [10–12]. Therefore, root elongation into deep soil is an important adaptive trait in crops when dealing with drought stress and defining the regulatory network could provide insights that could be exploited for enhanced drought tolerance [13]. The root apex is the major region that determines growth due to its high mitotic rate thereby providing cells to the meristem for growth of the root apex. Subsequently, these cells are displaced from the root apex and enter the elongation zone, where cells undergo longitudinal expansion to reach their final size and function in the differentiation zone [14]. Cell division and the longitudinal expansion into the elongation zone are tightly regulated by plant hormones. The roles of these hormones are cellular context-dependent and exhibit either synergistic or antagonistic effects [15]. These hormones also influence the construction of root systems to increase the capacity to acquire water and also the expression of tolerance-related genes in drought tolerant wheat varieties [16]. Predictability, the expression of these genes also differs between different zones of the root apex. For example, in barley roots adaptation to drought stress is controlled in a region specific manner [17]. Further, time-course assessment of primary maize roots revealed increased longitudinal expansion in region close to the apex but decreased in basal under decreasing water potential [18]. However, such spatio-temporal differences are often not taken account of in drought tolerance assessments. Therefore, any analyses focusing on the whole root apex analysis could “dilute” and indeed, miss region-specific roles of root apex, different zones of root apex in responses to drought stress in wheat.

Plants have been reported to benefit from prior exposure to a stress in order to improve tolerance to subsequent stresses [19]. Drought priming has been considered as one means to enhance plant tolerance to drought stress during seedling and grain filling stages in wheat [20,21]. During grain filling, primed plants showed higher grain yield and nitrogen use efficiency compared with non-primed plants under drought stress [22]. Primed plants could also maintained higher photosynthetic rates and antioxidant enzyme activities to reduce oxidative stress damage [20]. These effects could be linked to the higher levels of abscisic acid (ABA) and jasmonates acid (JA) that have been observed with drought priming which are linked to increased tolerance in wheat seedlings [23].

Our earlier study indicated that previous drought could prime wheat tolerance to recurrent drought stress to reduce grain yield loss and improve physiological performance [20]. Recently, we observed that primed wheat plants showed higher root length than non-primed plants when under drought stress [24]. This implies that drought priming aids in root elongation to reach the deeper water with recurrent drought stress by unknown mechanisms. Moreover, since the root elongation is tightly related to the cell division and elongation at different regions of root apex, particular zones of the root apex could have discrete roles in priming-induced root growth and drought tolerance. To date, there has been no study of the differing responses of different root apex zones in primed versus non-primed plants with re-occurring drought stress.

Herein we characterize the morphological and physiological mechanisms of root growth and plant tolerance to drought stress induced by drought priming. We describe spatial differences along root apex in both drought primed plants and non-primed plants with a secondary drought stress that demonstrate the role of key phytohormonal events.

2. Materials and methods

2.1. Experimental design

Uniform seeds of Yangmai 16 were surface sterilized with H2O2 for 10 min, and then washed 4 times with water. The seeds were germinated in dark at 20 °C until the emergence of the second leaf. Thereafter, uniform seedlings were transplanted into Hoagland culture solution in growth chamber at a day/night temperature of approximate 20 °C/16 °C, and with a 16 h/8 h light/dark cycle at 500 μmol m–2 s–1 light intensity supported by combination of Light Emitting Diode (LED) and sodium lamps.

At the three-leaf stage, half of the plants were drought primed through the addition of 5% PEG solution (φ = –0.37 MPa) for 6 h, and then allowed to recover for 6 days. Control non-primed plants were untreated. Thereafter, both primed and non-primed plants were either drought stressed with 15% PEG (φ = –0.78 MPa) for 24 h or kept under control conditions. Thus, four treatments were assessed: no priming + no drought stress (CK), priming + no drought stress (PC), priming + drought stress (PD), and no priming + drought stress (CD). In total 480 pots were used, each pot (320 mm × 240 mm × 125 mm) with 150 plants, and 40 pots were used as one replicate, three replicates were performed for each treatment.

2.2. Plant water status and contents of osmolytes

Leaf or root water potential was measured with a SKPM1456 pressure chamber (Skye Instruments Ltd., Powys, UK). The last fully expanded leaf was excised and placed in the pressure chamber with the cut-end protruding through the lid. Water potential was measured by pumping air into the chamber until sap protruded from the cut surface. For the root tissue, the shoots were cut off using razor blades at a distance of 40 mm from the base and the whole root used to measure root water potential. The osmotic potential was determined using a vapor pressure osmometer (Wescor 5600, Wescor Inc., Logan, UT, USA) at 25 °C. Proline content was determined using the ninhydrin coloring method [25]. Glycine betaine (GB) content was measured following the protocol [26].

2.3. Plant biomass, leaf photosynthesis and root growth rate

Ten plants of each replicates were harvested, and the roots and shoots were separated to measure fresh and dry weights. The net assimilation rate analysis was carried out on individual fully expanded leaves of all treatments at the last day of the drought stress using a LI-COR 6400 portable photosynthesis system (LI-COR, Lincoln, USA). Five measurements for each treatment were taken.

To monitor root growth rate, a very fine marker pen was used to mark 8 lines at equal distances from the tip of the primary root. Microscopy (OLYMPUS-SZ61) was used to image the roots just before the drought stress. After 24 h of drought stress, the root was imaged again. The images were analyzed using the ImageJ software.
software. The following equation was used to calculate the relative root growth rate:

Relative root growth rate \( \left( \text{mm} \text{ d}^{-1} \right) = \frac{\ln(b) - \ln(a)}{t} \)

where \( b \) refers to root length after 24 h, \( a \) refers to root length before treatments, and \( t \) refers to the growth period of 24 h. At least ten biological replicates were taken for measurement of root growth rate.

2.4. Transcriptome in different zones along root apex

The different zones of root apex were dissected using a computer visual assistant anatomic microscope. ‘Zone A’ is the section of top 1 mm from the root tip, ‘Zone B’ is the section of 8 mm long from the end of ‘Zone A’, and ‘Zone C’ is that 10 mm long from the end of ‘Zone B’, which roughly correspond to the meristem region, the cell division region and the elongation region, respectively. In total, around 2400 dissections of Zone A, 800 of Zone B, and 500 of Zone C were separately collected into 1.5 ml tubes for one replicate. The collected root tissues were immediately frozen in liquid nitrogen, and then stored at \(-80^\circ\text{C}\). Three biological replicates were taken for each treatment.

Total RNA was isolated using approximately 50 mg root tissue by OminiPlant RNA Kit (DNase I) (CoWin Biosciences) following the manufacturer’s protocol. Adapted ligation products were size selected by agarose gel electrophoresis, PCR amplified, and sequenced on an Illumina HiSeq 4000 system. The RNA was sequenced to a depth of approximately 31 million read-pairs per sample per lane, giving 2.07 billion, yielding 311 Gb Data. The clean reads were matched to the cDNA database (ftp://ftpensemblgenomes.org/pub/release-28/plants/fasta/triticum_aestivum/cdna) using TopHat v2.0.12/ Bowtie v2.2.3 software.

Gene abundances were quantified by software RNASeq by Expectation Maximization [27]. To identify differentially expressed genes (DEGs) across samples or groups, the edgeR package (http://www.r-project.org/) was used. The fold change (FC \( \geq 2 \)) and a false discovery rate (FDR) < 0.05 as the cut-off values to determine DEGs among the three zones of CK, PC, PD, and PC root apices. These DEGs were then subjected to enrichment analysis of Gene Ontology (GO) functions and KEGG pathways. PCA was performed with R package g models (http://www.r-project.org/).

Gene expression pattern analysis was used to cluster genes of similar expression patterns for zones CK-A, CK-B, CK-C, and the same zones in PD and CD treatments. The expression data were normalized and then clustered by Short Time-series Expression Miner software [28]. The clustered profiles with P-value \( \leq 0.05 \) were considered as significant profiles. The DEGs in all or each profile were subjected to GO and KEGG pathway enrichment analysis.

2.5. Quantitative Real-Time PCR (qPCR) analysis

The FastQuant RT Kit (KR106, TIANGEN) was used to synthesize first-strand cDNA from RNA. The cDNA samples were used to determine gene expression levels by qPCR with ABI step one plus (ABI, USA). The average threshold cycle was obtained to evaluate the relative expression levels using the 2\(^{-\Delta\DeltaCT}\) method. The primers sequences used in this study are listed in Table S4.

2.6. Proteome in different zones along root apex in wheat

The proteomic analysis was performed using the same sample as transcriptomic analyses except for the PC treatment. Total proteins were extracted and digested to peptide labeled with iTRAQ Reagents-SPlex (AB SCIEX, Foster City, CA) [29]. The iTRAQ assay was performed on an Easy-nLC 1000 system (Thermo Fisher Scientific, MA, USA) connected to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, MA, USA) equipped with an online nano-electrospray ion source. Full-scan MS (m/z 350–1550) were acquired with a mass resolution of 120 K, sequentially followed by high energy collisional dissociation MS/MS scans with a resolution of 30 K. The other parameters were: isolation window set as 1.6 Da, the automatic gain controls target set as 400 000, MS/MS fixed first mass set at 110, 10 precursors used for fragmentation in each scan cycle, normalized collision energy 30 eV, charge state exclusion settings unassigned 1, >8. Differentially regulated proteins were analyzed by Student’s \( t \) test, with the selection criteria of a fold change > 1.2 or < 0.83 (\( P < 0.05 \)).

3. Results

3.1. The effect of drought priming on plant water status and osmolyte content under recurrent drought stress

The imposition of recurrent drought stress decreased water and osmotic potential in both wheat leaves and roots (Fig. 1). Compared with CK (control), the loss in water potentials in PD (priming with subsequent drought) were not as severe as with CD (no priming with subsequent drought). Thus, leaf water potential in PD decreased by 98.2% compared to CK whilst with CD this was 138.2%. For root water potentials, with PD the decreased was 83.1% but with CD it was 113.3% compared to CK (Fig. 1A). Leaf osmotic potential decreased by 40.2% and 22.2%, while root osmotic potential decreased by 44.1% and 25.9% in PD and CD, in comparison to CK (Fig. 1B). Compared to CK, PC resulted in lower root osmotic potentials, but there were no differences in root and leaf water potentials and osmotic potential of leaf.

Measurements of proline and GB in leaves and roots also indicated a protective effect of priming. Protective proline content in leaves increased by 89.4% in PD which was greater than the 43.5% seen with CD when compared to CK. In roots, proline increased by 91.8% and 2.4% in PD and CD, respectively (Fig. 1C). Similarly, leaf GB content increased by 80.7% and 46.8%, and in root by 22.4% and 18.1% in PD and CD in relation to CK, respectively (Fig. 1D).

The expression levels of \( \Delta 1 \)-pyrroline-5-carboxylate synthetase (P5CS), a major enzyme catalyzing proline synthesis, were downregulated in leaves and roots in CD, compared to CK. No significant differences in expression levels of P5CS in PD and CK leaves and roots. Betaine aldehyde dehydrogenase (BADH) which the major enzyme of GB biosynthesis, did not differ significantly between CD and CK. However, BADH was highly expressed in PD and PC roots as compared with CK, but its expression in leaves barely changed with these three treatments (Fig. S1).

3.2. Drought priming promotes biomass accumulation and root growth under the recurrent drought stress

Leaf biomass was significantly decreased in CD as compared to CK (Fig. 2A). Under drought stress, the photosynthetic rate decreased by 38.3% and 46.1% in PD and CD, respectively in relation to CK (Fig. 2B). Root biomass was inhibited by drought stress as shown in Fig. 3A. The average root growth rate decreased by 49.9% and 76.3% in PD and CD as compared with CK, respectively (Fig. 3B). There were no significant differences in growth rate of the meristem zone between PD and CD (Fig. 3C). However, the relative growth rate of the elongation zone was inhibited by drought stress, but decreased only by 28.7% in PD as compared to 48.0% with CD in relation to CK, respectively (Fig. 3D).
3.3. The transcriptome of different zones under different treatments in wheat

Transcriptomic analyses of different root zones were undertaken. Compared to zone A, 16,195 and 31,911 differentially expressed genes (DEGs) were found in Zone B and Zone C under CK. When these zones were assessed under the context of the different drought treatments 10,824 and 27,071 DEGs in Zone B and Zone C under CD, 14,592 and 27,918 DEGs with PD, respectively (Table S2). When compared to CK 1280, 1676 and 2296 DEGs in Zone A, Zone B and Zone C, under PC were identified. DEG numbers were 9968, 11,789, 12,379 under CD and 12,525, 13,771, 15,546 under PD when compared with CK (Table S2).

Comparison of all the DEGs, revealed obvious spatial variations in the transcriptome along the root apex (Fig. 4). Focusing on these, 11,506 DEGs were gradually up-regulated (pattern 1), 11,259 DEGs down-regulated (pattern 2) from Zone A to Zone C. Examining the effect of drought treatment on these, 10,386 and 9907 DEGs were up-regulated, 9582 and 7064 DEGs were down-regulated in CD and PD, respectively (Fig. 4A). KEGG pathway assessment of the DEGs in different zones showed that the up-regulated genes (pattern 1) were mainly involved in translation, transcription, folding, sort-
ing and degradation, replication and repair. However, the down-regulated genes (pattern 2) were mainly classified as linked to metabolism, signal transduction and environmental adaptation (Fig. 4B).

When we reran the analyses focusing on the responses to different treatments (CK, PC, PD, and CD) the majority of DEGs were arranged into four patterns (Fig. 5). The least interesting were those DEG up or down regulated by drought stress with no significant differences between PD and CD (pattern 3 and pattern 4). However, other patterns showed up regulation by drought stress but with lower increase rate in PD than in CD (pattern 5) or were down regulated by drought stress with a less decrease rate in PD than in CD (pattern 6). Considering zonal differences, i.e. zones A, B and C, these could be related to 957, 1340, and 1445 DEGs in pattern 5; 1100, 1786, and 1974 DEGs in pattern 6, respectively (Fig. 5). KEGG pathways assignations for the DEGs showed that the folding, sorting and degradation, transcription, translation, replication and repair were the major pathways in pattern 5 (Fig. S2). However, pattern 6 suggested DEGs linked to metabolism, signal transduction and environmental adaptation (Fig. 6).

3.4. qPCR validation of genes expression profiles

The transcriptome (RNA-seq) results were validated for ten genes by real time quantitative qPCR analysis. The ten genes included three cell wall related genes (peroxidase P7-like, cellulose synthase-like protein D1 and expansin-B11-like), two genes encoding TFs (transcription factor bHLH69-like and Zinc finger CCCH domain-containing protein 43), two genes encoding plant hormone pathway (9-cis-epoxycarotenoid dioxygenase 1 and auxin-responsive protein IAA20) and three genes related to other pathways (calmodulin-like protein 5, proline-rich receptor-like protein kinase PERK8 and endonuclease 4-like). The qPCR results correlated with the transcriptome results (Pearson correlation 0.6655) (Fig. S3). Thus, the transcriptome results represent the responses of gene expressions in different zones of root apex to the drought priming under the later drought stress.

3.5. Comparative transcriptomic and post-transcriptional analysis

To further elucidate the key regulatory networks playing a role in the three zones along root apex, with a particular focus on priming, we considered post-translational events. Thus, we conducted a proteomic study based on iTRAQ technology of the same samples used for the RNA-seq experiments. We determined relative expression levels for more than 6200 proteins, corresponding to about 70% of all transcribed protein-coding genes. Over 99% of proteins identified by iTRAQ assay were also observed in the transcriptomic data suggesting the two omics provided complementary and cross-validatory results. However, when the expression levels of all of the components of the omics were compared correlations were generally poor. This was not the case for specific features. Thus, the co-analysis between proteomic data and the corresponding transcriptomic results indicated the key roles for plant hormone signaling transduction pathways in priming with a positive Pearson correlation coefficient 0.54 for these aspects (Table S2). Arginine and proline metabolism showed a significant negative correlation with Pearson correlation coefficient -0.53 (Table S2).

4. Discussion

It is known that an earlier moderate drought priming can increased tolerance to a reoccurring severe drought stress. This was exemplified by maintaining better leaf water status and pho-
tosynthesis under the drought in primed plants. In this study, root growth rates were less inhibited in primed plants than the non-primed plants which paralleled enhanced tolerance to the recurrent drought stress in wheat. Further evidence indicated that different root apex zones has discrete responses in priming-induced drought tolerance. This was indicated by spatial roots differences in the transcriptome and proteome profiles. The results lead us to propose a regulatory network of phytohor-

**4.1. Primed plants enhanced osmotic adjustment capacity under drought stress**

Plant water potential is an important trait to indicate plant water status, and osmotic potential tends to decrease to maintain...
Fig. 6. The KEGG pathway annotations of genes from the tendency analysis in Pattern 6 of three zones along root apex during drought stress. A, B and C denote different zones along root apex. CK, no drought priming + no drought stress; PC, priming + no drought stress; PD, priming + drought stress; CD, no priming + drought stress. Numbers in the bars represent the number of differentially expressed genes with clustered in the corresponding pathway.
cell turgor pressure, especially under drought stress [30,31]. Here, primed plant improved water movement to maintain higher plant water potential under drought stress. The accumulation of proline and GB protect cells from stresses by maintaining an osmotic balance with improved the cell membrane fluidity under drought stress [32,33]. In this current study, primed plants showed higher proline and GB contents in leaves and roots compared to non-primed plants, which contributed to lower osmotic potentials to maintain higher water potential.

BADH is the major enzyme catalyzing the biosynthesis of GB [34] and BADH transcript levels were higher in primed versus non-primed plants. This was consistent findings for drought tolerance in maize [35]. P5CS catalyzes the first step of proline synthesis and is required for stress-induced proline accumulation [36]. However, P5CS transcription in both leaves and roots was decreased in non-primed plants, but was insignificantly affected in primed plants, as compared with control. In maize, the high level of proline accumulating at the maize root tip at low water potential was not due to de novo synthesis at the root tip but most likely came from proline transport from other parts of the plant [37]. Therefore, the increased contents of proline in primed plants was unlikely to be the transcriptional activation of proline biosynthesis but arose from translocation to the root.

4.2. Primed plants exhibit less inhibition of root growth rate under drought stress

We observed that root growth in primed plants was less inhibited with improved root biomass and length compared to non-primed plants under reoccurring severe drought stress. This was consistent with our previous study where wheat plants primed at the vegetative growth stage had longer root lengths and enhanced tolerance to severe drought stress during grain filling [24]. Further analyses suggested that the elongation zone decreased much more than meristem zone under drought stress. This agreed with radial growth rates being decreased throughout the elongation zone in maize at low water potentials [18]. Crucially, this reduction in the length of the elongation zone was much less in primed plants than in non-primed plants.

4.3. Defining root zone specific gene expression with priming to maintain root growth

The analysis of drought responsive signatures along longitudinal root sections found that genes related to secondary metabolites biosynthesis, carbohydrate metabolism, amino acid metabolism and lipid metabolism were up regulated from Zone A to Zone C in controls. These trends were similar to what has been reported in barley with a developmental gradient towards the shoot, amino acid, carbon metabolism and defense response-related pathways with the highest activities in the root mature zone [17]. Genes involved in the cell wall loosening, ABA and ethylene signaling, and carbohydrate metabolism were highly expressed in elongation zones of the primary roots as with both maize and soybean [38]. The downstream target genes which most related to cell elongation and cell wall modification were more highly expressed in both Zones B and C of primed, compared with non-primed plants. Peroxidases, expansins and xyloglucan endotransglucosylase/hydrolases (XTHs) mediate the cell wall loosening process and can maintain plant growth under drought stress [39,40]. Peroxidase is both involved in scavenging hydrogen peroxide and in modifying the cell wall [40] whilst expansins are cell wall loosening proteins. XTHs are xyloglucan-metabolizing enzymes that are believed to be the important agents for controlling cell wall strength and extensibility [41]. In this study, peroxidase, expansins and XTHs were mostly down regulated by drought stress; while more highly expressed primed plants and this will contribute to root growth.

The DEGs between primed plants and non-primed plants were mapped onto the interaction network and visualized with Cytoscape (Fig. S3). A total of 20 genes were found as the hub genes including hormonal components (ABA receptor PYL4) along with key factors that could influence tolerance (e.g. dehydrins, aquaporin, antioxidant enzymes) and cell division (e.g. cyclin dependent kinases) (Table S3). Although such analyses are predictive, these hub-phytohormone genes could play critical roles in drought priming promoted root growth and require further study in root growth under drought stress.

Integrative transcriptomic-proteomic analyses between primed and non-primed plants showed a poor correlation. This indicated the complex and probably discrete transcriptomic and post-transcriptional regulatory mechanisms controlling priming. However, some transcript–protein correlations did indicate biological processes that are important in Zone B and Zone C of primed and non-primed plants under drought stress. Most importantly, there was a high correlation for transcripts–proteins involved in ‘plant hormone signal transduction’ as defined by KEGG pathways.

4.4. Plant hormone signaling transduction differs in root apex zones between primed and non-primed plants

The importance of phytohormone regulation was highlighted by our analyses of DEGs involved in pattern 5 and pattern 6 which were key in the three root apex zones' responses to drought priming. Plant hormones are the principal regulators of root growth with many genes involved in plant hormone biosynthesis and signaling pathways, are involved in root development [42]. Here, auxin, cytokinin (CTK), brassinosteroids (BR), ethylene, ABA, JA and salicylic acid (SA) were down regulated by drought stress, and crucially this reduction was much lower in PD than in CD in all the zones of root apex, especially in Zones B and C under drought stress.

Genes related to the auxin biosynthesis pathway are down-regulated by drought stress and are up-regulated after recovery [38]. Various members of the ARF family have been linked with enhanced drought tolerance in soybean roots [43]. In the present study, genes YUC and ABA aldehyde oxidase (AAO), which involved in auxin biosynthesis, as well as ARFs, AUX/IAA, amido synthetases (GH3) and small auxin-up RNA (SAUR) (all involved in auxin signaling), were higher expressed in primed plants than non-primed plants. This is highly likely to contribute to continued root growth with drought.

CTK is a negative regulator of primary roots elongation, acting in conjunction with auxin in Arabidopsis [44] and barley [45]. Thus, increasing CTK degradation in roots by expression of a cytokinin dehydrogenase (CKX) in transgenic lines promoted root elongation under drought stress [46]. In our study, CKX and A-type Arabidopsis response regulator A-ARR (A-ARR) were maintain higher levels in primed plants. These indicated that the CTK biosynthesis and signaling were inhibited. This would act with auxin to maintain root growth.

BR are known to participate in multiple developmental and physiological processes including as promoters of root growth and development in Arabidopsis [47,48]. Mutants impaired in BR biosynthesis or signal transduction display a short-root phenotype [49]. In our study, genes encoding brassinosteroid-insensitive 1 protein (BR1), benzoazepine receptor (BZR1) and touch 4 (TCH4) which involved in BRs signaling were down regulated by drought stress but less so in primed than non-primed plants.

The effects ethylene on plant root growth are not clear. Some studies found that ethylene inhibited of root growth [50,51] but, other studies have shown the opposite results [38]. It seems likely...
that an optimum level of ethylene is required for root elongation under drought stress [52,53]. Overexpression of the GmERF3 gene isolated from soybean could maintain root growth of tobacco under drought stress [54]. We found that genes encoding ACC synthase (ACS), enzyme for ethylene synthesis, and ERF were down regulated by drought stress but were more highly expressed in primed than non-primed plants. These are likely to contribute to maintenance of a basal ethylene levels and signaling to maintain root growth.

ABA accumulation is crucial for the maintenance of primary root elongation under drought stress [55] but higher levels is an inhibitor of primary root growth [56]. ABA regulates root growth through a hormonal network that includes CTK, ethylene and auxin [57]. In this current study, the ABA receptor and transcriptional factors of its signaling pathway were decreased in root zones by drought stress, while primed showed higher level than non-primed plants.

JA and SA play important roles in plant basal defense against biotic stress and are involved in abiotic stress responses [58]. 12-oxo phytodienoic acid (OPDA) reductase (OPR) is involved in the biosynthesis of JA [59] which acts to initiate the degradation of JA ZIM-domain (JAZ) proteins. JAZ proteins are transcriptional repressors of JA responses in Arabidopsis but over-expression of OsJAZ9 resulted in increased salt stress tolerance in rice [60]. We found that OPR and JAZ were higher expressed primed plants of zones B and C in PD.

5. Conclusions

Taken together this study proved some mechanistic insights into priming treatment to induce an adaptation response in wheat plants to better cope with the later severe drought stress. We have indicated the prominence of phytohormone regulation in different zones along root apex in controlling root growth behavior in the primed state. Thus, based on our data, we propose a model representing the summary of plant hormone signaling transduction in this study (Fig. 7). The model highlights genes that related to a coordinated yet complex interplay between hormones cell wall modifications are required for enhanced dehydration tolerance and less inhibition of root growth in primed plants under stress. Further validation of this model, could allow it to be exploited to increase drought stress in wheat through priming induced root growth.

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 appreciated the advice from Prof. Donglei Yang and Prof. Yufeng Wu from Nanjing Agricultural University for data analysis. This study was supported by the National Key Research and Development Program of China (2016YFD0300107), the National Natural Science Foundation of China (31771693, U1803235), the China Agriculture Research System (CARS-03), JCIC-MCP, the 111 Project (B16026), and the UK Biotechnology and Biological Sciences Research Council (BBSRC) Exchange Grant (BB/R02118X/1).

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2020.11.008.

References

The Crop Journal xxx (xxxx) xxx

ARTICLE IN PRESS

X. Wang, J. Chen, J. Ge et al.


