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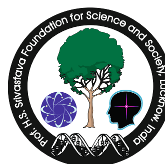

RESEARCH ARTICLE

Isolation and characterization of maize *ZmPP2C26* gene promoter in drought-response

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Abstract The clade A members of serine/threonine protein phosphatase 2Cs (PP2Cs) play crucial roles in plant growth, development, and stress response via the ABA signaling pathway. But little is known about other PP2C clades in plants. Our previous study showed that maize the *ZmPP2C26*, a clade B member of *ZmPP2Cs*, negatively regulated drought tolerance in transgenic *Arabidopsis*. However, the upstream regulatory mechanism of *ZmPP2C26* remains unclear. In the present study, the expression of *ZmPP2C26* gene in maize was analyzed by quantitative real time PCR (qRT-PCR). The results showed that the expression of *ZmPP2C26* in shoot and root was both significantly inhibited by drought stress. Subsequently, a 2175 bp promoter of *ZmPP2C26* was isolated from maize genome (P_{2175}). To validate whether the promoter possess some key *cis*-element and negatively drive *ZmPP2C26* expression in drought stress, three 5′-deletion fragments of 1505, 1084 and 215 bp was amplified from P_{2175} and were fused to β-glucuronidase (*GUS*) and luciferase gene (*LUC*) to produce *promoter::GUS* and *promoter::LUC* constructs, and transformed into tobacco, respectively. Transient expression assays indicated that all promoters could drive *GUS* and *LUC* expression. The GUS and LUC activity were both significantly inhibited by PEG-6000 treatment. Notably, the − 1084 to − 215 bp promoter possess one MBS element and inhibits the expression of *GUS* and *LUC* under drought stress. Meanwhile, we found that the 215 bp length is enough to drive *ZmPP2C26* expression. These findings will provide insights into understanding the transcription-regulatory mechanism of *ZmPP2C26* negatively regulating drought tolerance.

Keywords Maize · Serine/threonine protein phosphatase 2C · Promoter · Drought stress

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Introduction

Protein phosphorylation and dephosphorylation is a dynamic balance process and widespread in prokaryotes and eukaryotes. Plants can select phosphorylation or dephosphorylation of substrate molecules to realize the cascade of signals and enable plants to respond to any stimuli (Luan et al. 2003; Ma et al. 2009; Schweighofer et al. 2004). The protein phosphatases (PPs) catalyze the dephosphorylation and play crucial roles in plant growth and development. According to the difference of homology and substrate specificity, PPs are divided into eight families, including PP1, PP2A, PP2B, PP2C, PP4, PP5, PP6 and PP7. Among these PPs, PP2C, a kind of multifunctional monomer enzyme, belongs to serine/threonine PPs and specifically dephosphorylates the phosphorylated serine/threonine residues of proteins in vivo. PP2Cs have been implicated as a negative regulator of protein kinase cascades, which are activated by environmental stress in eukaryotes (Park et al. 2009; Rodriguez 1998).

Till date, there are 83, 80 and 130 PP2C members identified by bioinformatic analyses in *Arabidopsis*, rice and maize, respectively (Antoni et al. 2012; Wang et al.

2018). Likewise, PP2Cs are divided into 13 clades of A-K. The Clade A members of PP2Cs interact with PYR/PYL/RCAR ABA receptors, then act on SnRK2 in ABA signaling pathway, to regulate plant growth, development and stress response, such as seed size, dormancy, germination, and drought resistance (Bhaskara et al. 2012; Han et al. 2018; He et al. 2019; Komatsu et al. 2013; Lu et al. 2017; Nishimura et al. 2018; Park et al.2009; Xiang et al. 2017). However, the functions of clade B members are rarely reported. In *Arabidopsis*, AP2C1, a clade B PP2C, interacts with CBL-interacting protein kinase 9 (CIPK9) in the cytoplasm to dephosphorylate CIPK9 and thus negatively regulate plant tolerance to low-K^+ stress (Singh et al. 2018). Additionally, other clade B PP2Cs regulate seed germination, stomatal development, and defense response via mitogen-activated protein kinase (MAPK) signaling pathway in *Arabidopsis* (Brock et al. 2010; Julijia et al. 2010; Meskiene et al. 1998; Schweighofer et al. 2007; Shubchynskyy et al. 2017; Sidonskaya et al. 2016; Umbrasaite et al. 2010). In maize, some clade A PP2C members have also been characterized and confirmed to increase seed germination and regulate stress response (He et al. 2019; Hu et al. 2010; Xiang et al. 2017). However, the function of other clade PP2C members of maize remains obscure.

In our previous study, maize ZmPP2C26 was identified as clade B members of ZmPP2Cs and found to negatively regulate drought tolerance in transgenic *Arabidopsis* (Wang et al. 2018; Zhang et al. 2018). However, the upstream regulatory mechanism remains unknown. In this study, hence, the expression of *ZmPP2C26* in maize was analyzed by qRT-PCR. Subsequently, the *ZmPP2C26* promoter was isolated, truncated at 5′-end, fused with *GUS* and 1 *LUC* gene, which were used for promoter driven-activity evaluation to provide insights into exploring the potential upstream factors acting on ZmPP2C26 under drought stress.

Materials and methods

Plant materials and growth condition

The seeds of maize inbred lines 87-1 were germinated in a petri dish. Subsequently, the seedlings were transplanted into a plastic mesh grid for hydroponic culture at 28 °C under a photoperiod of 14 h light/10 h dark and used for PEG treatment.

Tobacco (*Nicotiana benthamiana*) seeds were evenly sown in the flowerpot with nutrient soil and allowed to germinate and grow at 25 °C for 2 weeks. The seedlings were then transplanted into some small pots, and grown in a tissue culture chamber under a 16 h light/8 h night for 6 weeks at 25 °C and used for *Agrobacterium* transformation.

RNA extraction and qRT-PCR ananlysis

At the three-leaf stage, maize seedings were divided into three groups and treated with 16% (w/v) PEG-6000 solution. At 0 (control), 3, 6, 9, 12, 24 and 48 h of treatment, the shoot and root were sampled and immediately ground in liquid nitrogen for RNA extraction with three replicates, respectively. Total RNA was extracted using RNAiso plus kit (TaKaRa, Japan), quantified using NanoDrop^{TM} OneC (ThermoScientific, USA), and reverse transcribed into cDNA using the PrimeScript^{TM} reagent kit (TaKaRa, Japan) according to the manufacturer's instruction. The specific primer pairs qPf/qPr and qGf/qGr (Table1) were designed using PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), synthesized at Sangon biotech (Shanghai, China), used to amplify 358 bp fragment of *ZmPP2C26* gene, and 171 bp fragment of *ZmGAPDH* gene that was used as internal reference, respectively. The cDNA samples were used as templates for qRT-PCR by using Cham^{Q} Universal SYBR qPCR Master Mix (Vazyme, Nanjing) in CFX96^{TM} Real Time System (Bio-Rad, USA). As described by Sun et al. (2020), the two-step temperature cycle was performed as follows: 95 °C for 30 s; 40 cycles of 95 °C for 10 s, 60 °C for 20 s; at the end of the last cycle, the temperature was increased to 95 °C at 0.5 °C/s, so that the melting curve could be calculated and used to differentiate specific and non-specific amplicons. The $2^{-\Delta\Delta CT}$ method of the CFX Manger^{TM} software version 2.0 (Bio-Rad, USA) was used to normalize the deferential gene expression between *ZmPP2C26* and *ZmGAPDH* gene.

Isolation and sequence analysis of *ZmPP2C26* promoter

The sequence of 2500 bp 5′ flanking region to start codon (ATG) of *ZmPP2C26* gene (GenBank accession. KJ855114.1) was retrieved from MaizeGDB and used as reference to design primers to amplify *ZmPP2C26* promoter from genomic DNA of maize inbred line 87-1. The specific primers PF0/PR (Table 1) was designed and used to amplify *ZmPP2C26* promoter. The PCR product was subcloned into the pMD19-T Vector (TaKaRa, Japan) and confirmed by sequencing. Finally, the 2175 bp fragment was obtained, considered as the full-length promoter and used for *cis*-acting element analysis by using online programs PlantCARE (https://www.plantcare.co.uk/, Lescot et al. 2002).

Table 1 The primers used in the study

Primer name	Sequence (5′-3′)	Description
qPf	GGGAGGACGAGAAGGAAAGG	qRT-PCR of *ZmPP2C26* gene
qPr	AACTGCACGATCACGACACT	
qGf	TGAATGGCAAGCTCACTGGT	qRT-PCR of *ZmGAPDH* gene
qGr	TGAATGGCAAGCTCACTGGT	
PF0	TCACTTTACCATTTTTATGCGGGA	Forward primer for P_{2175} amplification
PR	CGGGGCTAGGGTTTTTTTTTCTTTC	Reverse primer for P_{2175} amplification
gus-PF0	acgacggccagtgccaagcttTCACTTTACCATTTTTATGCGGGA	Forward primers for PCR of r *promoter::GUS* construct
gus-PF1	acgacggccagtgccaagcttGAGCAAATCGACTCCATCCTT	
gus-PF2	acgacggccagtgccaagcttAGCGTCGCGTCTATCCTTCTC	
gus-PF3	acgacggccagtgccaagcttCTACGAAACCGCAAAGTCCAT	
gus-PR	tgtgattgtgatgtatctagaCGGGGCTAGGGTTTTTTTTTCTTTC	Reverse primer for PCR of *promoter::GUS* construct
luc- PF0	ctatagggcgaattgggtacc TCACTTTACCATTTTTATGCGGGA	Forward primers for PCR of *promoter::LUC* construct
luc- PF1	ctatagggcgaattgggtaccGAGCAAATCGACTCCATCCTT	
luc- PF2	ctatagggcgaattgggtaccAGCGTCGCGTCTATCCTTCTC	
luc- PF3	ctatagggcgaattgggtaccCTACGAAACCGCAAAGTCCAT	
luc- PR	caggaattcgatatcaagcttCGGGGCTAGGGTTTTTTTTT	Reverse primer for PCR of *promoter*::LUC construct

Construction of *promoter*::*GUS* and *promoter::LUC* Plasmids

To validate the promoter driven-activity and explore the potential key segments of 2175 bp promoter, three 5′ deleted fragments of *ZmPP2C26* promoter in different size (− 1505, − 1084, and − 215 bp to − 1 bp; the “A” in “ATG” of *ZmPP2C26* was designated as + 1,) were amplified from 2175 bp promoter using the specific primers (Table 1) that were designed according to the position of *cis*-acting elements via CE Design V1.04 software. To generate *promoter::GUS* constructs, the PCR products were inserted into the *Xba* I/*Hind* III site of the pRI201-*GUS* plasmid to replace *35S* promoter using ClonExpress II One Step Cloning Kit (Vazyme, Nanjing). To generate *promoter::LUC* constructs, the PCR products were inserted into the *Kpn* I/*Hind* III site of pGreenII0800-*LUC* plasmid to drive *LUC* gene. In the pGreenII0800-*LUC* plasmid, there is a Renilla luciferase gene (*REN*) and *35S* promoter, which was used as reference for LUC activity test.

The reconstructed plasmids were transformed into *Agrobacterium tumefaciens* GV3101 strain by freeze and thaw method, and used for tobacco transformation. The pRI201-*GUS* and pGreenII0800-*LUC* vector both containing *35S* promoter was used as positive control for GUS and LUC activity analysis, respectively.

Tobacco transformation and stress treatment

The leaves of 7-weeks-old tobacco seedlings were used for *Agrobacterium* infection according the methods described by Yu et al. (2019). The GV3101 strains with recombinant plasmids were cultured overnight at 28 °C until OD_{600} reached at 0.5–0.7 using LB liquid medium containing 50 mg/L rifampicin and 50 mg/L kanamycin. The *Agro*-cells were centrifugated for 5 min at 8000 r/min and resuspended in the transformation buffer containing 10 mM MES, pH 5.6, 10 mM $MgCl_2$, and 100 µM acetosyringone, and used for infiltration of tobacco leaf abaxial surfaces. After *Agro*-infiltration, the plants were cultured 36–48 h at 25 °C. For drought stress, the infiltrated leaves were incubated in the liquid 1/2 MS medium supplemented with 20% (w/v) PEG-6000 for 2 h and 4 h. The infiltrated leaves and wild type leaves incubated in the liquid 1/2 MS medium without PEG were used as control. The leaves infiltrated by *Agro*-cells were sampled and used for further study.

Measurement of GUS activity

Histochemical GUS staining was performed by using GUS staining Kit (Coolaber, Beijing). Subsequently, the GUS activity was measured as described by Jefferson et al. (1987) and Hou et al. (2016) with minor modification. The leaves were sampled and ground in liquid nitrogen. The 100 mg ground powder was transferred into 1.5 ml tubes, suspended with 400 µL extraction buffer containing 50 mM sodium phosphate (pH 7.2), 0.1% Triton X-100, 0.1% 2-Hydroxy-1-ethanethiol, 10 mM EDTA (pH 8.0) and 1 mg/mL SDS, and centrifuged for 10 min at 12,000 r/min. The supernatant was transferred to 1 mM 4-methylumbelliferyl-β-glucuronide (4-MUG) (Solarbio, Beijing)

buffer at 37 °C for 4 h. The fluorescence was measured by using fluorescence spectrophotometer (Fluoroskan Ascent FL, Thermo, USA) at the excitation and emission wavelengths of 365 and 455 nm, respectively. Protein concentration was determined as described by Bradford (1976). The GUS activity was normalized with six MU standards (10 nM, 20 nM, 40 nM, 60 nM, 80 nM, 100 nM) under control conditions and calculated as nmol of 4-MU per mg protein per minute.

Measurement of LUC activity

The LUC activity was measured by using Dual Luciferase Reporter Gene Assay Kit (Beyontime, Shanghai). The 100 mg ground powder was suspended with 200 µL lysis buffer and centrifuged at 15,000 r/min for 5 min. The supernatant was transferred into 100 µL firefly luciferase detection reagent and used for LUC measurement on a multifunctional microplate reader. Subsequently, the 100 µL of REN test working solution was added into the above supernatant, which was used to determine REN activity. The relative activity of LUC was calculated as LUC/REN.

Result

Expression of *ZmPP2C26* was inhibited by PEG treatment

Gene expression patterns can provide important information for gene function. The *ZmPP2C26* expression in maize inbred lines 87-1 under PEG treatment was analyzed using qRT-PCR. The results showed that the expression of *ZmPP2C26* in maize shoot and root was significantly down-regulated by PEG-6000 treatment, and reached a minimum value at 12 h and 9 h of treatment, respectively (Fig. 1). The finding indicates that *ZmPP2C26* genes promoter may be inhibited by some factors under drought stress to down-regulate its expression.

Sequence and *cis*-elements of *ZmPP2C26* promoter

Based on the annotation information of maize genome, the 2175 bp promoter sequence of *ZmPP2C26* upstream of the ATG was cloned from maize 87-1 genomic DNA, suggested as a putative full-length promoter, and used to identify *cis*-acting regulatory elements (Figs. 2 ,3). Apart from the eleven copies of CAAT-box and seven copies of constitutive core elements TATA-box, in the 2175 bp sequence, twelve kinds of putative *cis*-acting elements including A-Box, ABRE, ABRE4, Box4, CGTCA-motif, DRE1, G-Box, GC-motif, MBS, MYB and Sp1 were identified in the 2175 bp sequence (Table 2).

Construction of *promoter::GUS* and *promoter::LUC* vectors

The specific primers (PF0/PR, PF1/PR, PF2/PR, PF3/PR) were used to amplified the 2175 bp promoter and three 5′-end deleted fragment (1505, 1084 and 215 bp) of *ZmPP2C26* from maize 87-1 genomic DNA (Fig. 2). As a result, these fragments were specifically amplified and perfectly matched to the reference sequence from maizeGDB, and named as P_{2175}, P_{1505}, P_{1084} and P_{215}, respectively (Fig. 3). Subsequently, these fragments were cloned into pRI201-*GUS* and pGreenII 0800-*LUC* plasmid to generate P_{2175}-GUS, P_{1505}-GUS, P_{1084}-GUS, P_{215}-GUS, P_{2175}-LUC, P_{1505}-LUC, P_{1084}-LUC and P_{215}-LUC plasmid, respectively (Fig. 4).

The − 1084 to − 215 bp is key region for expression-inhibition

The result of GUS staining showed that the leaf infiltrated with *Agrobacterium* containing reconstructed constructs exhibited blue color, which was similar to positive control that infiltrated with *35S-GUS* (Fig. 5a), indicating that these promoters could drive the gene expression in tobacco leaves. Meanwhile, the GUS and LUC activity assays exhibited that four promoters with different length could

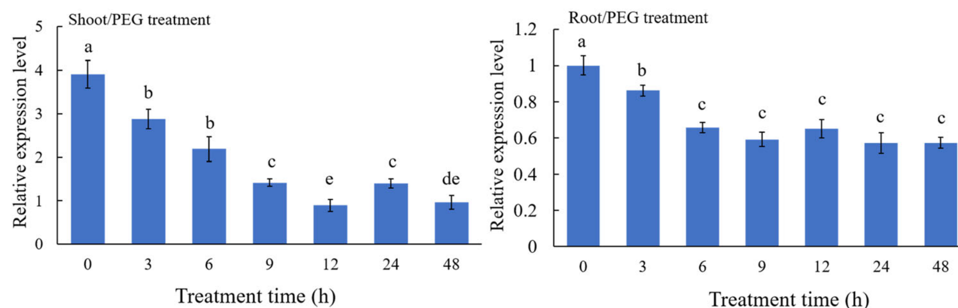

Fig. 1 Expression pattern of *ZmPP2C26* gene. Three-leaf stage of 87-1 seedlings were exposed to water solution supplemented with 16% PEG-6000. Data represent mean ± SD from three biological replicates (n = 3). Different lowercase letters indicate significant differences at $P < 0.05$

Fig. 2 The sequence of *ZmPP2C26* promoter. The ''A'' in ''ATG'' of *ZmPP2C26* gene is designated as ''+ 1''. The predicted *cis*-acting elements are shown in red color with underline. The core elements CAAT and TATA box are shown in dark grey and white grey background, respectively. The detailed description of elements is listed in Table 2. The black arrow under the sequence indicates the position of primers to amplify different deletion fragments

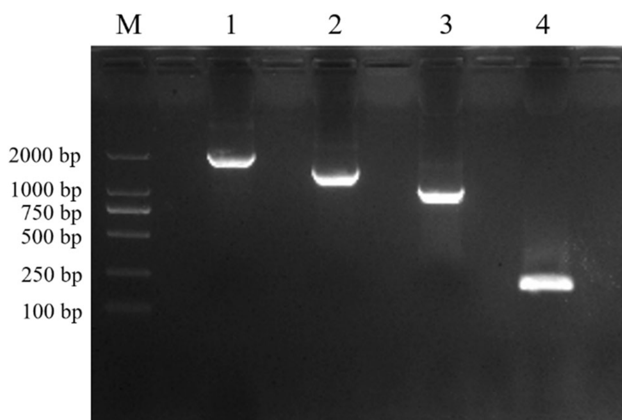

Fig. 3 Specific fragments of *ZmPP2C26* promoter and three 5′-end deletion fragments amplified by PCR. M: DNA 2000 bp marker. Lanes 1, 2, 3 and 4 represents 2175, 1505, 1084 and 215 bp promoter, respectively and 1.5% agarose gel separation

drive *GUS* and *LUC* expression in different level. However, the activities of GUS and LUC driven by P_{2175}, P_{1505} and P_{215} were stronger than that of P_{1084}. Interestingly, P_{215} showed highest driven-ability among these promoters. The GUS and LUC activities driven by P_{215} were corresponds to 9.4 and 12.9 folds to P_{1084}, respectively (Fig. 5b, c). These results suggest that the sequence of − 1084 to − 215 bp is key region to inhibit *ZmPP2C26* expression. It's also concluded that the − 215 bp sequence may be the core functional region of *ZmPP2C26* promoter, which contains one cope of GC-motif element, ABRE element, DRE1 element and core element TATA-box (Fig. 2).

The driven-activity of *ZmPP2C26* promoter was inhibited by drought stress

Our previous study showed that *ZmPP2C26* negatively regulated drought tolerance in *Arabidopsis* (Zhang et al. 2018). In order to investigate whether *ZmPP2C26* promoter is involved in drought stress, the tobacco leaves infiltrated with *Agrobacterium* were subjected to 20% PEG-6000. As shown in Fig. 6, the activity of GUS and LUC driven by *35S* promoter showed no significant difference after PEG-6000 treatment. However, at 2 and 4 h of PEG-6000 treatment, the activity of GUS and LUC driven by P_{2175}, P_{1084}, P_{1505} and P_{215} was significantly reduced compared to control (0 h). Meanwhile, P_{215} still showed highest driven-ability among these promoters, which was also observed before treatment. Notably, the sequence of − 1084 to − 215 bp contains one MBS elements that is MYB transcription factor binding site involved in drought-response (Fig. 2). Therefore, we speculate that the − 1084 to − 215 bp region of *ZmPP2C26* promoter may recruit potential transcription factors to inhibit *ZmPP2C26* expression under drought stress and negatively regulate drought tolerance in maize.

Discussion

Protein phosphorylation or dephosphorylation plays pivotal roles in various of signaling cascade and functional modification of transport proteins in plants (He et al. 2019; Lee

Table 2 The *cis*-acting elements of *ZmPP2C26* gene promoter

Cis element	Number	Description
AAGAG-motif	1	Mediate the regulation of gene expression in signal transduction pathway
A-Box	2	*cis*-acting regulatory element
ABRE	1	*cis*-acting element involved in abscisic acid responsiveness
ABRE4	1	*cis*-acting element involved in abscisic acid responsiveness
Box4	4	Part of a conserved DNA module involved in light responsiveness
CAAT-Box	11	Common *cis*-acting element in promoter and enhancer regions
CGTCA-motif	3	*cis*-acting regulatory element involved in the MeJA-responsiveness
DRE1	1	Dehydration-responsive element
G-Box	1	*cis*-acting regulatory element involved in light responsiveness
GC-motif	2	Enhancer-like element involved in anoxic specific inducibility
MBS	1	MYB binding site involved in drought response
MYB	1	MYB *cis*-acting element
Sp1	2	Light responsive element
TATA-Box	7	Core promoter element around − 30 of transcription start

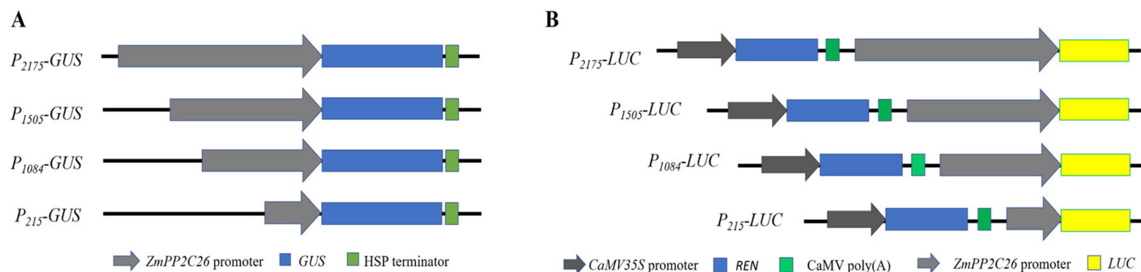

Fig. 4 The constructs of promoter deletions of *ZmPP2C26*. **a** Diagram of constructs of *promoter::GUS*. **b** Diagram of constructs of *promoter::LUC*. The Renilla luciferase (REN) driven by 35S promoter was used as reference for firefly luciferase (LUC) activity test. The number indicates the length of promoter

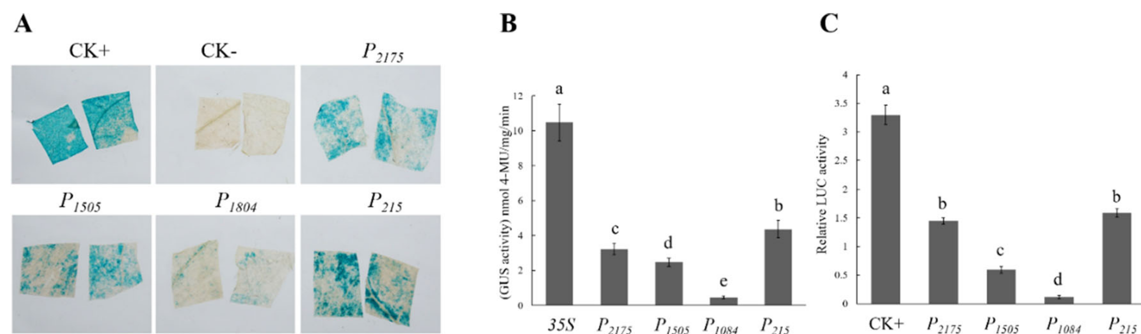

Fig. 5 The promoter activity during transient expression in tobacco leaves. P_{2175}, P_{1505}, P_{1084} and P_{215} indicate different length promoter. **a** GUS staining. CK+: infiltrated with *35S-GUS* plasmid as positive control; CK−: un-infiltrated wild type. **b** Fluorometric GUS analysis. **c** Detection of LUC activity. All values are means (± SE) of three biological replicates. Different lowercase letters indicate significant differences at $P < 0.05$

et al. 2009; Singh et al. 2018). PP2Cs catalyze protein dephosphorylation, which is important for plants growth, development, and stress response. The clade A members of PP2C are key factors in the ABA signaling pathway (Cheng et al. 1999; Yang et al. 2001). The PP2C activity is inhibited by binding ABA and its direct receptor PYL, which negatively regulates ABA signaling. (Lu et al. 2017; Née et al. 2017; Nishimura et al. 2018; Xiang et al. 2017). In our previous study, we found that *ZmPP2C26*, a member of clade B, negatively regulate drought tolerance in transgenic *Arabidopsis* (Zhang et al. 2018). Therefore, it is proposed that the upstream promoter of *ZmPP2C26* may play a crucial role in drought response in maize. To uncover the hypothesis, the *ZmPP2C26* promoter was cloned and evaluated for its driven-ability under drought stress.

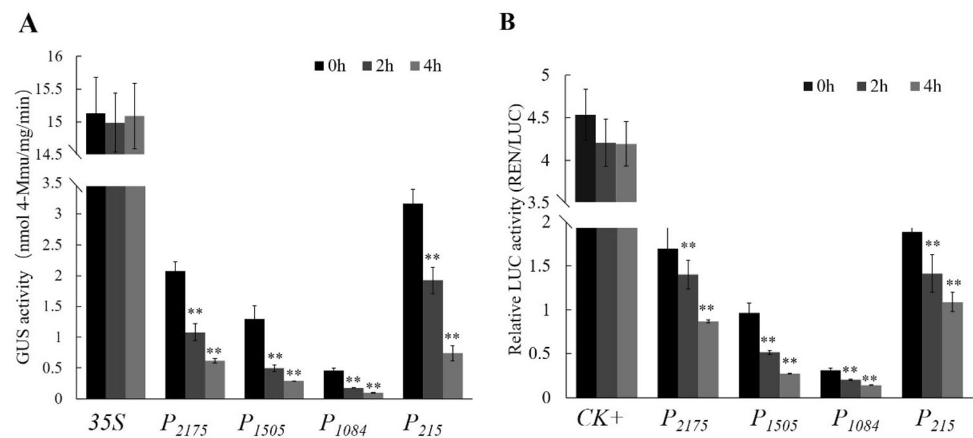

Fig. 6 Quantitative analysis of GUS and LUC activities under PEG-6000 treatment. **a** GUS activity. **b** LUC activity. The tobacco leaves were incubated in the liquid 1/2 MS medium supplemented with 20% (w/v) PEG-6000 for 2 and 4 h. The leaves incubated in the liquid 1/2MS medium were used as control. Values represent the mean ± standard deviation among replicates. $^*P < 0.05$, $^{**}P < 0.01$

Under drought stress, the expression of *ZmPP2C26* in root and shoot of maize seedlings was significantly down-regulated (Fig. 1), indicating that its promoter may be inhibited by drought stress. The finding further facilitates us to investigate the function of *ZmPP2C26* promoter. As well known, promoter possesses *cis*-acting elements to drive gene expression (Connors et al. 2002; Yu et al. 2019). In this study, *ZmPP2C26* promoter region contains 11 copies of CAAT-box and 7 copies of TATA-box, which are core elements for promoters. Meanwhile, some environmental stimuli responsive elements are found in the *ZmPP2C26* promoter, including MBS (related to drought stress), ABRE and ABRE4 (abscisic acid responsive elements), Box4, G-Box and Sp1 (light responsive elements), and CGTCA-motif (MeJA-responsive elements), which may contribute to the resilience of *ZmPP2C26* gene to stress response.

The analysis of 5′-end truncated fragments revealed that the sequence of − 215 bp is the key promoter region for *ZmPP2C26* expression (Fig. 5 and 6). The TATA-box in this region may be a crucial element for the promoter (Fig. 2). Moreover, the P_{1084} promoter showed lowest activity and P_{215} promoter activity was significantly higher than P_{1505} (Fig. 5 and 6). We found that there was an MBS element (MYB binding site) and no other *cis*-acting element related to drought stress in the − 1084 to − 215 region (Fig. 2). MYBs, an abundant kinds of transcription factors in plants, are found to regulate the plant tolerance to drought stress (Shan et al. 2012; Wu et al. 2019; Zhao et al. 2019). Therefore, we speculate that the sequence of − 1084 to − 215 bp fragment may be a key part for expression-inhibition under drought stress, and some MYBs may bind to this site to inhibit the *ZmPP2C26* expression under drought stress. In addition to MBS, ABRE (ABA-responsive element, − 1505 to − 1084 bp), ABRE4 (− 215 to − 1 bp) and DRE1 (dehydration-responsive element, − 215 to − 1 bp) in the *ZmPP2C26* promoter bonded by AREB/ABF or DREB transcription factors may also contribute for drought response through binding (Sharma et al. 2019; Takuya et al. 2010; Yoshihiro et al. 2003). Although they are not confirmed to negatively regulate *ZmPP2C26* expression under drought stress, these elements are candidates for further validation.

In summary, the − 215 bp length is enough to drive *ZmPP2C26* expression. Besides, the − 1084 to − 215 fragment of *ZmPP2C26* promoter may recruit potential transcription factors to inhibit *ZmPP2C26* expression under drought stress and negatively regulate drought tolerance in maize. These findings will provide insights into understanding the molecular mechanism of *ZmPP2C26* regulating drought tolerance.

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