



# The Aux/IAA protein *TaIAA15-1A* confers drought tolerance in *Brachypodium* by regulating abscisic acid signal pathway

Peisen Su<sup>1</sup> · Chao Sui<sup>1</sup> · Jingyu Li<sup>1</sup> · Kun Wan<sup>1</sup> · Haonan Sun<sup>1</sup> · Shuhan Wang<sup>1</sup> · Xiaoqian Liu<sup>1</sup> · Shangjing Guo<sup>1</sup>

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## Abstract

**Key message** Overexpression of the Aux/IAA protein *TaIAA15-1A* from wheat improves drought tolerance by regulating the ABA signalling pathway in transgenic *Brachypodium*.

**Abstract** Drought is a major abiotic stress that causes severe crop yield loss. Aux/IAA genes have been shown to be involved in drought stress responses. However, to the best of our knowledge, there has been little research on the molecular mechanism of the wheat Aux/IAA gene in the context of drought tolerance. In this study, we found that expression of the wheat Aux/IAA gene *TaIAA15-1A* was upregulated by PEG6000, NaCl, SA, JA, IAA and ABA. Transgenic plants overexpressing *TaIAA15-1A* showed higher drought tolerance than wild-type (WT) plants. The physiological analyses showed that the transgenic lines exhibited a higher survival rate, shoot length, and relative water content than the WT plants. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were enhanced in transgenic lines, causing a reduction in the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anion radical (O<sub>2</sub><sup>-</sup>) contents. Transcriptome analysis showed that *TaIAA15-1A* overexpression alters the expression of these genes involved in the auxin signalling pathway, ABA signalling pathway, phenolamides and antioxidant pathways. The results of exogenous ABA treatment suggested that *TaIAA15-1A* overexpression increased sensitivity to ABA at the germination and postgermination stages compared to WT plants. These results indicate that *TaIAA15-1A* plays a positive role in plant drought tolerance by regulating ABA-related genes and improving antioxidative stress ability and has potential application in genetically modified crops.

**Keywords** Wheat · Aux/IAA protein · *TaIAA15-1A* · Drought tolerance · Abscisic acid

## Introduction

Cereal crops inevitably face various abiotic stresses, such as drought, heat, salinity, and cold, during growth and development processes; these stresses greatly impact field productivity (Robles et al. 2018). In the long-term evolutionary interaction between plants and environmental abiotic stresses, plants have evolved complex resistance mechanisms, such as antioxidant systems, osmotic balance, and phytohormone signal transduction (Sallam et al. 2019). To date, hundreds

of genes, such as *TaPYL1-1B*, *TaSAP5*, *OsIAA18*, *TaNAC69-1*, and *OsIAA6*, have been shown to function in controlling drought tolerance in crops (Jung et al. 2015; Chen et al. 2016; Zhang et al. 2017; Wang et al. 2021; Mao et al. 2022).

Auxin has been reported to play an important role in plant stress defence responses (Mutka et al. 2013). In *Arabidopsis thaliana*, endogenous and exogenous auxin positively regulates drought resistance by modulating abiotic stress-related gene expression and reactive oxygen species (ROS) metabolism (Shi et al. 2014). A previous study showed that exogenous IAA treatment can alleviate drought stress and effectively enhance drought resistance in wheat (Liu et al. 2009). Plants regulate endogenous auxin homeostasis in response to drought stress (Du et al. 2013). As a key mediator, ABA has been shown to enhance the drought tolerance of plants via crosstalk with auxin signalling. For example, increasing evidence indicates that auxin homeostasis changes influence ABA synthesis and thus alter plant tolerance against drought stress (He et al. 2021; Zhang et al. 2021).

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✉ Peisen Su  
pssu2014@163.com

✉ Shangjing Guo  
guoshangjing@lcu.edu.cn

<sup>1</sup> College of Agronomy, Liaocheng University, Liaocheng 252059, People's Republic of China

In plants, auxin/indoleacetic acid (Aux/IAA) proteins are a large family and have been identified to play an important role in the auxin signalling pathway; they bind to TIR1/AFB, are ubiquitinated by the E3 ubiquitin ligase complex SCF<sup>TIR/AFBs</sup>, and are then degraded by the 26S proteasome, allowing ARF-mediated transcriptional regulation (Maraschin et al. 2009). A number of Aux/IAA genes have been shown to influence drought tolerance in crops; for example, overexpression of *OsIAA20* enhances transgenic rice tolerance against drought and salt stresses through the ABA pathway (Zhang et al. 2021). A previous study showed that *OsIAA18* overexpression in rice plants significantly improves salt and drought tolerance by upregulating ABA-regulated gene expression levels and improving ROS scavenging capacity (Wang et al. 2021). In addition, these genes, including *OsIAA6* and *OsIAA9* in rice (Jung et al. 2015) and *IAA5*, *IAA6*, and *IAA19* in *Arabidopsis* (Salehin et al. 2019), have also been reported to be involved in drought tolerance.

To date, the roles of some Aux/IAA genes in tolerance against drought stress have been widely shown; however, to the best of our knowledge, there has been little research on the molecular mechanism analysis of wheat Aux/IAA genes in the context of drought tolerance. In this study, a wheat Aux/IAA gene, *TaIAA15-IA*, was cloned, and the tolerance against drought stress conferred by *TaIAA15-IA* in transgenic *Brachypodium* was investigated. Expression pattern analysis showed that the expression level of *TaIAA15-IA* was upregulated by PEG6000, NaCl, SA, JA, IAA and ABA. Our results indicate that *TaIAA15-IA* enhances plant tolerance against drought stress by regulating ABA-related genes and improving antioxidative stress ability and has potential applications in crop improvement programs.

## Materials and methods

### Plant materials and abiotic treatments

The bread wheat (*Triticum aestivum* L.) cultivar “SuMai3” and *Brachypodium distachyon* “Bd21” were used in this study. The WT “Bd21” and *TaIAA15-IA*-overexpressing transgenic *Brachypodium* lines were grown at 22–25 °C with a photoperiod of 16/8 h in a greenhouse. The seedlings of WT and transgenic *Brachypodium* lines at the three-leaf and one-heart stage were stressed by withholding watering for 21 days under controlled greenhouse conditions and then rewatered for 7 days.

### Cloning and transformation of *TaIAA15-1A*

The leaves of “SuMai3” seedlings at the three-leaf and one-heart stage were harvested and used for total RNA extraction. The RNA was reverse-transcribed into cDNA for gene

amplification. The full-length sequence of *TaIAA15-IA* (TraesCS1A02G118400) was obtained from Ensembl plants and used to design specific primers. Then, the open reading frame (ORF) of *TaIAA15-IA* was cloned into the pCambia1300-35S-GFP vector. The vector was transformed into *Agrobacterium* and then used for genetic transformation as previously described (Vogel et al. 2008).

### Expression analysis of *TaIAA15-1A* in various stress treatments

The three-leaf wheat leaves were harvested at 0, 6, 12, 24, 48, and 72 h after 20% (w/v) PEG 6000 treatment and at 0, 3, 6, 12, and 24 h after 200 mM NaCl, 1.5 mM SA, 200 μM MeJA, 200 μM IAA, and 200 mM ABA treatment. Total RNA was extracted from all samples using TRIzol reagent (Transgen) and then reverse-transcribed into cDNA, which was used as a template for *TaIAA15-IA* gene expression analysis. The expression levels of *TaIAA15-IA* were determined by quantitative RT–PCR using the Roche LightCycler @480 system (Roche, Germany). The wheat *18S rRNA* gene and *Brachypodium distachyon* *BdUBC18* gene were used as the internal references. The relative expression levels of genes under different stress treatments were calculated using the  $2^{-\Delta\Delta CT}$  method. The sequence details of the primers used for qRT–PCR are listed in Table S1.

### Transcriptional analysis by RNA-sequencing

Leaves of the WT plants “Bd21” and *TaIAA15-IA* overexpression transgenic lines were harvested from three-week-old plants, and the total RNA was extracted from each biological replicate using RNAPrep Pure Plant Kit (TIANGEN). The RNA samples were sent to Metware company (Wuhan) for construction of cDNA libraries and RNA sequencing. The RNA-seq was performed using an Illumina HiSeq<sup>TM</sup>2000. Then, the clean reads of RNA-seq was mapped to the *Brachypodium* reference genome sequences ([http://plants.ensembl.org/Brachypodium\\_distachyon/Info/Index](http://plants.ensembl.org/Brachypodium_distachyon/Info/Index)). Subsequently, the transcripts were assembled, and the fragments per kilobase of exon per million fragments (FPKM) values were used to measure transcript abundance. Comparisons of *TaIAA15-IA* transgenic lines and WT plants were used to identify differentially expressed genes (DEGs). The DESeq2 package was used to perform the DEGs analyses as previously described (Varet et al. 2016). The DEGs were identified as significantly enriched or depleted under  $\log_2$ Fold Change $\geq 1$  and false discovery rate (FDR)  $< 0.05$ . Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis (<https://www.genome.jp/kegg/>) were performed on the DEGs.

## Exogenous ABA treatments

The sterilized seeds of WT and *TaIAA15-1A* overexpression lines (*TaIAA15-OE-1*, *TaIAA15-OE-2* and *TaIAA15-OE-3*) were placed on damp filter paper containing water or 2.5  $\mu\text{M}$  ABA solution. Then, these seeds were germinated for 5 days at 25 °C with a photoperiod of 16/8 h in a growth chamber. The seed germination rates of WT and *TaIAA15-1A* overexpression lines were counted and assayed with three biological replicates. The seeds of WT and *TaIAA15-1A* overexpression lines (*TaIAA15-OE-1*, *TaIAA15-OE-2* and *TaIAA15-OE-3*) that were germinated on damp filter paper containing water were transplanted to 1/2 MS medium with 2.5 mM ABA. The shoot lengths of the WT and *TaIAA15-1A* overexpression lines were measured after 1 week. All measurements were made with three biological replicates.

## Measurements of physiological parameters

The seedlings of WT and *TaIAA15-1A* overexpression lines (*TaIAA15-OE-1*, *TaIAA15-OE-2* and *TaIAA15-OE-3*) under control and drought treatment were collected after 21 days and used to measure physiological parameters related to abiotic stress, including relative water content (RWC), malondialdehyde (MDA), soluble total sugars, proline content,  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$  and antioxidant enzymes. The RWC was measured by the formula  $\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100\%$ , where FW, DW and TW represent fresh weight, dry weight and turgid fresh weight, respectively (Zhou et al. 2014). The MDA content was measured using the thiobarbituric acid method as previously described (Heath and Packer 1968). Soluble total sugar and proline contents were measured as previously described (Spiro 1966; Bates et al. 1973).  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  levels were visualized by nitroblue tetrazolium (NBT) and 3-diaminobenzidine (DAB) staining (Gay et al. 1999; Tian et al. 2013). The antioxidant enzyme activity (SOD, CAT, POD) was measured as described previously (Dhindsa et al. 1981; Aebi 1984; Chance and Maehly 1955).

## Measurements of IAA and ABA contents

The leaves of WT and *TaIAA15-1A* overexpression lines (*TaIAA15-OE-1*, *TaIAA15-OE-2* and *TaIAA15-OE-3*) under normal growth conditions were collected and used to analyse IAA and ABA contents as previously described (Xiao et al. 2018). The contents of IAA and ABA were measured through (ultra performance liquid chromatography, UPLC) (ExionLC™ AD, <https://sciex.com.cn/>) and (Tandem Mass Spectrometry, MS/MS) (QTRAP® 6500+, <https://sciex.com.cn/>). All treatments were performed in triplicate.

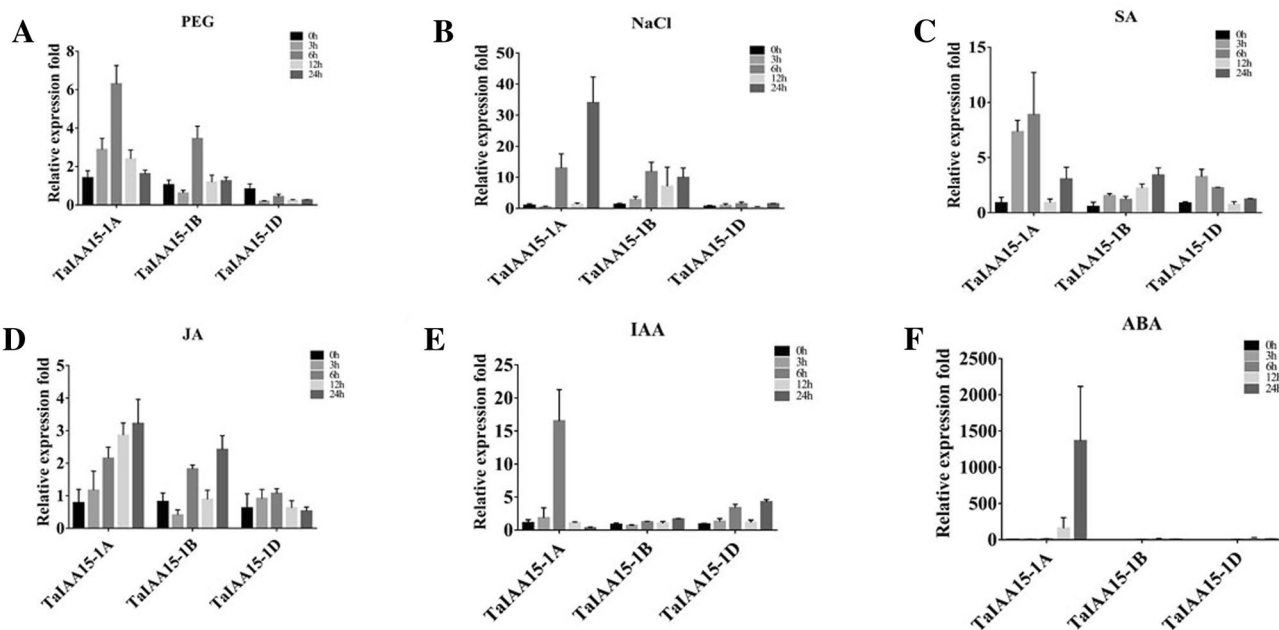
## Results

### Expression patterns of *TaIAA15-1A*, *-1B* and *-1D* under various stress treatments

To investigate the expression patterns of *TaIAA15-1A*, *-1B* and *-1D* in response to stress-related signalling, we detected the expression levels of *TaIAA15-1A*, *-1B* and *-1D* under different stress treatments (PEG6000, NaCl, SA, MeJA, IAA, and ABA) through qRT-PCR. The results showed that the expression levels of *TaIAA15-1A* and *TaIAA15-1B* were induced upon treatment with PEG6000 and NaCl, and the expression levels peaked at 6 h after PEG6000 treatment (Fig. 1A). NaCl stress induced *TaIAA15-1A* to a peak value at 24 h after treatment initiation (Fig. 1B). *TaIAA15-1A* expression levels were significantly higher than *TaIAA15-1B*, and *TaIAA15-1D* under PEG6000 and NaCl treatments. Then, we examined the expression levels of *TaIAA15-1A*, *-1B* and *-1D* under various plant hormone treatments (SA, MeJA, IAA, and ABA). As shown in Fig. 1C–F, we found that *TaIAA15-1A* expression was responsive to treatment with different plant hormones and exhibited approximately eightfold upregulation at 6 h after SA treatment (Fig. 1C). JA treatment induced *TaIAA15-1A* to a peak value at 24 h (Fig. 1D), and IAA stress induced the highest gene expression levels at 6 h after stress initiation (Fig. 1E). However, *TaIAA15-1A* expression remained relatively unchanged from 0 to 6 h after the initiation of ABA treatment but exhibited an approximately 1000-fold upregulation at 24 h. The expression levels of *TaIAA15-1B* and *TaIAA15-1D* were slightly altered under IAA and ABA treatments (Fig. 1F).

### *TaIAA15-1A* enhanced drought tolerance in transgenic *Brachypodium*

To clarify the abiotic stress tolerance function of *TaIAA15-1A* in wheat, we generated transgenic *TaIAA15-1A* overexpression lines in the *Brachypodium* Bd21 background and genotyped three independent homozygous overexpression transgenic lines by monitoring GFP using fluorescence microscopy. In addition, we also confirmed the expression levels of *TaIAA15-1A* in three independent homozygous overexpression transgenic lines (approximately three-fold increases in *TaIAA15-1A* expression were observed for each of three overexpression lines) (Fig. S1). Subsequently, we examined drought tolerance in *TaIAA15-1A* overexpression lines and the WT control. Under normal conditions, we did not observe significant phenotypic differences between the *TaIAA15-1A* overexpression lines and WT control. Under drought conditions,



**Fig. 1** The expression analysis of *TaIAA15-1A*. *TaIAA15-1A* expression levels were measured with different stress treatments including 20% (w/v) PEG 6000, 200 mM NaCl, 1.5 mM SA, 100  $\mu$ M MeJA, 100  $\mu$ M IAA, and 100  $\mu$ M ABA. The wheat gene *18S rRNA* was used

as an endogenous control. The relative expression levels of *TaIAA15-1A* was calculated using formula  $2^{-\Delta\Delta CT}$ . Values are presented as mean  $\pm$  SD

the *TaIAA15-1A* overexpression lines exhibited significantly higher tolerance to drought stress than WT plants after 21 days of drought treatment (Fig. 2A). We also found that the survival rate of WT plants was only 36%, whereas the survival rates of the three independent overexpression transgenic lines were 74, 73.9, and 74% (Fig. 2B).

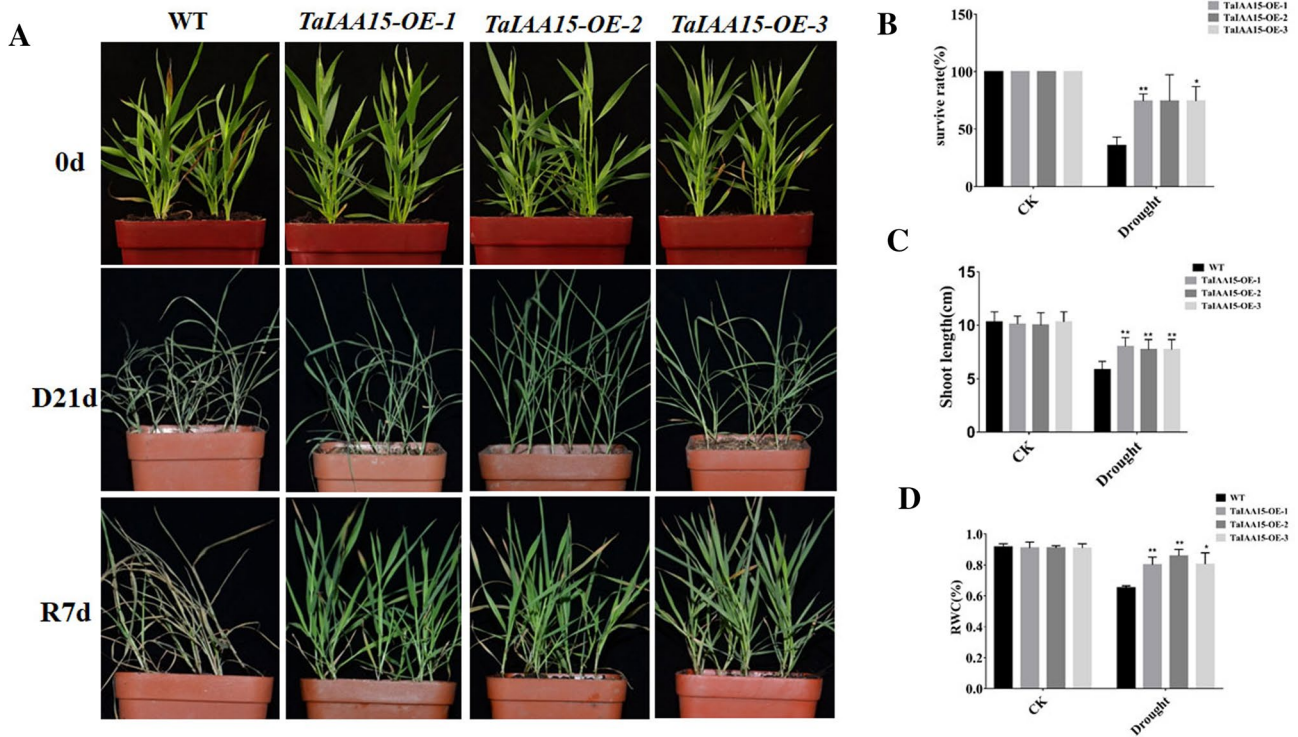
Then, we measured the shoot lengths and RWC between the overexpression transgenic lines and WT plants under normal and drought stress conditions. The results showed that no visible shoot length or RWC differences were observed between transgenic lines and WT plants under normal conditions, whereas transgenic lines exhibited longer shoot lengths and a higher RWC than WT plants under drought stress (Fig. 2C, D). To further explore the tolerance mechanism by which *TaIAA15-1A* contributes to drought stress, we compared physiological parameters between *TaIAA15-1A* transgenic lines and WT plants under normal and drought stress conditions. The results showed that transgenic lines had a lower MDA content (Fig. 3A). Taken together, these results suggested that *TaIAA15-1A* overexpression conferred drought tolerance in *Brachypodium* by influencing the

levels of osmotic and oxidative stress tolerance-related metabolites.

### **TaIAA15-1A overexpression influences ROS scavenging**

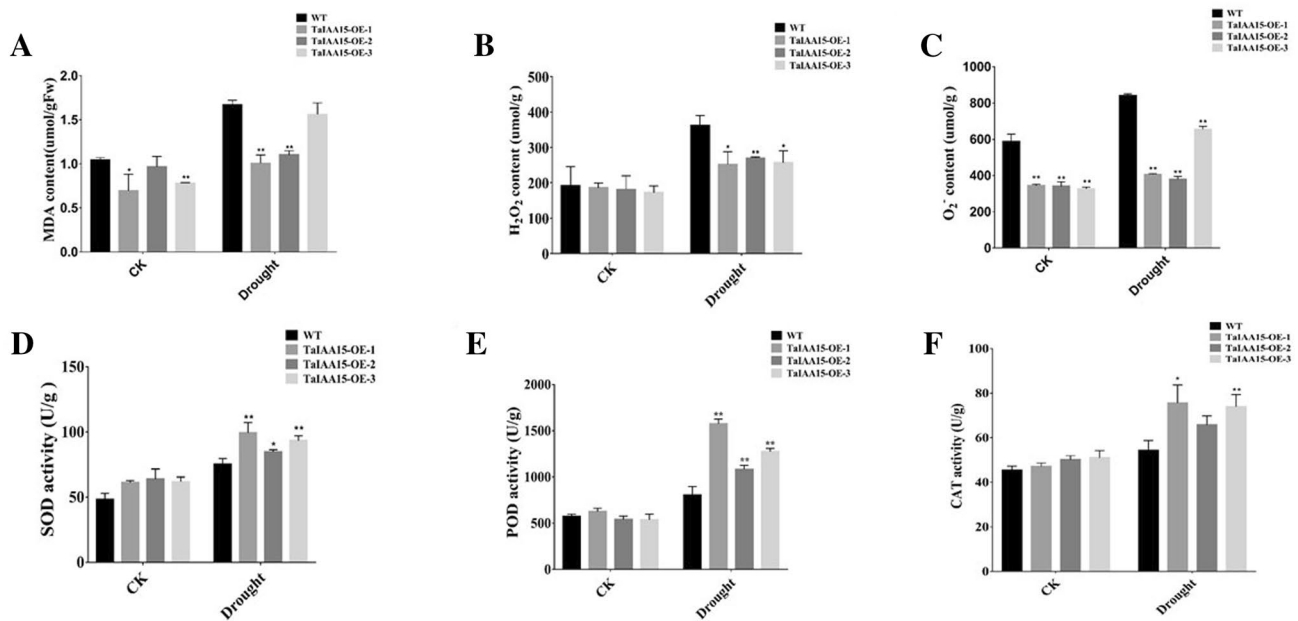
The function of *TaIAA15-1A* in reducing ROS levels was examined in transgenic lines under drought treatment. First, we determined the levels of  $O_2^-$  and  $H_2O_2$  for comparison between.

transgenic lines and WT plants. As shown in Fig. 3B, the accumulation of  $H_2O_2$  was not significantly different between the transgenic lines and WT plants under normal conditions; however, the  $O_2^-$  contents in the transgenic lines were significantly lower than those in the WT plants (Fig. 3C). Under drought stress, we found that the levels of  $O_2^-$  and  $H_2O_2$  in the transgenic lines were significantly lower than those in the WT plants (Fig. 3B, C). Next, the activities of some antioxidative enzymes, including SOD, POD, and CAT, were examined, and the results indicated that the SOD, POD, and CAT activities in the transgenic lines were higher than those in the WT plants (Fig. 3 D-F).



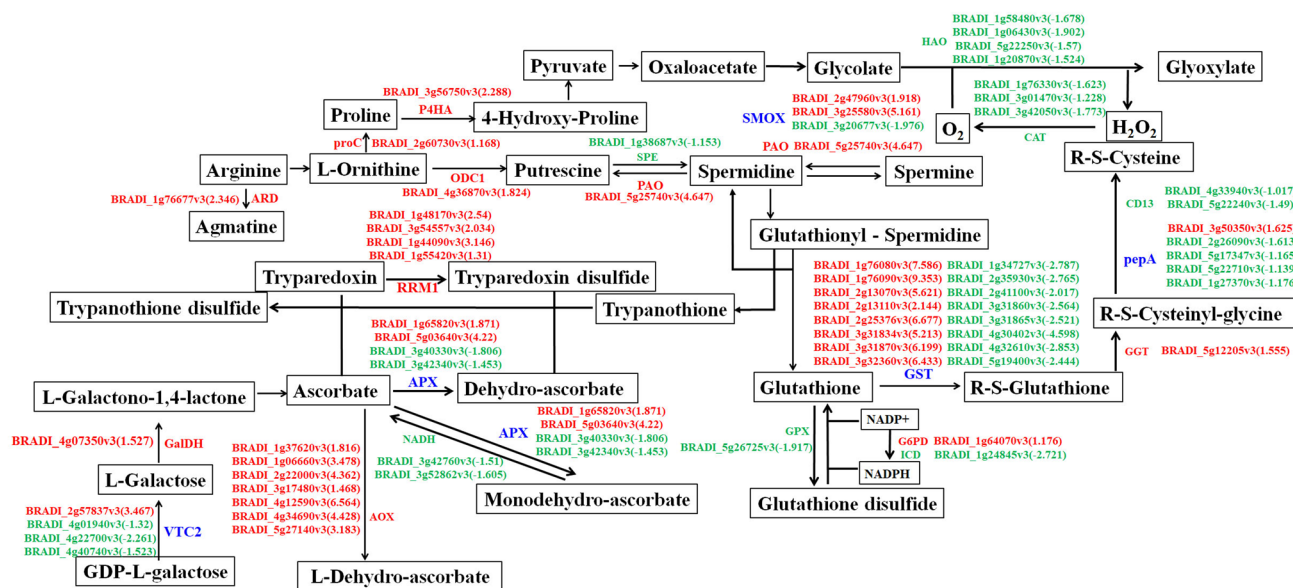
**Fig. 2** *TaIAA15-1A* overexpression improved the drought tolerance. **A** The phenotype of *TaIAA15-1A*-overexpressing transgenic and WT plants (*Brachypodium* Bd21) under drought stress. The transgenic lines and WT plants were treated with drought stress for 21 days followed by 7 days of re-watering. **B–D** The survival rates, shoot length,

and relative water content of *TaIAA15-1A*-overexpression seedlings and WT plants under drought stress. Data are presented as mean ± SE of three biological replicates. \* and \*\* indicate a significant difference compared with WT plants (\**P* < 0.05; \*\**P* < 0.01)



**Fig. 3** Physiological and biochemical indices between WT plants and *TaIAA15-1A* transgenic lines. **A** MDA content in *TaIAA15-1A*-overexpression seedlings and WT plants under drought stress. **B–C** The content of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anion radical

(O<sub>2</sub><sup>-</sup>). **D–F** The activity of SOD, POD, and CAT. Data are presented as mean ± SE of three biological replicates. \* and \*\* indicate a significant difference compared with WT plants (\**P* < 0.05; \*\**P* < 0.01)



**Fig. 4** *TaIAA15-1A* overexpression altered expression of polyamine and antioxidant-related genes. The polyamine and antioxidant-related genes are listed next to the arrow with green (down-regulated) and red (up-regulated) color-scale. *ODC* ornithine decarboxylase; *proC* pyrroline-5-carboxylate reductase; *P4HA* prolyl 4-hydroxylase; *ARD* arginine decarboxylase; *SPE* spermidine synthase; *PAO*, polyamine oxidase; *G6PD*, glucose-6-phosphate 1-dehydrogenase; *ICD* isocitrate dehydrogenase; *GPX* glutathione peroxidase; *GST* glutathione S-transferase; *GGT* gamma-glutamyl transpeptidase; *CD13* aminopeptidase N; *pepA* leucyl aminopeptidase; *RRM1*, ribonucleoside-diphosphate reductase subunit M1; *APX* ascorbate peroxidase; *VTC2* GDP-L-galactose phosphorylase; *GalDH* galactose dehydrogenase; *AOX*, ascorbate oxidase; *NADH* monodehydroascorbate reductase; *HAO* (S)-2-hydroxy-acid oxidase; *CAT*, catalase (color figure online)

## **TaIAA15-1A improves drought tolerance by regulating phenolamides and antioxidant pathways**

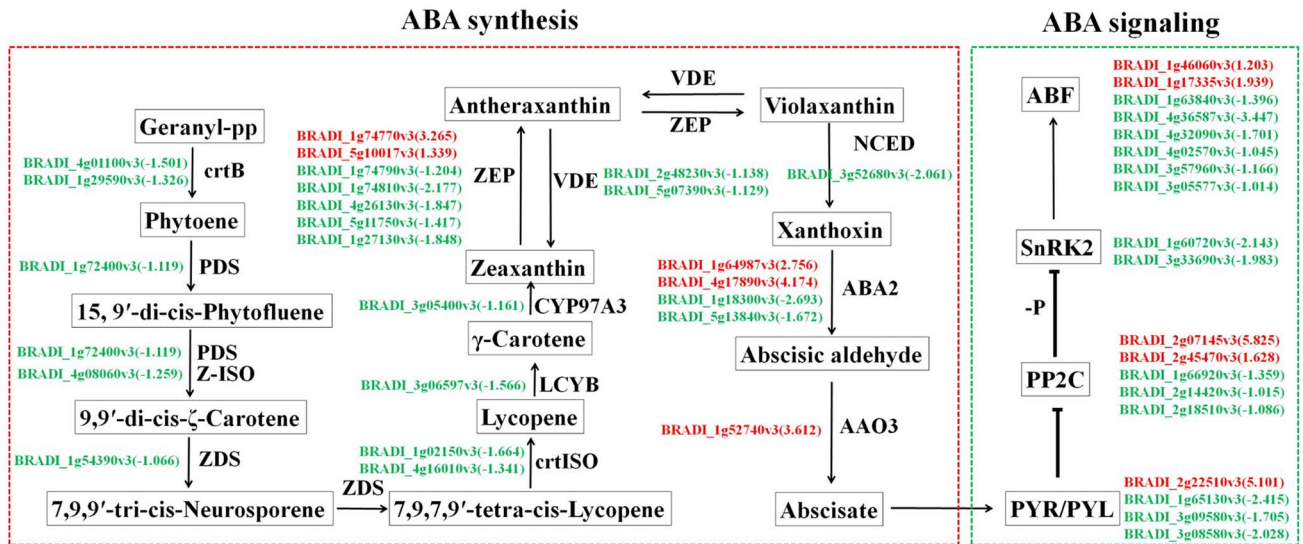
TO study genes potentially regulated by *TaIAA15-1A*, the transcriptome analysis was performed. The results showed that *TaIAA15-1A* overexpression modulated both phenolamides and antioxidant pathways. Based on the DEGs and KEGG pathways, we generated a network of signalling pathways involved in phenolamides and antioxidant pathways (Fig. 4). The results showed that some DEGs were enriched in phenolamides and antioxidant pathways, among which ornithine decarboxylase (*ODC*), pyrroline-5-carboxylate reductase (*proC*), prolyl 4-hydroxylase (*P4HA*), arginine decarboxylase (*ARD*), polyamine oxidase (*PAO*), glucose-6-phosphate 1-dehydrogenase (*G6PD*), gamma-glutamyl transpeptidase (*GGT*), ribonucleoside-diphosphate reductase subunit M1 (*RRM1*), galactose dehydrogenase (*GalDH*), and ascorbate oxidase (*AOX*) were upregulated in transgenic lines. In contrast, spermidine synthase (*SPE*), isocitrate dehydrogenase (*ICD*), glutathione peroxidase (*GPX*), aminopeptidase N (*CD13*), monodehydroascorbate reductase (*NADH*), (S)-2-hydroxy-acid oxidase (*HAO*), and catalase (*CAT*) were

significantly downregulated in transgenic lines compared with WT plants (Fig. 4).

significantly downregulated in transgenic lines compared with WT plants (Fig. 4).

## **TaIAA15-1A overexpression affects drought tolerance via the ABA signalling pathway**

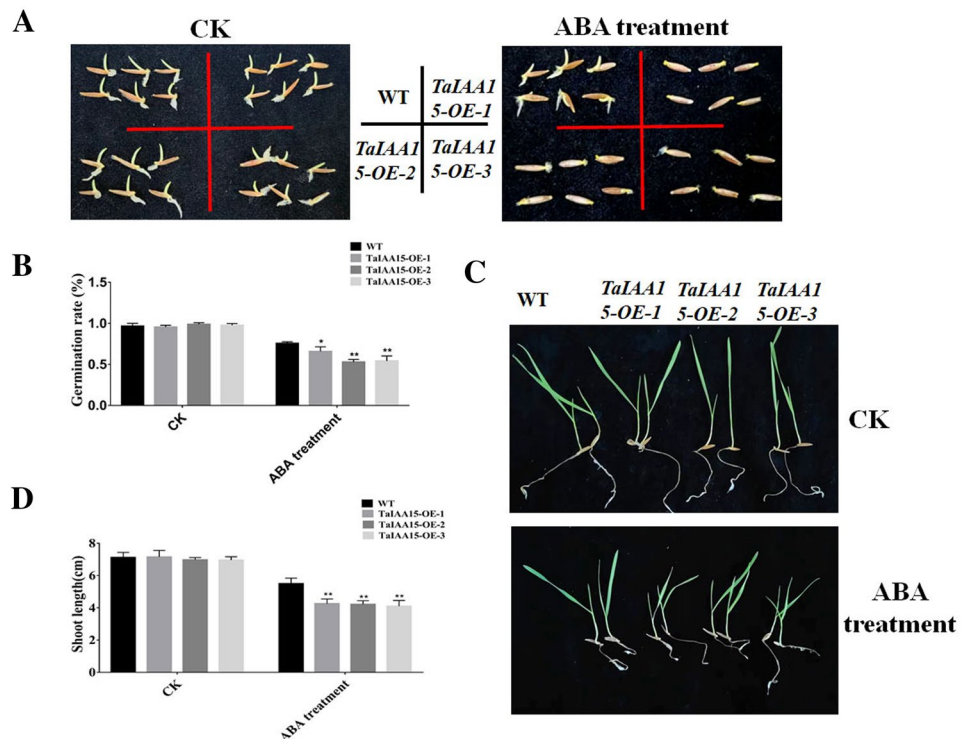
Previous transcriptome analysis showed that the ABA biosynthesis signalling pathway was influenced in *TaIAA15-1A* overexpression transgenic lines compared with WT plants. Based on the DEGs and KEGG pathways, we generated a network of signalling pathways involved in ABA biosynthesis (Fig. 5). The results showed that some DEGs were enriched in the ABA biosynthetic pathway, including 15-cis-phytoene synthase (*ctrB*), 15-cis-phytoene desaturase (*PDS*), zeta-carotene isomerase (*Z-ISO*), zeta-carotene desaturase (*ZDS*), polycopene isomerase (*ctrISO*), lycopene beta-cyclase (*LCYB*), zeaxanthin epoxidase (*ZEP*), beta-ring hydroxylase (*CYP97A3*), violaxanthin de-epoxidase (*VDE*), 9-cis-epoxycarotenoid dioxygenase (*NCED*), xanthoxin dehydrogenase (*ABA2*), abscisic-aldehyde oxidase (*AAO3*), abscisic acid receptor PYR/PYL family (*PYR/PYL*), protein phosphatase 2C (*PP2C*), serine/threonine-protein kinase SRK2 (*SnRK2*), and ABA responsive element binding factor (*ABF*). qRT-PCR analysis



**Fig. 5** Effect overview of *TaIAA15-1A* overexpression on ABA biosynthesis and signal transduction pathway in transgenic *Brachypodium* plants. The ABA-related genes are list next to the arrow with green (down-regulated) and red (upregulated) color-scale. *crtB* 15-cis-phytoene synthase; *PDS* 15-cis-phytoene desaturase; *Z-ISO* zeta-carotene isomerase; *ZDS* zeta-carotene desaturase; *crtISO* prolycopene isomerase; *LCYB* lycopene beta-cyclase; *ZEP* zeaxanthin

epoxidase; *CYP97A3* beta-ring hydroxylase; *VDE* violaxanthin de-epoxidase; *NCED* 9-cis-epoxycarotenoid dioxygenase; *ABA2* xanthoxin dehydrogenase; *AAO3* abscisic-aldehyde oxidase; *PYR/PYL* abscisic acid receptor PYR/PYL family; *PP2C* protein phosphatase 2C; *SnRK2* serine/threonine-protein kinase SRK2; *ABF* ABA responsive element binding factor (color figure online)

**Fig. 6** ABA treatment influenced germination and growth of *TaIAA15-1A* overexpression transgenic lines and WT plants. **A** Seed germination phenotype of *TaIAA15-1A* overexpression transgenic lines and WT plants under normal conditions and ABA treatment. **B** Germination rates of *TaIAA15-1A* overexpression transgenic lines and WT plants under normal conditions and ABA treatment. **C** Growth performance of *TaIAA15-1A* overexpression transgenic lines and WT plants under normal conditions and ABA treatment. **D** Statistical analysis of the shoot lengths and root lengths under normal conditions and ABA treatment. Values are presented as mean ± SD. (\**P* < 0.05; \*\**P* < 0.01, *t* test)



results supported the expression levels of genes detected from RNA-seq (Fig. S2).

### TaIAA15-1A affected seedling growth under ABA treatment

To further test the connection between *TaIAA15-1A* and the ABA signalling pathway, we treated *TaIAA15-1A* overexpression transgenic lines and WT plants with exogenous ABA solution and observed its effects on germination and shoot growth. The seeds of transgenic lines and WT plants were germinated in 1/2 MS medium containing 0 and 2.5  $\mu\text{M}$  ABA. The results showed that the germination rates and shoot lengths of the transgenic lines and WT plants were similar in the absence of ABA treatment (Fig. 6). However, compared with WT plants, the germination rates and shoot lengths of the transgenic lines were significantly reduced under ABA treatment (Fig. 6B–D). The results suggested that *TaIAA15-1A* overexpression increased transgenic lines hypersensitive to ABA, compared with WT plants, indicating that *TaIAA15-1A* may positively regulate ABA signalling in *Brachypodium*.

Subsequently, we measured the IAA and ABA contents in *TaIAA15-1A* overexpression lines and WT plants. The results showed that the IAA contents were decreased, and accumulation of high ABA contents was observed in the *TaIAA15-1A* overexpression lines compared with WT (Fig. S3). It is hypothesized that *TaIAA15-1A* overexpression enhanced drought tolerance of transgenic lines by regulating the crosstalk of ABA and IAA biosynthesis.

### TaIAA15-1A overexpression alters the expression profiles of bZIP transcription factors

In plants, basic region/leucine zipper motif (bZIP) transcription factors have been reported to be involved in various processes, including flower development, tolerance against abiotic stress, and pathogen defence (Zulfiqar et al. 2016). As ABA signalling pathway genes, ABFs are a group of transcription factors that belong to the bZIP transcription factor family (Jakoby et al. 2002). In our study, we checked the expression levels of the bZIP gene family (35 members) in our RNA-seq data between *TaIAA15-1A* transgenic *Brachypodium* lines and WT plants. The results showed that all bZIP genes were DEGs (23 downregulated and 12 upregulated) (Fig. S4A). Seven bZIP genes were further selected to confirm the expression trends of RNA-seq by qRT-PCR, and the results supported the expression levels of genes detected from RNA-seq (Fig. S4B).

## Discussion

Drought stress is a widespread and serious abiotic stress that causes yield and economic losses in wheat (Robles et al. 2018). At present, some studies have identified many drought tolerance genes, such as *TaPYL1-1B*, *OsIAA18*, *OsIAA6*, and *OsIAA20* (Jung et al. 2015; Wang et al. 2021; Zhang et al. 2021; Mao et al. 2022). However, information regarding drought the tolerance mechanisms of Aux/IAA proteins is limited in wheat. Therefore, a functional study of wheat Aux/IAA genes involved in tolerance to abiotic stress is needed. In this study, we characterized the role of a wheat Aux/IAA gene, *TaIAA15-1A*, in response to drought stress, as this abiotic stress causes severe yield reduction. The results showed that *TaIAA15-1A* contributes positively to plant tolerance against drought stress by influencing ABA-related genes.

Beyond their role in plant development and growth, Aux/IAA proteins have been known to function in the plant response to abiotic stresses, and some studies have shown that overexpression of Aux/IAA proteins can improve plant resistance to abiotic stresses. For example, a previous study showed that *OsIAA6* and *OsIAA9* respond to drought tolerance (Jung et al. 2015). Overexpression of *OsIAA20* and *OsIAA18* has been shown to enhance the resistance of rice to drought and salt stresses (Wang et al. 2021; Zhang et al. 2021). In *Arabidopsis*, *IAA5*, *IAA6*, and *IAA19* have also been reported to be involved in drought tolerance (Salehin et al. 2019). These studies suggest that Aux/IAA proteins can positively regulate plant resistance to various abiotic stresses; they are consistent with our findings in the present study, wherein we found that overexpression of a wheat Aux/IAA gene, *TaIAA15-1A*, improved the resistance of transgenic *Brachypodium* to drought stress.

Excessive ROS (particularly  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ ) accumulation accompanies abiotic stresses in plants, which results in lipid peroxidation and damage to cell membrane permeability and integrity (Baxter et al. 2013). Strong ROS scavenging ability plays an important role in improving resistance to abiotic stresses in plants. Previous studies have shown that auxin signalling and ROS might interact in plant responses to abiotic stress (Yuan et al. 2013). For example, overexpression of *OsIAA18* enhanced rice tolerance to drought and salt stresses by improving antioxidant enzyme activities and reducing ROS accumulation (Wang et al. 2021). In our study, we found that *TaIAA15-1A* overexpression improved drought tolerance by improving antioxidant activity (SOD, CAT, and POD), thereby reducing ROS levels ( $\text{H}_2\text{O}_2$  and  $\text{O}_2^-$ ), which suggested that *TaIAA15-1A* regulates antioxidant-related gene expression, thereby enhancing drought



tolerance. Future research will explore the tolerance mechanisms through which the interaction between auxin and ROS mediated by *TaIAA15-1A* regulates drought resistance in wheat.

In plants, the ABA signalling pathway has been reported to regulate multiple biological processes, including stress responses and plant growth and development (Wolters and Jürgens 2009). In *Arabidopsis* and rice, the positive function of ABA in altering plant resistance to abiotic stress has been recently described (Li et al. 2021; Zhang et al. 2020). For example, exogenous ABA treatments can enhance plant resistance to drought stress by improving antioxidative enzyme activities (Awan et al. 2021). In wheat, overexpression of the ABA receptor genes *TaPYL4* and *TaPYL1-1B* confers wheat tolerance to drought stress and promotes grain production under drought conditions (Mega et al. 2019; Mao et al. 2022). The crosstalk between ABA and auxin has been reported to contribute to plant tolerance to abiotic stress. For example, the auxin biosynthesis gene AMIDASE 1 (AMI1) is known to function in coordinating the trade-off between stress responses and growth by balancing auxin with ABA homeostasis (Pérez-Alonso et al. 2021). *OsIAA20* and *OsIAA18* have been implicated in mediating drought stress tolerance through the ABA pathway (Wang et al. 2021; Zhang et al. 2021). In contrast, relatively little research has focused on the function of AUX/IAA genes in controlling drought resistance via the ABA pathway in wheat. In our study, we found that *TaIAA15-1A* was upregulated by exogenous ABA treatment and that *TaIAA15-1A* overexpression improved drought tolerance via the canonical ABA signalling pathway through PYR/PYL, PP2C, SnRK2, and ABFs (Fig. 5). Thus, the combined evidence from our results in concert with that from previous studies suggests that *TaIAA15-1A* might improve drought tolerance by activating the ABA signalling pathway. Further study is warranted to explore the regulatory tolerance mechanisms through which AUX/IAA regulates these genes in the ABA signalling pathway.

## Conclusion

In this study, we characterized the function of the Aux/IAA gene *TaIAA15-1A* in response to drought stress in wheat. The results demonstrated that the overexpression of *TaIAA15-1A* significantly improved tolerance to drought stress in transgenic *Brachypodium* by activating the auxin signalling pathway, ABA signalling pathway, phenolamides and antioxidant pathways, resulting in a change in ABA-related gene expression and lower ROS accumulation. Our findings will deepen the understanding

of drought tolerance mechanisms and have high application value in drought-tolerant wheat variety cultivation in the future.

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**Author contributions** PS and SG conceived and designed the experiments; PS performed most experiments; CS performed exogenous ABA treatments; YJL performed expression analysis. KW, HS, SHW, XQL performed physiological and biochemical indice measures; PS wrote and revised the manuscript. All authors have read and approved the final manuscript.

**Data availability** All data generated or analyzed during this study are included in this published article and its supplementary information files.

## Declarations

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

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