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ORIGINAL ARTICLE

Expression analysis and promoter methylation under osmotic and salinity stress of *TaGAPC1* in wheat (*Triticum aestivum* L)

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Abstract Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC) catalyzes a key reaction in glycolysis and encoded by a multi-gene family which showed instability expression under abiotic stress. DNA methylation is an epigenetic modification that plays an important role in gene regulation in response to abiotic stress. The comprehension of DNA methylation at promoter region of *TaGAPC1* can provide insights into the transcription regulation mechanisms of plant genes under abiotic stress. In this study, we cloned *TaGAPC1* genes and its promoters from two wheat genomes, then investigated the expression patterns of *TaGAPC1* under osmotic and salinity stress, and analyzed the promoter sequences. Moreover, the methylation patterns of promoters under stress were confirmed. Expression analysis indicated that *TaGAPC1* was induced inordinately by stresses in two wheat genotypes with contrasting drought tolerance. Several stress-related *cis*-acting elements (MBS, DRE, GT1 and LTR et al.) were located in its promoters. Furthermore, the osmotic and salinity stress induced the demethylation of CG and CHG nucleotide in the promoter region of *Changwu134*. The

methylation level of CHG and CHH in promoter of *Zhengyin1* was always increased under stresses, and the CG contexts remained unchanged. The cytosine loci of stress-related *cis*-acting elements also showed different methylation changes in this process. These results provide insights into the relationship between promoter methylation and gene expression, promoting the function investigation of *GAPC*.

Keywords *Cis*-elements · DNA methylation · Gene expression · Promoter · Stress · Wheat

Introduction

DNA methylation is a ubiquitous modification of genomes in eukaryotes (Zilberman et al. 2007). The most common DNA methylation is the modification of cytosine C5, and it could reach up to 25 % in the plant genome (Steward et al. 2002). There are three types of cytosine methylation in plants: CG, CHG and CHH (H means any nucleotide except guanine), while in animals methylation occurs almost only in CG sequence context (Henderson et al. 2010). As a crucial epigenetic mark in plant, DNA methylation can regulate the genomic activity by three ways: de novo methylation, maintaining and removing methylation (Liu et al. 2015). Recently, it is revealed that genome methylation was always involved in response to external factors and contributed to the evolution in plant genomes. At least three genes had been proved to be affected by DNA methylation under heat stress in Arabidopsis (Boyko et al. 2010; Naydenov et al. 2015). The level of DNA methylation that existed in genome fragment of maize hybrids was lower than that of its corresponding mid-parents, and researchers conjectured that there is an increasing trend of demethylation in the hybrids relative to their parents (Sun et al. 2015).

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Wheat (*Triticum aestivum* L) is one of the world's most important food crops that provide dietary carbohydrates for numerous people of the world. Water-deficit and salinity are the major abiotic factors that affect wheat production worldwide. Growth and development of plants are regulated by several proteins and genes under abiotic stresses (Li et al. 2015). Some functional genes changed in methylation patterns under abiotic stress in wheat (Sun et al. 2014; Xu et al. 2013). DNA methylation exists not only in transposable elements and repeat sequences but also in transcriptional regions of promoter (Jeon et al. 2015). And it had been proved that methylation interfered in the combination between *cis*-acting elements and transcription factors directly (Inamdar et al. 1991). So study on the cytosine methylation of promoter region is significant for understanding the mechanism of stress response gene in plant.

GAPC (glyceraldehyde-3-phosphate dehydrogenase, cytosolic) was once considered as a simple "housekeeping" protein and had been used as a reference in gene expression analysis (Nicholls et al. 2012). In recent years, many researches had revealed that GAPC played a positive role in responding to stresses in plants (Arumugam Pillai et al. 2002; McLoughlin et al. 2013). There are multiple genes encoding GAPC isozymes in Arabidopsis, rice and maize (Manjunath and Sachs 1997; Marri et al. 2005; Zhang et al. 2011). The Arabidopsis thaliana genome contains four phosphorylating glycolytic *GAPC* genes (*GAPC1*, *GAPC2*, *GAPCp1*, and *GAPCp2*) (Guo et al. 2014). 6 *GAPC* genes were identified in wheat cv. *Chinese Spring* previously, and *TaGAPC1* (Traes_7DL_961822B36.2) was strongly induced by PEG (polyethylene glycol) treatment (Zeng et al. 2016). Here, *TaGAPC1* and its promoter were cloned, and the expression patterns under osmotic and salinity stress of *TaGAPC1* were investigated. Meanwhile, the stress-related *cis*-acting elements were identified from the promoters. Moreover, the methylation changes on the promoter under osmotic and salinity were analyzed with bisulfite sequence. Those studies expected to provide further insight into the molecular mechanisms of wheat tolerance and a new evidence for the correlation between promoter methylation and gene expression under abiotic stress.

Materials and methods

Primer design

The sequence of Traes_7DL_961822B36.2 was used as a query to search against the *Triticum aestivum* databases by Ensembl Plants (http://plants.ensembl.org/index.html). The specific primers used for gene and promoter cloning, qRT-PCR were designed by Primer Premier 5.0 and DNAMAN 6.0 based on *cv. Chinese Spring* sequence that differs from other *TaGAPC*s (Zeng et al. 2016). The wheat *β-actin* gene (AB181991) was

used as reference for qRT-PCR. Methyl Primer Express v1.0 was used to detect the CpG islands in promoters and design the Nested amplification primers of bisulfite sequencing. Specific primer sequences were given in Table 1. Primers *TaGAPC1-1FR-mF* and *TaGAPC1-1FR-mR* were used for the first round of Nested PCR of region I, and *TaGAPC1-1SR-mF* and *TaGAPC1-1SR-mR* were employed in the second round. *TaGAPC1-2FR-mF* and *TaGAPC1-2FR-mR* were used for the first round of Nested PCR of region II, and *TaGAPC1-2SR-mF* and *TaGAPC1-2SR-mR* were employed in the second round.

Plant materials and growing conditions

Wheat cv. *Changwu134* (drought-tolerant) (Weng et al. 2015) and *Zhengyin1* (drought-susceptible) (Ma et al. 2015) were used in this study. The seeds were provided by the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau of the Institute of Soil and Water Conservation at the Chinese Academy of Sciences. Seeds were disinfected with 0.1 % HgCl$_2$ for 15 min and germinated on wet filter paper for 8 h at 22 °C dark, then put the germinated seeds on a petri dish (45 cm in diameter), containing half-strength Hoagland. The wheat grains were kept under a 12 h photoperiod (light/dark temperature 22 °C) for 14 days. At the two-leave stage, the solution were replaced by 20 % PEG8000 and 250 mM NaCl. The leaves were harvested and rapidly frozen in liquid nitrogen and stored at −80 °C for extraction of total RNA and genomic DNA. All experiments were repeated three times.

Homologous cloning of *TaGAPC1s* and sequence analysis

TaGAPC1s were amplified from wheat cDNA with PCR. The PCR reaction were performed in a final volume of 20 μL using the following PCR program: an initial denaturation step (94 °C, 5 min) was followed by 32 cycles (94 °C, 30 s; 56 °C, 45 s; 72 °C, 1 min 10 s) and a final elongation step (72 °C, 10 min). The PCR products were checked using 2 % agarose gel electrophoresis and purified, then cloned into a pMD19T (simple) (TaKaRa, Japan) vector. Several clones of each reaction were sequenced (Invitrogen, USA).

The exon-intron structures of gene were determined by aligning their coding sequences with their corresponding genomic sequences and a diagram was generated through the online Gene Structure Display Serve (http://gsds.cbi.pku.edu.cn). The sequence of genes and promoters was compared by DNAMAN6.0. The putative proteins were confirmed using the Pfam database (http://pfam.sanger.ac.uk/) and the putative localization of protein was identified using the PSORT prediction server (http://www.genscript.com/psort.html). Domains of the proteins were analyzed by the InterProScan (http://www.ebi.ac.uk/cgi-bin/iprscan/). GAPCs from Arabidopsis thaliana were searched from

Table 1 Primer sequences used for qRT-PCR analysis, promoter cloning and bisulfite sequencing

Purpose	Primer name	Sequence (5′~3′)	Annealing temperature (°C)	Product (bp)
Quantitative RT-PCR	*TaGAPC1*-qF *TaGAPC1*-qR	TGTGGATGTGTCAGTTGT CAGCCTTGATAGCCTTCT	56	132
	β-Actin-qF *β-Actin*-qR	CGACTCTGGTGATGGTGTGAG AGCAAGGTCCAAACGAAGGA	56	85
Gene cloning	*TaGAPC1*-gF *TaGAPC1*-gR	ATGGGCAAGATTAAGATCGGAAT CTACTGAGTCTTGGCCATGTGGCGGA	55	1014
Promoter cloning	*TaGAPC1*-pF *TaGAPC1*-pR	CGGCGAGTAAGCAAAGGG TCAGCAAAGAACAATCACCATC	58	972
Bisulfate PCR	*TaGAPC1*-1FR-mF *TaGAPC1*-1FR-mR	GGGGTTGTTTGTATTTTTAGTGTTAT CATATTTACTTTCCCTCCRATACCRA	(60~47) 55	636
	TaGAPC1-1SR-mF *TaGAPC1*-1SR-mR	GTTATYGTTTTTGTTATTGTTGTTGTYG CTAAAAACAACTCCAATAAAACRACCC	(60~50) 55	452
	TaGAPC1-2FR-mF *TaGAPC1*-2FR-mR	GGGTYGTTTTATTGGAGTTGTTTTTAG ACAATCACCATCAAATCAAAAT	(62~47) 55	605
	TaGAPC1-2SR-mF *TaGAPC1*-2SR-mR	TTTATGGGTTTTTTTTTTTTTGTT ACCCATAACRAAAAAATAAAACTAC	(60~50) 55	405

NCBI and phylogenetic tree was constructed by MEGA5.0 using the neighbor-joining (NJ) method.

Quantitative real-time PCR

Frozen tissues were ground in liquid nitrogen and total RNA was isolated using the RNAiso plus reagent (TaKaRa, Japan) as per the manufacturer's specifications and treated with RNase-free DNase I (Invitrogen, USA) for 15 min to degrade any residual genomic DNA. For real-time PCR analysis, first-strand cDNAs were synthesized from DNaseI-treated total RNA using PrimeScript^{TM} RT-PCR Kit (TaKaRa, Japan) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was carried out by BioEasy Master Mix (SYBR Green, BioFlux) according to the manufacturer's instructions on the Bio-Rad CFX96 system (Bio-Rad Laboratories, USA). The PCR thermal cycle was set up as follows: 95 °C for 1 min; 40 cycles of 94 °C for 15 s, 56 °C for 15 s and 72 °C for 30s; And melting curve analysis. The quantitative analysis was accomplished with the $2^{-\triangle\triangle CT}$ method by Bio Rad CFX Manager software and the relative expression graphs of *TaGAPC1* were drawn by Origin 9.0. The wheat *β-actin* gene was used as an internal control. Three biological replicates were set up and all experiments were repeated three times.

Cloning of promoters and sequence analysis

Genomic DNA isolated by the CTAB (Hexadecyltrimethy Ammonium Bromide) method (Attitalla 2011) and the PCR reaction of promoter cloning were performed in a final volume of 20 μL using the following PCR program: an initial denaturation step (94 °C, 5 min) was followed by 32 cycles (94 °C, 30 s; 58 °C, 45 s; 72 °C, 1 min) and a final elongation step (72 °C, 10 min). The PCR products were checked using 2 % agarose gel electrophoresis and purified, then cloned into a pMD19T (simple) (TaKaRa, Japan) vector. Several clones of each reaction were sequenced (Invitrogen, USA).

PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al. 2002) and PLACE (http://www.dna.affrc.Go.jp/PLACE/) were used to detect *cis*-elements of promoters.

Bisulfite sequencing and nested PCR

Genomic DNA was extracted with CTAB method. 1 μg DNA was modified via the EZ DNA Methylation-Gold^{TM} kit (ZYMO RESEARCH, http://zymoresearch.com). The first round of Nested PCR reactions were performed in a final volume of 20 μL containing 2 μL bisulfite-treated DNA, 2 μL 10× buffer (Mg^{+2} plus), 0.8 μL dNTP, 1 μL primers, and 2.5 Units of hot-start Taq DNA polymerase (TaKaRa, Japan) with the following PCR (Touchdown PCR) program: an initial denaturation step (94 °C, 5 min) was followed by 16 touch-down cycles (cycles of 94 °C, 30 s; 60 °C to 47 °C (Region I) or 62 °C to 47 °C (Region II), 45 s, 1 °C decrease after each cycle; 72 °C, 1 min 20 s followed by 24 additional cycles of: 94 °C, 30 s; 55 °C, 45 s, 72 °C, 1 min 20 s), with a final elongation step (72 °C, 10 min). The second round of Nested PCR reactions were performed in a final volume of 50 μL containing 1 μL product of the first wheel DNA, 5 μL 10× buffer (Mg^{+2} plus), 4 μL dNTP, 1 μL primers,

and 2.5 Units of hot-start Taq DNA polymerase with another PCR (Touchdown PCR) program: an initial denaturation step (94 °C, 5 min) was followed by 11 touch-down cycles (cycles of 94 °C, 30 s; 60 °C to 50 °C, 45 s, 1 °C decrease after each cycle; 72 °C, 1 min followed by 29 additional cycles of: 94 °C, 30 s; 55 °C, 45 s, 72 °C, 1 min), with a final elongation step (72 °C, 10 min). Size, quality and quantity of the PCR products were checked using 2 % agarose gel electrophoresis.

The PCR products were extracted using the Biospin Gel Extraction Kit (BioFlux) and ligated with the pMD™19–T Vector (TAKARA). Transferred to E.coli (DH5α) cells, at least 15 clones per insert were processed for sequencing (Invitrogen, USA). Sequences of which the cytosine transformation efficiency <97 % were removed by BiQ Analyzer software (Bock et al. 2005). The ratio of C methylation at each CG dinucleotide, and CHG and CHH trinucleotide was calculated and transformed into a percentage using KISMETH software (http://katahdin.mssm.edu/kismeth/revpage.pl) (Gruntman et al. 2008).

Statistical analysis

All of the data obtained in the study were subjected to statistical analysis by one-way ANOVA using SPSS v20. Data were considered statistically significant at $P \leq 0.05$.

Results

Sequence analysis of *TaGAPC1* genes and GAPC proteins

The length of *TaGAPC1* cDNA were 1014 bp (Fig. 1a), and 337 amino acids were encoded by this sequence. The sequence was submitted to GeneBank and obtained an accession number of KU933269. *TaGAPC1* was comprised of 11 exons and 10 introns (Fig. 2b). Alignment of GAPCs were given in Fig. 2a. The results revealed that the similarity of TaGAPC1 with AtGAPCs were over 90 %. NAD (P) binding domain (InterPro: IPR020828) and Glyceraldehyde 3-phosphate dehydrogenase, catalytic domain (InterPro: IPR020829) were detected in GAPCs (Fig. 2a). The prediction of protein subcellular locations showed that TaGAPC1 were cytoplasmic with 69.6 % possibility.

To further confirm the subfamily of this gene and discover the potential functions, the evolutionary relationship between the putative protein and other GAPDHs from Arabidopsis thaliana were analyzed by MEG 5.0 software. Result revealed that the TaGAPC1 belonged to clade of plant GAPCs, according to a previously described classification method used for Arabidopsis GAPDH family (Fig. 3) (Martin and Cerff 1986).

Expression profiles of *TaGAPC1* in wheat

To reveal the expression of *TaGAPC1* in response to osmotic and salinity stress, qRT-PCR of *TaGAPC1* was performed after treated with 20 % PEG8000 and 250 mM NaCl for 24 h in two wheat cultivars, respectively. The expression of *TaGAPC1* was upregulated significantly under PEG and NaCl treatment in *Changwu134*, peaking at 24 h (25-fold) and 6 h (13-fold), respectively (Fig. 4a). However in *Zhengyin1*, PEG and NaCl treatment resulted in upregulation of *TaGAPC1* slightly, only reaching 3-fold and 6-fold, respectively, peaking at hours 6 and 12, respectively (Fig. 4b).

Isolation the upstream region of *TaGAPC1s* genes from two wheat cultivars

To investigate the function of promoter in gene expression and ensure the accuracy of upstream sequences in two cultivars, the sequences were cloned by specific primers. The length of these two promoter sequences were 972 bp (Fig. 1b), which registered in the GeneBank (Accession number: KX196174). Moreover, the sequence alignment revealed that these two sequences from *Changwu134* and *Zhengyin1* shared 97.74 % identity (Supplementary fig. 1). Two sequences were analyzed in detail by PLACE and PlantCARE database, and the crucial elements that respond to stress and hormone were focused and shown in Table 2. The *cis*-acting elements that respond to drought and salinity (G-box, MBS, DRE, CGTCA-box, CBF, GT1) (Li et al. 2013a; Sun et al. 2010; Tao et al. 2015), ABA (CE1, ERE) (Luo et al. 2013; Niu et al. 2002), MeJA (CGTCA-box, TGACG-motif) (Rouster et al. 1997), low temperature (TATA-box, LTRECOREATCOR15, DRE and MYC) (Baker et al. 1994; Li et al. 2013b) and light (CATT-motif, Box I, GT1-motif and G-box) (Luo et al. 2013; Roy et al. 2012) were determined in both two wheat cultivars.

Bisulfite sequence result of promoter in two regions

The bisulfite sequencing PCR (BSP) was used to examine the cytosine methylation status of two regions (I and II) at the promoter region. As showed in Fig. 2b, region I covered from 208 to 596 bp, and region II covered from 714 to 1119 bp. The BSP result of two regions in normal growth of *Changwu134* and *Zhengyin1* displayed a cytosine methylated map in Fig. 5a, b. The results showed that the region I were heavily (>60 %) CG cytosine methylated and moderately (>20 %) CHG cytosine methylated, but slightly (<20 %) CHH cytosine methylated in studied two cultivars. It was obvious that the methylation rate ranking of the three cytosine methylation types in promoter region I was CG > CHG > CHH, which follow the same pattern of average methylation levels (Cokus et al. 2008). But in region II, it was irregular that all of the cytosine were slightly (<20 %) methylated in region II, even approaching to

Fig. 1 Determination of electrophoresis result for homologous cloning and Nested PCR for bisulfite sequencing. cw is *Changwu134* and zy is *Zhengyin1*; M: DNA Marker DL2000. **a**. Amplification of *TaGAPC1*. **b** Amplification of promoter region for *TaGAPC1*. **c, d** Nested PCR results of region I and region II, respectively, the first two lane is the first round result and last two lane is the second round result
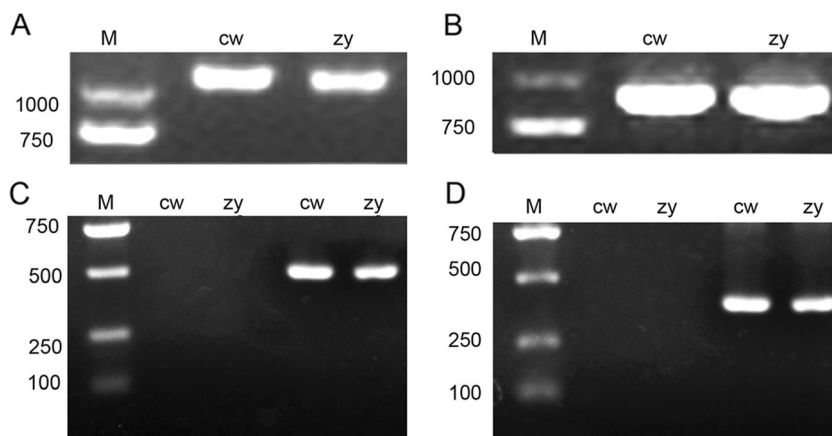
zero approximately. It is revealed that the cytosine methylation mainly occurred in the upstream of promoter.

The methylation rate of promoters under 20 % PEG8000 and 250 mM NaCl conditions were also detected. The results showed that the CG and CHG context was demethylated under PEG and NaCl stress in *Changwu134* (Fig. 4c). CG methylation rate of PEG group were lower than CK group (0.85-fold), and CHG methylation rate of NaCl group were also lower than CK (0.67-fold). But the cytosine methylation level was increased in *Zhengyin1* (Fig. 4c), the CG context methylation level was constant and CHG and CHH was increased slightly under PEG and NaCl treatment. The C methylation change of

cis-elements which involved in stress response was given in Table 3. The cytosines of G-box, MBS, ABRERATCAL, MYBCORE and MYCCONSENSUSAT were demethylated by stresses in *Changwu134*. The cytosines of CE1 were demethylated by all the stresses in two wheat genotypes.

Discussion

Water deficit and salinity stress have adverse effects on the growth and production of crops. Many efforts have been made
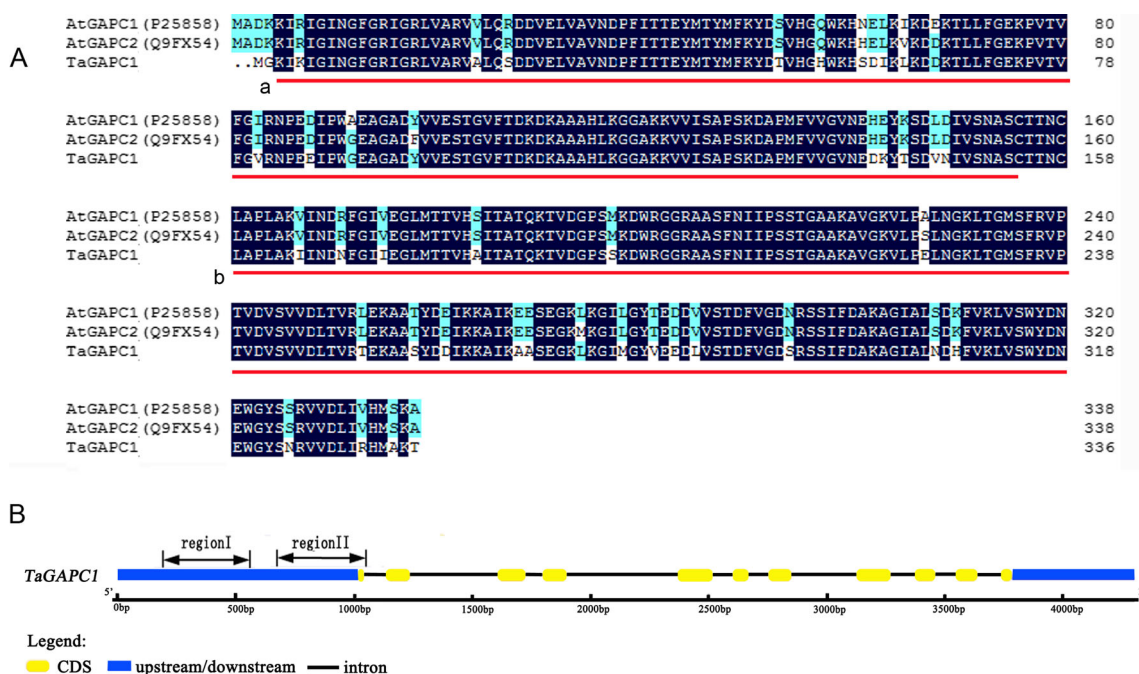

Fig. 2 **a** Multiple sequence alignment of TaGAPC1 and GAPC proteins from Arabidopsis thaliana. (Consensus: represents the same deoxynucleotide among these sequences. The identical, 75 and 50 % deoxynucleotide similarity is indicated by mazarine, *pink* and *green* color. Red underlined *a*, Glyceraldehyde 3-phosphate dehydrogenase, NAD (P) binding domain (IPR020828); Red underlined *b*, Glyceraldehyde 3-phosphate dehydrogenase, catalytic domain (IPR020829)). **b** Schematic diagram for exons/introns and upstream/downstream structures of *TaGAPC1* (Exons, introns, upstream/downstream are indicated by *yellow boxes*, *black horizontal lines*, and *blue boxes*, respectively. The position of two regions about bisulfite sequencing given in *arrows*)

Fig. 3 Phylogenetic analysis of TaGAPC1. Numbers above or below branches of the tree indicate bootstrap values. The accession number of the GAPC proteins is as follows: AtGAPC1 (P25858.2), AtGAPC2 (Q9FX54.1), AtGAPA1 (P25856.3), AtGAPA2 (Q9LPW0.1), AtGAPB (P25857.2), AtGAPCp1 (Q9SAJ6.1), AtGAPCp2 (Q5E924.1)
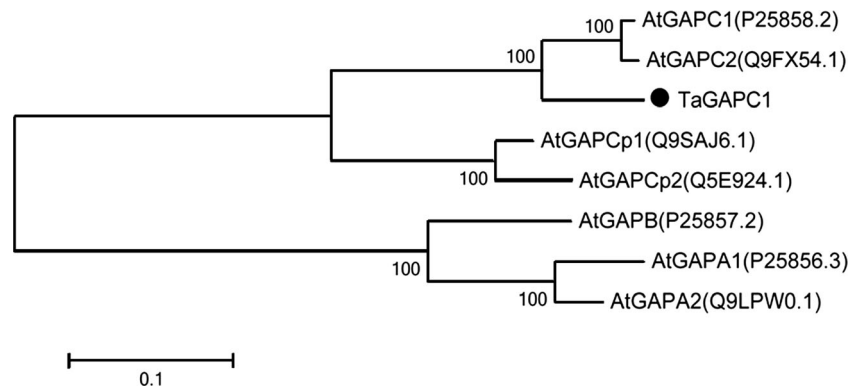
to elucidate the mechanisms of stress response in crops. The fine tuning of gene expression in response to abiotic stress was a key strategy for crops to cope with the changing environment (Petrillo et al., 2014). Recent researches have investigated variety functions of GAPC proteins in plants, including the stress-response (Guo et al. 2012; Hancock et al. 2005). In poaceae

plants, *OsGAPC2* had been proven that involved in responding to drought, salt, heat and ABA stresses (Zhang et al. 2011), and *ZmGAPC1* was response to anoxia (Manjunath and Sachs 1997). In this research, phylogenic analysis investigate that TaGAPC1 belonged to one clade of AtGAPC1 and AtGAPC2, which was involved in responding to heat,
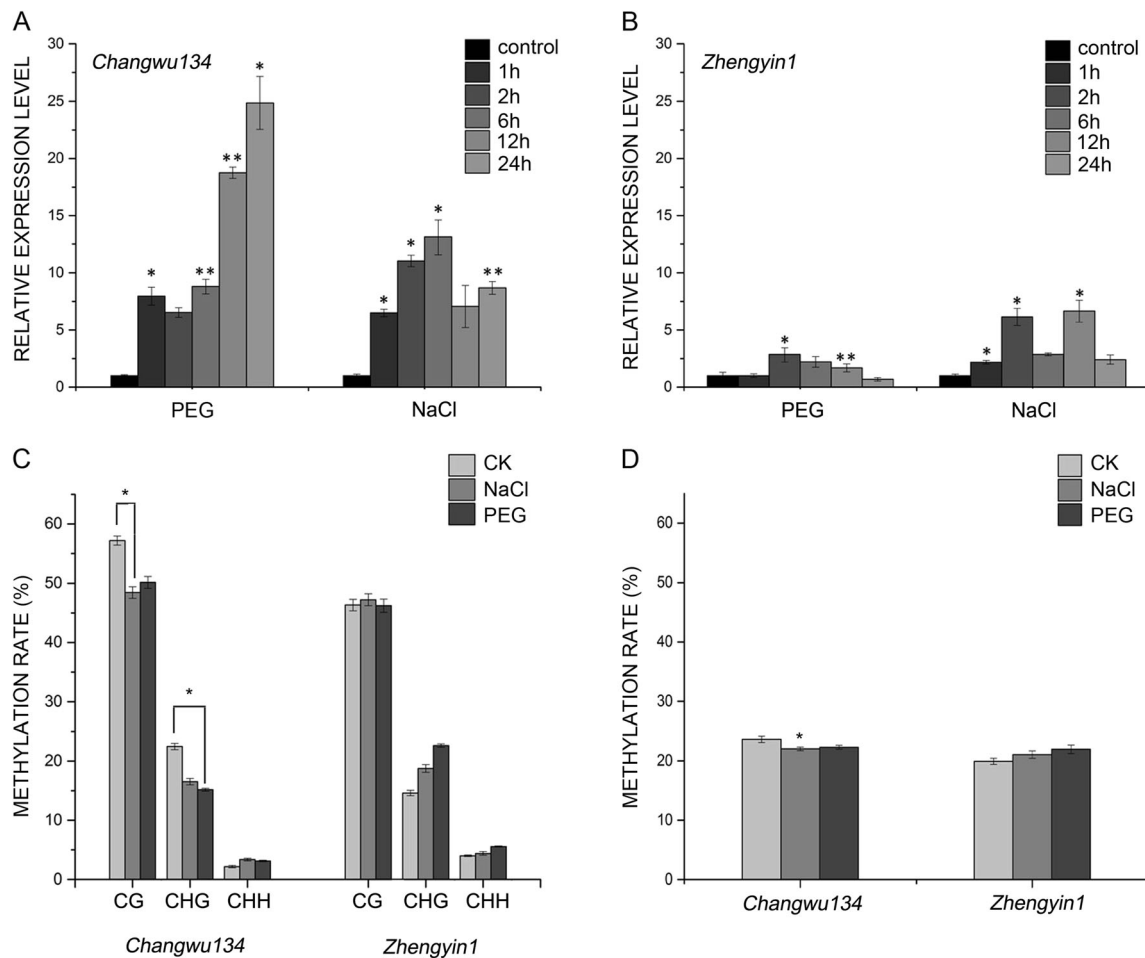

Fig. 4 **a**, **b** The transcript abundance of *TaGAPC1* after exposed to 20 % PEG8000 and 250 mM NaCl for 1, 2, 6, 12, and 24 h in *Changwu134* and *Zhengyin1*. Standard deviation (SD) is displayed on the bar chart, significant differences were detected by the Student's *t* test (**Significant at $P \leq 0.01$ level; *significant at $P \leq 0.05$ level). **c** CG, CHG and CHH context methylation change (in percentage) under stresses. **d** Total methylation changes of promoter in *Changwu134* and *Zhengyin1*. Data are shown as the mean ± SD. Significant differences were detected by the Student's *t* test, ** $P \leq 0.01$ were shown in the graph

Table 2 The *cis*-acting of *TaGAPC1* gene promoters

Name	Sequence	Number		Function
		a	b	
ABRELATERD1	ACGTG	2	2	Dehydration responsive element
ABRERATCAL	MACGYGB	5	4	ABA responsive element
ACGTATERD1	ACGT	6	6	Dehydration and etiolation responsive element
ARE	TGGTTT	2	2	Essential for the anaerobic induction
ARR1AT	NGATT	4	4	Salinity-responsive element
CATT-motif	GCATTC	1	1	Part of a light responsive element
CBFHV	RYCGAC	5	5	Dehydration responsive element
CE1	TGCCACCGG	1	1	*Cis*-element involved in ABA responsiveness
CGTCA-motif	CGTCA	3	3	*Cis*-element involved in the MeJA-responsiveness
DRECRTCOREAT	RCCGAC	4	4	*Cis*-elements involved in abiotic stress
ERELEE4	AWTTCAAA	1	1	Ethylene responsive element
GT1-motif	GGTTAA	1	1	Light responsive element
G-box	CACGTC	2	2	*Cis*-element involved in light and drought responsiveness
GAG-motif	GGAGATG	1	0	Part of a light responsive element
LTRECOREATCOR15	CCGAC	4	4	Low-temperature responsive element
MBS	CAACTG	1	1	MYB binding site
MYCCONSENSUSAT	CANNTG	5	5	Dehydration, ABA, cold and light responsive element
MYB	GGATA/CCWACC/AACGG/CNGTTR	5	3	Drought-responsive element
Sp1	GGGCGG/CC(G/A) CCC	5	6	Light responsive element
TC-rich repeats	ATTTTCTCCA	1	1	*Cis*-acting element involved in defense and stress responsiveness
WRKY71OS	TGAC	5	5	Biotic stress responsive element

a represent *Changwu134* and b represent *Zhengyin1*; R = A/G;Y = C/T;W = A/T;N = A/C/G/T;M = A/C; B = C/G/T

anaerobic, salt and drought (Guo et al. 2012; Yang et al. 1993; Zhang et al. 2011). So study on the expression of *GAPC* gene under abiotic stress was significant for understanding the functions of stress response in wheat. Further result of qRT-PCR confirmed that the expression of *TaGAPC1* was strongly induced by stresses in drought-tolerant wheat genotype and slightly induced in drought-sensitive wheat genotype. It was indicated that *TaGAPC1* may play some roles of stress response via unknown mechanism in wheat.

Epigenetic modification contributes to the expression divergence of functional gene expression, including histone acetylation and DNA methylation (Hu et al., 2013; Xu et al., 2013). Growing evidences from recent studies have suggested that demethylation of promoter region could enhance the expression of genes when plants exposed to stresses (Paszkowski and Whitham, 2001; Choi and Sano, 2007). It has been found that hypomethylation and hypermethylation are more frequent by drought stress in drought-tolerant and drought-sensitive rice genotypes, respectively (Gayacharan and Joel, 2013). In recent researches, numerous diversely methylated regions associated with differential expression of genes were found important for abiotic stress response in some rice cultivars with different

resistance (Garg et al., 2015). Moreover, in plants, the three patterns of DNA methylation (CG, CHG, and CHH) were accomplished, maintained, and demethylated by different enzymes or protein families, also being affected by respective regulatory pathways (Law and Jacobsen 2010; Stroud et al. 2014). So study on the methylation of gene's upstream region is significant for understanding the mechanism of stress response gene in plants. In present study, CG and CHG context of promoter region were demethylated by stresses in drought-tolerant wheat. However in drought-susceptible wheat, CG context was maintained methylation and CHG (CHH) methylation was established by their pathways. This difference may is a reason why gene transcribe significantly in drought-tolerant wheat.

The *cis*-elements, which presented in the promoter region of stress-responsive genes, play a crucial role in interacting with transcription factors to regulate the gene expression under stress (Bjornson et al. 2016). The *cis*-elements were the key factors in regulating of promoter activity, so the methylation of their sequences worth been further explored. In this study, the prediction results of promoters revealed that there were regulatory elements that response to abiotic stress, such
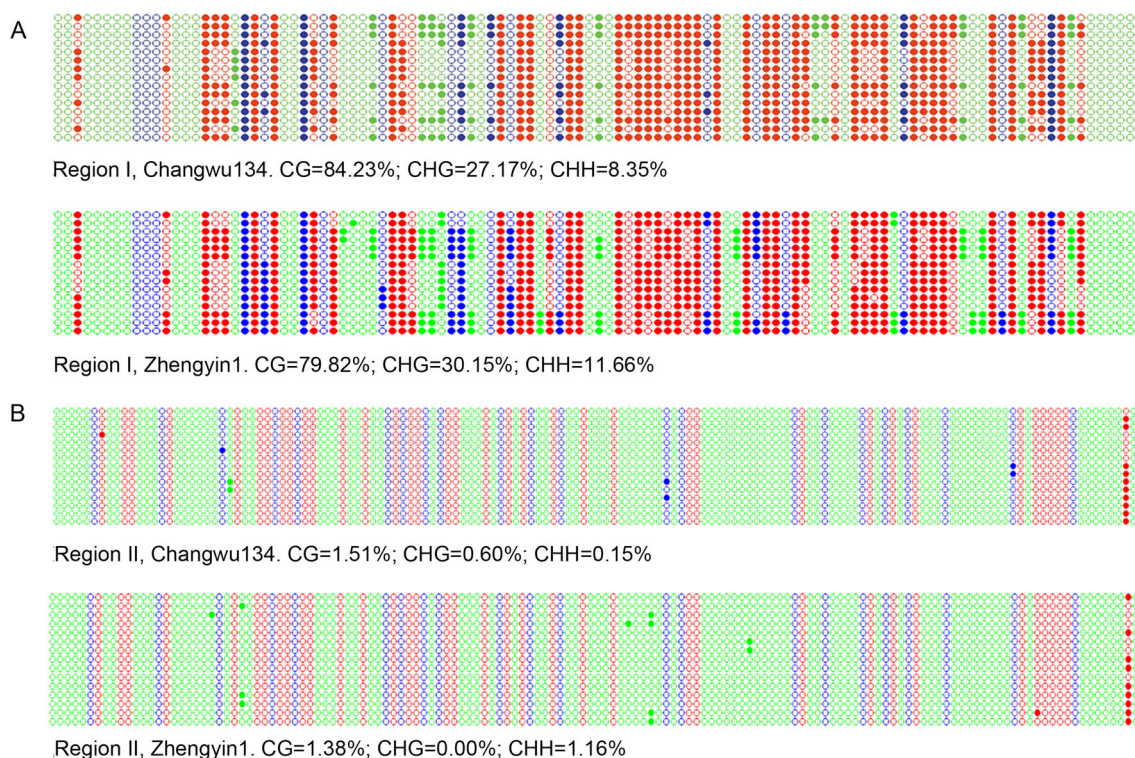
Region I, Changwu134. CG=84.23%; CHG=27.17%; CHH=8.35%

Region I, Zhengyin1. CG=79.82%; CHG=30.15%; CHH=11.66%

Region II, Changwu134. CG=1.51%; CHG=0.60%; CHH=0.15%

Region II, Zhengyin1. CG=1.38%; CHG=0.00%; CHH=1.16%

Fig. 5 Bisulfite sequencing for cytosine methylation of two regions (I and II) within the promoter of *TaGAPC1* in plants under normal conditions of two wheat cultivars. All three types of cytosine residues, CG (*red circles*), CHG (*blue circles*), and CHH (*green circles*) were shown in the map. *Filled* and *empty circles* denote methylated and unmethylated cytosines, respectively. A row represents a sequencing result of a positive clone. **a** Cytosine methylation maps for region I in *Changwu134* and *Zhengyin1* orderly. **b** Cytosine methylation maps for region II in *Changwu134* and *Zhengyin1* orderly

as low temperature, ABA, salinity, and drought (Fig. 4). The statistics result summarized the methylation frequency of *cis*-elements which related to stress tolerance (Table 3) (Wang et al. 2014). Some of the stress-induced alterations in C methylation occurred at sites that located within known *cis*-elements, such as G-box and MBS (drought and salinity response), were demethylated in *Changwu134*, but established in *Zhengyin1*; CE1 (ABA response) was reduced by osmotic stress and lower by salinity stress. It was conclusion that *TaGAPC1* was responding to both osmotic and salinity stress

in drought-tolerant wheat genotypes, indicating that there may some unknown pathways for combining transcriptional regulatory proteins, which can recognize methylation of cytosine in the *cis*-elements of promoter regions.

In conclusion, the *TaGAPC1* gene was expressed differentially under stress condition in wheat genotypes with contrasting drought tolerance. Further results revealed that the methylation patterns of promoter region, which induced by stress differentially in wheat genotypes with contrasting drought tolerance, were an important regulatory mechanism

Table 3 Cytosine methylation frequency of cis-elements under stresses

Cis-elements	Sequence	Methylation frequencies					
		CW-ck (%)	*CW*-PEG (%)	*CW*-NaCl (%)	*ZY*-ck (%)	*ZY*-PEG (%)	*ZY*-NaCl (%)
G-box	**CACGTC**	60.00	43.59	45.45	33.33	47.62	52.38
MBS	**CAACTG**	100.00	92.86	45.45	0.00	72.73	14.29
ABRERATCAL	MA**C**GYGB	59.38	27.59	29.41	25.00	37.50	18.75
MYBCORE	**C**NGTTR	87.50	14.29	45.45	62.50	62.50	85.71
MYCCONSENSUSAT	**C**ANNTG	81.25	58.82	63.64	75.00	87.50	50.00
CE1	TG**CC**A**C**CGG	77.78	66.67	54.55	100.00	75.00	9.52

CW represents the wheat *Changwu134* and *ZY* represents the wheat *Zhengyin1*; ck denote that the group without any stress; PEG denote that the group with 20 % PEG8000 for 24 h and NaCl denote that the group with 250 mM NaCl for 24 h. **C** was the cytosine which been investigated methylation frequency

for sensing and responding to the stress conditions. Our investigation also suggested the potential role of some specific loci methylation change on *cis*-elements for gene responding to stresses. These findings allow us to further known the function of *TaGAPC1* genes, and understand of epigenetic regulation of gene expression in abiotic stress responses.

Compliance with ethical standards

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

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