Functions of OsDof25 in regulation of *OsC4PPDK*

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Received: 12 March 2015 / Accepted: 31 July 2015 / Published online: 3 September 2015 © The Author(s) 2015

Abstract  Relative little is known about the functions of the so-called Dof zinc factors in plants. Here we report on the analysis of OsDof25 and show a function in regulation of the important C4 photosynthesis gene, *OsC4PPDK* in rice. Over-expression of *OsDof25* enhanced the expression of *OsC4PPDK* in transient expression experiments by binding in a specific way to a conserved Dof binding site which was confirmed by yeast and in vitro binding studies. Expression studies using promoter GUS plants as well as qPCR experiments showed that *OsDof25* expressed in different tissues including both photosynthetic and non-photosynthetic organs and that expression of OsDof25 was partially overlapping with the *OsC4PPDK* gene. Conclusive evidence for a role of OsDof25 in regulation of *C4PPDK* came from loss-of-function and gain-of-function experiments with transgenic rice, which showed that down-regulation or over-expression of *OsDof25* correlated with *OsC4PPDK* expression and that OsDof25 has functions as transcriptional activator.

Keywords  Dof · *OsC4PPDK* · Transcription factor · Rice

Abbreviations

Dof DNA binding with one finger
GUS β-Glucuronidase
MS Murashige–Skoog
*OsC4PPDK* Pyruvate orthophosphate dikinase

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world because it feeds more than half of global population and is daily carbohydrate source in many countries. Traditional breeding only results in an annual yield increase of about 1% whereas this should be higher to keep in pace with the increase in demand. Much is expected from molecular breeding as a tool to combine the best genes and alleles in novel plant types but to enable this we need new information about which genes and alleles are responsible for which traits. To enable this process, many national and international genetics and genomics projects on rice were initiated in the last decade and as results the genomes of indica and japonica have been completely or partially sequenced (Matsumoto et al. 2005), a large number of T-DNA insertion or transposon-based tagged mutants have been produced (Hirochika et al. 2004; Jung and An 2013; Priya and Jain 2013), genome wide
expression profiles have been obtained by using microarray and SAGE (Bao et al. 2005; Li et al. 2006). All these approaches have provided very useful information and resources enabling functional genomics with rice. However, the function of the majority of rice genes has not been experimentally demonstrated (Zhang et al. 2006). In particular knowledge on functions of transcription factors and other regulatory proteins could be relevant for improvement of rice since some of them are related to important traits in cereals. Examples of such transcription factors are Opaque2 from maize that is a regulator of seed-storage protein deposition and TB1 which plays a major role in tiller number and apical dominance in maize (Hwang et al. 2004). In rice, MOC1 was shown to be important in determining tiller number (Li et al. 2003) and OsSPL14 was shown to be involved in tiller number and grain per panicle number (Luo et al. 2012).

Transcription factors play key roles in regulating gene expression at the transcriptional level. According to the rice transcription factors database, there are at least 63 transcription factor families in the rice genome (Gao et al. 2006). One of them is the Dof gene family (for reviews see Yanagisawa 2002; Noguero et al. 2013) which has important functions in responses to plant hormones such as gibberellins (Washio 2001; Mena et al. 2002) and auxin (DePaolis et al. 1996; Kisu et al. 1998), stress responses (Zhang et al. 1995), flowering time (Li et al. 2009; Corrales et al. 2014), tissue specific expression (Yanagisawa 1998; Plesch et al. 2001) and photosynthesis (Yanagisawa and Sheen 1998; Yanagisawa 2000). Dof transcription factors have one copy of the Dof zinc finger domain (hence the name of DNA binding with only one finger), which normally resides in the N-terminal region, but the sequences outside the Dof domains are very diverse (Riechmann and Ratcliffe 2000; Yanagisawa 1998, 2002). Most of the Dof domain proteins recognise an AAAG motif or the reverse complement CTTT as core sequence element in DNA binding assays in vitro (Yanagisawa 2002), except for AOBP, a protein from pumpkin, which recognises an AGTA repeat as core binding sequence (Kisu et al. 1998).

ZmDof1 was found to be associated with expression of multiple genes involved in carbon fixation in maize (Yanagisawa 2000), and was shown to be able to activate expression of the OsC4PPDK promoter by binding to the AAAG motif (Yanagisawa 2000). OsC4PPDK (pyruvate orthophosphate dikinase) catalyses the conversion of pyruvate into phosphoenolpyruvate (Edwards et al. 1985). This is one of the key steps in the C4 photosynthesis pathway, since PPDK can regenerate the primary CO2 acceptor phosphoenolpyruvate (PEP) which is the substrate for an important step in carbon fixation in mesophyll cell chloroplasts (Chollet et al. 1996). Since carbon fixation is the most important process underlying yield in cereals, we wanted to study whether Dof transcription factors can be used as a tool to modify OsC4PPDK expression. As a first step, we cloned OsDof25 from rice which together with OsDof24 is the closest homolog to ZmDof1 on the protein level and investigated its function in more detail using loss-of-function and gain-of-function studies supported by in vitro binding studies. The results show novel insight in regulation of OsC4PPDK by OsDof25 and will ultimately contribute to understanding the regulation of C3 and C4 photosynthesis genes in rice.

Materials and methods

Sequence alignments and phylogeny analysis

The sequence of the ZmDof1 protein was obtained from GenBank ABF51012 (Yanagisawa 2001; Cavalar et al. 2007). All protein sequences of rice Dof transcription factors were downloaded from the rice transcription factor database (http://ricetfdb.bio.uni-potsdam.de/v2.1/) (Riano-Pachon et al. 2007). The amino acid sequences of ZmDof1 and the rice Dof proteins were aligned using ClustalX (2.0). The Neighbor-Joining algorithm implemented in MEG4 (Tamura et al. 2007) was used for the phylogenetic tree assay. Two hundred bootstrapped data sets were used to estimate the confidence of each clade tree.

RNA isolation, RT-PCR, gene cloning and sequence analysis

Flag leaves harvested 10 DAF from rice hybrid Liangyou 2186 (Oryza sativa L. ssp. indica) were ground in liquid nitrogen and total RNA was extracted by using Trizol according to the manufacturer’s instructions (Invitrogen). Genomic DNA contaminants were removed from RNA samples by incubating with DNA-free™ (Ambion) at 37 °C for 30 min. First-strand cDNA was synthesised starting from 1 μg of total RNA with SuperScript III reverse transcriptase (Invitrogen) as described by the manufacturer.

The RT-PCR reactions were performed based on single-strand cDNA. The primer OsDof25-F1 and OsDof25-R1 (Table S3) were used to get the cDNA of OsDof25. PCR conditions were 5 min of initial denaturation at 98 °C, 36 cycles of denaturation at 98 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 45 s, followed by a final extension step at 72 °C for 10 min. The PCR products were separated in a 1× TBE, 1 % agarose gel.

To confirm the identity of the amplified sequences, the PCR products were cloned in pCR-Blunt II-TOPO (Invitrogen) and sequenced commercially (BaseClear, Leiden, The Netherlands). The sequences of OsDof25 were further analysed using DNAMAN.
Construction of effector and reporter plasmids, transient transformation of rice protoplasts and GUS assays

An effector plasmid, Pro35S-OsDof25, was made by insertion of an Xhol–KpnI fragment from pCR-blunt II-TOPO-OsDof25 into expression vector pRT100 (Töpfer et al. 1987). For the loss-of-function analysis, GUS reporter plasmids carrying different lengths of the OsC4PPDK promoter were generated by insertion of different PCR fragments of the OsC4PPDK promoter using the BamHI and NcoI sites into vector pGusXX (Pasquali et al. 1994). For the gain-of-function analysis, wild type sequence and mutant fragments from –385 to –274 of OsC4PPDK promoter were obtained by PCR and then digested with NotI and inserted into plasmid pGusXX-47 (Pasquali et al. 1994) which has a minimal TATA box containing fragment from the CaMV 35S promoter. Reporter plasmids ProPPDK-A::GUS, ProPPDK-B::GUS, ProPPDK-C::GUS, ProPPDK-D::GUS and ProPPDK-E::GUS (Fig. 6) represent the fragment containing wild type CTTO motif, mutant motifs GTTT, CATT, CTAT and CTTA respectively. The primers used to construct the reporter plasmids construction are listed in Table S3.

Starting material for the protoplast experiments were 2 weeks old seedlings. Seeds of rice cultivar Minghui 86 (Oryza sativa L. ssp. indica) were allowed to imbibe in water at darkness at room temperature for 3 days. Next, the seeds were sown in soil and grown at 26 °C, 80 % humidity and 12/12 dark/light period, with a light intensity (photosynthetically active radiation value) of 180 μmol/ m²/s. Protoplast isolation and transfection was essentially performed as described by Chen et al. (2006). Cotransformations were performed with 4 μg reporter plasmids and 6 μg effector plasmids using a PEG-based transformation method. Co-transformations with empty overexpression vectors served as controls. After incubation in 1.5 ml W5 buffer in a dark room at 28 °C overnight, protoplasts were harvested and total protein were isolated and then frozen in liquid nitrogen. GUS activity assays were performed as described by Van der Fits and Meemlelink (1997) and protein concentrations were measured using the Bradford protein assay reagent (BioRad). For the loss-of-function analysis, wild type sequence and mutant fragments from –385 to –274 of OsC4PPDK promoter were obtained by PCR and then digested with NotI/SpeI and inserted into plasmid pGusXX-47 (Pasquali et al. 1994) which has a minimal TATA box containing fragment from the CaMV 35S promoter. Reporter plasmids ProPPDK-A::GUS, ProPPDK-B::GUS, ProPPDK-C::GUS, ProPPDK-D::GUS and ProPPDK-E::GUS (Fig. 6) represent the fragment containing wild type CTTO motif, mutant motifs GTTT, CATT, CTAT and CTTA respectively. The primers used to construct the reporter plasmids construction are listed in Table S3.

Recombinant OsDof25 protein expression and electrophoretic mobility shift assays (EMSA)

The fragment of OsDof25 cDNA was fused in-frame with the GST sequence in expression vector pGEX-KG (Guan and Dixon 1991) by sub-cloning BamHI–EcoRI fragments from construct Pro35::OsDof25. In order to extract recombinant protein, 5 ml of overnight cultures of BL21 (DE3 pLys) (Novagen) carrying pGEX-KG-OsDof25 plasmids were used to inoculate 500 ml LB medium containing 200 μg/ml carbenicillin and 50 μg/ml chloramphenicol, which was incubated at 37 °C to OD600 0.5. Next, protein synthesis was induced by the addition of solid IPTG to final concentration of 1 mM and cultures were incubated for 4 h at 29 °C. The harvested cells were suspended in 20 ml PBS and frozen in liquid nitrogen. After thawing pellets at 37 °C, the bacteria were lysed by sonication (eight times 10 s burst; 5 s pause between bursts), and centrifuged (at 18,000 rpm for 30 min at 4 °C), then the supernatant was filtered through a 0.45 μm membrane. Protein purification was performed using PolyPrep Chromatography columns (Biorad 731-1550) containing 0.5 ml settled Glutathion-Sepharose 4B beads (Amersham Biosciences). Columns were first washed two times with 10 ml PBS before bacterial extract was passed through. After binding, columns were washing with 10 ml PBS and bound proteins were eluted in 2.5 ml (10 × 0.25 ml) glutathion elution buffer (100 mM glutathione, 500 mM Tris–HCL pH 8.0). Eluted protein was concentrated using Microcon centrifugal filter devices (Millipore) according to manufacturer’s instruction and the protein content was determined by the method of Bradford. The GST-OsDof25 fusion protein was stored at –80 °C in 10 % glycerol.

All EMSA reactions contained 100 ng poly-(dl-dC)-poly-(dl-dC) (Amersharm-Pharmacia) and 1 ng of 32P end-labeled probe (~108 cpm/μg) in nuclear extraction buffer (Green et al. 1987). Labeled probes were incubated with GST-OsDof25 proteins together at room temperature 30 min and then were loading on a native 4 % polyacrylamide (30:0.8) gel in 0.5 × TBE while under current. Probes used in labeling originated from annealed oligonucleotides are listed in Table S2.

DNA binding specificity of OsDof25 proteins in yeast one-hybrid assay

Fragments P3 and P6 from the C4PPDK promoter, containing the putative Dof binding motif CTTT and its mutant derivative CTAT, respectively, were obtained by annealing the primers listed in Table S2 and then cloned into yeast integrative vector pN1T-HIS3NB (GenBank Accession AY061966; Ouwerkerk and Meijer 2011) between NotI and SpeI sites. The resulting plasmids ProOsC4PPDK-WT::HIS3 and ProOsC4PPDK-MU::HIS3 were confirmed by sequencing. Next, the HIS3 reporter-containing fragments were excised with SaeI–NcoI and introduced into yeast strain Y187 (MATa, ura3-52, his3-Δ200, ade2-101, trpl-901, leu2-3, 112, met+ , gal4 gal80, URA3;GAL1_UAS; GAL1_TATA-lacZ; Clontech) (Meijer et al. 1998; Ouwerkerk and Meijer 2001, 2011) resulting in yeast strains
YPO101 is a control strain used in yeast one-hybrid assays. Y187:ProOsC4PPDK-WT and Y187:ProOsC4PPDK-MU.

An EcoRI/BamHI fragment from pRT100-0sDof25 containing the OsDof25 ORF was inserted into pACTIIa (Meijer et al. 1997) to generate plasmid pACTIIa-OsDof25, which has a fusion between OsDof25 and the GAL4 AD in order to carry out DNA binding assays in a yeast one-hybrid system. For this, plasmid pACTIIa-OsDof25 was introduced into yeast strains Y187:ProOsC4PPDK-WT, Y187:ProOsC4PPDK-K-MU and YPO101. The empty plasmid pACTIIa was transformed into the same strains as a negative control.

Yeast transformations were performed as earlier described (Ouwerkerk and Meijer 2001). The transformed yeast cells were plated on CM/−Leu−His+Met+Ade+Trp medium and incubated at 30°C and usually after 4 or 5 days colonies appeared. Next, the resulting colonies were streaked on CM/−Leu+His+Met+Ade+Trp medium, including different concentrations of 3-AT. The plates were incubated at 30°C for 1 week before scoring. All handling with yeast were as described earlier (Meijer et al. 2000; Ouwerkerk and Meijer 2001, 2011).

Sub-cellular localisation of OsDof25 protein

The ORF of OsDof25 was amplified with primers OsDof25-F2 and OsDof25-R2 (Table S3) from the Topo vector. The PCR product was fused in frame to the N-terminus of the green fluorescent protein (GFP) gene to generate plasmid Pro35S::OsDof25::GFP. Vector pTH2 (Pro35S::GFP) was used as a control (Chiu et al. 1996). In this plasmid the S65T sGFP gene is driven by the CaMV 35S promoter and no specific localisation signals are present. Protoplasts isolated from 2 weeks old rice seedlings were transiently transformed using a PEG-mediated method (Chen et al. 2006). The transformed protoplasts were incubated at 28°C overnight in K3 buffer and then checked for expression using a then observed using a Nikon Eclipse Ci fluorescence microscope. Excitation and emission filters for GFP detection were Ex470-490/DM505/BA520-560.

Binary vector construction and plant transformation

To generate a OsDof25 (LOC_Os09g29960) promoter GUS fusion construct, a 2368 bp DNA sequence upstream of the predicted OsDof25 translation start site was amplified by PCR from genomic DNA isolated from Minghui 86 using Phusion polymerase (Invitrogen) and primers ProOsDof25-F and ProOsDof25-R (Table S3). The PCR product was subsequently cloned into vector pCR2.1 Topo (Invitrogen) for sequence analysis and then inserted into vector pHABIA-1391Z (GenBank Accession AF234312) resulting in a fusion with the GUS reporter gene. An OsC4PPDK promoter GUS fusion construct (ProOsC4PPDK::GUS) was generated by insertion of a 2572 bp EcoRI–NcoI fragment OsC4PPDK promoter, into pCAMBIA-1391Z. The resulting constructs were used for rice transformation.

To construct an OsDof25 vector for overexpression, the full length coding sequence was amplified by PCR with primers OsDof25-F5 and OsDof25-R5 (Table S3) and then inserted as NcoI/BamHI fragment between the GOS2 promoter and nos terminator in binary vector pCAMBIA-1300intC (GenBank Accession AF294978). The resulting construct was used for rice transformation. The expression of OsDof25 was down-regulated by an RNAi approach based on generating transgenic plants equipped with a pHANNIBAL silencing vector (Wesley et al. 2001). Specific OsDof25 sense and anti-sense sequences were obtained by PCR and then inserted into pHANNIBAL in a two-step cloning procedure. First, the anti-sense fragment of OsDof25 was generated using primers OsDof25-F6 and OsDof25-R6 (Table S3), then digested by Clal/BamHI and inserted into pHANNIBAL vector between the same sites. Second, the sense fragment of OsDof25 was generated using primers OsDof25-F7 and OsDof25-R7 (Table S3) and then digested with XhoI/KpnI, and inserted into pHANNIBAL already carrying the anti-sense fragment. The resulting plasmid was digested with SalI/Spel and the generating fragments was inserted into pCAMBIA-1300intC between SalI and Xbal. The resulting binary vector was used in rice transformation.

The OsC4PPDK promoter (LOC_Os05g33570) was obtained by PCR on genomic DNA isolated from indica rice cultivars Minghui 86 and SE21S using primers ProOsC4PPDK-F and ProOsC4PPDK-R (Table S3). To confirm the identity of the amplified sequences, PCR products were cloned in pCR-Blunt II-TOPO vector (Invitrogen) and sequenced by BaseClear (Leiden, The Netherlands).

Transformation of the japonica rice cultivar Zhonghua 11 with the above described binary vector constructs was performed as described by Scarpella (Scarpella et al. 2000) except that A. tumefaciens LBA4404 was used for all rice transformations. Prior to growth in the greenhouse, transgenic seedlings were selected on a half-strength Murashige–Skoog medium supplied with 0.7 % type I agarose (Sigma) and 25 μg/ml hygromycin. By using Southern blotting with hpt as a probe, we identified single copy T-DNA lines.

Detection of GUS expression in transgenic rice, cytological techniques and microscopy

Histochemical detection of GUS activity, cytological techniques and microscopy were performed as described
Expression assays of OsDof25 and OsC4PPDK using qPCR

To study the expression profile of OsDof25 and OsC4PPDK, a qPCR analysis was done on a collection of nine tissues, including 2-week old seedlings and eight different tissue samples from mature Minghui 86 plants, including stems, roots, sheath, flag leaves, penultimate leaves and panicles at 10 DAF (days after flowering). For the expression analysis of OsDof25 and OsC4PPDK in over-expression and RNAi transgenic lines, total RNA was isolated from 10 DAF flag leaves using Trizol kit (Invitrogen). RT reactions were performed with SuperScript™ II reverse transcriptase (Invitrogen) following the manufacturer’s instructions. Reactions were performed in an optical 96-well plate with an ABI PRISM® 7500 Real-time PCR System (Applied Biosystems) by using SYBR® Green to monitor dsDNA synthesis. All reactions contained 12.5 μl 2× SYBR® Green Master Mix Reagent (Applied Biosystems), 2.0 ng cDNA and 10 pmol of each gene-specific primer in a final volume of 20 μl. Thermal cycling was as follows: 50 °C for 2 min; 95 °C for 10 min; 50 cycles of 95 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s. Relative expression levels of reporter and target genes were calculated using the 2−ΔΔCt method (Livak and Schmittgen 2001) using rice Actin1 and Ubiquitin as internal control. An overview of primers used in qPCR is shown in Table S4.

Accession numbers

Sequence data from this article can be found in the EMBL/GenBank databases with accession codes KC996732, KC996733 and KP772258 for the OsC4PPDK promoter, the OsDof25 cDNA clone and the OsDof25 promoter, respectively.

Results

Phylogenetic analysis of the japonica rice Dof transcription factor family and identification of OsDof24 and OsDof25 as closest homologs of ZmDof1

A bioinformatics analysis of the japonica rice genome identified 30 Dof transcription factor genes (Oryza sativa) (Lijavetzky et al. 2003). All Dof transcription factor genes have been assigned to a chromosomal map position (Fig. 1; Table S1) which is based on the TIGR database (http://plantsdb.bio.uni-potsdam.de/v3.0/) (Ouyang et al. 2007). To determine if there are any paralogous gene pairs we checked their chromosomal locations in relation to the history of genome duplications (Yu et al. 2005). The chromosomal map presented in Fig. 1 shows that the rice Dof genes are not generally clustered and that the pair OsDof7, −8, −9−18 and the pair OsDof24/25 are very likely paralogues because they are located in duplicated regions on chromosomes 2/4 and 8/9 respectively.

Alignments of the amino acid sequences of the Dof domains of all 30 Dof proteins, which were downloaded from the transcription factor database (Riano-Pachon et al. 2007), showed that the Dof domains were highly conserved but that outside of the Dof domain there is little conservation (Yanagisawa 2002). Next, we performed a phylogenetic analysis with the rice Dof family and ZmDof1. As shown in Fig. 2, there are two Dof proteins from rice, OsDof24 and OsDof25, in the same clade with ZmDof1. Given the role of ZmDof1 in regulation of the photosynthesis genes PEPC and C4PPDK, it may very well be that OsDof25 is also involved in regulation of OsC4PPDK.

In order to further study the functions of OsDof25, this gene was cloned from the Chinese super-hybrid rice combination Liangyou 2186 (GenBank Accession KC996733). Sequence analysis showed that there are two extra serine residues on position 97 compared with the sequence downloaded from the plant transcription factor database (http://plantsdb.bio.uni-potsdam.de/v3.0/). Likely these two serines do not have an serious effect on functionality of the Dof domain of OsDof25 since they are outside this domain (data not shown).

OsDof25 is an activator of the OsC4PPDK promoter

To determine whether OsDof25 can regulate the expression of OsC4PPDK an experimental set-up was designed based on transient expression assays in rice protoplasts using OsDof25 effector constructs and OsC4PPDK promoter reporter constructs. For this, a 2.5 kb promoter fragment of OsC4PPDK was cloned from rice cultivar SE21S and Minghui86 (Oryza sativa L. ssp. indica) which are the parents of hybrid rice combination Liangyou 2186. Sequencing and alignments showed that there is no difference between the OsC4PPDK promoters from cultivars SE21, Minghui86 (GenBank Accession KC996732) and Nipponbare. The reporter ProPPDK::GUS was constructed by introducing a 2.5 kb OsC4PPDK promoter fragment fused to the GUS reporter gene as a transcriptional fusion. Plasmid Pro35S::OsDof25, carrying the ORF of OsDof25 under the control of the CaMV 35S promoter was used as effector. As shown in Fig. 3, a combination of this reporter and effector resulted in a doubled GUS activity compared...
to the empty effector plasmid pRT100. Thus the results strongly suggested that OsDof25 can activate expression of the OsC4PPDK promoter in vivo.

It has been reported that most of Dof transcription factors will recognise the sequence AAAG or its reverse complementary sequence, CTCT, as an essential DNA binding motif (Yanagisawa and Izui 1993; Yanagisawa and Schmidt 1999; Yanagisawa 2002). We used PLACE (Higo et al. 1998) to search for cis-acting elements, in the 2.5 kb OsC4PPDK promoter fragment, and as a result 16 putative binding motifs (AAAG or CTTT) were identified which are indicated in Fig. 3a. To further study the function of the putative Dof binding sites identified in the PPDK promoter, a loss-of-function experiment was designed based on a series of five deletion constructs (ProPPDK-D1::GUS to ProPPDK-D5::GUS) fused to the GUS reporter gene (Fig. 3a). As shown in Fig. 3b, only co-transfection with reporter ProPPDK-D5::GUS and effector Pro35S::OsDof25 did not result in an increase of relative GUS activity, but co-transfection with the other reporters showed a similar increase of relative GUS activity compared to the full length OsC4PPDK promoter. The difference between ProPPDK-D4::GUS and ProPPDK-D5::GUS is that there is a 111 bp deletion from $-385$ to $-274$, which contains one putative Dof binding motif with the consensus sequence CTCT (Fig. 3a) which in principle could be binding site for the OsDof25 protein. Taken together, the results showed that OsDof25 can activate a series of OsC4PPDK promoter deletion constructs. Up to coordinate $-385$, deletion had little effect on activation by OsDof25. In turn, when a 111 bp fragment spanning from $-385$ to $-274$ bp containing a putative Dof binding motif was deleted, activation by OsDof25 decreased from twofold to only onefold. Although it seems that this particular region in the OsC4PPDK promoter is important in regulation we do not exclude that there are important elements further upstream in the promoter which also contribute to expression together with the region from $-385$ to $-274$ bp.
OsDof25 recognises the OsC4PPDK promoter through a Dof binding motif

The transient experiments assays indicated that OsDof25 is able to activate the OsC4PPDK promoter through a 111 bp fragment (−385 to −274) containing a putative Dof protein binding site. To confirm whether OsDof25 regulates the OsC4PPDK promoter by interacting directly with this specific sequence element, a series of electro mobility shift assays (EMSAs) were conducted. For this, OsDof25 was expressed and purified from E. coli as recombinant protein. Next, three different parts of the fragment from −385 to −274 of OsC4PPDK promoter with or without the CTTT motif were used as probes (Table S2). In order to determine the specificity of the interaction of OsDof25 protein with the Dof binding motif, a set of four different mutant oligonucleotides was designed and used as EMSA probes (Fig. 4a; Table S2). The EMSA assay of OsDof25 protein with wild type probe P3 produced a distinct complex (Fig. 4b) which could be competed away with a range of unlabelled P3 oligonucleotide (Fig. 4c). On the other hand, when OsDof25 protein was incubated with mutant probes P4, P5, P6 and P7 or two different wild type probes derived from the OsC4PPDK promoter (stretching from −385 to −274) without a Dof binding site, than the protein-DNA complex did not appear (Fig. 4b). In conclusion, the EMSAs confirmed that OsDof25 is indeed able to interact specifically with the OsC4PPDK promoter through the Dof binding site at position −291 bp.

Furthermore, we also studied the interaction between OsDof25 and the OsC4PPDK promoter using a yeast one-
hybrid system. For this, probes P3 and the mutant P6 (Table S2), were inserted upstream of the reporter gene HIS3 in the integrative vector pINT1-HIS3NB (Meijer et al. 1998; Ouwerkerk and Meijer 2001) which was transformed into yeast resulting in strains Y187:ProOsC4PPDK-WT and Y187:ProOsC4PPDK-MU. OsDof25 was expressed in yeast using a GAL4 AD vector. Because of the fusion with the GAL4 Activation Domain (AD), the resulting vector (pACTIIa-OsDof25) can be used for detection of DNA binding of OsDof25 protein to a target sequence without OsDof25 having its own activation domain. Detection of such interaction is via activation of a HIS3 reporter gene on yeast cells growing on a minimal medium lacking histidine but containing a minimal concentration (5 mM) of 3-AT which is a competitive inhibitor of His3p activity. Figure 5a, shows the growth results of all four combinations of effector and reporters on histidine-containing medium as control to show that none of the effectors has any negative effects on growth. As shown in Fig. 5b, the combination of pACTIIa-OsDof25 and the reporter preceded by the OsC4PPDK promoter fragment grew well on minimal medium lacking histidine (Fig. 5b) whereas pACTIIa as empty control did not result in any growth. However, the strain with mutant fragment P6 did not grow on the same minimal medium in combination with either pACTIIa-OsDof25 or pACTIIa (Fig. 5b). Thus, the results from the yeast one-hybrid system show that OsDof25 indeed

Fig. 3 Loss-of-function analysis of the OsC4PPDK promoter in rice protoplasts. a Schematic representation of truncated promoter-GUS constructs that were used in transient expression assays with rice protoplasts. Promoter lengths are indicated on the left. Negative numerals indicate the nucleotide position relative to the transcriptional start site. Putative Dof binding sites, AAAG or CTTT, as predicted by the PLACE database are indicated by black bars. Numbers of the putative binding motif within truncated regions are indicated. b The effects of OsDof25 overexpression and the mapping of OsDof25-binding fragments on the OsC4PPDK promoter were tested using an overexpression construct Pro35S::OsDof25 which was co-transformed into rice protoplasts with a series of OsC4PPDK promoter GUS constructs. The empty effector plasmid pRT100 was used as a control. Relative GUS activities were normalised for total protein. GUS activities of co-transformation with Pro35S::OsDof25 are indicated in black and columns representing empty effector plasmids are blank. The bar graphs are based on the mean values of three independent transformations of each construct combination and error bars represent means and standard deviations (SDs) of biological replicates. The data were analysed using ANOVA followed by Bonferroni corrections. Asterisks indicate significant differences (p < 0.05) compared with the untransformed controls.
recognizes the −285 to −274 fragment of OsC4PPDK promoter which has a putative Dof binding motif. The specificity of OsDof25 is demonstrated by the lack of activation of a mutant fragment where a sequence CTTT was converted into CTAT.

Initially, a loss-of-function approach identified a 111 bp fragment in the OsC4PPDK promoter which is important for regulation by OsDof25. Further EMSAs and yeast one-hybrid studies confirmed specific binding of OsDof25 with a CTTT sequence in this fragment. In order to further confirm the interaction of OsDof25 protein with this fragment in planta another series of GUS reporter plasmids were made which encompassed a series of five constructs (ProPPDK-A::GUS, ProPPDK-B::GUS, ProPPDK-C::GUS, ProPPDK-D::GUS and ProPPDK-E::GUS) for a gain-of-function approach in combination with transient expression in protoplasts. These constructs were based on the 111 bp fragment from −385 to −274 bp and represented the wild type fragment and four mutants in the CTTT motif (Table S2). As shown in Fig. 6, the wild type fragment (construct ProPPDK-A::GUS) was activated by OsDof25 by fivefold, but when either of the four mutant fragments are used, the ratio of induction drops down to twofold as the empty constructs. Together, the results show that OsDof25 can interact in vitro and in vivo specifically with a Dof binding motif close to the ORF of OsC4PPDK and may play an important role in planta regulation too.

Since OsDof24 is the closest homologue in the phylogeny tree with OsDof25 (Fig. 2), we also tested if this gene is able to activate the same OsC4PPDK promoter constructs in planta as used in the loss-of-function and gain-of-function analyses. As shown in Fig. S1, the effects of OsDof24 expression in the protoplast system are essentially the same as with OsDof25 (Figs. 3, 6).
Sub-cellular localisation of OsDof25 transcription factor in rice protoplasts

The subcellular localisation of OsDof25 protein was studied using transient expression of a GFP-tagged OsDof25 fusion protein in rice protoplasts. For this, the ORF of OsDof25 was fused in frame to the N-terminus of the GFP gene to generate plasmid Pro35S::OsDof25-GFP. The empty plasmid pTH2 (Pro35::GFP) was used as a control (Chiu et al. 1996). Fluorescence was detected using confocal laser scanning microscopy (CSLM). As shown in Fig. 7, GFP-tagged OsDof25 was specifically localised in the cell nucleus, whereas the control GFP protein was localised in both cytoplasm and the nucleus. This experiment confirmed that the OsDof25 protein is a nuclear-localised protein which is consistent with a function as transcription factor.

Expression profiling of OsDof25 and OsC4PPDK

The expression profile of OsDof25 was studied using qPCR and promoter GUS plants. The qPCR assays showed that the expression of OsDof25 is not strictly tissue-specific, but has highest expression in penultimate leaves at 10 days after flowering (DAF) and 2 week old seedlings, followed by flag leaves at 10 DAF, panicle, leaf sheath and stem and lowest expression in roots (Fig. 8a). To further study the expression pattern of OsDof25 and the possible overlap with OsC4PPDK in more detail, we generated ProOsDof25::GUS and ProOsC4PPDK::GUS transgenic lines. For both OsDof25 and OsC4PPDK promoters, the GUS signal was observed in leaves and florets as well as in germinating seeds but there was no GUS signal detected in radicles which is in accordance to the qPCR data for OsDof25. For OsC4PPDK, GUS activity was also detected in immature seeds, where no expression for OsDof25 could be detected (Fig. 8b).

Taken together, the results demonstrated that OsDof25 is expressed in different tissues and at different developmental stages. The GUS signals detected in ProOsDof25::GUS rice are in accordance with the expression profiles of OsDof25 determined by qPCR and overlap with those of OsC4PPDK, indicating that OsDof25 may play an important role in rice growth and development besides playing a key role on the transcription of OsC4PPDK but we cannot rule out entirely different functions.

Functions of OsDof25 in regulation of OsC4PPDK expression in rice

The regulation of OsC4PPDK by OsDof25 was further studied using loss-of-function and gain-of-function
approaches in transgenic rice. To determine whether OsDof25 is required for expression of the OsC4PPDK gene, we tried to down-regulate OsDof25 expression using RNA interference (RNAi) with the pHANNIBAL system (Wesley et al. 2001). For this, a binary vector was made carrying an inverted repeat of a specific part of the OsDof25 and used in rice transformation. Five independent single copy transgenic lines were identified using Southern blotting analysis (Fig. S2a) and used in further analysis. Among the five lines transformed with the OsDof25 silencing construct, two lines, numbers #5 and #30, showed a decrease in OsDof25 mRNA level as shown by qPCR analysis (Fig. S2b) but no obvious phenotype was visible. Next, the T1 generation of RNAi-OsDof25 line #5 and line #30 were analysed in more detail for the effect on OsC4PPDK expression. Both RNAi lines showed a reduction in the level of OsC4PPDK mRNA compared to the control lines which were non-transgenic lines separated from the T0 (Fig. S2c).

The RNAi experiments demonstrated that OsDof25 plays a role in the expression of OsC4PPDK. To determine whether an elevated level of OsDof25 expression is sufficient for activating the expression of OsC4PPDK, transgenic rice plants were made, which were transformed with a binary vector carrying the OsDof25 gene driven by the constitutive GOS2 promoter (De Pater et al. 1992; Ouwerkerk et al. 2001). Three independent single copy number lines were selected using Southern blotting (Fig. S3a) and qPCR assays showed that all three transgenic lines had significantly elevated OsDof25 mRNA levels compared to the control lines (Fig. S3b). These lines did not show obvious differences in phenotype but analysis of the expression level of OsC4PPDK by qPCR showed significant up-regulation (Fig. S3c). Since OsC4PPDK is important in photosynthesis we also checked photosynthetic capacity in the OsDof25 overexpressors and RNAi lines but we did not observe a significant and large difference with the wild type lines (Fig. S4). Taken together, both loss-of-function and gain-of-function studies with OsDof25 showed a clear and opposite effect as activator of OsC4PPDK expression which confirmed the results obtained from the EMSA, yeast one-hybrid assay and transient expression experiments in rice protoplasts where we showed binding of OsDof25 with the OsC4PPDK promoter.

**Discussion**

In a comparative study between Dof genes from rice and maize we identified the paralogous pair OsDof24 and OsDof25 as orthologues of ZmDof1. Little is known about the precise functions of Dof genes although some members were
characterised in rice (Gaur et al. 2011; Nie et al. 2013), Arabidopsis, Sorghum (Kushwaha et al. 2011), tomato (Cao et al. 2013), Brachypodium (Hernando-Amado et al. 2012) and soybean (Guo and Qiu 2013). In rice for OsDof12 (Li et al. 2009), a function in flowering time was described in detail. OsDof24 is also named OsDof25 because of a different nomenclature (Santos et al. 2012) and has been implicated in regulation of genes involved in carbon and nitrogen metabolism but this is a different gene than the OsDof25 gene described here. Other Dof genes such as BPBF from barley (Diaz et al. 2002) and several genes from Brachypodium (Hernando-Amado et al. 2012) are expressed during grain development and seem to play a role in this process but this seems not be the case for OsDof25 showing that although Dof genes bind similar cis-acting elements, they may still have functions in different tissues and biological processes. More than half of the Dof members in Arabidopsis are expressed in vascular tissues and may have roles in vascular development and functioning such as short or long-distance signaling (Le Hir and Bellini 2013). We found that OsDof25 was also expressed in vascular tissue, which suggests that this gene could also play a role in development of vascular tissues or in regulation of genes involved in vascular loading or unloading or other processes acting in this tissue.

In maize, Dof1 is known to have functions in regulation of C4PPDK (Yanagisawa 2000) which plays an important role in C4 photosynthesis (Chollet et al. 1996). Since OsDof25 is a close homologue of ZmDof1 which is involved in regulation of PPDFK, we conducted several studies to check whether this gene–gene interaction is conserved in rice. In order to understand the expression of the mechanism by which OsDof25 potentially could regulate OsC4PPDK, we delineated the OsC4PPDK promoter and identified a minimal fragment with only one Dof binding site that still gave activation by OsDof25 in a transient expression analysis. Functionality of this site in regulating OsC4PPDK expression was proven by mutation analysis that showed reduced binding in in vitro and in yeast one-hybrid experiments which was next confirmed with the transient protoplast experiments. Important data for a role of OsDof25 in regulation of OsC4PPDK came from a loss-of-function and gain-of-function analysis where down-regulated or up-regulated OsDof25 expression correlated with lower or higher OsC4PPDK expression respectively. Furthermore, no adverse phenotypical effects were seen in these transgenics which also increases the usefulness in biotechnological applications. Because OsDof24 is closest to OsDof25, we also did some analyses on this gene and found that it is also able to regulate the OsC4PPDK promoter in rice protoplasts in the same way as OsDof25 did. However, although we did not check for OsC4PPDK expression in OsDof24 overexpression rice transgenics, we came to the conclusion that these genes will have different downstream targets since these plants showed an obvious delay in flowering time and strong effects on flowering time genes were observed (Yu et al., unpublished results).

The overlap in expression patterns of the OsDof25 and OsC4PPDK genes in green tissues is confirmed by the promoter GUS analyses and is in accordance with the transgenic experiments that show that OsDof25 is a key activating regulator of OsC4PPDK. Future experiments will have to show whether this is also the case for other photosynthesis genes in either the C3 or C4 pathways. Since OsDof25 is also expressed in non-photosynthetically active tissues, this activator will likely also be involved in completely other pathways. Although rice is not a typical C4 plant like maize or Sorghum since it does not have the typical Kranz anatomy to capture CO2 nor does rice have the accompanying photosynthetic capacity, C4 photosynthesis genes are present (Bao et al. 2005; Song et al. 2010). Chinese hybrid rice combinations can show a slightly elevated photosynthetic capacity and it has been speculated that enhanced activity of C4 photosynthesis enzymes is responsible for this phenomenon (Bao et al. 2005; Song et al. 2010) but it needs still to be investigated if the Dof genes such as OsDof25 are involved in any differential expression of downstream targets. We also observed that the photosynthetic capacity of OsDof25 over-expression and RNAi lines was not different from the controls. However, transgenic overexpression studies with the maize PEPC and PPDFK genes in rice and wheat showed a clear effect on photosynthetic capacity and certain yield components such as increased grain yield also because of increased tillering (Ku et al. 2001; Zhang et al. 2014). However, these experiments are quite different than our set-up where OsC4PPDK was higher expressed due to overexpression of an upstream regulator which may mean that OsC4PPDK expression is not higher in all cell types.

To summarise, in this study we confirmed an in planta interaction between the cis-element of the OsC4PPDK promoter and OsDof25 and we confirmed that this gene is an activator of OsC4PPDK. However, since photosynthetic capacity is not increased due to OsDof25 over-expression, likely also other genes will be required to achieve this and increase yield in this way.

Acknowledgments We thank E. Schrijnemakers for excellent plant care. Y.Z. was supported by the CAS-KNAW Joint Ph.D. Training Programme (05-PhD-02) of the Royal Netherlands Academy of Arts and Sciences (KNAW). M.W., Z.Z. and P.B.F.O. were supported by the KNAW Programme Scientific Alliance (04-PSA-BD-04) and by the KNAW China Exchange Programmes (CEP) 04CD022, 06CD0033, 07CDP005, 08CDP042 and 10CDP023.

Author contributions M.W., Z.Z. and P.B.F.O. designed the project. Z.Y. and P.B.F.O. designed the research and wrote the article. Z.Y. and N.I.V. performed experiments and M.W. and P.B.F.O. contributed to analysis and interpretation of results.
Compliance with ethical standards

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

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References

Bao JY, Lee SG, Chen C, Zhang XQ, Zhang Y, Liu SQ, Clark T, Wang J, Cao ML, Yang HM, Wang SM, Yu J (2005) Serial analysis of gene expression study of a hybrid rice strain (LYP9) and its parental cultivars. Plant Physiol 138:1216–1231

Cao ZH, Zhang SZ, Wang RK, Zhang RF, Hao YJ (2013) Genome wide analysis of the apple MYB transcription factor family allows the identification of MdOMYB121 gene conferring abiotic stress tolerance in plants. PLoS ONE 8:e69955

Cavalar M, Philippen Y, Kreuzaler F, Peterhansel C (2007) A drastic reduction in DOF1 transcription levels does not affect C4-specific gene expression in maize. J Plant Physiol 164:1665–1674

Chen SB, Tao LZ, Zeng LR, Vega-Sanchez ME, Umemura K, Wang GL (2006) A highly efficient transient protoplast system for analyzing defence gene expression and protein–protein interactions in rice. Mol Plant Pathol 7:417–427

Chiu WL, Niwa Y, Zeng W, Hirano T, Kobayashi H, Sheen J (1996) Engineered GFP as a vital reporter in plants. Curr Biol 6:325–330

Chollet R, Vidal J, Oleary MH (1996) Phosphoenolpyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. Ann Rev Plant Physiol Plant Mol Biol 47:273–298

Corrales AR, Nebauer SG, Carrillo L, Fernandez-Nohales P, Marques J, Chollet R, Vidal J, Oleary MH (1996) Phosphoenolpyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. Ann Rev Plant Physiol Plant Mol Biol 47:273–298

De Paolis A, Sabatini S, DePascalis L, Costantino P, Capone I (1996) A rolB regulatory factor belongs to a new class of single zinc finger plant proteins. Plant J 2:837–844

DePilos A, Sabatini S, DePascalis L, Costantino P, Capone I (1996) A rolB regulatory factor belongs to a new class of single zinc finger plant proteins. Plant J 2:837–844

Diaz I, Vicente-Carbajosa J, Abraham Z, Martinez M, Isabel-La Moneda I, Carbonero P, O’Gara J (2002) The GAMYB protein from barley interacts with the DOF transcription factor BPBF and activates endosperm-specific genes during seed development. Plant J 29:453–464

Edwards GE, Nakamoto H, Burnell JN, Hatch MD (1985) Pyruvate, Pi dikinase and NADP-malate dehydrogenase in C-4 photosynthesis—properties and mechanism of light dark regulation. Ann Rev Plant Physiol Plant Mol Biol 36:255–286

Gao G, Zhong YF, Guo AY, Zhu QH, Tang W, Zheng WM, Gu XC, Wei LP, Luo JC (2006) DRFT: a database of rice transcription factors. Bioinformatics 22:1286–1287

Gaur VS, Singh US, Kumar A (2011) Transcriptional profiling and in silico analysis of DOF transcription factor family gene for understanding their regulation during seed development of rice Oryza sativa L. Mol Biol Rep 38:2827–2848

Green PL, Kay SA, Chua NH (1987) Sequence-specific interactions of a pea nuclear factor with light-responsive elements upstream of the rbcS-3A gene. EMBO J 6:2543–2549

Guan KL, Dixon JE (1991) Eukaryotic proteins expressed in Escherichia coli: an improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. Anal Biochem 192:262–267

Guo Y, Qiu LJ (2013) Genome-wide analysis of the DoF transcription factor gene family reveals soybean-specific duplicate and functional characteristics. PLoS ONE 8:e67889

Hernando-Amado S, Gonzalez-Calle V, Carbonero P, Barrero-Silicia C (2012) The family of DOF transcription factors in Brachypodium distachyon: phylogenetic comparison with rice and barley DOFs and expression profiling. BMC Plant Biol 12:202

Higo K, Uegawa Y, Iwamoto M, Higo H (1998) PLACE: a database of plant cis-acting regulatory DNA elements. Nucl Acids Res 26:348–359

Hirochika H, Guiderdoni E, An G, Hsing YL, Eun MY, Han CD, Upadhyaya N, Ramachandran S, Zhang QF, Pereira A, Sundaresan V, Leung H (2004) Rice mutant resources for gene discovery. Plant Mol Biol 54:325–334

Hwang YS, Ciceri P, Parsons RL, Moose SP, Schmidt RJ, Huang N (2004) The maize O2 and PBF proteins act additively to promote transcription from storage protein gene promoters in rice endosperm cells. Plant Cell Physiol 45:1509–1518

Jung KH, An G (2013) Functional characterization of rice genes using a gene-indexed T-DNA insertional mutant population. Methods Mol Biol 955:67–70

Kisu Y, Ono T, Shimofurutani N, Suzuki M, Esaka M (1998) Characterization and expression of a new class of zinc finger protein that binds to silencer region of ascorbate oxidase gene. Plant Cell Physiol 39:1054–1064

Ku MS, Cho D, Li X, Jiao DM, Pinto M, Miyao M, Matsuoka M (2001) Introduction of genes encoding C4 photosynthesis enzymes into rice plants: physiological consequences. Novartis Found Symp 236:100–111

Kushwaha H, Gupta S, Singh VK, Rastogi S, Yadav D (2011) Genome-wide identification of DoF transcription factor gene family in sorghum and its comparative phylogenetic analysis with rice and Arabidopsis. Mol Biol Rep 38:5037–5053

Le Hir R, Bellini C (2013) The plant-specific DOF transcription factors family: new players involved in vascular system development and functioning in Arabidopsis. Front Plant Sci 4:164

Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li J (2003) Control of tillering in rice. Nature 422:618–621

Li L, Wang XF, Stolc V, Li XY, Zhang DF, Su N, Tongprasit W, Li SG, Cheng ZK, Wang J, Deng XW (2006) Genome-wide transcription analyses in rice using tiling microarrays. Nat Genet 38:124–129

Li D, Yang C, Li X, Gan Q, Zhao X, Zhu L (2009) Functional characterization of rice OsDoF12. Planta 229:1159–1169

Lijavetzky D, Carbonero P, Vicente-Carbajosa J (2003) Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis DOF gene families. BMC Evol Biol 3:17

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408

Luo L, Li W, Miura K, Ashikari M, Kyozuka J (2012) Control of tiller growth of rice by OsSPL14 and strigolactones, which work in two independent pathways. Plant Cell Physiol 53:1793–1801

Matsumoto T, Wu JZ, Kanamori H, Katayose Y, Fujisawa M, Namiki N, Mizuno H, Yamamoto K, Antonio BA, Baba T et al (2005) The map-based sequence of the rice genome. Nature 436:793–800

Meijer AH, Obermeier T, van Dijk EL, Qin L, Taal AJ, Rhee S, Harrington SE, McCouch SR, Schilperoort RA, Hoge JHC
(1997) Transcriptional repression by Oshox1, a novel homeodomain leucine zipper protein from rice. Plant J 11:263–276

Meijer AH, Ouwerkerk PBF, Hoge JHC (1998) Vectors for transcription factor cloning and target site identification by means of genetic selection in yeast. Yeast 14:1407–1415

Meijer AH, Schouten J, Ouwerkerk PBF, Hoge JHC (2000) Yeast as versatile tool in transcription factor research. In: Gelvin SB, Schilperoort RA (eds) Plant molecular biology manual, 2nd edn, suppl IV. Kluwer Academic Publishers, Dordrecht, pp 1–28

Mena M, Cejudo IF, Isabel-Lamonedo I, Carbonero P (2002) A role for the DOF transcription factor BPBF in the regulation of gibberellin-responsive genes in barley aleurone. Plant Physiol 130:111–119

Nie DM, Ouyang YD, Wang X, Zhou W, Hu CG, Yao J (2013) Genome-wide analysis of endosperm-specific gene expression in rice. Gene 530:236–247

Noguero M, Atif RM, Ochatt S, Thompson RD (2013) The role of the DNA-binding One Zinc Finger (DOF) transcription factor family in plants. Plant Sci 209:32–45

Ouwerkerk PBF, Meijer AH (2001) Yeast one-hybrid screening for DNA–protein interactions. Curr Protoc Mol Biol Chap 12, Unit 12.12

Ouwerkerk PBF, Meijer AH (2011) Yeast one-hybrid screens for detection of transcription factor DNA interactions. Methods Mol Biol 678:211–227

Ouwerkerk PBF, De Kam RJ, Hoge JHC, Meijer AH (2001) Glucocorticoid-inducible gene expression in rice. Planta 213:370–378

Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The TIGR rice genome annotation resource: improvements and new features. Nucl Acids Res 35:D883–D887

Pasquali G, Ouwerkerk PBF, Memelink J (1994) Versatile transformation vectors to assay the promoter activity of DNA elements in plants. Gene 149:373–374

Plesch G, Ehrhardt T, Mueller-Roemper B (2001) Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. Plant J 28:455–464

Priya P, Jain M (2013) RiceSRTFDB: a database of rice transcription factors containing comprehensive expression, cis-regulatory element and mutant information to facilitate gene function analysis. Database (Oxford): bat027

Riano-Pachon DM, Ruzicic S, Dreyer I, Mueller-Roemper B (2007) PlantTFDB: an integrative plant transcription factor database. BMC Bioinformatics 8:42

Riechmann JL, Ratcliffe OJ (2000) A genomic perspective on plant transcription factors. Curr Opin Plant Biol 3:423–434

Santos LA, de Souza SR, Fernandes MS (2012) OsDof25 expression alters carbon and nitrogen metabolism in Arabidopsis under high N-supply. Plant Biotechnol Rep 6:327–337

Scarpella E, Rueba S, Boot KJ, Hoge JHC, Meijer AH (2000) A role for the rice homeobox gene Oshox1 in provascular cell fate commitment. Development 127:3655–3669

Song GS, Zhai HL, Peng YG, Zhang L, Wei G, Chen XY, Xiao YG, Wang L, Chen YJ, Wu B et al (2010) Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol Plant 3:1012–1025

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Toepfer R, Matzeit V, Gronenborn B, Schell J, Steinbiss HH (1987) A set of plant expression vectors for transcriptional and translational fusions. Nucl Acids Res 15:5890

Van der Fits L, Memelink J (1997) Comparison of the activities of CaMV 35S and FMV 34S promoter derivatives in Catharanthus roseus cells transiently and stably transformed by particle bombardment. Plant Mol Biol 33:943–946

Washio K (2001) Identification of Dof proteins with implication in the gibberellin-regulated expression of a peptidase gene following the germination of rice grains. Biochim Biophys Acta Gene Struct Expr 1520:54–62

Wesley SV, Helliwell CA, Smith NA, Wang MB, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27:581–590

Yanagisawa S (1998) Dof proteins: involvement of transcription factors with a novel DNA-binding domain in tissue-specific and signal-responsive gene expression. Seikagaku 70:280–285

Yanagisawa S (2000) Dof1 and Dof2 transcription factors are associated with expression of multiple genes involved in carbon metabolism in maize. Plant J 21:281–288

Yanagisawa S (2001) The transcriptional activation domain of the plant-specific Dof1 factor functions in plant, animal, and yeast cells. Plant Cell Physiol 42:813–822

Yanagisawa S (2002) The Dof family of plant transcription factors. Trends Plant Sci 7:555–560

Yanagisawa S, Izui K (1993) Molecular cloning of 2 DNA-binding proteins of maize that are structurally different but interact with the same sequence motif. J Biol Chem 268:16028–16036

Yanagisawa S, Schmidt RJ (1999) Diversity and similarity among recognition sequences of Dof transcription factors. Plant J 17:209–214

Yanagisawa S, Sheen J (1998) Involvement of maize Dof zinc finger proteins in tissue-specific and light-regulated gene expression. Plant Cell 10:75–89

Yu J, Wang J, Lin W, Li SG, Li H, Zhou J, Ni PX, Dong W, Hu SN, Zeng CQ et al (2005) The genomes of Oryza sativa: a history of duplications. PLoS Biol 3:266–281

Zhang B, Chen W, Foley RC, Buttner M, Singh KB (1995) Interactions between distinct types of DNA binding proteins enhance binding to ocs element promoter sequences. Plant Cell 7:2241–2252

Zhang JW, Li CS, Wu CY, Xiong LZ, Chen GX, Zhang QF, Wang SP (2006) RMD: a rice mutant database for functional analysis of the rice genome. Nucl Acids Res 34:D745–D748

Zhang H, Xu W, Wang H, Hu L, Li Y, Qi X, Zhang L, Li C, Hua X (2014) Pyramiding expression of maize genes encoding phospho-enolpyruvate carboxylase (PEPC) and pyruvate orthophosphate dikinase (PPDK) synergistically improve the photosynthetic characteristics of transgenic wheat. Protoplasma 251:1163–1173