Isolation and identification of a vegetative organ-specific promoter from maize

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Received: 3 March 2018 / Revised: 28 April 2018 / Accepted: 3 May 2018 / Published online: 4 June 2018
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Abstract To avoid the unregulated overexpression of the exogenous genes, specific or inducible expression is necessary for some exogenous genes in transgenic plants. But little is known about organ- or tissue-specific promoters in maize. In the present study, the expression of a maize pentatricopeptide repeat (PPR) protein encoding gene, *GRMZM2G129783*, was analyzed by RNA-sequencing data and confirmed by quantitative real time PCR. The results showed that the *PPR_{GRMZM2G129783}* gene specifically expressed in vegetative organs. Consequently, a 1830 bp sequence upstream of the start codon of the promoter for *GRMZM2G129783* gene was isolated from maize genome (*P₁₈₃₀*). To validate whether the promoter possesses the vegetative organ-specificity, the full-length and three 5'-end deletion fragments of *P₁₈₃₀* of different length (1387, 437, and 146 bp) were fused with glucuronidase (GUS) gene to generate *promoter::GUS* constructs and transformed into tobacco. The transient expression and fluorometric GUS assay in transgenic tobacco showed that all promoter could drive the expression of the *GUS* gene, the – 437 to – 146 bp region possessed some crucial elements for root-specific expression, and the shortest and optimal sequence to maintain transcription activity was 146

and 437 bp in length, respectively. These results indicate that the promoter of the *PPR_{GRMZM2G129783}* gene is a vegetative organ-specific promoter and will be useful in transgenic modification of commercial crops for moderate specific expression after further evaluation in monocotyledons.

Keywords Maize · Pentatricopeptide repeat protein · Promoter · Vegetative organ specificity

Introduction

Transgenic technology has emerged as an effective way for improvement of plant properties, which is difficult to be improved in conventional breeding. However, the uncontrolled and unregulated overexpression of the exogenous genes in all tissues during the whole growth stage is not only unnecessary, but also consumes too much matter and energy within the cells (Hsieh et al. 2002; Karim et al. 2007; Xu et al. 2006; Zhou et al. 2013). To date, the *CaMV35S* and maize ubiquitin promoter are widely used in transgenic modification for dicots and monocots transformation, respectively. As constitutive promoters, however, they drive the transgene expression in all tissues at all developmental stages and frequently cause the growth retardation in plants (Cornejo et al. 1993; Han et al. 2015; Karim et al. 2007; Odell et al. 1985; Zhao et al. 2007).

To eliminate the strong expression of transgene in all tissues, some exogenous genes are suggested to be expressed only in vegetative organs of transgenic crops. This heavily depends on an appropriate promoter that drives the exogenous genes expression at the desired level or tissue and plays pivotal roles in efficient production of transgenic plants (Han et al. 2015; Tao et al. 2015).

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Therefore, discovery and function validation of specific or inducible promoter is necessary for plant genetic transformation. For example, green-tissue specific promoter *prbcS* (promoter of ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit gene) and leaf-/stem-specific promoter *pST-LSI* (promoter of 10 kDa protein in the oxygen-evolving complex of photosystem II gene) were successfully used to drive the expression of the *Bt* gene and confer insect resistance to transgenic tobacco and potato (Christov et al. 1999; Naimov et al. 2003; Zaidi et al. 2005). Moreover, many tissue-specific and inducible promoters have been identified, such as seed-specific promoter (Pan et al. 2015), pollen-specific promoter (Wang et al. 2017), drought-, salinity- or pathogen-inducible promoters (Hou et al. 2016; Li et al. 2017; Tavakol et al. 2014; Zhang et al. 2016). However, the knowledge of tissue-specific promoter in maize is still limited.

The pentatricopeptide repeat (PPR) proteins are distinguished by the presences of a 35-amino acid tandem degenerate motif (Li et al. 2012; Lurin et al. 2004; Mingler et al. 2006; O'Toole et al. 2008). PPRs constitute an extraordinarily large family in the plant kingdom and are composed of more than 400 members in vascular plants. For example, 450 members from *Arabidopsis* and 477 members from rice (O'Toole et al. 2008), 521 members from maize (Wei and Han 2016) and more than 800 members from *Selaginella* (Banks et al. 2011) were predicted. PPRs were described as specific RNA-binding proteins and participated in the RNA metabolism of genes in the mitochondria or chloroplasts (Andrés-Colás et al. 2017; Dai et al. 2018; Filipovska and Rackham 2013; Guillaumot et al. 2017; Miranda et al. 2017; Zhang et al. 2017). Recent studies reported that PPRs also played key roles in plant growth and development, such as seed and pollen development (Dai et al. 2018; Liu et al. 2017a; Zhang et al. 2017), male sterility (Liu et al. 2017b), as well as stress tolerance (Jiang et al. 2015; Lv et al. 2014; Tan et al. 2014). However, the function of the *PPR* genes promoter remains obscure. In our previous study, we have found that a member of maize *PPR* gene family, registered as *GRMZM2G129783* in MaizeGDB database (<http://archive.maizegdb.org/>), showed vegetative organ-specific expression. It is hypothesized that the promoter of the *PPR_{GRMZM2G129783}* gene is a tissue-specific promoter. Hence, isolation and identification of the *PPR_{GRMZM2G129783}* promoter will provide insights into understanding the transcriptional regulation of maize *PPR* genes and the promoter resources for plant transgenic modified breeding.

Materials and methods

Expression analysis of the *PPR_{GRMZM2G129783}* gene

The expression data of the *PPR_{GRMZM2G129783}* gene in root, leaf, stem, tassel, anther, silk, seed, endosperm, embryo, and pericarp of different development stages published by Stelpflug et al. (2016) based on RNA-sequencing (RNA-seq) was obtained from the MaizeGDB database and used for tissue specific expression analysis. Subsequently, the quantitative real time PCR (qRT-PCR) was performed to analyze the expression of the *PPR_{GRMZM2G129783}* gene. The seeds of maize inbred line B73 were germinated in petri dish and 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. At the three-leaf stage, the leaves, stem and roots from the seedlings of same size were sampled and ground in liquid nitrogen, separately, with three replications. Total RNA was extracted by using RNAiso plus kit (TaKaRa, Japan), removed probable genomic DNA by RNase-free DNase, quantified by NanoDrop ONE (ThermoScientific, USA), and used for synthesizing the cDNA by using a PrimeScript™ reagent kit (TaKaRa, Japan). A pair of specific primers (q1s/q1r) of the *PPR_{GRMZM2G129783}* gene and a pair of specific primers (q2s/q2r) of the internal reference gene, *ZmActin*, were designed by the Primer-BLAST software (<http://www.ncbi.nlm.nih.gov/tools/primer-blast>) and synthesized at Invitrogen (USA) (Table 1). The qRT-PCR was conducted using SYBR® Green (Takara, Japan) on CFX96™ Real-Time System (Bio-Rad, USA). The two-step temperature cycle was as follows: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 61 °C (*PPR_{GRMZM2G129783}*) or 58 °C (*ZmActin*) for 30 s. At the end of the last cycle, the temperature was increased to 95 °C by 0.5 °C s⁻¹, so that a melting curve could be calculated and used to differentiate between specific and non-specific amplicons. The 2^{-ΔΔCT} method was used to normalize the expression differentiation between the *PPR_{GRMZM2G129783}* gene and the internal reference (Livak and Schmittgen 2001).

Isolation and sequence analysis of the *PPR_{GRMZM2G129783}* promoter

The chromosomal location of the *PPR_{GRMZM2G129783}* gene was analyzed based on the position information in the MaizeGDB database. The sequence of the 2000 bp 5' flanking to start codon (ATG) of the *PPR_{GRMZM2G129783}* gene was retrieved from the MaizeGDB and used for *cis*-acting elements analysis through online program PlantCARE (<http://www.plantcare.co.uk/>, Lescot et al. 2002).

Table 1 The primers used in the present study

Primer name	Sequence (5'–3')	Description
q1f	TACGTGCTCCTGTGCAACAT	qRT-PCR primer for the <i>PPR_{GRMZM2G129783}</i> gene
q1r	CCAGCCATGAACCTGTGGAT	
q2f	CTGAGGTTCTATTCCAGCCATCC	qRT-PCR primer for the <i>ZmActin</i> gene
q2r	CCACCACTGAGGACAACATTACC	
P1	CATGCATGCAGCGGAAGTGAATCAGACCG	Forward primer to amplify the 1830 bp promoter with primer Pr
P2	CATGCATGCCGAACAGTCACGGCAGC	Forward primer to amplify the 1387 bp promoter with primer Pr
P3	CATGCATGCGGAAAAACAAACGGAGCCTT	Forward primer to amplify the 437 bp promoter with primer Pr
P4	CATGCATGCTATCCATCCCTACCTAATC	Forward primer to amplify the 146 bp promoter with primer Pr
Pr	GCTCTAGACATTGAGCGGCGGAGGGGAT	Reverse primer for every promoter amplification
gf	GCTAACGTATCCACGCCGTA	PCR amplification of the <i>GUS</i> gene to identify transgenic line
gr	CATGAAGATGCGGACTTGCG	

The nucleotides underlined are restriction *Sph* I site (GCATGC) and *Xba* I site (TCTAGA)

Construction of promoter::*GUS* plasmids

To validate the function of the *PPR_{GRMZM2G129783}* promoter, the full length and three 5' deleted fragments of different length of the *PPR_{GRMZM2G129783}* promoter were amplified by PCR from the genomic DNA of the maize inbred line B73, using the primers listed in Table 1. The primers were designed according to the *cis*-acting elements position in the full-length region (Fig. 1). Every forward primer contained restriction enzyme *Sph*I site and reverse primer contained restriction enzyme *Xba* I site. To generate the *pPPR_{GRMZM2G129783}::GUS* constructs, the PCR products were cloned into the *Sph* I/*Xba* I site of the pRI201-*GUS* plasmid (TaKaRa, Japan) to replace the constitutive promoter *CaMV35S*. The destination vectors were

confirmed by double restriction digestion and sequencing. The pRI201-*GUS* vector containing *CaMV35S* promoter upstream from the *GUS* was used as a positive control (35S-*GUS*). The regenerated constructs and control plasmid were transformed into the *Agrobacterium tumefaciens* strain EHA105 by freeze and thaw method and used for the tobacco transformation.

Transient expression and stable transformation in tobacco

The GUS transient expression assay was performed as described by Zhang et al. (2016) with minor modification. *Agrobacterium* EHA105 containing either control or recombinant plasmid was cultured in YEP liquid medium

Fig. 1 Sequence of the *PPR_{GRMZM2G129783}* gene promoter. The “A” in “ATG” of the *PPR_{GRMZM2G129783}* gene is designated as “+ 1”. The predicted *cis*-acting elements are shown in red color with underline. The core elements TATA box and CAAT box are shown in dark grey and white grey background, respectively. The detailed description of elements is listed in Table 2. The green arrow under the sequence indicates the position of primers to amplify different deletion fragments

with 50 mg/L rifampicin and 50 mg/L kanamycin at 28 °C until OD₆₀₀ reached at 0.6. The cultures were centrifuged for 5 min at 8000 r/min to collect *agro*-cells. Subsequently, the *agro*-cells were re-suspended using transformation buffer (50 mM MES, pH 5.6, 2 mM Na₂PO₄, 0.5% glucose), and used for leaf infiltration. The abaxial surfaces of tobacco (*Nicotiana benthamiana*) leaves were infiltrated using needleless syringe with re-suspended *agro*-cells, cultured 2–3 days at 25 °C and subsequently these agro-infiltrated leaves were used for histochemical GUS staining.

To quantify promoter activity, the *P*₁₈₃₀-*GUS*, *P*₁₃₈₇-*GUS*, *P*₄₃₇-*GUS* and *P*₁₄₆-*GUS* plasmid, as well as 35S-*GUS* (positive control), were transformed into the tobacco variety Wisconsin 38 (*Nicotiana tabacum*). The seeds of Wisconsin 38 were surface-sterilized with 75% ethanol for 1 min, 5% NaClO for 15 min, and washed 4 times with sterile water. The sterile seeds were germinated on Murashige and Skoog (MS) medium at 25 °C, 16 h light/8 h dark for 4 weeks. The *Agrobacterium* transformation of tobacco leaf disks was conducted as described by Voelker et al. (1987). The leaf disks were placed into *Agrobacterium* liquid for 10 min, cultured 3 d in the dark and transferred to the MS medium supplemented with 1.0 mg/L 6-benzylaminopurine (6-BA) and 0.1 mg/L 1-naphthylacetic acid (NAA) plus 100 mg/L kanamycin for re-differentiation of plantlets (Fig. 2). The regenerated T₀ plants with kanamycin resistance were identified by PCR amplification of a 647 bp fragment of the *GUS* gene with primer gf/gr

(Table 1) and GUS staining, and transferred into soil, producing T₁ and T₂ seeds by bagged-self pollination. The homozygous lines with a single insertion of re-constructed plasmid were screened for further analysis through segregation ratio analysis.

Histochemical and fluorometric GUS analysis

Histochemical GUS staining was performed to test the expression of re-constructed plasmid as described by Hou et al. (2016) with minor modification. The samples were soaked in GUS staining buffer containing 50 mM sodium phosphate buffer (pH 7.2), 0.1% Triton X-100, 10 mM EDTA (pH 8.0), 2 mM potassium ferrocyanide, 2 mM potassium ferricyanide and 10 mg/mL X-Gluc (Sangon, China), and then incubated at 37 °C for overnight. Subsequently, the samples were decolorized in 75% ethanol and photoed. Fluorometric GUS assay was conducted to detect the GUS activity driven by 5' deleted promoter as described by Hou et al. (2016) and Zhang et al. (2016) with minor modification. Briefly, every 100 mg fresh sample was homogenized at 4 °C in extraction buffer containing 50 mM sodium phosphate buffer (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 then centrifuged 10 min at 12,000 r/min. The supernatant was used to measure the GUS activity using reaction buffer containing 1 mM 4-MUG (Sigma, USA) at 37 °C. After 4 h, the 0.2 mmol Na₂CO₃ was added into solution to terminate reaction. The

Fig. 2 The process of tobacco transformation using *Agrobacterium*-mediated leaf disk transformation. **a** Leaf disks culture after transformation; **b** callus production from the edge of leaf disk; **c** screening on the kanamycin medium; **d** differentiation and regeneration of plantlets; **e** root regeneration; **f** plants culture in soil

fluorescence was detected using fluorescence spectrophotometer at the wavelengths of 365 and 455 nm. Protein concentration in supernatant was measured as described by Bradford (1976). Finally, the GUS activity was normalized using 4-MUG standard and calculated per mg protein.

Statistical analysis

All data were presented as mean values $\pm$ SD (standard deviation), and the statistical significance among biological replicates was tested by the Student's *t* test with IBM-SPSS software (<http://www-01.ibm.com/software/analytics/spss/>).

Results

Expression pattern of *PPR_{GRMZM2G129783}* gene

From the RNA-seq data, it was found that the *PPR_{GRMZM2G129783}* gene dominantly expressed in leaf, highly expressed in the internode although weakly expressed in the whole seeds before 12 days after pollination (DAP), but there was no expression in roots, endosperm and embryo beginning at the 14 DAP (Fig. 3). The results of the qRT-PCR also showed the similar trend with RNA-seq result. The expression of the *PPR_{GRMZM2G129783}* gene in leaf reached more than 2.6 times than that in stem, whereas no expression was detected in roots (Fig. 4). These results suggested that the *PPR_{GRMZM2G129783}* promoter specifically drive gene expression in vegetative organs.

Bioinformatic analysis of the *PPR_{GRMZM2G129783}* promoter

The *PPR_{GRMZM2G129783}* gene was located on the maize chromosome no.6 from the 106466750 to the

106469829 bp. Based on the information from MaizeGDB database, the primers were screened and designed to amplify the full length of the *PPR_{GRMZM2G129783}* promoter. The 1830 bp upstream to ATG of the *PPR_{GRMZM2G129783}* gene was successfully amplified from genomic DNA, confirmed by sequencing and alignment against the reference sequence from MaizeGDB and named *P₁₈₃₀* (Figs. 1, 5). Apart from the twenty-three copies of constitutive core elements TATA box and thirteen copies of CAAT box, in the 1830 bp sequence, there were eleven copies of nine kinds of light-responsive *cis*-acting elements (one copy of CATT-motif, ATCT-motif, ATC-motif, GAG-motif, Box 4, Sp1, Box II, MNF1 and three copies of G-Box). Meanwhile, the promoter had two MeJA responsive elements (CGTCA-motif and TGACG-motif) and some abiotic stress elements (one copy of MBS, ARE and TC-rich repeats). Moreover, it also possessed one protein binding site (Box III), circadian control element and Skn-1 motif required for endosperm expression (Fig. 1; Table 2). Therefore, the 1830 bp full-length sequence was submitted to GenBank with an accession number KX665160.

Generation of constructs and *GUS* transient expression

Specific primers (P1/Pr, P2/Pr, P3/Pr, and P4/Pr) were designed, and used to amplify the full-length sequence (– 1830 bp), as well as the – 1387, – 437, and – 146 bp 5'-end deleted sequences of the *PPR_{GRMZM2G129783}* promoter (the “A” in “ATG” of the *PPR_{GRMZM2G129783}* gene designated as “+ 1”) from the genomic DNA (Fig. 1). As a result, specific fragments of – 1830, – 1387, – 437, and – 146 bp were amplified with the respective primers from the genomic DNA (Fig. 5). The sequencing results perfectly matched to the bioinformatics prediction. Therefore, these sequences were named as *P₁₈₃₀*, *P₁₃₈₇*, *P₄₃₇*, and *P₁₄₆*, respectively, and cloned into pRI201-*GUS* plasmid,

Fig. 3 Expression pattern of the *PPR_{GRMZM2G129783}* gene in different organs of maize, profiled using RNA-seq

Fig. 4 The expression pattern of the *PPR_{GRMZM2G129783}* gene in root, stem and leaf detected by qRT-PCR

Fig. 5 Specific fragments of 5'-end deletion promoter amplified by PCR from the genomic DNA. M: DNA2000 molecular marker; lanes 1, 2, 3, and 4: the 1830, 1387, 437, and 146 bp of the promoter

resulting in *P₁₈₃₀-GUS*, *P₁₃₈₇-GUS*, *P₄₃₇-GUS* and *P₁₄₆-GUS* plasmid (Fig. 6). The *GUS* transient expression showed that the leaf infiltrated with *Agrobacterium* containing constructs exhibited blue color after *GUS* staining, which was similar with positive control (infiltrated with *35S-GUS*). But there was no color change for negative control (wild type) (Fig. 7), suggesting that the promoter could drive the expression of the *GUS* gene in tobacco.

Identification of the transgenic tobacco

To identify the transgenic plants, a 647 bp fragment of the *GUS* gene was amplified from every plant using primer gf/gr (Table 1). The results exhibited that five, three, four, four, and six transgenic lines transformed by the expression vectors *P₁₈₃₀-GUS*, *P₁₃₈₇-GUS*, *P₄₃₇-GUS*, *P₁₄₆-GUS* and *35S-GUS* (positive control), respectively, were identified as transgenic lines (Fig. 8).

The – 437 to – 146 fragments is crucial for root-specific expression

The results of histochemical *GUS* staining showed that the – 1830, – 1387 and – 437 bp of the *PPR_{GRMZM2G129783}* promoter could drive the expression of the *GUS* gene in the root, stem and leaf, which was similar with the constitutive promoter *35S*. However, the – 146 bp promoter only drove the expression of the *GUS* gene in stem and leaf (Fig. 9a). The fluorometric *GUS* assay showed that the *GUS* activity in root, stem and leaf driven by the – 1830 bp promoter was lower than that driven by the *35S* promoter and – 1387 bp 5'-end deleted promoter, and the *GUS* activity driven by the – 437 bp promoter in the stem and leaf was higher than that driven by the – 1830 and – 146 bp promoter. Although the – 146 bp 5'-end deleted promoter kept low activity, there was detectable *GUS* accumulation in stem and leaf (Fig. 9b). These results indicate that the sequence of – 437 to – 146 possesses some crucial elements for root-specific expression.

Discussion

The PPRs function in the RNA metabolism, including RNA binding, cleavage, stabilization, splicing, and locate in the mitochondria or chloroplasts (Andrés-Colás et al. 2017; Dai et al. 2018; Filipovska and Rackham 2013; Guillaumot et al. 2017; Miranda et al. 2017; Zhang et al. 2017). PPRs are an extraordinarily large family and composed of more than 400 members in vascular plants (Banks et al. 2011; O'Toole et al. 2008; Wei and Han 2016), and play crucial roles in plant growth, development, and stress response (Dai et al. 2018; Jiang et al. 2015; Liu et al. 2017a; Liu et al. 2017b; Lv et al. 2014; Tan et al. 2014; Zhang et al. 2017). We found that the maize *PPR* gene, *GRMZM2G129783*, showed vegetative organ-specific

Fig. 6 Diagram of the 5'-end deletion promoter of *PPR_{GRMZM2G129783}* fused with the *GUS* gene. The number indicates the length of promoter

Fig. 7 GUS staining of leaves from transient expression tobacco. WT: un-infiltrated wild type; CK+: infiltrated with 35S-*GUS* plasmid as positive control; *P₁₈₃₀*, *P₁₃₈₇*, *P₄₃₇* and *P₁₄₆* indicate *P₁₈₃₀*-*GUS*, *P₁₃₈₇*-*GUS*, *P₄₃₇*-*GUS* and *P₁₄₆*-*GUS* plasmid, respectively

expression, which was confirmed by RNA-seq and qRT-PCR (Figs. 3, 4). The results suggest that the promoter of the *PPR_{GRMZM2G129783}* gene is an organ-specific promoter.

The promoter requires *cis* elements to drive the gene expression. The promoter length and composition of the *cis*-elements affect the gene expression. Usually, a promoter possesses core promoter elements, such as TATA- and CAAT-box, and other inducible or specific *cis*-acting elements. In the study, besides the abundance of TATA- and CAAT-box, there are lot of light responsive elements in the *PPR_{GRMZM2G129783}* promoter, including CATT-, ATCT-, ATC-, GAG-motif, Box 4, Sp1, Box II, MNF1 and G-box (Fig. 1; Table 2), which was found in many other green tissue-specific promoters (Eckes et al. 1986; Kyo-zuka et al. 1993; Kagaya et al. 1995). Together with the expression profiles from RNA-seq and qRT-PCR, we conclude that the promoter of the *PPR_{GRMZM2G129783}* gene

is a vegetative tissue-specific promoter. Likewise, it also includes some stress responsive elements, such as MBS involved in drought-inducibility and TC-rich repeats involved in defense and stress responsiveness, suggesting that the expression of the *PPR_{GRMZM2G129783}* gene may be induced by the stress. This speculation need to be elucidated in the further study.

The analysis of 5' truncated segments of the *PPR_{GRMZM2G129783}* gene revealed that the sequence of – 437 to – 146 bp was crucial for root expression (Fig. 9). However, only four types of *cis*-acting elements including GAG-motif, box 4, CAAT- and TATA-box were found in this region (Fig. 1). The TATA-box is core promoter element around transcription start site. The CAAT-box is common *cis*-acting element in the promoter and enhancer regions. The GAG-motif and box 4 are light responsive elements. Thus, it is speculated that the

Fig. 8 PCR identification of transformed plants by amplification of the 647 bp of the *GUS* gene fragment. **a**, **b**, **c**, **d** and **e** represent specific fragments amplified from the T_0 regenerated plants transformed with P_{1830} -*GUS*, P_{1387} -*GUS*, P_{437} -*GUS*, P_{146} -*GUS* and 35S-

GUS plasmid, respectively. M: DNA2000 molecular marker; WT: untransformed wild type plants used as negative control; lane CK +: the plasmid P_{1830} -*GUS* used as positive control; L1 to L22 indicate the independent transgenic line, respectively

Fig. 9 Histochemical and fluorometric *GUS* analysis of transgenic tobacco. The *GUS* staining (**a**) and *GUS* enzyme activity (**b**) in root, stem and leaf of transgenic tobacco. P_{1830} , P_{1387} , P_{437} and P_{146} indicate P_{1830} -*GUS*, P_{1387} -*GUS*, P_{437} -*GUS* and P_{146} -*GUS* plasmid, respectively. WT: untransformed wild type plants used as negative control; CK+: the plants transformed using 35S-*GUS* used as positive control

Table 2 Putative *cis*-elements of the *PPR_{GRMZM2G129783}* gene promoter

<i>Cis</i> element	Number	Description
CATT-motif	1	Light responsive
ATCT-motif	1	
ATC-motif	1	
GAG-motif	1	
Box 4	1	<i>cis</i> -acting regulatory element involved in the MeJA-responsiveness
Sp1	1	
Box II	1	
MNF1	1	
G-box	3	MYB binding site involved in drought-inducibility
CGTCA-motif	1	
TGACG-motif	1	
MBS	1	
Skn-1 motif	4	<i>cis</i> -acting regulatory element required for endosperm expression
ARE	2	
Box III	1	<i>cis</i> -acting regulatory element essential for the anaerobic induction
circadian	1	
TC-rich repeats	1	Protein binding site
CAAT-box	13	
TATA-box	23	<i>cis</i> -acting regulatory element involved in circadian control
		<i>cis</i> -acting element involved in defense and stress responsiveness
		Common <i>cis</i> -acting element in promoter and enhancer regions
		Core promoter element around – 30 of transcription start

The position of every *cis*-acting element is shown in Fig. 1

sequence between – 437 and – 146 bp may contain some unknown *cis*-acting elements that are crucial for root-specific expression. Moreover, the activity of the – 437 bp promoter is higher than that of the – 1830 and – 146 bp promoters and corresponds to about 30–50% of 35S promoter (Fig. 9). It is well known that the strong expression of exogenous genes might be harmful and unnecessary for plant growth and development, moderate expression of transgene could avoid the unregulated overexpression, and small promoter size was beneficial for transgenic modification in plants (Han et al. 2015; Karim et al. 2007; Xu et al. 2006; Zhou et al. 2013). Therefore, the – 437 bp of the *PPR_{GRMZM2G129783}* promoter is an optimal promoter for moderate vegetative organ-specific expression.

Conclusion

The promoter of the *PPR_{GRMZM2G129783}* gene is a vegetative organ-specific promoter. The – 146 bp of the promoter is sufficient to drive the gene expression. The sequence from – 437 to – 146 bp possesses some crucial elements for root-specific expression. The promoter is useful in transgenic modification of commercial crops for vegetative-specific expression after further evaluation in monocotyledons.

Acknowledgements This work was supported by the National Key Science and Technology Special Project (2016ZX08003004-005) and

the National Natural Science Foundation of China (31071433). The authors thank the technical support from Key Laboratory of Biology and Genetic Improvement of Maize in Southwest Region.

Compliance with ethical standards

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

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