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A bZIP transcription factor GhVIP1 increased drought tolerance in upland cotton

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Abstract

Background Cotton is extremely affected by severe natural stresses. Drought is one of the most serious abiotic stress that adversely influences cotton growth, productivity, and fiber quality. Previous studies indicate that basic leucine-zipper (bZIP) transcription factors are involved in the response of plants to various stresses. However, the molecular function and regulatory mechanism of *GhVIP1* in response to drought stress are still unknown.

Results In this research, *GhVIP1* was cloned from a drought-tolerant variety. Expression of *GhVIP1* was up-regulated in response to multiple abiotic stresses, especially under drought stress. And *GhVIP1* was highly expressed in the root, stem, and 10 days post-anthesis ovule. Inhibiting the expression of *GhVIP1* in cotton using the virus-induced gene silencing method resulted in higher electrical conductivity in leaves, but lower water content under drought stress compared with the WT plant. Overexpression of *GhVIP1* in *Arabidopsis* enhanced plant drought tolerance through increasing the seed germination rate and improving the development of root. The exogenous expression of *GhVIP1* up-regulated the transcription of genes associated with drought response and proline biosynthesis during drought stress in *Arabidopsis*.

Conclusion In summary, these results indicated that *GhVIP1* played a positive role in plants' response to drought stress. The use of GhVIP1 via modern biotechnology might facilitate the improvement of drought tolerance in cotton cultivars.

Keywords Cotton, GhVIP1, Drought stress, Proline Biosynthesis

Introduction

Along with global warming and climate change, drought has become a major severe abiotic stress to plant. It adversely influenced the whole process of plants growth and development by changing the cell membrane structure and permeability, reducing the leaf water potential and transpiration rate, inhibiting the photosynthesis, and disrupting the normal metabolism in plant. Accordingly, plants have evolved a range of complex regulatory mechanisms to cope with the harsh environmental stress of water shortage. Among them, transcriptional factors played a key role in regulating expression levels of a series downstream genes in response to drought stress. Transcription factors including WRKY, NAC, AP2, MYB, and bZIP play a crucial role in the process of plants response to drought stress (Golldack et al. 2011; Baldoni et al. 2015; Li et al. 2021).

As a paramount commercial crop, cotton is the main source of cellulosic fiber oil and biofuel. However, cotton, like other plants is extremely affected by severe natural stresses. Drought is known as one of the most serious abiotic stresses that adversely influences cotton growth,

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productivity, and fiber quality. To improve the drought resistance of cotton, many efforts have been made to better understand the mechanism of drought tolerance. Drought-associated candidate genes have been explored and isolated using modern biotechnologies, including whole genome sequencing (WGS) / whole genome re-sequencing (WGR), genome-wide association studies (GWAS), and bulked segregant analysis (BSA-Seq). Integrating genome modification technologies including transgenic approaches and CRISPR/Cas9 genome editing systems, drought-tolerant cotton materials were developed. Hou et al. have revealed that the genetic bases of drought tolerance through genotyping 319 upland cotton accessions. Twenty single nucleotide polymorphism (SNPs) traits and 4 candidate genes related to drought-tolerance have been identified using GWAS and RNA-seq techniques (Hou et al. 2018). Traits from natural populations formed from 200 representative cotton accessions under drought stress were investigated on 119 images from an automatic phenotyping platform, and genes related to drought resistance, such as *GhRD2*, *GhNAC4*, *GhHAT22*, and *GhDREB2* were identified and selected by integrating digital phenotyping, GWAS analysis, and transcriptome data (Li et al. 2020). Additionally, earlier studies demonstrated that *GhNAC2*, *GhWRKY21*, *GhNAC072*, *GbWRKY1*, and *GhWRKY33* play a key role in drought tolerance in cotton, which have proved that transcription factors were also significantly important in the response of cotton to drought stress (Gunapati et al. 2016; Wang et al. 2021; Mehari et al. 2021; Luo et al. 2020; Wang et al. 2019). Thus, it is feasible to improve drought tolerance in cotton by regulating the gene expression of transcription factors.

VirE2 interacting protein 1 (VIP1), is a basic leucine-zipper (bZIP) transcription factor, which was first identified in *Arabidopsis* (Tzfira et al. 2000). VIP1 acts as a bridge between VirE2 and nuclear importin α, it is involved in the formation of the T-DNA complex, and mediates the transportation of T-DNA in the cytoplasm to the plant nucleus (Citovsky et al. 2004; Djamei et al. 2007). Besides vital roles in *Agrobacterium*-mediated transformation, VIP1 is associated with other functions such as plant defense responses, sulfur utilization, starch accumulation, metal-binding, touch-induced root waving, in particular, the abiotic stress response (Wu et al. 2010; Zhang et al. 2019; Tsugama et al. 2016; Chen et al. 2016; Tsugama et al. 2018). VIP1 encodes a functional bZIP transcription factor and regulates the expression of downstream stress-related genes through binding to the VIP1 response element (VRE) in the promoters of the stress-related genes. VRE is a DNA hexamer motif, which is known as the specific binding site of the VIP protein (Pitzschke et al. 2009). Tsugama et al. have suggested that the binding of VIP1 enhances the promoter activities of *CYP707A1/3* genes and is involved in the osmosensitivity signaling process in *Arabidopsis*. Liu et al. has found that *OsbZIP81,* a homolog of *Arabidopsis VIP1*, was largely induced by MeJA and PEG6000 treatments. OsbZIP81 positively affects the endogenous JA level via regulating the expression level of *OsPIOX*, which belongs to the α-linolenic acid metabolism pathway (Liu et al. 2019). A research group recently reported that *BnMYB44* and *BnVIP1* are candidate hub genes that contribute to drought and salt stress response in *Brassica napus*. The expression level of *BnMYB44* and *BnVIP1* were quickly and significantly induced and up-regulated under drought conditions in a stress-tolerant cultivar (Shamloo-Dashtpagerdi et al. 2018). Additionally, *MYB44* promoters are activated by the VIP1 transcription factor through binding to VREs, and the expression level of *MYB44* has been increased in plants overexpressing *VIP1* (Pitzschke et al. 2009). Xu et al. has suggested that AtVIP1 is phosphorylated by MPK6 and responded to ABA signaling. The drought tolerance was significantly improved in transgenic plants overexpression of *AtVIP1* (Xu et al. 2015).

It is clear that VIP1 as a transcriptional regulator, controls stress-related gene expression by binding to VRE and acts as key player to cope with severe abiotic and biotic stresses. However, the molecular function and regulatory mechanism of *GhVIP1* in cotton especially in response to drought stress are still unknown. In this study, *GhVIP1* has been isolated from a drought tolerance cotton variety and the structure, expression pattern, and hereditary character of *GhVIP1* has been investigated. The molecular function of *GhVIP1* has been verified in cotton and *Arabidopsis* using transient virus induced gene silencing (VIGS) and stable transformation methods. This research expands the knowledge of the function of *GhVIP1* in response to drought and will facilitate new approaches to improve drought tolerance in cotton cultivars.

Results

Characterization of GhVIP1 in cotton

In this study, *GhVIP1* was isolated from *Gossypium hirsutum* cv Zhong H177, a drought-tolerant germplasm. The open reading frame (ORF) of *GhVIP1* was 1 002 bp in length and encoded a protein of 333 amino acid residues. There were four nucleotide variations between the sequence of *GhVIP1* from Zhong H177 (tolerant) and TM-1 (sensitive), which caused a difference of one amino acid (Additional file 1, Fig. 1a). Results of a phylogenetic analysis indicated that the VIP1 protein from other plants contained a single bZIP domain, which

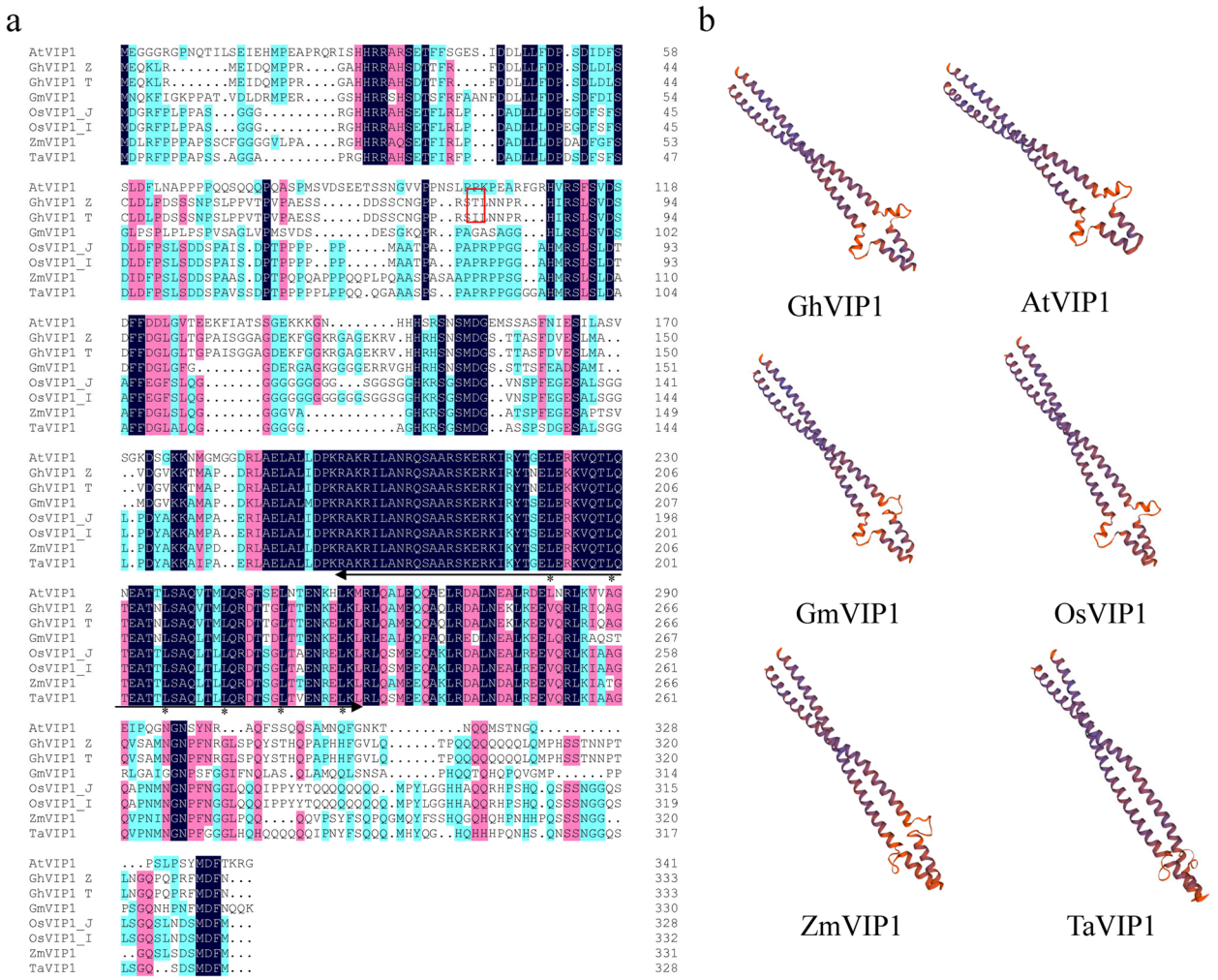

Fig. 1 Sequence alignment and structure prediction of VIP1 from different plant species. **a** Protein sequence alignment of VIP1 proteins from different plant species, including *Arabidopsis* (NP_564486.1), *Glycine max* (NP_001237194.2), *Oryza sativa Japonica* group (XP_015618202.1), *Oryza sativa Indica* Group (EAY82358.1), *Zea mays* (NP_001151391.2), and *Triticum aestivum* (AHY03428.1). The black shadows represented the same amino acid sequences. The conservative bZIP motifs were marked by a double-headed arrow. "*" represented the leucine: one leucine occurred in every six amino acid. **b** 3D structure prediction of VIP1 proteins

was the most conservative region among VIP1 proteins (Fig. 1a). The 3D structures of the conserved domains in VIP1 proteins were predicted by SWISS-MODEL online software. VIP1 proteins from different plants had conserved topology structures (Fig. 1b). *Cis*-regulatory elements of promoter regions played a vital role in regulating downstream gene expressions. Thus, *cis*-regulatory elements of *GhVIP1* promoter regions were predicted and results indicated that 1, 1, 5, 2, and 9 *cis*-elements responded to defense and stress, drought, abscisic acid, MeJA, and light, respectively. The results of topology prediction, phylogenetic analysis, and promoter analysis suggested that VIP1 proteins were evolutionarily conserved and were involved in stress and phytohormone responses.

Subcellular localization and expression pattern of GhVIP1 in different tissues and responding to stresses

The subcellular localization of a protein has a strong correlation with its function. The online prediction result from WOLFPSORT software suggested that GhVIP1 was localized in the nucleus and cytoplasm. To determine the subcellular localization of GhVIP1, the vector pBI121-GhVIP1-GFP was constructed. Results indicated that GhVIP1 was localized in the nucleus and cell membrane (Fig. 2). To improve the understanding of the important roles of GhVIP1 in cotton, the expression pattern of *GhVIP1* in different tissues and in response to stress were analyzed using qRT-PCR. The results showed that *GhVIP1* was highly expressed in the root, stem, and ovule at

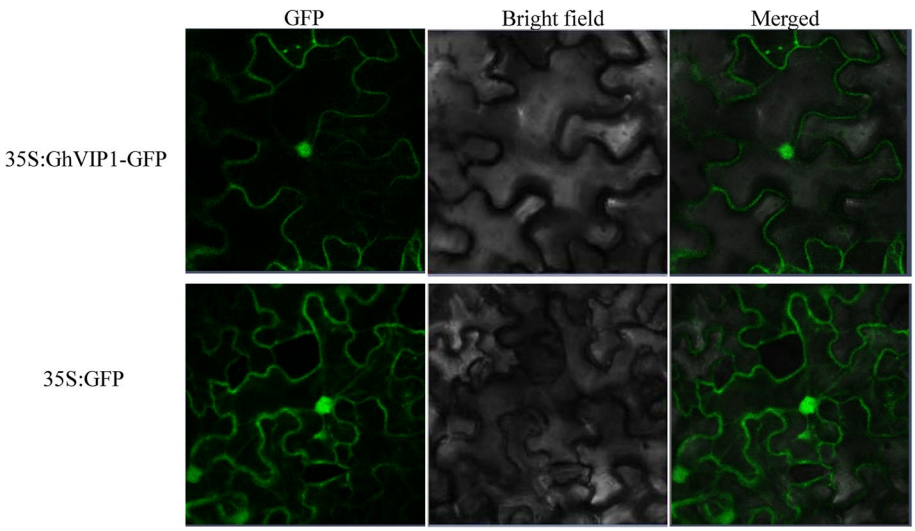

Fig. 2 Subcellular localization of GhVIP1 in tobacco leaves

10 days post-anthesis (DPA), and the expression levels in the leaf and ovule at 25 DPA were low (Fig. 3a). Additionally, *GhVIP1* was up-regulated under cold, drought, and salt stresses compared with normal conditions. Furthermore, changes in the expression level appeared as early as the 3 h after stress treatment. The transcript of *GhVIP1* was not induced under heat stress (Fig. 3b).

Functional evaluation of *GhVIP1* in drought response

To investigate the function of *GhVIP1* in response to drought stress in further detail, VIGS technology was used to generate *GhVIP1*-silenced cotton seedlings, and the *PDS* gene was used to monitor the VIGS efficiency. The recombinant vector of TRV2:*GhVIP1* was constructed (Fig. 4a). When an albino phenotype appeared in the true leaves of cotton seedlings inoculated with Agrobacteria containing the *PDS* gene (TRV:*PDS*), the expression level of *GhVIP1* was decreased in the silenced plants (TRV: *GhVIP1*) compared with the control (TRV:00) (Fig. 4b, c). There was no obvious phenotypic difference under normal conditions between TRV:*GhVIP1* and control plants. For the silenced and control plants which were transferred to the 10% PEG6000 solution, the leaves of TRV:*GhVIP1* exhibited an obvious yellow and wilting phenotype compared with the control plants (Fig. 4d). In addition, the electrical conductivity (EC) of leaves from VIGS and control plants were measured. The detached leaves from silenced lines showed higher EC values than the control under drought stress (Fig. 4e). Overall, the current results indicated that *GhVIP1* might be involved in responding to drought stress in cotton.

Ectopic expression of GhVIP1 enhanced *Arabidopsis* tolerance to drought

Earlier studies suggested that *GhVIP1* played a vital role in coping with drought stress. Transgenic *Arabidopsis* lines overexpressing *GhVIP1* were generated by flower soaking to further investigate the drought resistant function of *GhVIP1*. The exogenous *GhVIP1* gene was driven by 35S promoters and the kanamycin resistant gene was used as a marker for screening (Fig. 5a). After kanamycin selection and qRT-PCR analysis, two homozygous overexpression lines OE1 and OE2, with relatively higher *GhVIP1* expression levels in *Arabidopsis* were selected (Fig. 5b). There was no obvious morphological difference between transgenic plants and wild type (Col-0) under normal conditions. The germination rate and root length of transgenic plants and WT on 1/2 MS agar media containing mannitol were measured to explore whether *GhVIP1* was involved in the response to drought stress in *Arabidopsis.* Seeds of the two overexpression lines and WT were surface sterilized and symmetrically sown on 1/2 MS agar media with or without the addition of 200 mmol·L^{-1} mannitol. As shown in Fig. 5, all transgenic and WT plants were germinated and showed similar growth status on normal plates. However, the OE1 and OE2 plants exhibited better growth status and higher germination rates than that of WT plants on 1/2 MS medium supplemented with 200 mmol·L^{-1} mannitol (Fig. 5c). Additionally, under drought treatment, the germination rate of WT was approximately 50% of the transgenic lines (Fig. 5d). The transgenic and WT seeds were then sowed vertically on 1/2 MS medium with or without 200 mmol·L^{-1} mannitol to analyze root development. Length and thickness were

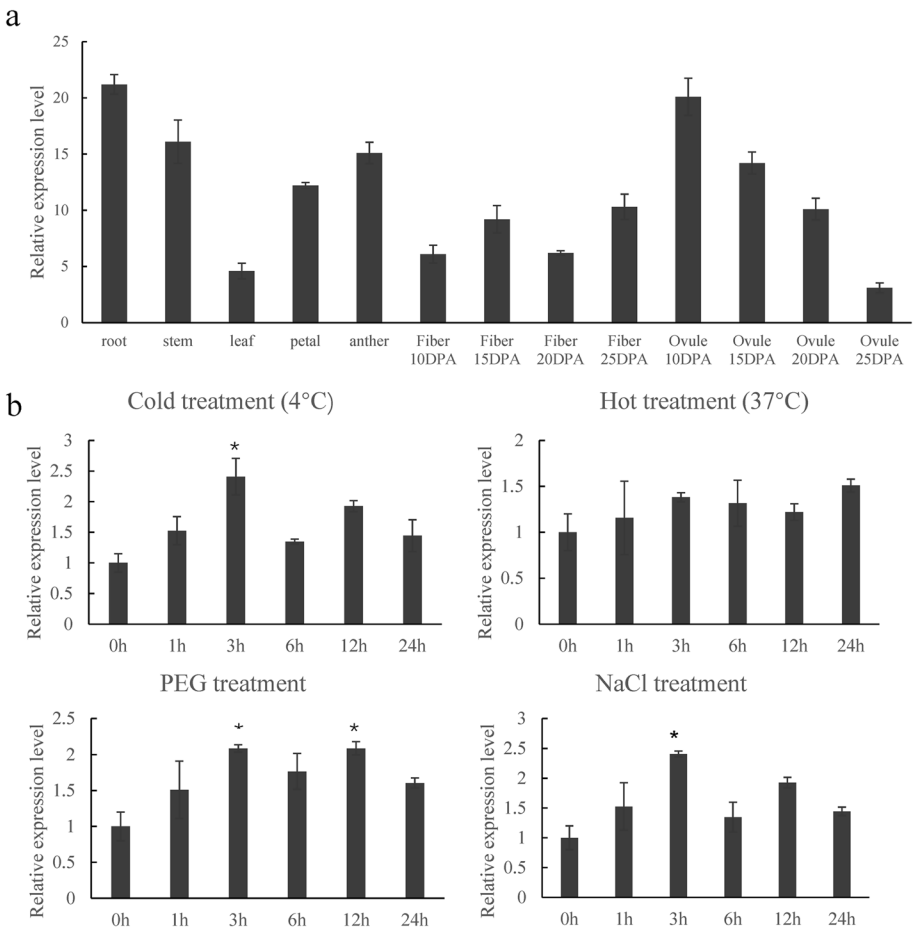

Fig. 3 Expression pattern of *GhVIP1* gene in different tissues and under different stresses. **a** Analysis of *GhVIP1* expression in different tissues. **b** Analysis of *GhVIP1* expression under cold, heat, PEG, and NaCl treatment. 0 h was the control and "*" represented a significant difference at 0.05 probability level by *t*-test

used as a standard to measure the root development. On normal medium, there was no significant difference in the root growth. However, the roots growth of transgenic and WT plants were both inhibited under drought treatment, but the roots of transgenic lines were longer and thicker than that of the WT on 1/2 MS medium containing 200 mmol·L^{-1} mannitol (Fig. 6a and b). To verify the function of *GhVIP1* in *Arabidopsis* drought resistance during the vegetative growth stage, transgenic and WT seeds were sown in soil. Plant seedlings of similar growth status were selected and irrigated with 15% PEG6000 to simulate drought conditions. The results showed that after 10 days of the drought treatment, the leaves of WT plants began to wilt and were less green. whereas the growth of *Arabidopsis* plants overexpressing *GhVIP1* were less affected (Fig. 6c). These results indicated that *GhVIP1* could enhance drought tolerance by increasing seed germination rate and improving root development.

Overexpression of *GhVIP1* in transgenic *Arabidopsis* regulated drought-related gene expression

To explore a possible mechanism for *GhVIP1*-mediated tolerance to drought in transgenic plants, the expression level of drought-related genes including *P5CS1*, *P5CS*, *DREB1*, *RAB18*, *RAD29*, and *COR15A* were measured under drought stress using qRT-PCR. Results indicated that *P5CS1* and *P5CS* which are key enzymes in the proline biosynthetic pathway were significantly upregulated under drought stress in transgenic plants compared with WT. The expression level of *P5CS1* increased gradually and reached the maximum at 24 h, whereas that of *P5CS* increased rapidly at the beginning of drought stress (Fig. 7). The transcript of *RAB18* was induced more strongly at 6 h and 12 h after drought stress in transgenic plants than in WT, whereas the expression pattern of *RAB18* was the opposite at 24 h after drought stress. The expression level of *DREB1*, *RAD29*, and *COR15A* was induced more remarkablely at 3 h after drought stress in transgenic plants than in WT, whereas

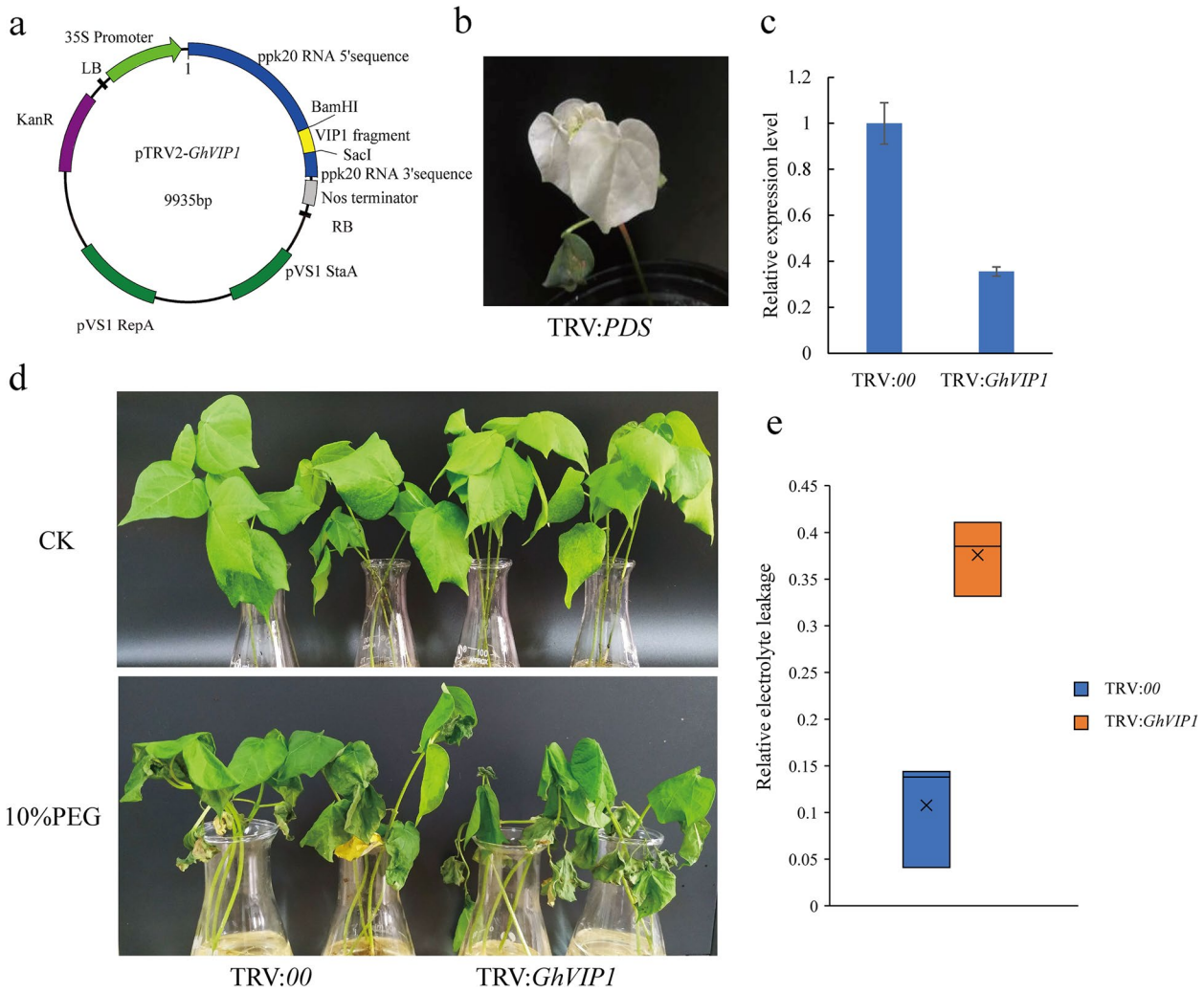

Fig. 4 Silencing of *GhVIP1* reduced the tolerance to drought stress in cotton. **a** Vector structures of TRV2:*GhVIP1*. **b** Albino phenotype of TRV:*PDS*. **c** Detection of silencing efficiency. **d** Drought tolerance assay of *GhVIP1*-silenced seedlings compared with the control. **e** Relative electrical conductivities analysis on the detached leaves of TRV:*00* and TRV:*GhVIP1* cotton seedlings under drought stress

the transcription level of genes were decreased after 12 h under drought condition (Fig. 7). Overall, *GhVIP1* might be involved in the coping mechanism of plant to drought stress by regulating the expression pattern of genes related to the proline biosynthetic pathway.

Discussion

The bZIP family of transcription factors comprises one of the largest transcription factor families in plants and is evolutionarily conserved in eukaryotic organisms (Droge-Laser et al. 2018; Ye et al. 2022). Earlier studies indicated that a total of 197 bZIP transcription factor members were identified in *G. hirsutum* genome, and could be divided into 13 subfamilies through phylogenetic analysis based on conserved motifs and gene structures (Zhang et al. 2022a). The bZIP transcription factors are involved in a plethora of functions in various biological processes, especially in response to abiotic stresses (Wang et al. 2020). Zhang et al. has cloned and characterized the bZIP transcription factor *GhABF3,* and has suggested that the gene is involved in the drought and salt stress response in plants. Overexpression of the *GhABF3* gene has up-regulated the *RD29B*, *RAD18*, and *CHS* genes, increased root length, reduced the degree of cellular oxidation, and decreased leaf water loss and wilting, which has resulted in the improvement of drought and salinity tolerance in cotton and *Arabidopsis* (Zhang et al. 2022b). *GhABF2* has also been induced by drought and ABA treatment and the overexpression of *GhABF2* in cotton has enhanced plant drought and salt tolerance

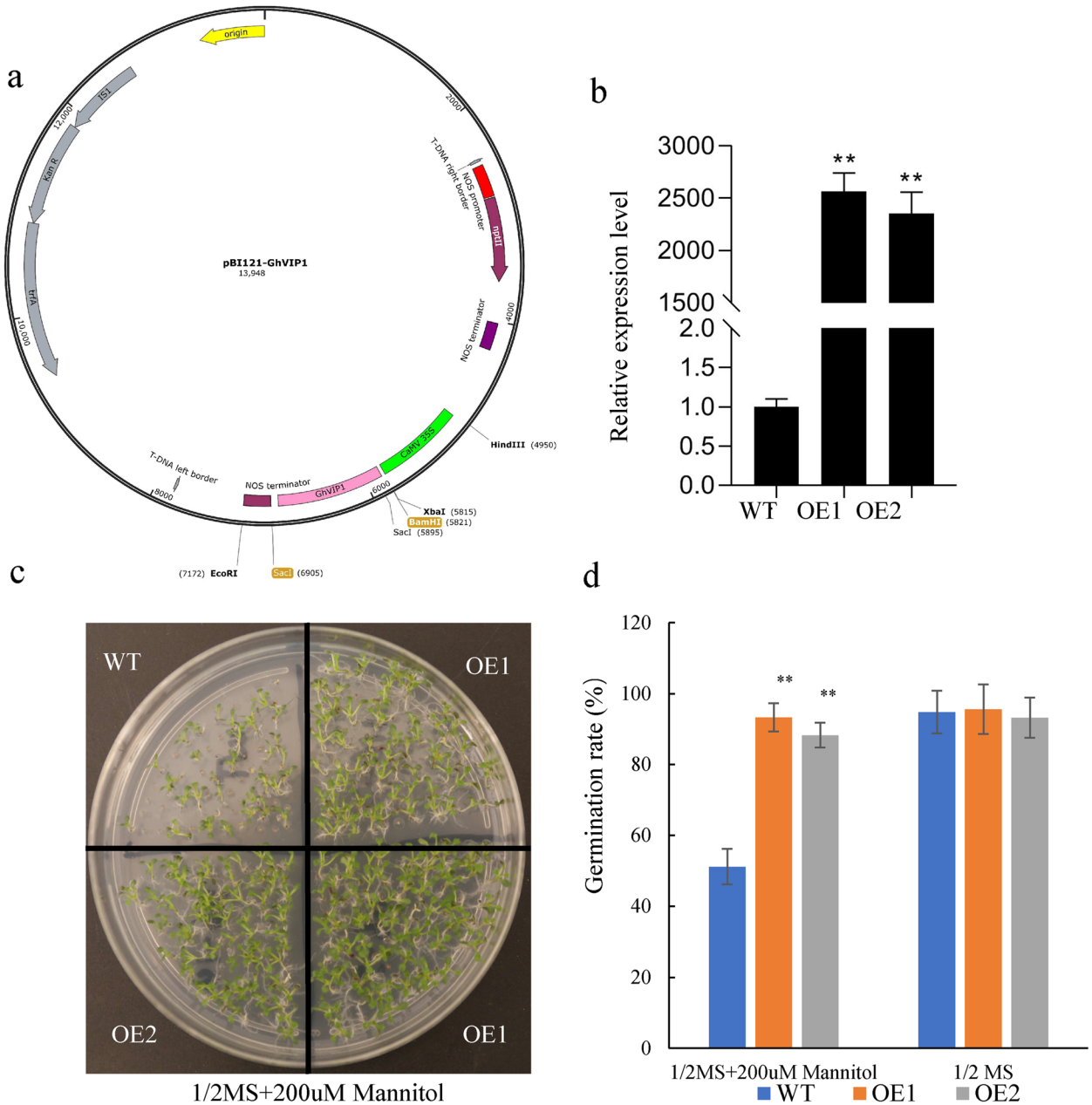

Fig. 5 Verification and function analysis of *GhVIP1* transgenic plants. **a** Construction of overexpression vector. **b** The expression level of exogenous *GhVIP1* gene in transgenic plants were detected by qRT-PCR. WT was used as control and "**" represented a significant difference at 0.01 probability level. **c** Germination of *GhVIP1* overexpressed *Arabidopsis* and WT plant under drought stress. **d** Statistic analysis of root length of transgenic plants and WT on 1/2 MS agar media with or without 200 mM mannitol. "**" represented a significant difference at the 0.01 probability level

via increasing the content and activity of proline, superoxide dismutase (SOD) and catalase (CAT) (Liang et al. 2016). In our study, VIP1 proteins, as transcription factors of the bZIP, are involved in plant growth and development via regulating abiotic and biotic stress related genes in plants. GhVIP1 plays an essential role in the drought response in plants by activating the expression of genes related to the proline biosynthetic pathway.

Previous studies have showed that the expression of *VIP1* in plants is induced by various stresses and phytohormones. *BnVIP1* expression level has increased rapidly and reached the highest at 3 h after salt stress in a salt-tolerant cultivar and shows significantly response to drought stress and reaches the maximum level at 6 h after drought stress (Shamloo-Dashtpagerdi et al. 2018). Sarraf et al. have indicated that the

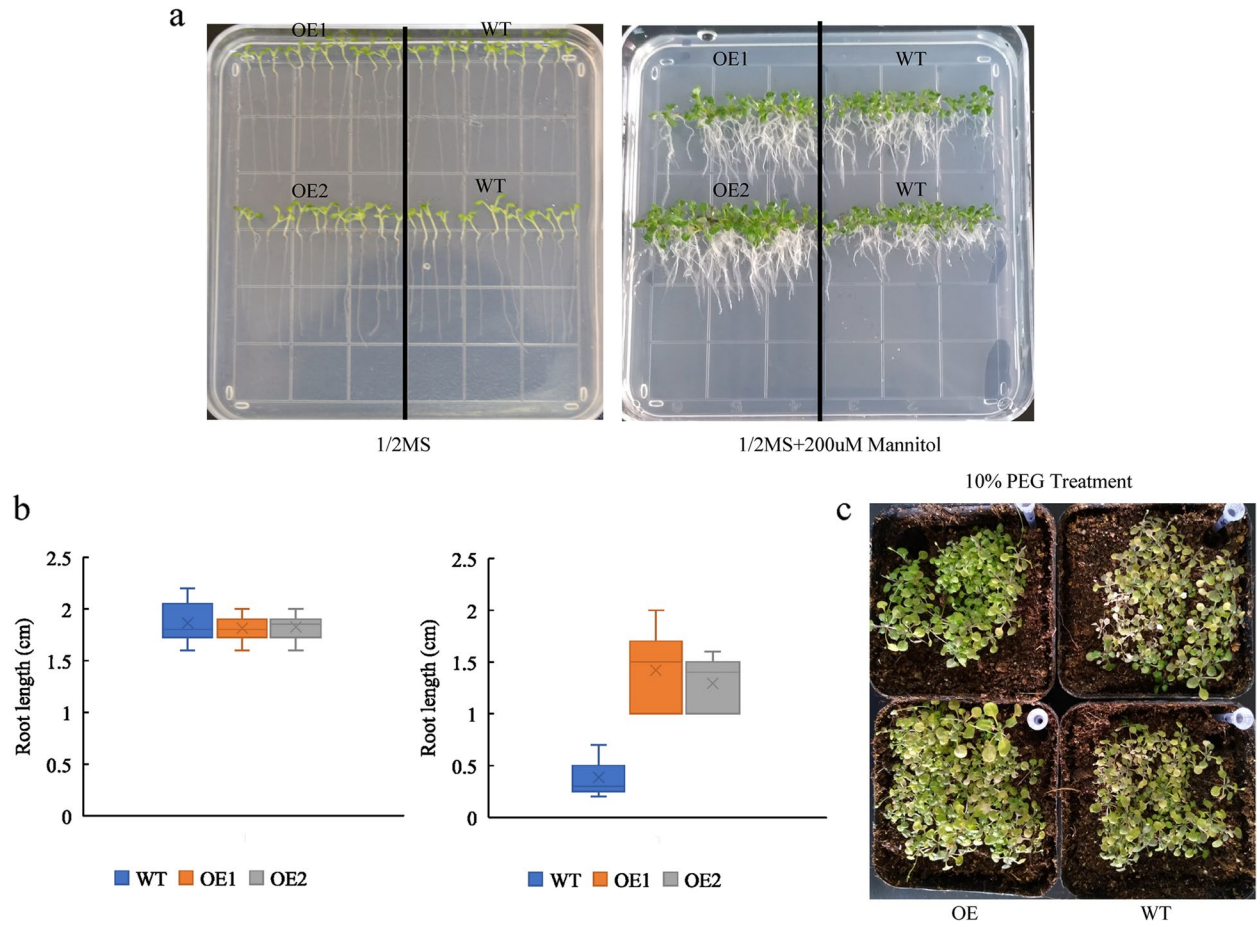

Fig. 6 *GhVIP1* overexpression *Arabidopsis* increased the root length and drought tolerance. **a** Transgenic plants overexpressing *GhVIP1* and WT plants on 1/2MS with or without 200 mmol·L^{-1} mannitol. **b** Analysis of root length of transgenic plants and WT under normal condition and drought stress. **c** Phenotypic comparison of plants with ectopic expression of *GhVIP1* and WT *Arabidopsis* under 10% PEG treatment

expression levels of *HvVIP1* are stable in various barley tissues and are induced by *Agrobacterium tumefaciens* and *Fusarium culmorum,* and a strong correlation has been identified between *HvVIP1* and *PR* genes including *HvPR1*, *HvPR3,* and *HvPR10* (El Sarraf et al. 2019). ABA and MeJA are important phytohormones, play a key role in responding to various stresses. Several genes that participate in the response to stress by plants regulating the expression of genes related to the ABA or MeJA pathways. It has been reported that the expression of *VIP1* genes was induced by ABA and MeJA. The transcription levels of *OsbZIP81.1* and *OsbZIP81.1*, which are two homologs of *AtVIP1* in rice, are strongly induced by MeJA, PEG6000, and *Agrobacterium* infection (Liu et al. 2019). In this study, *GhVIP1* has been rapidly and significantly induced by cold, drought, and salt treatment. The margin in the increase of *GhVIP1*-relative expression level is not huge, which consists with previous studies. It might be that GhVIP1, as a functional transcription factor, strongly enhances the expression level of multiple genes which harbor several VRE motifs, but does not play a direct role. *GhVIP1* was not remarkablely induced by treatment with ABA in cotton probably because there were differences in the expression patterns of *VIP1* among plants.

The VIP1 protein acts as a transcriptional factor and regulates downstream genes mainly through binding to VREs at promoters. VRE contains a DNA hexamer motif and the combination of VIP1-VRE significantly enhances the expression level of genes with promoters that harbor several VRE motifs (Lacroix and Citovsky 2013). Earlier studies verified that promoters of the *Trxh8* and *MYB44* genes have six and three copies of VRE, respectively, and their expression levels have been activated by VIP1 in a VRE-dependent manner in *Arabidopsis* (Pitzschke et al. 2009). Liu et al. have discovered a novel binding motif of OsVIP1 via ChIP-Seq, named as OVRE. The expression level of *OsLOX5*, *OsHI-LOX*, *OsAOC*, and *OsPIOX* genes

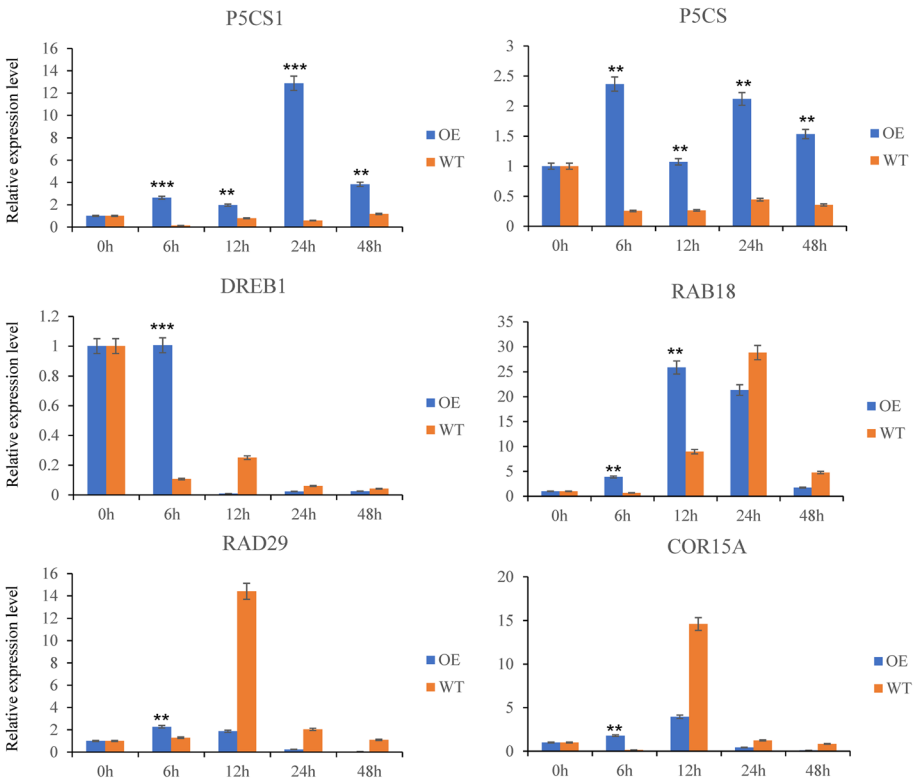

Fig. 7 The expression pattern of drought tolerance associated genes in *GhVIP1* overexpression plants under drought stress. The expression data of 6 h, 12 h, 24 h, 48 h were normalized to that of 0 h. "**" and "***" represented a significant difference between WT and GhVIP1 overexpression plants at 0.01 and 0.001 probability level by *t*-test, respectively

were directly regulated by OsbZIP81.1, respectively (Liu et al. 2019). In this study, the relationship between *GhVIP1* and genes involved in the drought response including *P5CS1*, *P5CS*, *DREB1*, *RAB18*, *RAD29*, and *COR15A* has been analyzed using qRT-PCR. Although the expression pattern of the genes mentioned above was not identical, all of the genes have been detected a rapidly induction in transgenic plants under drought stress. Additionally, the binding motifs of GhVIP1 and the evolutionary conservation in binding motifs among bZIP transcriptional factors in cotton have not been well studied. Research of transcription factor-bound *cis*-acting elements in the bZIP transcriptional factor has important impact and provides new insights into further studies of bZIP family in plants.

The growth and production of cotton are adversely affected by drought stress and many efforts have been made to manage the threat of drought stress. Previous studies have indicated that drought tolerance is a complicated process that included stress sensing and multiple stress responses (Mahmood et al. 2019). The stress signaling pathway involved in the cotton drought response mainly includes the mitogen-activated protein kinase (MAPK) signaling pathway, Ca^{2+} signaling pathway, and phytohormone-mediated signaling pathway (Wang et al. 2016; He et al. 2013; Luo et al. 2020). Several stress responses including morpho-physiological responses, biochemical, and cellular responses, and antioxidant defense are known to minimize or reduce the damage caused by drought (Singh et al. 2022). Li et al. have demonstrated that stomatal movement including the closure speed, length, and width of stomata are involved in plant tolerance to drought stress (Li et al. 2022). Overexpression of *SNAC1* remarkablely improved root development including root biomass and root length in cotton which results in increased tolerance to drought in cotton (Liu et al. 2014). In this study, the roots of transgenic plants overexpressing *GhVIP1* are obviously longer and thicker than that of the WT grown in 1/2 MS medium containing 200 mmol·L^{-1} mannitol, which was potentially important in the response to drought stress.

Results have indicated that *P5CS1* and *P5CS* genes are significantly upregulated under drought stress in transgenic plants compared with WT. *P5CS1* and *P5CS* which are rate-limiting enzymes in the proline biosynthetic pathway. Proline accumulation is a major metabolic adaptation of plants in response to multiple environmental stresses (Feng et al. 2016; Shrestha et al.

2022). Increasing proline accumulation and the application of exogenous proline has been verified to improve plant drought tolerance (Ashraf and Foolad 2007). Chen et al. have indicated that overexpression of the *FRIGIDA* gene improves drought tolerance by up-regulating *P5CS1* expression level and proline accumulation under drought stress (Chen et al. 2018). Li et al. has suggested that OsERF71 plays a positive role in drought-induced proline biosynthesis by increasing the expression level of *OsP5CS1* and *OsP5CS2* genes which enhanced the tolerance of rice to drought (Li et al. 2018). Taken together, we propose that GhVIP1 improves cotton drought tolerance by up-regulating the expression level of genes involved in proline biosynthesis and by increasing the length and biomass of roots under drought stress. These findings have broadened our knowledge of the function of GhVIP1 involved in cotton drought tolerance. However, the molecular mechanism and regulation relationship underlying *GhVIP1* and genes associated with drought tolerance need further characterization.

Conclusion

In this study, the data has suggested that *GhVIP1* plays a positive role in the regulation of drought tolerance in cotton. *GhVIP1* expression has induced by various abiotic stresses and the GhVIP1 protein is located in the nucleus and cell membrane. Silencing of *GhVIP1* in cotton reduces plant drought tolerance and *GhVIP1* overexpressing *Arabidopsis* lines exhibites improved tolerance to drought. *GhVIP1* participates in the drought response by increasing the length and biomass of root in transgenic plants, and by up-regulating the expression level of genes involved in proline biosynthesis.

Methods and materials

Plant materials and treatment conditions

Tetraploid upland cotton Zhong H177 which is known as a drought-tolerant cultivar, and TM-1 were used for gene cloning. The seeds were soaked overnight in water and cultured on absorbent paper at 37 °C for 24 h in the dark. The germinated seeds were planted in nutrient soil and grown in incubators under conditions of 28 °C (day) /25 °C (night) with a light cycle of 16 h/8 h. Three true-leaf stage cotton seedlings were selected and treated with drought stress, in which 15% PEG 6000 and 200 $mmol{\cdot}L^{-1}$ NaCl were used for simulating drought and salt environment, respectively. For cold and hot treatment, cotton seedlings were transferred to a chilling chamber (4 °C) and hot chamber (37 °C), respectively. Normal treatment was applied on the control. The leaves of plant seedings were harvested at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h after stress treatment and frozen in liquid nitrogen. The treatment had at least three biological repeats and each repeat had more than five plants.

RNA extraction, gene cloning, and bioinformatic analysis

Total RNA was extracted using the RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China) and then cDNA was synthesized using a HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed on a QuantStudio TM 6 Flex qPCR System (Applied Biosystems, Carlsbad, CA, USA) using ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China). The relevant primers used in this study were listed in Additional file 2. A 50 μL reaction system was used to clone the *GhVIP1* gene. The reaction included 10×PCR buffer for KOD-Plus-Neo, 0.2 $mmol{\cdot}L^{-1}$ dNTPs, 1.5 $mmol{\cdot}L^{-1}$ $MgSO_4$, 0.3 $\mu mol{\cdot}L^{-1}$ forward and reverse primers, 1.0 U KOD-Plus-Neo (TOYOBO, Osaka, Japan), and 500 $mmol{\cdot}L^{-1}$ cDNA. PCR products were sequenced by Sangon Biotech. The optimal sequences of VIP1 proteins from *Arabidopsis* (NP_564486.1), *Glycine max* (NP_001237194.2), *Oryza sativa Japonica* group (XP_015618202.1), *Oryza sativa Indica* group (EAY82358.1), *Zea mays* (NP_001151391.2), and *Triticum aestivum* (AHY03428.1) were obtained by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments and predictions of conversed motifs were performed using the DNAMAN and CD-Search, respectively. The 3D structures of VIP1 proteins were generated by SWISS-MODEL online software (https://swissmodel.expasy.org/). *Cis*-regulatory elements of the promoter were predicted by PlantCARE.

Subcellular localization of GhVIP1

To predict the subcellular localization of GhVIP1, the WOLFPSORT online software (https://wolfpsort.hgc.jp) was used. The pBI121 vector containing the *GhVIP1* gene (without the stop codon) was used for transient expression and was transformed to tobacco leaf cells by the *Agrobacterium* infiltration method. The empty vector was used as a negative control. The fluorescence signals of subcellular localization were observed using a laser confocal scanning microscope Olympus FV1200 (Olympus, Tokyo, Japan) with the excitation and emission wavelength of 488 nm and 510 nm, respectively.

Agrobacterium-mediated virus-induced gene silencing analysis

A 272 bp GhVIP1 fragment was amplified and cloned into TRV2 vectors, forming the recombinant vector of TRV:*GhVIP1* through a ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China). Then recombinant vector of TRV2:*GhVIP1* and TRV2:*PDS,* as well as the empty vector were transformed into an *Agrobacterium tumefaciens* strain GV3101 using the freeze thaw method. Activated *Agrobacterium* containing TRV2 vectors and the

TRV1 vector were mixed in a 1:1 ratio, and then the mixture were infiltrated into the cotyledons of Zhong H177 seedlings. The silence efficiencies of VIGS were detected by qRT-PCR when the TRV2:*PDS* plants exhibited an albino phenotype. The target gene-silenced cotton plants were treated with drought stress (15% PEG). VIGS experiments were repeated at least three times and each repeat used more than 15 plants.

Transformation of *Arabidopsis*

The full-length ORF of *GhVIP1* was inserted into the binary vector pBI121 containing the cauliflower mosaic virus (CaMV) 35S promoter to construct 35S:GhVIP1. An *Agrobacterium* (Strain GV3101) harboring the recombinant construct vector was transformed into *Arabidopsis* (Colombia-0; WT) via the floral dip method. The seeds of transformants were first screened on 1/2 MS medium with 50 $mg{\cdot}L^{-1}$ kanamycin. Then PCR was used to verify the transformants and qRT-PCR was applied to detect the expression level of the exogenous *GhVIP1* gene. Descendants from self-fertilization whose segregation ratio consisted with the genetic laws of Mendel and with correct expression of the exogenous *GhVIP1* were efficiently selected to produce homozygous T3 lines for futher experiments. Two independent homozygous lines of transgenic plants were used to analyze growth and morphological differences. For the germination assay, seeds of transgenic lines and WT were sterilized firstly by 70% ethanol for 1 min and then by 10% NaClO for 5 min. Sterilized seeds were washed three times using sterile water and then spread on the 1/2 MS medium with or without 200 $mmol{\cdot}L^{-1}$ mannitol at 25 °C day/23 °C night by 16 h/8 h light–dark. After 10 days, the growth status and germination rate of transgenic and WT plant under normal or simulated drought stress conditions were calculated and determined. For root lengths measurement, the seed sterilization method and medium formula were the same as described previously. The seeds were sowed vertically on normal and drought stress media. The length and thickness of roots were observed and photographed. To study drought stress response during the vegetative growth stage, seeds of transgenic *Arabidopsis* and the control were directly sown in the nutrient soil. The drought treatment was similar to that of cotton seedlings described previously.

Abbreviations

ABA	Abscisic acid
WGS	Whole genome sequencing
WGR	Whole genome re-sequencing
GWAS	Genome-wide association studies
BSA-Seq	Bulked segregant analysis
VIP1	VirE2 interacting protein 1
VRE	VIP1 response element
VIGS	Virus-induced gene silencing
EC	The electrical conductivity
SOD	Superoxide dismutase
CAT	Catalase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-023-00148-9.

Additional file 1: Fig. S1. Sequence differences of *GhVIP1* genesfrom drought tolerant and sensitive varieties.

Additional file 2: Table S1. The primer sequences used in our study.

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Authors' contributions

Wang HM conceived and designed the experiments, Zhao P and Xu YW performed the experiments and analyzed the data, Zhao P wrote the paper, Zhao YL revised the manuscript, Chen W and Sang XH prepared the materials. All of the authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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