Transcription factor ZmPLATZ2 positively regulate the starch synthesis in maize

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Abstract

Maize is one of the three major crops worldwide based on its yield and quality. Starch is crucial to both the yield and quality of maize as it accounts more than 60% of the seed weight, and its structure influences the quality of the crop. Starch synthase I (SSI) contributes to the majority of the starch synthase activity in the maize endosperm. An in-depth understanding of the starch synthesis regulatory mechanism would provide opportunities for improving the yield and quality of maize. In this study, *ZmPLATZ2*, a plant AT-rich sequence and zinc-binding protein (PLATZ) transcription factor related to starch synthesis, was selected based on co-expression analysis. The semiquantitative RT-PCR and qRT-PCR assays revealed that *ZmPLATZ2* had a high expression in the endosperm, and reached the peak at 12 days after pollination (DAP). Different treatments demonstrated that *ZmPLATZ2* was downregulated by the presence of sucrose. Subsequent transactivation and subcellular localization analyses showed that *ZmPLATZ2* was localized in the nuclei without transactivation. Yeast one-hybrid and transient expression in maize endosperm indicated that *ZmPLATZ2* could bind to the promoters of *ZmSSI*, *ZmISA1*, and *ZmISA2* and increase their gene expression. After *ZmPLATZ2* overexpression in rice, four starch synthesis genes were significantly upregulated in the transgenic plant, including the *OsSSI* gene. In vitro DAP-seq data showed that *ZmPLATZ2* could bind to the CAAAAAAA element. In conclusion, our data support that *ZmPLATZ2* binds to the CAAAAAAA element in the *ZmSSI* promoter and mediates the Glu signal pathway.

Keywords Transcription factor · Starch · Maize

Abbreviations

AGPase Adenosine diphosphate glucose pyrophosphorylase
 DAP Days after pollination

DAP-qPCR DNA affinity purification sequencing-quantitative real-time polymerase chain reaction
 DAP-seq DNA affinity purification sequencing
 DBE Starch debranching enzyme
 DEGs Differentially expressed genes
 GO Gene ontology
 MS Murashige and Skoog medium
 SBE Starch branching enzyme

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SS	Starch synthase
TFs	Transcription factors
TG	Transgenic
WT	Wide-type

Introduction

Maize (*Zea mays*) is an important crop that contributes to solving the food security problem (Godfray et al. 2010). Starch accounts for more than half the weight of the seed; therefore, increasing the starch content is an essential measure for improving the yield. Nevertheless, the mechanism of starch biosynthesis is unclear as it includes a series of complex metabolic processes. Four types of enzymes are involved in the starch biosynthesis reaction: adenosine diphosphate glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) (Leterrier et al. 2008; Zhou et al. 2016; Hennen-Bierwagen et al. 2008). AGPase is the first key enzyme in starch synthesis that involved in ADP-glucose production (Huang et al. 2014; Li et al. 2018). SS mainly participates in amylopectin synthesis. *IbSSI*, a soluble starch synthase I gene, is involved in the synthesis of amylopectin chains and alters the content and structure of starch in the sweet potato (Wang et al. 2017). SBE is an essential factor in generate different heteromorphous starch granules (Wang et al. 2018a, b). The maize *sbe2b* (amylose-extender, ae) mutant shown that the amylose and resistant starch content increase 60% and 40% respectively (Li et al. 2008). DBE is responsible for generated linear starch fraction (Reddy et al. 2018). These four enzymes, during the seed filling period, influence the starch content and accumulation rate.

Transcription factors (TFs) regulate the expression patterns of target genes by binding to the *cis*-elements and play a vital role in starch synthesis. One example is that of ZmMYB14, which directly binds to the *Bt1* promoter through a TAACTG motif of promote *BT1* expression (Xiao et al. 2017). ZmABI4 binds to the CACCG sites of the *ZmSSI* promoter to mediate the ABA signal pathway (Hu et al. 2012). *ZmEREB94* and *ZmEREB156* are induced by sucrose and ABA, and are involved in the starch biosynthetic and metabolic pathways (Li et al. 2017b; Huang et al. 2016).

Nowadays, several TFs appear at one branch of the evolution tree. For example, the nuclear receptor family is absent in plants and yeast but is found in animals (Bennett et al. 2017), whereas the homeobox family has been found in animals and plants but not in yeast. The plant AT-rich sequence and zinc-binding protein (PLATZ) TF family is present only in plants and was first found in peas (Nagano et al. 2001). Previous studies of the PLATZ family are rare. However, it was recently established that PLATZ1 is significant in

seed germination and seedling establishment and mediates the ABA, GA, and ethylene signaling pathways in cotton (Zhang et al. 2018). In addition, ORESARA15, a number of the PLATZ TF family, functions as a coordinator in leaf growth and senescence in *Arabidopsis* (Kim et al. 2018). The PLATZ TF family is also involved in grain development. The rice GL6, belonging to the PLATZ family, participates in rice grain development by interacting with C53 to negatively regulate grain numbers (Wang et al. 2019). Mutation in *ZmPLATZ12* results in a floury endosperm, which indicates that *ZmPLATZ12* plays a crucial role in endosperm development and storage reserve filling (Li et al. 2017a).

In previous study, we identified a gene, *GRMZM2G31165*, via co-expression analysis. This gene encoded a TF containing a PLATZ domain, named ZmPLATZ2. The assays used determined that ZmPLATZ2 was able to bind to the *SSI*, *SSIIa*, *SSIV*, *SSV*, *ISA1*, and *ISA2* promoters. In addition, ZmPLATZ2 increased the promoter activities of *Sh2*, *Bt2*, *GBSSI*, *SSI*, and *SSIIIa*. Meanwhile, we found that ZmPLATZ2 expression was stimulated when treated with glucose. In addition, the transcription factor identified in our study could regulate maize starch synthesis. Our study will contribute to elucidate the starch synthesis mechanism and improve yield by molecular technology.

Materials and methods

Co-expression analysis of the *ZmPLATZ2* gene and starch synthesis related gene

Co-expression analysis of the data that include seven different period endosperms from Stelpflug et al. (2016) was used to select a candidate gene (GRMZM2G311656) coding a PLATZ protein. Twenty-seven maize starch synthesis related genes, which possessed a high expression in maize seed, were considered as the guide genes (Crofts et al. 2017). The Pearson correlation coefficient (PCC) between ZmPLATZ2 transcription factor and starch synthesis related genes were calculated by excel, and cytoscape_3.7.2 was use to display the co-expression analysis results.

Plant materials

Maize (*Z. mays*) inbred line 18-599 was grown during the summer field season at the Sichuan Agricultural University Farm. Developing kernels from self-pollinated plants were collected at specified days after pollination (DAP) for quantitative real-time PCR. The roots, stems, leaves, embryos, endosperms, and seeds were harvested at 15 DAP for semi-quantitative RT-PCR. All samples were frozen in liquid N₂ and stored at −80 °C for further use. Endosperms that used

in transient expression and ABA, sucrose and glucose treatment were harvested 9 or 10 DAP, as described previously (Xiao et al. 2017).

Transcript level measurement by RT-PCR and semiquantitative RT-PCR

Total RNA was extracted from the developing kernels by using TRIzol (Invitrogen, China) and RNase-free DNase to remove the genomic DNA. The 2 ng DNase treated RNA was converted into cDNA using reverse transcription PCR (Takara, Dalian, China). The transcription level of *ZmPLATZ2* in various tissues was measured by semiquantitative RT-PCR and the temporal expression of *ZmPLATZ2* in the seed was analyzed by qRT-PCR. For RT-PCR, the reaction mixture was composed of cDNA first-strand template, primer mix, and SYBR Green mix (Takara, Dalian, China). *ZmACTIN* was used as the internal control. Gene specific primers sets used for qRT-PCR are list in Supplementary Table S1.

Gene cloning and bioinformatics analysis of *ZmPLATZ2*

ZmPLATZ2 was cloned from the maize inbred line Mo17 based on the B73 reference genome using a primer designed by Primer 5 software. The *ZmPLATZ2* gene comprised 930 base pairs (bp) and encodes 309 amino acids. SMART (<http://smart.embl-heidelberg.de/>) analysis was used to predict the protein domain. PSIPRED analysis (<http://bioinf.cs.ucl.ac.uk/psipred/>) was used to predict the secondary domain. Primers were list in Supplementary Table S1.

Influence of ABA, sucrose, and glucose treatment on the expression level of *ZmPLATZ2*

At 9–10 DAP, the maize kernels were surface sterilized with 75% ethanol and split in half under sterile conditions. Murashige and Skoog (MS) liquid medium was used to analyze *ZmPLATZ2* expression in the maize endosperm in response to sucrose (200 μ M), ABA (100 μ M), glucose (200 μ M), and 100 μ M ABA + 200 μ M sucrose, as described previously (Li et al. 2017b; Acevedo-Hernández et al. 2005). The treated samples were harvested at different times post-exposure: 0.5, 1, 3, 6, 12, 24, and 48 h, and *ZmPLATZ2* expression was analyzed using qRT-PCR.

Subcellular localization

The full-length ORF of *ZmPLATZ2* was cloned into the transient expression vector p2300-eGFP at the *Bam*HI/*Xba*I site using a ClonExpress II one-step cloning kit (Vazyme, Nanjing, China). Onion epidermal cells were peeled and cultured on Hypertonic MS medium for 4 h. A 0.9 μ g sample of plasmid per vector was used to coat the gold powder, which was bombarded into the cells by a helium biolistic gun transformation system. The bombarded samples were then cultured for 24 to 48 h at 28 °C, as described in a previous study (Zhang et al. 2016). The samples were observed under BX61 fluorescent microscopy. The p2300-eGFP vector was used as the control. Primers were list in Supplementary Table S1.

Transient expression assays in maize endosperm

For the qualitative transactivation assay, the test vector was generated by inserting the starch synthesis promoter into the *Pst*I and *Bam*HI sites of the pBI221 vector. The ubi-*ZmPLATZ2* effector construct was created by cloning its coding sequences into the *Bam*HI and *Sac*I sites. Gus, driven by a ubiquitin promoter, was used as the internal control. For analysis of the promoter activity in the endosperm, the test construct and internal control plasmid were combined at a molar ratio of 2:1. For analysis of the effect of *ZmPLATZ2* on the expression of different types of starch synthesis, the molar ratio of the test construct, effector construct, and internal control plasmid was 2:1:1. Endosperm at 9 or 10 DAP were sterilized with 75% (v/v) ethanol and cultivated on MS medium containing 8.5‰ agar and 12% sucrose for 4 h prior to bombardment using a helium biolistic gun transformation system. The bombarded endosperms were cultivated for 24 h to analyze LUC and GUS reporter gene expression. The independent experiments consisting of three replicates were conducted. Primers were list in Supplementary Table S1.

Transcription activation

The yeast transactivation assay, using Yeast two-hybrid system, was performed as described in a previous study (Chen et al. 2016). The full-length ORF of *ZmPLATZ2* was cloned into a DNA-binding domain vector (pGBKT7) with *Nde*I and *Bam*HI sites and transformed into yeast AH109, harboring the *LacZ* and *Trp* reporter genes. The empty vector, pGBKT7 was used as the negative control. Primers were list in Supplementary Table S1.

Generation of rice transgenic plants

The ORF of *ZmPLATZ2* was cloned into an overexpression vector, pCAMBIA1301 with *Hind*III and *Eco*RI restriction

enzymes. This recombination construct, pCAMBIA1301-*ZmPLATZ2*, harbored a Gus reporter gene and overexpressed a full-length cDNA of *ZmPLATZ2* driven by ubiquitin promoter and was subsequently transformed into rice using *Agrobacterium*-mediated transformation. Nipponbare was used as the wide-type (WT) plant and *ZmPLATZ2* as the transgenic (TG) plant, which were confirmed by histochemical staining of Gus activity and PCR. Then, qRT-PCR was used to test the expression levels of the starch synthesis genes in the TG and WT rice.

Yeast one-hybrid assay

Yeast one-hybrid assays were performed as described in a previous study (Chen et al. 2016). The full-length *ZmPLATZ2* was cloned into the *NdeI* and *BamHI* sites of the pGADT7-Rec2 (Takara, Dalian, China) vector. The promoters of *Bt2*, *Sh2*, *GBSSI*, *SSI*, *SSIIa*, *SSIIa*, *SSIV*, *SSV*, *ISA1*, *ISA2*, and pullulanase were cloned into pHis2 with *RcoRI* and *SacI* using a ClonExpress II one-step cloning kit (Vazyme, Nanjing, China). Primers were list in Table S1.

Protein expression in *E. coli* and purification

The full-length cDNA sequence of *ZmPLATZ2* was cloned into the pET32a vector between the *BamHI* and *HindIII* sites to generate *His::PLATZ2*. Then, the fusion vectors were transformed into BL21(DE3) in *E. coli*. Bacteria, containing the construct, were grown at 37 °C to OD600=0.6, then the fusion protein was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside at 16 °C for 8 h. The His-PLATZ2 was purified using Nickle-IDA agarose beads following the manufacturer's instructions. Primers were list in Table S1.

DAP-seq and DAP-qPCR

The total DNA of Mo17 and purified His-PLATZ2 was used for the DAP assays. The total DNA was broken into 300–500 bp fragments using an ultrasonic crusher. The His fusion protein was purified on Nickle-IDA agarose beads (GenStar, Beijing, China). His-PLATZ2 and DNA fragments were co-incubated for 12 h. The incubation buffer included 50 mM Tris, 1 mM EDTA, and 100 mM KCl, adjusted to a pH of 7.0 by HCl. 5% glycerol, 0.1% Triton X-100, 1 mM DTT. After incubation, the Nickle-IDA agarose beads were washed three to five times using the incubation buffer. Then, 100 μl of 5 M NaCl was added to the bead solution and incubated for 2 h to relieve the crosslinking of Nickle-IDA agarose beads and DNA fragments. The phenol–chloroform method was used to extract the DNA fragments. The fragments were sequenced with a Hiseq2500 system (Novogene, Beijing, China). An empty carrier vector served as the negative control. The DNA fragments prepared in DAP-seq were used for qPCR using SSI-CH1 primer pairs. Three replications were performed for each sample and the input sample served as the negative control in DAP-qPCR.

Results

Co-expression of *ZmPLATZ2* with maize starch synthesis genes

Co-expression analysis between starch synthesis related genes and *ZmPLATZ2* is shown in Fig. 1. The results shown that SBE1, AGPLS4, Sh2, Bt2, SS1, GBSS1, SS3b-1 and SS2c have a PCC absolute value greater than 0.83 with *ZmPLATZ2*. The PCC between other genes and *ZmPLATZ2* is small than 0.8.

Fig. 1 Co-expression analysis of *ZmPLATZ2* and starch synthesis related genes based on the expression data of Stelpflug et al. (2016). The vertical distance from gene to Y axis indicates the size of PCC value

ZmPLATZ2 was cloned from the maize inbred line Mo17 based on the B73 reference genome. The primary sequence analysis showed that the *ZmPLATZ2* gene comprised 894 bp and encoded a 33.679 kDa protein composed of 297 amino acids (Fig. 2a). Analysis using the PSIPRED software revealed that the n-termini of the *ZmPLATZ2* protein consists of an α -helix (Fig. 2a). Using domain analysis with

Expression pattern

To determine the expression pattern of *ZmPLATZ2*, semi-quantitative RT-PCR and RT-PCR were performed. The

different organs of *ZmPLATZ2* expression were examined by semiquantitative RT-PCR. *ZmPLATZ2* was expressed mainly in the seed, with higher expression in the endosperm (Fig. 3a). RT-PCR was performed with *Actin* as the internal reference gene to determine expression pattern of the different stages of *ZmPLATZ2*. The expression of *ZmPLATZ2* was highest at 12 DAP and decreased rapidly post 12 DAP (Fig. 3b).

To analyze the expression regulation of *ZmPLATZ2* in greater detail, a series of treatments were performed. Maize endosperms, collected at 9 or 10 DAP, were exposed to glucose (200 μ M), sucrose (200 μ M), ABA (100 μ M), and 100 μ M ABA + 200 μ M sucrose. The concentration used in each treatment was based on those reported in previous studies (Acevedo et al. 2005; Hu et al. 2011).

As shown in Fig. 3c, *ZmPLATZ2* expression was upregulated more than twofold after 0.5 h with glucose treatment and the expression of *ZmPLATZ2* decreased by approximately 50% after 6 h when exposed to ABA and sucrose separately, whereas the treatment of ABA together with sucrose remitted this decrease. Therefore, we hypothesized that the expression of *ZmPLATZ2* was strongly upregulated by glucose and temporarily downregulated by sucrose, which was complemented by adding ABA.

ZmPLATZ2 binds to the starch synthetic genes promoter

To analyze the interaction of *ZmPLATZ2* with the promoters of the genes involved in the synthesis of starch, a yeast one-hybrid experiment was performed. The constructs of pGADT7-*ZmPLATZ2* and pHis2, harboring the promoter

of the key gene in starch synthesis, were co-transformed into the yeast strain Y187 using. *ZmPLATZ2* bound strongly to the promoters of *SSI* and *SSIIa* and showed binding to the promoters of *SSIV*, *ISA2*, and *SSV* (Fig. 4). Therefore, *ZmPLATZ2* can bind to the promoters of *SSI*, *SSIIa*, *SSIV*, *ISA2*, and *SSV* genes and may regulate their transcription.

Transient expression of *ZmPLATZ2* in maize endosperm

Transient expression analysis was undertaken to analyze the regulatory role of the *ZmPLATZ2* TFs in starch synthesis gene expression. Luc/(4-0) H Gus was used to detect whether *ZmPLATZ2* affected promoter activity. The promoter activities of the four starch synthetic genes were significantly upregulated by at least a two-fold threshold (Fig. 5). These upregulated genes included *Sh2* (AGPase large subunit), *Bt2* (AGPase small subunit), *SSI* (starch synthesis I), and *SSIIa* (starch synthesis IIIa) which was more than twofold. Meanwhile, *WX*, *ISA1*, and *ISA2* promoter activities were significantly upregulated by *ZmPLATZ2*, although these were less than two-fold.

Transcription factor characterization

PLATZ2 is a novel class of plant-specific zinc-dependent DNA-binding TF; therefore, how it regulates the downstream processes was investigated via the analysis of the subcellular location of *ZmPLATZ2* by the transient expression of the *ZmPLATZ2*-GFP protein in onion epidermal cells. The results showed that PLATZ2 was localized in

Fig. 3 Analysis of the expression pattern of *ZmPLATZ2*. **a** Expression analysis of *ZmPLATZ2* in different organs of maize. R root, St stem, L leaf, 10ds 10-day seed, F filament, An anther, Em embryo, En endosperm, SC seed coat. **b** Expression analysis of *ZmPLATZ2* at different stages of seed development. The data represents the

mean $\pm$ standard deviation (SD) of three biological replicate experiments. **c** Treatment of glucose. **d** Treatment of ABA, sucrose, and ABA + sucrose. The treated samples were harvested at different time points postexposure: 0.5, 1, 3, 6, 12, 24, and 48 h. The data are given as the means $\pm$ SD of three replicates

Fig. 4 Binding characteristics of ZmPLATZ2 transcription factors with the starch synthesis gene promoter

Fig. 5 ZmPLATZ2 promotes the promoter activities of starch synthesis genes. **a** Schematic of the vector constructs used for transient expression. **b–j** The results of the transient expression in maize endosperms. The data are reported as the means $\pm$ standard errors (SEs) of three replicates. A one-sided paired t-test (** $P < 0.01$) was used to analyze the significance of the differences between with and without effector vectors

Fig. 6 Cellular localization and transcription activity assays of ZmPLATZ2 in yeast strain AH109. **a** Schematic diagram of the yeast two-hybrid assay. **b** Yeast screened on SD/-Trp medium in accordance with the structure outlined in A; Yeast screened on SD/-Trp-His-Ura medium with X- α -gal in accordance with the structure outlined in A. **c** Schematic diagram of the subcellular localization assay. **d** Results of ZmPLATZ2-eGFP and 35S-eGFP under GFP field, bright, and in the merged image

the nuclei, which is consistent with the role of ZmPLATZ2 being a TF (Fig. 6d).

ZmPLATZ2 localizes to the nucleus and acts as a transcription factor. To examine its transcription activity, a fused vector, pGBKT7-ZmPLATZ2, was generated and transformed into the yeast strain AH109, with the transformants of pGBKT7, pGBKT7-Lam, and pGBKT7-53 as the negative controls and pGBKT7-Gal as the positive control. Each of the transformants was confirmed by PCR. All the yeast cells grew well on the SD/-Trp medium. Only the positive control had α -galactosidase activity on the SD/-Trp/-His/-Ura/X- α -GAL medium (Fig. 6b); therefore, ZmPLATZ2 had no transcription activity in the yeast cells.

Analysis of ZmPLATZ2 influences the starch synthesis gene in transgenic rice

To further study the effect of ZmPLATZ2 on starch synthesis in vivo, overexpressing rice plants were obtained using *Agrobacterium*-mediated transformation and a stable overexpression line was obtained. The transgenic rice was confirmed by Gus staining (Fig. 7a), DNA detection (Fig. 7b), and transcript detection (Fig. 7c). The main starch synthesis genes were measured in transgenic rice using qRT-PCR. The expression levels of SSI, BEIIb, BT1, SSIIa, and Wx were significantly upregulated 3- to 10-fold in TG rice compared to those in WT rice (Fig. 7d). These results indicate that ZmPLATZ2 could regulate the starch synthesis in rice.

Fig. 7 Overexpression of ZmPLATZ2 in rice. **a** Gus staining analysis of transgenic (TG) and wild-type (WT). **b** DNA detection of transformed plants. **c** Transcript detection of transformed plants. **d** qRT-PCR analysis of starch synthesis expression in WT and TG rice. The

data are reported as the means $\pm$ SEs of four replicates. A one-sided paired t-test ($*P < 0.01$) was used to analyze the significance of the differences between TG and WT

DAP-Seq identifies genomic sites are directly bound by ZmPLATZ2

To assess the target genes of the ZmPLATZ2 protein, we performed a DAP-seq experiment using His antibodies against His-tagged ZmPLATZ2 protein (Fig. 8h) and chromatin extracted from Mo17 (Fig. 8i). The empty vector was used as the negative control.

The results of the distribution of binding peaks in the ZmPLATZ2 DAP-seq experiment showed that approximately 91% of the ZmPLATZ2 binding sequences were located in the intergenic. Of these, 7% were located in the promoter regions (-2000 bp to the TSS) (Fig. 8a). We analyzed the peak width and distance between each peak summit and the TSS of its nearest gene to survey the promoter regions of ZmPLATZ2 binding. The peak widths of ZmPLATZ2 were mainly concentrated in the 300 to 400 bp region (Fig. 8b) and there was a peak in the gene promoter regions (Fig. 8c). Therefore, the ZmPLATZ2 peaks were highly focused in the 300 to 400 bp region, upstream of the TSSs.

By GO (Gene Ontology) classification of DEGs (Differentially Expressed Gene), we found that ZmPLATZ2 was mostly bound to the nucleic acid activity and cellular nitrogen compound biosynthesis (Fig. 8d). CAAAAAAA was identified as a statistically defined motif (P -value = $1E-138$; Fig. 8e). We identified the same motif with DAP-seq results from the promoter of ZmSSI, and then DAP-qPCR showed that the ZmSSI promoter was pulled down 15-fold compared to the control (Fig. 8f). Meanwhile, the promoter of *OsSSI* and *ZmSSI* has the same CAAAAAC/GTTTTTG motif in same site of promoter (Fig. 8g). These results suggested that CAAAAAAA was a highly conserved binding motif for ZmPLATZ2.

Discussion

PLATZ1 is the first reported member of the PLATZ TF family, which was identified from the pea (*Pisum sativum*) (Nagano et al. 2001). The first reported member in maize was ZmPLATZ12(*fl3*), which was specifically expressed in

Fig. 8 Profiles of ChIP-seq analysis. **a** Distribution of ZmPLATZ2 binding regions in the maize genome. **b** Peak width of ZmPLATZ2 in the ChIP-seq. **c** Distribution of ZmPLATZ2 binding sites corresponding to the –5000 to +3000 bp regions flanking the TSS. **d** GO classification of DEGs between ZmPLATZ2 and PET32a. **e** The

most prominent motif was identified as ZmPLATZ2 binding motif. **f** In vitro pull down of target DNA by ZmPLATZ2. His-ZmPLATZ2 or His alone were incubated with DNA for 12 h, pulled down, and qPCR was performed for the ZmSSI gene. **g** *OsSSI* and *ZmSSI* has the same CAAAAAC/GTTTTTG motif in promoter

the maize endosperm starchy cell and regulated many tRNAs and 5S RNA by interactions with RPC53 (Wang et al. 2018a, b).

Semiquantitative RT-PCR showed that *ZmPLATZ2* was mainly expressed in the endosperm and further series experiments showed that *ZmPLATZ2* harbored the PLATZ domain and was located in the nucleus. These results showed that *ZmPLATZ2* performs its function in the endosperm as a TF. However, no transcription activity was found in *ZmPLATZ2*. Thus, *ZmPLATZ2* interacted with other proteins to regulate the target gene, such as *ZmPLATZ12* (*fl3*). *ZmPLATZ* proteins are usually involved in the adjustment of RNAPIII transcription, whereas *ZmPLATZ2* could not interact with RPC53 or TFC1, which is similar to most of the *ZmPLATZ* proteins (Wang et al. 2018a, b). We attempted to discover

the key protein that interacted with *ZmPLATZ2* using yeast two-hybrid assays, but no results were obtained.

Yeast one-hybrid experiment of the *ZmPLATZ2* bound promoter of starch synthesis genes revealed centrally that *SSIIa*, *SSI*, *SSIV*, *ISA2*, and *SSV* promoters had a *ZmPLATZ2* binding motif. Our analysis of the transient expression in endosperm supported the role of regulating the *Sh2*, *Bt2*, *SSI*, and *SSIIa* genes. Therefore, we speculate that *ZmPLATZ2* can bind to the *ZmSSI* promoter, and thus promote *ZmSSI* expression. The analysis of the starch synthesis gene expression in TG rice revealed that the *OsSSI* gene was significantly upregulated. Meanwhile, the transcript levels of the *OsSBEIIb*, *OsSSIIa*, and *OsWX* genes had markedly higher overexpression in *ZmPLATZ2* TG rice than that of WT rice. The same effect has been

reported for the overexpression of ZmbZIP22 in maize and rice (Dong et al. 2019); therefore, these results in rice are credible. SSI has no isoforms in rice, unlike the other types of SS (Fujita et al. 2003); therefore, SSI might play an important role in starch synthesis. Meanwhile, we found that the promoter of *OsSSI* and *ZmSSI* has the same CAA AAC/GTTTTTG motif with the same flanking sequence and the distance of the motif to TSS is 157 bp in rice and 115 bp. Therefore, ZmPLATZ2 could bind to the same highly conservative motif in maize and rice. The mechanism by which ZmPLATZ2 regulates starch synthesis is different from that of the other PLATZ proteins bound with RPC53 or TFC1. Therefore, ZmPLATZ2 might regulate the downstream gene as a TF by binding with the promoter (Supplementary Table S2).

Transcription factor participate in hormone signal pathway (Lian et al. 2020; Chen et al. 2019). Different treatments of endosperms showed that ZmPLATZ2 was positively induced by glucose and negatively regulated by ABA and sucrose. In the present study, we found that the glucose-induced ZmPLATZ2 acted as a positive regulator of starch synthesis. ZmPLATZ2 was downregulated by ABA and sucrose, whereas the expression of ZmPLATZ2 after treatments was the same with CK. However, further studies are required to determine the mechanism underlying ZmPLATZ2 induced by glucose and the role that ZmPLATZ2 plays in the ABA and sucrose pathways.

Starch synthesis is crucial for grain weight. Many previous studies have shown that hormones can induce gene expression by regulating the TF. For example, ZmABI4 could bind to the CACCG motif of *ZmSSI*, and thus mediate the ABA pathway (Hu et al. 2012). In conclusion, we identified a new gene, ZmPLATZ2, which functions in the SSI transcription machinery mediated in glucose. In addition, we observed that ZmPLATZ2 is induced by glucose and binds to the CAAAAC/GTTTTTG motif of *ZmSSI* to promote expression. Although the detailed mechanism as to how ZmPLATZ2 regulates the expression of the starch synthesis genes and what role it plays in the ABA and sucrose pathways remains unclear, our data contribute toward the discovery of this process. Therefore, the exploitation of ZmPLATZ2 is a potential approach for regulating the expression of *ZmSSI* involved in the glucose pathway.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10725-020-00687-0>.

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