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OsDOG, a gibberellin-induced A20/AN1 zinc-finger protein, negatively regulates gibberellin-mediated cell elongation in rice

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ABSTRACT

The A20/AN1 zinc-finger proteins (ZFPs) play pivotal roles in animal immune responses and plant stress responses. From previous gibberellin (GA) microarray data and A20/AN1 ZFP family member association, we chose *Oryza sativa dwarf rice with overexpression of gibberellin-induced gene* (*OsDOG*) to examine its function in the GA pathway. *OsDOG* was induced by gibberellic acid (GA_3) and repressed by the GA-synthesis inhibitor paclobutrazol. Different transgenic lines with constitutive expression of *OsDOG* showed dwarf phenotypes due to deficiency of cell elongation. Additional GA_1 and real-time PCR quantitative assay analyses confirmed that the decrease of GA_1 in the overexpression lines resulted from reduced expression of *GA3ox2* and enhanced expression of *GA2ox1* and *GA2ox3*. Adding exogenous GA rescued the constitutive expression phenotypes of the transgenic lines. *OsDOG* has a novel function in regulating GA homeostasis and in negative maintenance of plant cell elongation in rice.

Introduction

The A20 zinc-finger domain was first identified in the protein encoded by a tumor-necrosis-factor-inducible gene, A20, in human endothelial cells (Opipari et al., 1990). The AN1 domain was first found at the C terminus of AN1, a ubiquitin-like protein from *Xenopus laevis* (Rebagliati et al., 1985). Proteins containing A20 and/or AN1 zinc-finger domains, referred to as the A20/AN1 zinc-finger proteins (ZFPs), have been found in 22 organisms, including protists, fungi, plants and animals (Vij and Tyagi, 2008). In animals, the role of some A20/AN1 ZFPs in regulating immune responses has been well characterized, including ZNF216 and AWP1 in humans (Duan et al., 2000; Huang et al., 2004; Scott et al., 1998), and ZNF216 in mice (Hishiya et al., 2006).

In plants, genome-wide surveys have revealed several plant species with A20/AN1 ZFP genes inducible by abiotic stresses; examples are 18 rice genes, 14 Arabidopsis genes and 13 tomato genes encoding A20/AN1 ZFPs and 19 poplar genes and 11 maize genes encoding proteins containing at least one AN1 zinc finger (Jin et al., 2007; Solanke et al., 2009; Vij and Tyagi, 2006). Some of the A20/AN1 ZFP genes play an important role in stress tolerance. For example, transgenic plants overexpressing *OsiSAP1* or *OsiSAP8* conferred tolerance to cold, drought and salt stresses (Kanneganti and Gupta, 2008; Mukhopadhyay et al., 2004). Moreover, overexpression of the rice A20/AN1 ZFP gene *ZFP177* in tobacco enhanced tolerance to both low and high temperature stresses while increasing sensitivity to salt and drought stresses (Huang et al., 2008). Although abundant research has shown that A20/AN1 ZFPs may play crucial roles in plant stress responses, little is known about their functions in regulating plant development.

Gibberellins (GAs) regulate several plant growth and development processes such as seed germination, stem elongation, leaf expansion and reproductive development (Sun and Gubler, 2004). Dwarfism is a typical phenotype for mutants with defects in GA-synthesis enzymes such as GA20ox and GA3ox (Sakamoto et al., 2004). Overexpression of *GA2ox1* or *GA2ox3* can inhibit stem elongation in transgenic rice (Sakai et al., 2003; Sakamoto et al., 2001). Moreover, factors that modify GA expression may also affect plant height. For example, *elongated uppermost internode* (*EUI*) encodes a cytochrome P450 monooxygenase that epoxidizes GAs; and *eui1* plants show greatly elongated internodes, especially the uppermost ones (Luo et al., 2006; Zhu et al., 2006). Mutations affecting GA signaling pathways may also alter plant height—mutants with impaired GA signaling are dwarf, whereas those with constitutively

Abbreviations: EST, expressed sequence tag; GA, gibberellin; GUS, β-glucuronidase; OsDOG, Oryza sativa dwarf rice with overexpression of gibberellin-induced gene; PAC, paclobutrazol; PI, propidium iodide; RNAi, RNA interference; ZFP, zinc-finger protein.

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active GA responses display a slender phenotype (Sun and Gubler, 2004).

In this paper, we describe an A20/AN1 ZFP gene from rice, *Oryza sativa dwarf rice with overexpression of gibberellin-induced gene* (*OsDOG*), which negatively regulates GA-mediated cell elongation in rice.

Materials and methods

Plant materials and treatments

Rice variety Zhonghua 10 (*Oryza sativa* L. ssp. *japonica* cv. Zhonghua 10, ZH10) was used in this study. Rice plants were grown on half-strength Murashige and Skoog (MS) medium with 0.7% agar (pH 5.8) under continuous light at 30 °C in a plant growth-chamber or in the field under natural conditions. T_2 and T_3 generations of transgenic rice lines were used in the phenotype analysis and physiological experiments.

RNA extraction and semi-quantitative or real-time PCR

RNA was extracted from rice tissues by use of TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR involved a two-step method: synthesizing the first-strand cDNA with AMV or M-MLV reverse transcriptase (Promega, USA) following the manufacturer's instructions, and amplifying the cDNAs with specific primers. LA Taq polymerase (Takara Bio Inc., Japan) was used for semi-quantitative RT-PCR, and SYBR® Green Realtime PCR Master Mix (Toyobo Co., LTD., Japan) was used for real-time PCR. The primers for *OsDOG* were 5′-CCTCGCTAACCCATTCCCAAAA-3′ and 5′-CCCTCTTTCCATCCAACTTCC-3′, and for *Tubulin* were 5′-TCAGATGCCCAGTGACAGGA-3′ and 5′-TTGGTGATCTCGGCAACAGA-3′. The real-time PCR primers for *OsDOG* were 5′-TGTGAATTGCCCTGACAGCT-3′ and 5′-TGGACCTACCAATAATGCCAG-3′. The real-time PCR primers for *GA3ox2*, *GA2ox1*, *GA2ox2* and *OsActin1* (the internal reference) were the same as those used by Dai et al. (2007).

Construction of OsDOG-overexpressing and RNA-interference (RNAi) rice lines

To generate the overexpression rice lines, the cDNA fragment of *OsDOG* containing the full-length open reading frame (ORF) was cloned with the primers 5′-GGATCCTCTCTCGGA TTCGATCAT-3′ (*Bam*H I site underlined) and 5′-GGTACCCCCTTGTATTATCCTTTTAG-3′ (*Kpn* I site underlined) and were inserted downstream of a maize Ubiquitin promoter in the pUN1301 vector (Ge et al., 2004).

For the RNAi rice construct, the specific fragment of *OsDOG* was amplified with the primers 5′-GGGTACCACTAGTCGTTAGGTTCTAAAAGGA-3′ (*Kpn* I and *Spe* I restriction site underlined) and 5′-GGATCCGAGCTCTTTCCATCCAACTTC-3′ (*Bam*H I and *Sac* I restriction site underlined) and was then inserted into the RNAi vector pTCK303 (Wang et al., 2004). The rice transformants were created as described (Ge et al., 2004).

Cell-length analysis in rice internodes

The middle parts of the internodes were separated from culms of mature rice and stained with propidium iodide (PI). The inner layer of parenchyma cells of the internodes was examined under a laser scanning confocal microscope (LSM 510, Zeiss, Oberkochen, Germany).

Construction of pOsDOG::GUS and histochemical localization of GUS

The promoter 2 kb upstream of the start codon of *OsDOG* was amplified with the primers 5′-GAGCTCGAAAGGGAGCAGAAGCAGCAG-3′ (*Sac* I site underlined) and 5′-TCTAGAGAGG CGAGGAGAGGGTGAGTC-3′ (*Xba* I site underlined) and then inserted into the 5′-end of the β-glucuronidase (GUS) gene (gusA) in pCAMBIA1301 to generate a vector containing *pOsDOG::GUS*. The construct was then introduced into rice plants and GUS signals were detected as described (Ge et al., 2004).

Quantitative analysis of endogenous GA_1

Shoots of four-leaf-stage seedlings of *OsDOG*-overexpressing lines and the wild type were harvested and ground with use of a mortar and pestle. The homogenized tissue was incubated in 80% acetone for 1 h at 25 °C. The extract was centrifuged at 10,000 × *g* for 5 min, and the supernatants were evaporated under vacuum to a volume of 50 μL (aqueous phase). After adding 350 μL distilled water, the aqueous phase was extracted with ethyl acetate. Particles were removed by centrifugation, and the ethyl acetate-soluble fraction containing GAs underwent high-performance liquid chromatography (Agilent Technologies 1200 series) for purification with a reverse-phase column (Eclipse XDB-C18). After silylation with 20 μL pyridine and 40 μL N,O-bis-(trimetylsilyl) trifluoroacetamide for 40 min at 65 °C, the purified fraction underwent gas chromatography–mass spectrometry with selected ion monitoring with use of a gas chromatography–mass spectrometer (Trace 2000 series, ThermoQuest) equipped with a capillary column (DB-5; Agilent Technologies) after derivatization. The GA_1 level was analyzed by use of ^{2}H-labeled GA_1 as described (Varbanova et al., 2007).

Effect of exogenous GA on the elongation of the second-leaf sheaths, shoots and internodes

Rice seeds were surface sterilized and planted on half-strength MS medium containing optimal concentrations of GA_3 or paclobutrazol (PAC) and incubated under continuous light at 30 °C for 8 d before the length of the second leaf sheath or the shoot length was measured or under continuous darkness at 30 °C for 14 d before the length of the second lower internodes was measured.

Results

Analysis of OsDOG characteristics

Using cDNA microarray analysis, we previously discovered that GA treatment in rice increased the expression of the expressed sequence tag (EST) p782a09 (Wang et al., 2005). This EST corresponds to LOC_Os08g39450 on chromosome 8 at location 24, 821, 089-24, 822, 306 in the rice genome and was designated *Oryza sativa dwarf rice with overexpression of gibberellin-induced gene* (*OsDOG*) on the basis of the phenotypes of its transgenic rice lines. We further confirmed the effect of GA on the expression of *OsDOG* using real-time PCR. The mRNA level of *OsDOG* increased with GA_3 treatment and decreased with PAC, an inhibitor of gibberellin synthesis (Fig. 1A and B). The putative transcript of *OsDOG* is 1218 bp and consists of a 513-bp coding region, a 73-bp 5′ untranslated region and a 632-bp 3′ untranslated region. The putative OsDOG protein contains 170 amino acids (aa) with a predicted molecular weight of 18.144 kDa (http://www.gramene.org). Because A20/AN1 ZFPs exist universally across diverse organisms (Vij and Tyagi, 2008), we analyzed the phylogenetic relation of OsDOG and its orthologs using Mega 4.0 software (Tamura et al., 2007) and found that A20/AN1 ZFPs from plants and animals

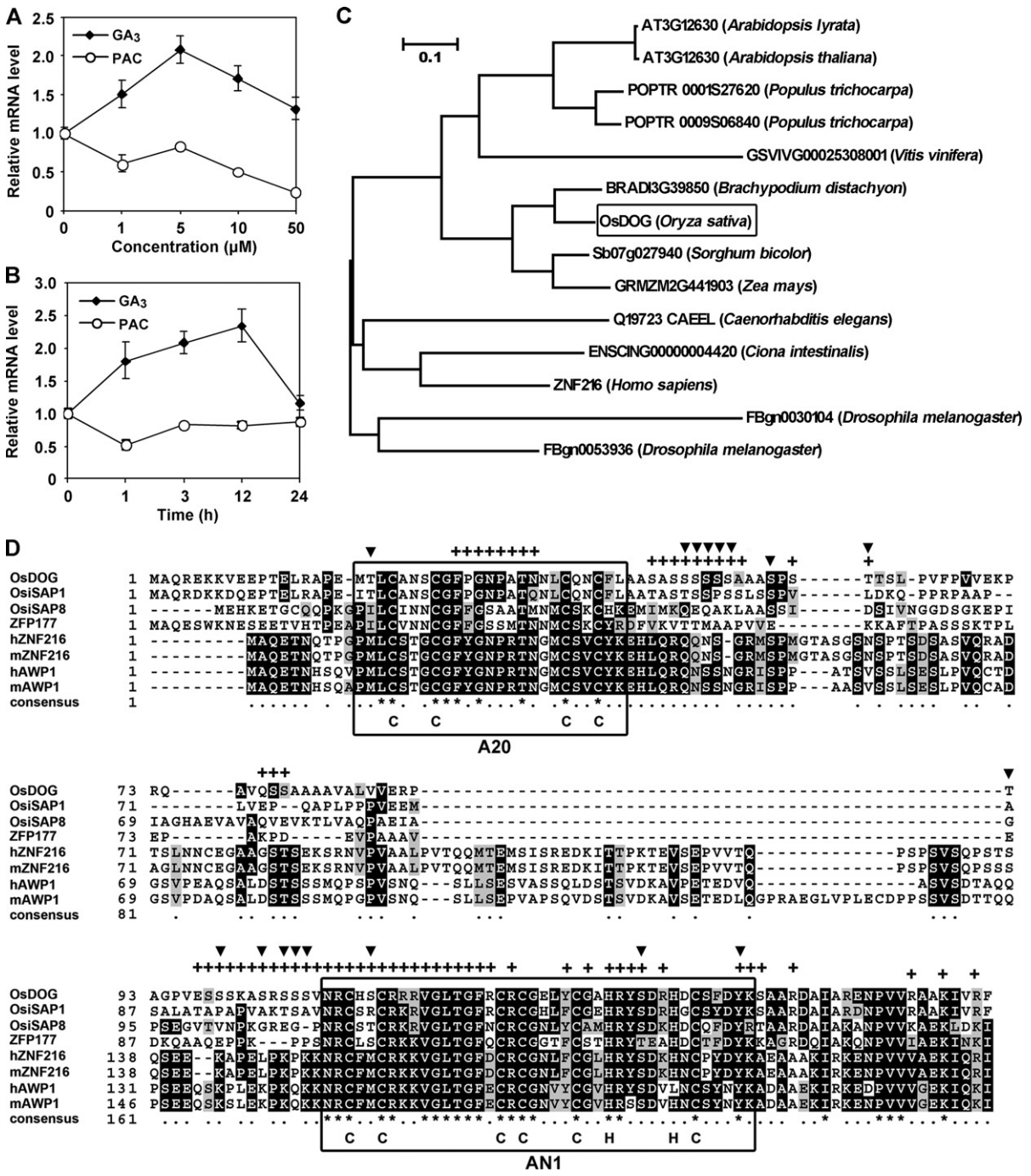

Fig. 1. Sequence characteristics and gibberellic acid (GA) response of *OsDOG*. (A) and (B) Expression of *OsDOG* in response to exogenous GA_3 and GA synthesis inhibitor paclobutrazol (PAC). Real-time PCR analysis of mRNA level of *OsDOG* in seedlings treated with 0, 1, 5, 10 or 50 μM GA_3 or PAC for 3 h (A), or 5 μM of GA_3 or PAC for different times (B). The expression was normalized to that of *Osactin1*. The mRNA levels of material not treated by GA_3 or PAC were set at 1. Error bars indicate ± SD. (C) Phylogenetic analysis of OsDOG and its orthologs. The un-rooted tree was generated by Mega 4.0 with the neighbour-joining method. The scale bar 0.1 marks 0.1 amino acid substitution per site. (D) Amino acid alignment of OsDOG (gi|75147526), OsiSAP1 (gi|158513706), OsiSAP8 (gi|35187687), ZFP177 (gi|30908921), human ZNF216 (hZNF216, gi|5174755), mouse ZNF216 (mZNF216, gi|25091739), human AWP1 (hAWP1, gi|49065378) and mouse AWP1 (mAWP1, gi|11121500). Clustal X2.0 was used for the alignment. The boxes indicate the A20 and AN1 zinc-finger domains. Solid triangles above the sequence of OsDOG indicate phosphorylation sites predicted by NetPhos 2.0. Cross symbols above the sequence of OsDOG indicate DNA-binding residues predicted by DNABindR. Conserved cysteine residues in A20 and cysteine and histidine residues in AN1 are listed at the bottom.

belong to different clusters. In plants, OsDOG and its orthologs from monocotyledons belong to one subcluster, and the orthologs from dicotyledons form another subcluster (Fig. 1C). OsDOG shows sequence similarities to several reported A20/AN1 ZFPs, including OsiSAP1 (Mukhopadhyay et al., 2004), OsiSAP8 (Kanneganti and Gupta, 2008), and ZFP177 (Huang et al., 2008) from rice, ZNF216 (Scott et al., 1998) and AWP1 (Duan et al., 2000) from humans and mice (Fig. 1D). OsDOG contains one A20-type zinc-finger (aa 19-43, CX3CX10CX2C) (Znf_A20, SM00259) at its N terminus and one AN1-type zinc-finger (aa 111-151, CX2CX10CXCX4CX2HX5HXC) (Znf_AN1, SM00154 and PF01428) at its C terminus (Fig. 1D). OsDOG also has DNA binding sites predicted by DNABindR (Yan et al., 2006), and phosphorylation sites predicted by use of NetPhos 2.0 (Blom et al., 1999) (Fig. 1D).

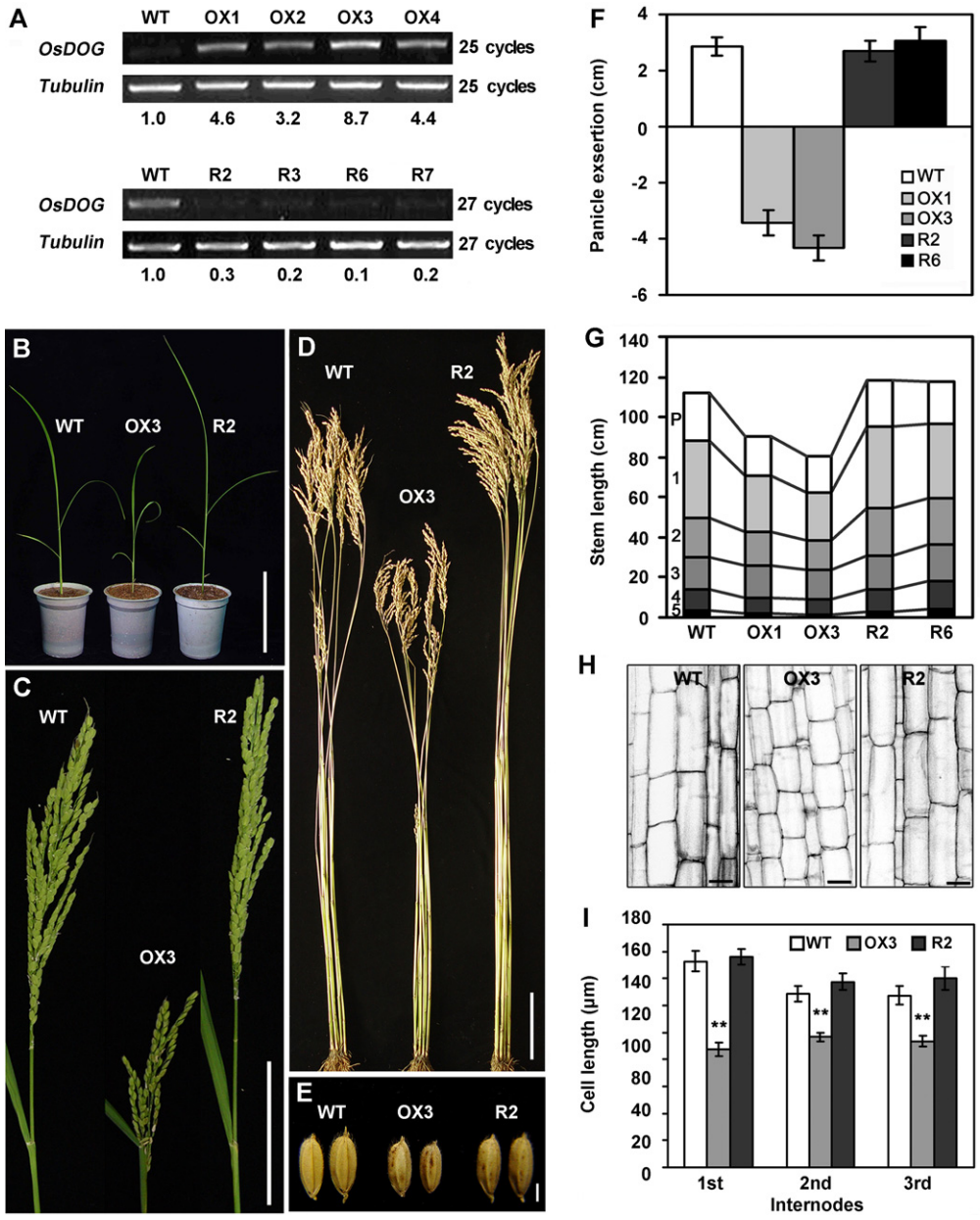

Fig. 2. Phenotypes of *OsDOG*-transgenic rice lines. (A) Semi-quantitative RT-PCR analysis of expression of *OsDOG* in the overexpression lines (OX1-4) and RNAi lines (R2, R3, R6 and R7). *Tubulin* was used as an internal reference. The cycle numbers of the PCR reactions are marked at the right of the images. The relative expression level of *OsDOG* is normalized to *Tubulin* and that in wild type was set at 1. (B–E) Gross morphology of *OsDOG*-overexpressing line OX3, RNAi line R2, and wild-type plants. (B) 14-d-old seedlings. Bar = 10 cm. (C) Panicle-exsertion features. Bar = 10 cm. (D) Culms at the mature stage. Bar = 10 cm. (E) Mature seeds. Bar = 20 mm. (F) Statistics for panicle exsertion of *OsDOG*-overexpressing lines, RNAi lines, and wild-type plants. Error bars indicate ± SE. (G) Internode length at the mature stage. P: panicles, 1–5: internodes from top to bottom. (H) Confocal microscopy of the inner layer of parenchyma cells of the first internodes from the overexpression line OX3, RNAi line R2, and wild type at the mature stage stained with propidium iodide. Bar = 50 μm. (I) Statistics for cell length of the first, second and third internodes of the *OsDOG*-overexpressing line OX3, RNAi line R2, and wild type. $^{**}P < 0.01$ (*t*-test).

OsDOG-overexpressing plants display a dwarf phenotype

To analyze the function of *OsDOG*, we generated multiple overexpression and RNAi transgenic rice lines and examined the expression level of *OsDOG* in the transgenic lines by RT-PCR. In the overexpression lines OX1-4, the mRNA level was 3.2–4.7 times that of the wild type. In the RNAi lines R2, R3, R6 and R7, the mRNA level was 10–30% that of the wild type (Fig. 2A). The overexpression lines exhibited dwarf phenotypes at the seedling stage, whereas the phenotype of the RNAi lines did not significantly differ from that of the wild type (Fig. 2B). At the reproductive stage, the inhibited elongation of the first internodes of the overexpression lines resulted in panicles that did not completely emerge from the leaf sheath (Fig. 2C). The panicle exsertion (the length of the stem exposed between the ear and the flag leaf sheath) of the overexpression lines was much shorter than that of the wild type (Fig. 2F). At the mature stage, the overexpression lines had shorter panicles and internodes (Fig. 2D and G) and smaller seeds (Fig. 2E) as compared with wild-type plants. The length of cells in the first, second, and third internodes of mature overexpression lines were shorter than that of the wild type (Fig. 2H and I). The plant height and internodes of RNAi lines were slightly longer than that of wild type (Fig. 2D and G) because of the longer cell length in the internodes (Fig. 2H and I). Thus, *OsDOG* negatively regulates the internode length by suppressing cell elongation rather than cell proliferation.

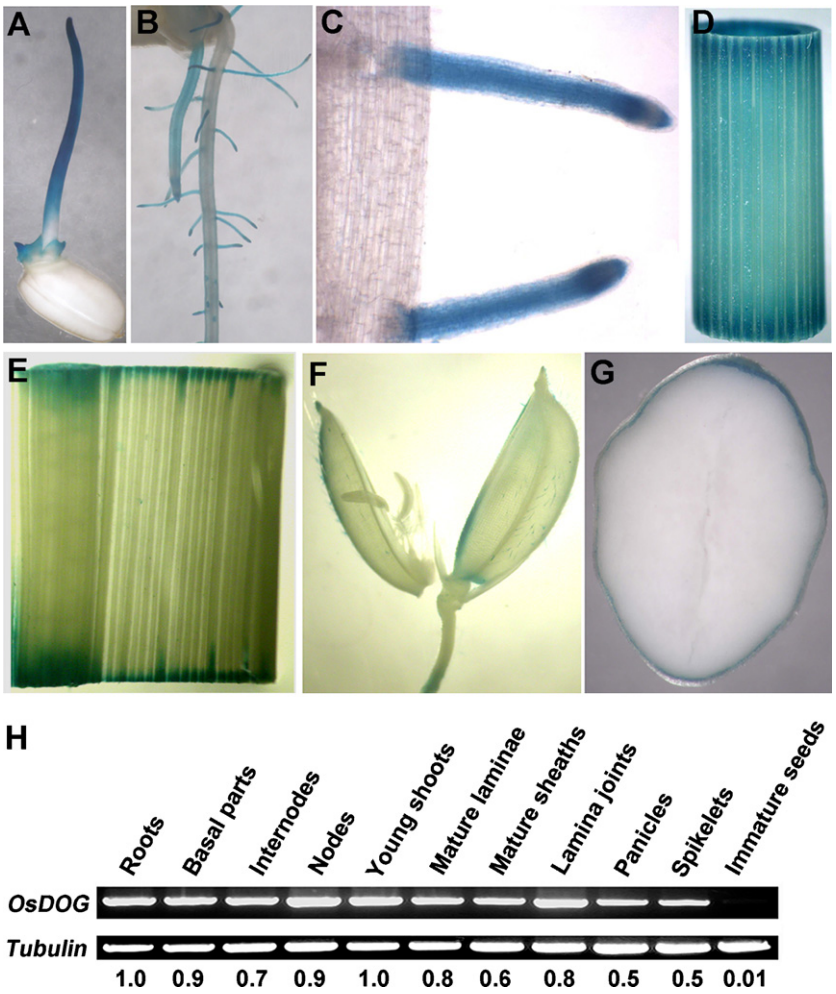

Fig. 3. The expression pattern of the mRNA of *OsDOG*. (A–G) The expression of *pOsDOG::GUS* fusion gene. GUS signals were detected in elongating coleoptiles (A), rapidly growing roots (B and C), stems (D), leaves (E), lemmas and glumellules (F) and aleurone layers (G), and not in stamens and pistils (F). (H) Semi-quantitative RT-PCR of the expression of *OsDOG* in various organs. *Tubulin* was used as the internal reference. The expression of *OsDOG* was normalized to *Tubulin* and that in roots was set at 1.

Expression pattern of OsDOG

To determine the expression pattern of *OsDOG*, we transformed the *pOsDOG::GUS* construct into rice. GUS staining was found in almost all types of rice tissues, including roots, shoots, stems, leaves, lemmas, glumellules and aleurone layers of mature seeds (Fig. 3A–G). Furthermore, its expression level was higher in elongating coleoptiles (Fig. 3A), the elongating zone of roots and root caps (Fig. 3B and C) and young stems (Fig. 3D). No expression was detected in stamens and pistils (Fig. 3F). 3 independent *pOsDOG::GUS* transgenic lines were analyzed and showed identical expression pattern of *OsDOG*. The expression pattern was confirmed by RT-PCR (Fig. 3H). Collectively, *OsDOG* is a constitutively expressing gene with increased expression in rapidly growing tissues.

Overexpression of OsDOG decreases GA_1 level in transgenic plants

The overexpression of GA-induced *OsDOG* in rice resulted in a dwarf phenotype, so we examined the level of endogenous GA_1, the major bioactive GA in rice (Yamaguchi, 2008). In *OsDOG*-overexpressing lines, the endogenous GA_1 decreased to about one-half the level in wild-type plants (Fig. 4A). *GA3ox2* encodes GA 3-oxidase, which converts GA_{20} to GA_1; *GA2ox1* and *GA2ox3* encode GA 2-oxidases, which transfer GA_1 and its immediate precursors into inactive forms (Yamaguchi, 2008). We tested the expression of *GA3ox2*, *GA2ox1* and *GA2ox3* in the overexpression lines and the wild type by real-time PCR. The expression level of *GA2ox1* and *GA2ox3* was increased, but that of *GA3ox2* was decreased in the overexpression lines as compared with wild-type plants (Fig. 4B). These results indicate that the overexpression of *OsDOG* in the transgenic lines resulted in lower conversion rates from GA_{20} to GA_1 through GA 3-oxidases and more inactive forms of GA from GA_1 by GA 2-oxidases. The altered expression of these GA metabolism genes directly lead to lower GA_1 content in *OsDOG*-overexpressing lines. We also found that the rice *eui1* mutation decreased the expression of *OsDOG* by half as compared with the wild type (Fig. 4C). Because *eui1* mutants have opposing phenotypes as compared with *OsDOG*-overexpressing lines, *OsDOG* acts negatively in cell elongation by regulating the GA metabolism pathway.

GA treatment rescues the phenotypes of OsDOG-overexpressing lines

The second leaf sheaths and shoots of *OsDOG*-overexpressing lines were less elongated than that of the wild type. To test whether GA_3 treatment could rescue this phenotype, we treated the transgenic lines and the wild type with various concentrations of GA_3 (up to 100 μM) and examined the length of the second leaf sheaths.

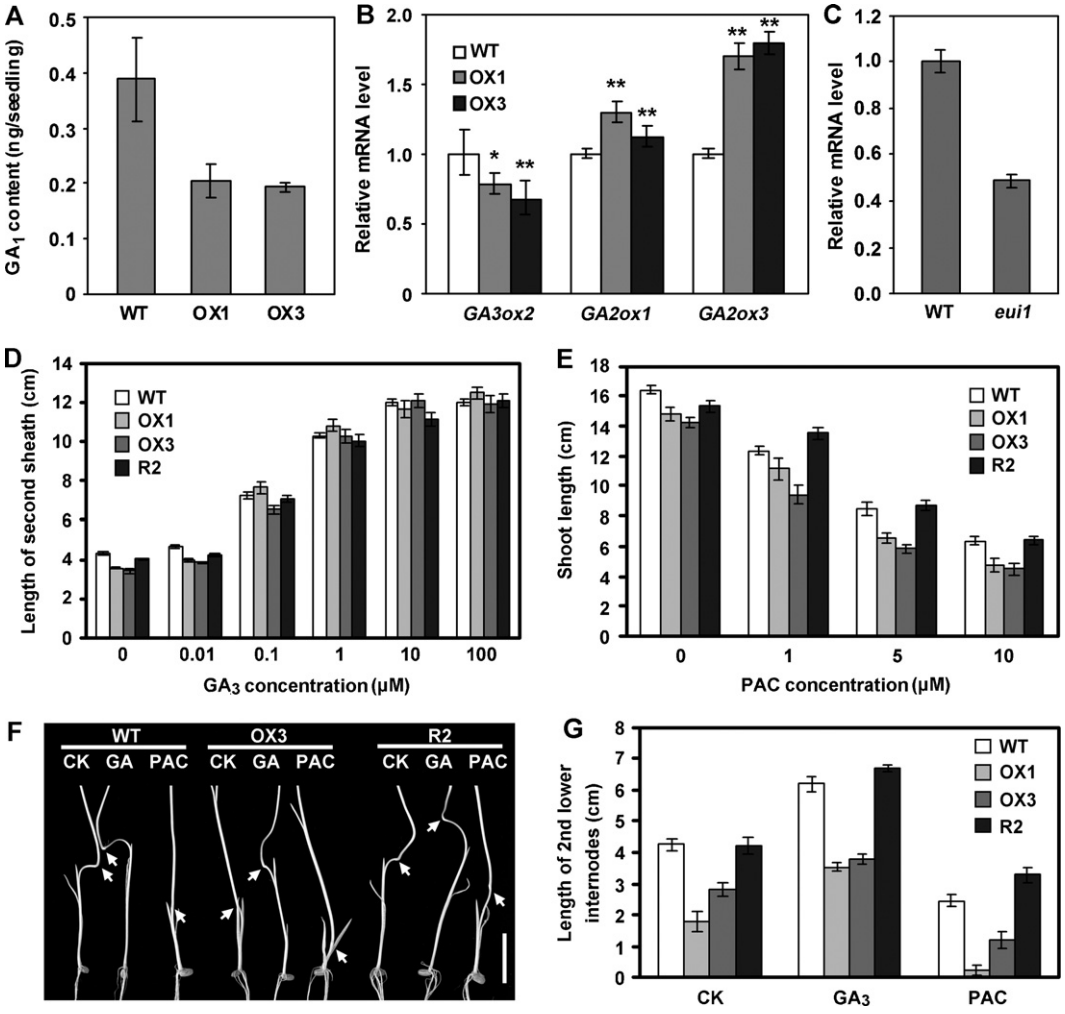

Fig. 4. Endogenous GA_1 level and the response to GA_3 and PAC in *OsDOG*-overexpressing lines. (A) The endogenous level of GA_1 in 14-d-old *OsDOG*-overexpressing lines OX1 and OX3 and the wild type determined by gas chromatography–mass spectrometry. Data are means ± SE. (B) The mRNA level of *GA3ox2*, *GA2ox1* and *GA2ox3* examined by real-time PCR in *OsDOG*-overexpressing lines OX1 and OX3 and the wild type. $^{*}P < 0.05$; $^{**}P < 0.01$ (*t*-test). (C) The mRNA level of *OsDOG* determined by real-time PCR in *eui1* mutant and the wild type. Expression level in (B) and (C) was normalized to that of *Osactin1*. Transcript levels of the wild type were set at 1. Error bars indicate ± SD. (D) Length of the second leaf sheaths from overexpression lines OX1 and OX3, RNAi line R2, and the wild type with GA_3 treatment. (E) Shoot length of the overexpression lines OX1 and OX3, RNAi line R2, and the wild type with PAC treatment. (F) Phenotype of *OsDOG*-overexpressing line OX3, RNAi line R2, and the wild type in response to GA_3 (1 μM) and PAC (1 μM) grown in the dark. The arrows indicate the third nodes from the bottom. CK: untreated control. Bar = 2 cm. (G) Length of the second lower internodes shown in (F). Error bars represent ± SE.

The results showed that 1 μM GA_3 could fully rescue the less-elongated phenotype of second leaf sheaths in the overexpression lines (Fig. 4D). In contrast, PAC treatment (up to 10 μM) reduced the shoot length of the overexpression lines, with a similar outcome in the wild type (Fig. 4E). In addition, the length of second leaf sheaths and shoots of RNAi lines was near that of the wild type and showed similar responses to GA and PAC treatment (Fig. 4D and E).

Internodes of rice seedlings grown in darkness consistently express unusual elongation as compared with those grown under light (Yamamuro et al., 2000). Skotomorphogenesis assays of the *OsDOG*-overexpressing lines showed that their internodes were not as elongated as those of the wild type when grown in the dark but were similar to those of PAC-treated dark-grown wild-type plants (Fig. 4F and G). When treated with GA, overexpression lines grown in the dark showed a similar internode length as that of untreated dark-grown wild-type plants (Fig. 4F and G). However, RNAi lines and wild-type plants did not differ in internode length (Fig. 4F and G). Therefore, cell elongation defects in the *OsDOG*-overexpressing lines can be rescued by exogenous GA_3.

Discussion

Our present study suggests a novel role of the A20/AN1 ZFP genes in regulating plant growth. *OsDOG*, a member of the A20/AN1 ZFP gene family, negatively regulates GA-mediated cell elongation in rice.

A novol role of A20/AN1 ZFP gene family in GA-mediated plant growth regulation

Several ZFPs are believed to be involved in GA-mediated plant growth regulation. *OsLOL2* encodes a protein with 2 LSD1-like zinc-finger domains. Antisense of *OsLOL2* reduced GA_1 content and plant height (Xu and He, 2007). RAMY contains a zinc finger with a CXCX4CX2H consensus sequence that can bind to the O2S box of the a-amylase gene promoter. *RAMY* mRNA accumulation occurs before that of *Amy2* mRNA accumulation with GA treatment (Peng et al., 2004). Rice YAB1 is one of the YABBY proteins that belong to the zinc-finger superfamily. Overexpression of *YAB1* induces a dwarf phenotype and reduces GA_1 level. YAB1 negatively regulates GA_1 level by directly binding to the promoter of *GA3ox2* (Dai

et al., 2007). Recent research has also suggested that a zinc-finger protein Double B-box 1a inhibits the expression of *GA2ox1* and *GA2ox8* but promotes that of *GA3ox1* and *GA20ox1* under blue light (Wang et al., 2010). *OsDOG* encodes an A20/AN1 zinc-finger protein containing an A20 zinc-finger at its N terminus and an AN1 zinc-finger at its C terminus. Genes of the A20/AN1 ZFP family have been shown involved in animal immune responses and plant stress responses (Heyninck and Beyaert, 2005; Hishiya et al., 2006; Huang et al., 2004, 2008; Kanneganti and Gupta, 2008; Mukhopadhyay et al., 2004). We discovered that *OsDOG* was induced by GA and suppressed by PAC (Fig. 1A and B) and that overexpression of *OsDOG* conferred dwarf phenotypes (Fig. 2B and D) and lower GA_1 level (Fig. 4A) from decreased expression of *GA3ox2* and increased expression of *GA2ox1* and *GA2ox3* (Fig. 4B). These results suggested a novel role of the A20/AN1 ZFP gene family in GA-mediated growth regulation. Knockdown of *OsDOG* expression by RNAi conferred phenotypes similar to that of the wild type. Similarly, knockout mutants of some AN1 ZFPs in Arabidopsis did not differ from the wild type in phenotype (Jin et al., 2007). Because there are 18 and 14 A20/AN1 ZFPs in rice and Arabidopsis, respectively (Vij and Tyagi, 2006), redundant function may exist between OsDOG and its homologs.

OsDOG acts as a negative regulator in GA-mediated cell elongation

Our data suggest that *OsDOG* negatively regulates internode elongation by suppressing cell elongation (Fig. 2D and G–I), and the reduction in elongation of leaf sheaths and internodes of the overexpression lines could be rescued by exogenous GA treatment (Fig. 4D–G). Contrary to the greatly elongated first internodes in the *eui1* mutant (Luo et al., 2006; Zhu et al., 2006), with *OsDOG* expression reduced (Fig. 4C), the panicles of *OsDOG*-overexpressing lines could not be completely exserted out of the leaf sheath (Fig. 2C and F). Correspondingly, the GA_1 level in *OsDOG*-overexpressing lines was lower than that in the wild type (Fig. 4A) but increased in the *eui1* mutant (Luo et al., 2006; Zhu et al., 2006). The greatly elongated uppermost internodes in the *eui1* mutant were due to excessive cell elongation (Luo et al., 2006; Zhu et al., 2006). In contrast, the less-elongated internodes of *OsDOG*-overexpressing lines were due to inhibited cell elongation (Fig. 2H and I). These data demonstrate that *OsDOG* plays a negative role in regulating GA-mediated cell elongation.

OsDOG is involved in feedback regulation of GA homeostasis

GA homeostasis is maintained via feedback and feed-forward regulation of GA metabolism. GA20ox, GA3ox and GA2ox are the major targets in GA homeostasis regulation (Yamaguchi, 2008). However, the molecular mechanism of GA homeostasis regulation is not fully understood. Several genes were reportedly involved in feedback regulation of GA metabolism. These genes were involved in GA feedback regulation by directly or indirectly regulating 2-oxoglutarate-dependent dioxygenases. RSG is involved in negative feedback regulation of GA homeostasis by directly binding to NtGA20ox1 (Fukazawa et al., 2010). AT-hook protein of GA feedback regulation binds to the promoter of *AtGA3ox1* to negatively regulate GA feedback (Matsushita et al., 2007). OsYAB1 acts negatively in GA feedback regulation by directly binding to the promoter of *GA3ox2* and suppressing its expression (Dai et al., 2007).

The expression of *OsDOG* increased in response to GA treatment and then returned to the normal value (Fig. 1A and B). Interestingly, in response to PAC treatment, *OsDOG* expression was the reverse (Fig. 1A and B). *OsDOG*-overexpressing lines showed dwarf phenotypes and decreased GA_1 level (Fig. 4A). These results suggest that *OsDOG* may be involved in the feedback regulation of GA level. The expression of *OsDOG* in rapidly growing tissues (Fig. 3A–D) provided a spatial possibility for *OsDOG* in regulating GA metabolism enzyme genes, which are mainly expressed in those tissues (Kaneko et al., 2003). The decreased expression of *GA3ox2* and increased expression of *GA2ox1* and *GA2ox3* in the overexpression plants (Fig. 4B) were the direct reason for decreased GA_1 level. OsDOG may play a role in feedback regulation of GA homeostasis. It may function by DNA or protein binding through A20 and/or AN1 zinc fingers to regulate GA metabolism genes. However, further research is needed to illuminate the molecular mechanism of OsDOG in regulating GA content.

In conclusion, we have demonstrated that *OsDOG*, an A20/AN1 ZFP gene, plays a novel role in GA homeostasis and cell elongation by regulating the expression of GA metabolism genes in rice.

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