Master's Thesis of Science in Agriculture

Molecular Characterization of *OsERF83*, a Vascular Tissue-Specific Transcription Factor Gene, Conferring Drought Tolerance in Rice

벼의 가뭄 저항성 증진에 관여하며 관다발 특이적 발현패턴을 보이는 전사인자 유전자 *OsERF83*의 분자적 특성 규명

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Se Eun Jung

Department of International Agricultural Technology

Graduate School of International Agricultural Technology

Seoul National University

Molecular Characterization of OsERF83, a Vascular Tissue-Specific Transcription Factor Gene, Conferring Drought Tolerance in Rice

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By
Se Eun Jung

Supervised by
Prof. Ju-Kon Kim

Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

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Approved as a qualified thesis
for the Degree of Master of Science in Agriculture
by the committee members

Chairman **Choonkyun Jung, Ph.D.**

Member **Ju Kon Kim, Ph.D.**

Member **Jang-Kyun Seo, Ph.D.**

Abstract

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Se Eun Jung

Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

Abiotic stresses severely affect plant growth and productivity. To cope with abiotic stresses, plants have evolved tolerance mechanisms that are tightly regulated by reprogramming transcription factors (TFs). APETALA2/ethylene-responsive factor (AP2/ERF) transcription factors are known to play an important role in various abiotic stresses. However, our understanding of the molecular mechanisms remains incomplete. In this study, we identified the role of *OsERF83*, a member of the AP2/ERF transcription factor family, in response to drought stress. OsERF83 is a transcription factor localized to the nucleus and induced in response to various abiotic stresses, such as drought and abscisic acid (ABA). Overexpression of *OsERF83* in transgenic plants ($OsERF83^{OX}$) significantly increased drought tolerance, with higher photochemical efficiency in rice. $OsERF83^{OX}$ was also

associated with growth retardation, with reduced grain yields under normal growth conditions. *OsERF83* is predominantly expressed in the vascular tissue of all organs. Transcriptome analysis revealed that *OsERF83* regulates drought response genes, which are related to the transporter (*OsNPF8.10, OsNPF8.17, OsLH1),* lignin biosynthesis (*OsLAC17, OsLAC10, CAD8D),* terpenoid synthesis (*OsTPS33, OsTPS14, OsTPS3),* cytochrome P450 family (*Oscyp71Z4, CYP76M10*), and abiotic stress-related genes (*OsSAP, OsLEA14, PCC13-62). OsERF83* also up-regulates biotic stress-associated genes, including *PATHOGENESIS-RELATED PROTEIN* (*PR*), *WALL-ASSOCIATED KINASE* (*WAK*), *CELLULOSE SYNTHASE-LIKE PROTEIN E1* (*CslE1*), and *LYSM RECEPTOR-LIKE KINASE* (*RLK*) genes. Our results provide new insight into the multiple roles of *OsERF83* in the cross-talk between abiotic and biotic stress signaling pathways.

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Keywords: drought tolerance, plant growth, *OsERF83,* ERF transcription factors, vascular tissue, *Oryza sativa,* CRISPR/rCas9

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Introduction

Abiotic stresses, including drought, cold, salinity, and nutrient stress, adversely affect the cellular homeostasis of plants and ultimately impair their growth and productivity. Among these abiotic stresses, extreme heat and water deficits frequently damage plants (Zandalinas *et al.*, 2018). As extreme weather disasters associated with climate change have become steadily more common, crop losses have also increased over the past several decades (Lesk *et al.*, 2016; Mickelbart *et al.*, 2015). Rice (*Oryza sativa.* L.) productivity is severely affected by drought because rice typically requires more water than other crops (Barnabas *et al.*, 2008). It is, therefore, essential to develop rice with enhanced tolerance to drought and heat stress.

To avoid drought stress, plants have evolved a series of sophisticated strategies. Plants respond to drought stresses through stress-specific signaling pathways, leading to morphological, physiological, and biochemical changes. Under drought stresses, plants first perceive external signals through sensors, and then various transcription factors are induced by the transduction of signals (Atkinson and Urwin, 2012; Hirayama and Shinozaki, 2010). These drought-responsive transcription factors, such as members of the AP2/ERF, MYB, bZIP, and NAC families, regulate drought-inducible genes, and consequently, plants show a tolerance to abiotic stresses (Hirayama and Shinozaki, 2010).

The *OsERF83* gene belongs to the APETALA2/ethylene-responsive factor (AP2/ERF) family, which is only present in the plant kingdom (Nakano *et al.*, 2006). The AP2/ERF superfamily commonly possesses a highly conserved AP2 DNA-binding domain, and can be classified into four subfamilies: APETALA2 (AP2), related to abscisic acid insensitive 3/viviparous 1 (RAV), ethylene-responsive factor

(ERF), and dehydration-responsive element binding protein (DREB) (Sakuma *et al.*, 2002). In the AP2/ERF superfamily, the ERF family has a single DNA-binding AP2 domain and is classified into ten subgroups (I–X) based on putative functional motifs (Nakano *et al.*, 2006). Many TFs belonging to the ERF family have been reported that specifically bind to the GCC box (AGCCGCC), an ethylene-responsive element (ERE) (Nakano *et al.*, 2006), to play a vital role in plant development, abiotic and biotic stresses, and hormonal signal transduction (Cheng *et al.*, 2013; Lorenzo *et al.*, 2003; Mizoi *et al.*, 2012; Muller and Munne-Bosch, 2015; Oh *et al.*, 2009; Xie *et al.*, 2019). Recently, *OsERF48* and *OsERF71* were shown to be involved in drought tolerance in rice by enhancing root growth (Jung *et al.*, 2017) and altering the root structure (Lee *et al.*, 2016), respectively. Additionally, overexpression of *OsEREBP1* confers tolerance to both biotic and abiotic stresses (Jisha *et al.*, 2015). In Arabidopsis, *AtERF019* has been reported to play an important role in plant growth and drought tolerance by delaying plant growth and senescence (Scarpeci *et al.*, 2017). However, despite increasing evidence that *OsERF* genes enhance drought tolerance, the molecular mechanisms have not yet been entirely elucidated.

TFs belonging to group IX are associated with defense against various pathogens (Nakano *et al.*, 2006). For example, overexpression of *AtERF1* and *ORA59* enhances resistance to necrotrophic pathogens in Arabidopsis. *AtERF1* is a regulator of ethylene responses after pathogen attack, and *ORA59* is an essential integrator of jasmonic acid (JA) (Berrocal-Lobo *et al.*, 2002; Pre *et al.*, 2008). In rice, *OsERF92* negatively regulates tolerance to *Magnaporthe oryzae* and salt stress, which is integrated into the cross-talk between biotic and abiotic stress signaling networks (Liu *et al.*, 2012). *OsERF83*, which belongs to Group IX, is induced by drought, high salinity, low temperature, and ABA treatments (Oh *et al.*, 2009). A previous study demonstrated that *OsERF83* is a transcription factor that positively regulates

resistance to *Magnaporthe oryzae* and is induced by multiple phytohormone treatments, such as methyl jasmonate, ethephon, and salicylic acid. Furthermore, several *PATHOGENESIS-RELATED* (*PR*) protein genes, including *PR1*, *PR2*, *PR3*, *PR5*, and *PR10*, are up-regulated in *OsERF83* overexpression ($OsERF83^{OX}$) transgenic rice (Tezuka *et al.*, 2019). These results prompted us to study the function of *OsERF83* in biotic and abiotic stresses. In this study, we found that *OsERF83* confers drought tolerance and positively regulates abiotic and biotic stress-associated genes.

Materials and methods

1. Plant materials

To generate *OsERF83* overexpressing plants *(OsERF83*^{OX}), the coding sequence of *OsERF83* (Os03g0860100, LOC_Os03g64260) was amplified from rice (*Oryza sativa* L. ssp. *Japonica* cv. Dongjin). The Japonica rice cultivar Dongjin (*Oryza sativa.* L.) was used as the non-transgenic control. The amplified *OsERF83* coding sequence was cloned into rice transformation vector *p700* carrying *GOS2* promoter for constitutive expression using the Gateway system (Invitrogen) (Figure 3). The final constructs were introduced into *Agrobacterium tumefaciens* LBA4404 by triparental mating and transformed into rice (*Oryza sativa* cv. Dongjin). Copy numbers were determined in T0 plants through Taq-Man PCR as described by (Bang *et al.*, 2015).

For the generation of knock-out (*OsERF83*^{KO}) mutants, we used the web-based tool CRISPR RGEN Tools (http://www.rgenome.net/, accessed on 18 May 2017). The method was performed as described previously (Chung *et al.*, 2020).

2. Stress treatments

To confirm the expression levels of the *OsERF83* gene under various abiotic stresses and phytohormone treatments, non-transgenic (NT) plants (*Oryza sativa* L. ssp. *Japonica* cv. Dongjin) were grown in soil for 2 weeks under standard greenhouse conditions (16 h light/8 h dark cycles at 28–30 °C). For stress treatments, the soil was moved from the roots of the seedlings, and drought stress was induced by air-drying the seedlings, while salinity stress and ABA treatment were imposed by incubating

the seedlings in water containing 400 mM NaCl and 100 μM ABA, respectively at 28 °C. Low-temperature stress was induced by incubating the seedlings in water at 4 °C.

3. Subcellular localization of OsERF83

To confirm the subcellular localization of OsERF83, we fused the coding regions of *OsERF83* without the stop codon to the *GFP*. The cassette was driven by a 35S promoter and inserted into the pHBT vector (GenBank accession number EF090408) using the In-fusion system (Clonetech, California, USA). *35S::NF-YA7-mCherry* was used as the control for nuclear localization (Lee *et al.*, 2015). The final construct (*35S::OsERF83-GFP*) and the control vector (*35S::NF-YA7-mCherry*) were transfected into protoplasts (*Oryza sativa* cv. Dongjin) using a PEG-mediated protoplast transformation system (Zhang *et al.*, 2011). The methods of rice protoplast preparation and transient gene expression were performed as described previously (Redillas *et al.*, 2019). GFP and mCherry signals were observed 12 h after transfection using a confocal laser scanning microscope (Leica TCS SP8 STED, Wetzlar, Germany). Images were processed using Leica LAS AF Lite software. GFP was excited at 488 nm and the emitted light was detected between 512 and 560 nm. mCherry was excited at 587 nm and the emitted light was detected at 610 nm.

4. RNA extraction, and quantitative real-time PCR (qRT-PCR) analysis

To investigate the spatial and temporal expression patterns of *OsERF83*, total RNA was isolated from the roots, stem, leaf, and flowers from different developmental

stages of rice plants using a Hybrid-R RNA purification kit (GeneAll, Seoul, Korea) according to the manufacturer's instructions. To measure the transcript levels of *OsERF83*, total RNA samples were extracted from the shoots and roots using a Hybrid-R RNA purification kit (GeneAll, Seoul, Korea) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized with Oligo-dT and random primers using RevertAid M-MuLV Reverse Transcriptase (Thermo Scientific, Massachusetts, USA). qRT-PCR was carried out using 2x qRT-PCR Pre-mix with 20x EvaGreen (SolGent, Seoul, Korea) and ROX dye (Promega, Madison, WI, USA). The amplification reactions were performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s in a 10 μL volume mix containing 0.5 μL EvaGreen Mix. The rice *Ubiquitin1* (AK121590, Os06g0681400) transcript was used as a normalization control, and two biological and three technical replicates were analyzed for all qRT-PCRs (Table 3).

5. Histochemical GUS assay

The amplified promoter region of *OsERF83* was linked to the *GUS* reporter gene in a rice transformation vector using the Gateway system (Invitrogen, Carlsbad, CA, USA). The resulting plasmid was introduced into *A. tumefaciens* strain LBA 4404 by triparental mating and transformed into rice. To detect GUS staining, we used 5-day-old, 2-week-old, and 1-month-old seedlings of the *OsERF83::GUS* transgenic lines. The GUS staining solution contained 4 mM 5-bromo-4-chloro-3-indolyl-glucuronide cyclohexylamine salt (Gold Biotechnology, Missouri, USA), 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $C_6FeK_4N_6$, 0.1% Triton X-100, 10 mM EDTA, and 100 mM phosphate buffer. Seedlings were placed into GUS staining solution and then vacuum infiltrated for 30 min. Seedlings were then incubated at 37 °C in a dark

chamber overnight. Chlorophyll was removed by washing with 70% (*v*/*v*) ethanol. We used Paraplast sections for longitudinals and cross-sections.

6. RNA-sequencing analysis

Three-week-old NT and *OsERF83* overexpression myc-tagged transgenic (*OsERF83-MYC*^{OX}) plants were grown under standard greenhouse conditions (16 h light/8 h dark cycles at 28–30 °C). Total RNA was extracted from 3-week-old transgenic (*OsERF83-cMYC*^{OX}) and non-transgenic (NT) roots and leaves using an RNeasy plant kit (Qiagen, Hilden, German). Two biological replicates were used for RNA-seq. Library construction and next-generation sequencing (NGS) were performed by the Macrogen facility (Seoul, Korea). To make expression profiles for *OsERF83-cMYC*^{OX} plants, the fragment per transcript kilobase per million fragments mapped reads (FPKM) values for each transcript were normalized with the FPKM values. The expression level of each transcript was expressed as the fragment per transcript kilobase per million fragments mapped reads (FPKM) value, which was calculated based on the number of mapped reads.

7. Drought stress treatment and measurement of chlorophyll fluorescence

We evaluated the drought tolerance of transgenic, knock-out, and non-transgenic (*Oryza sativa* L. ssp. *Japonica* cv. Dongjin) plants grown in a greenhouse at the vegetative stage. Transgenic, knock-out and non-transgenic seeds were germinated on Murashige and Skoog (MS) medium (Duchefa Biochemie, Haarlem, Netherlands) with 3% sucrose in the dark for 3 days at 28 °C and transferred into light conditions

for 1 day. Thirty seedlings from each transgenic, knock-out mutant, and non-transgenic plant were transplanted into ten soil pots (4 × 4 x 6 cm, three plants per pot) within a container (59 × 38.5 × 15 cm) and grown for 5 weeks in greenhouse conditions (16 h light/8 h dark cycles at 28–30 °C). Drought stress was simultaneously imposed by withholding water and rewatering. Drought-induced symptoms were monitored by imaging plants at the indicated time points using an a5000 camera (Sony, Tokyo, Japan). Soil moisture was measured at the indicated time points using an SM 150 soil moisture sensor (Delta T Devices, Cambridge, United Kingdom).

To evaluate the phenotypes of transgenic, knock-out, and non-transgenic plants exposed to drought conditions, we measured the chlorophyll fluorescence (F_v/F_m) and the performance index (PI_{total}). To measure F_v/F_m and PI_{total}, 4-week-old plants were transplanted into 15 cm diameter x 14 cm tall pots within another larger container (66 × 45.3 × 22.5 cm) and grown for 2 weeks. After each plant was subjected to drought stresses, F_v/F_m and PI_{total} were measured using the Handy-PEA fluorimeter (Plant Efficiency Analyzer; Hansatech Instruments, King's Lynn, Norfolk, United Kingdom) in dark conditions to ensure sufficient dark adaptation (at least 1 h). Nine leaves of each line were measured and calculated using the Handy PEA software (version 1.31) and analyzed according to the equations of the JIP test (Redillas *et al.*, 2011).

8. Statistical analysis

All data are represented as mean ± standard deviation. Each data point was compared with the control separately to determine whether they were significantly different from each other, using Student's *t*-test (* $p < 0.05$, ** $p < 0.01$). Data were analyzed by Microsoft Excel software.

9. Accession Numbers

Genes from this article can be found in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/, accessed on 19 November 2020) with the following accession numbers: *OsERF83* (Os03g0860100), *OsNPF8.10* (Os01g0142800), *OsNPF8.17* (Os10g0112500), *OsLHT1* (Os08g0127100), *OsMSL38* (Os11g0282700), *OsMyb* (Os01g0298400), *OsTPS3* (Os02g0121700), *OsCPS4* (Os04g0178300), *OsLAC17* (Os10g0346300), *OsLAC10* (Os02g0749700), *OsCAD8D* (Os09g0400400), *OsSAP* (Os09g0425900), *PCC13-62* (Os04g0404400), and *OsOPR4* (Os06g0215900).

Results

1. OsERF83 is a drought-inducible transcription factor

In our previous rice 3′-tiling microarray analysis, *OsERF83* was induced by drought treatments (Oh *et al.*, 2009). Based on the results, we investigated the function of *OsERF83* in response to drought. We analyzed the expression patterns using quantitative real-time PCR (qRT-PCR) (Figure 1a). We treated 2-week-old rice seedlings with various stresses, including drought, high salinity, low temperature, and ABA. The transcript level of *OsERF83* was significantly induced in both leaves and roots under drought and high salinity conditions, and the degree of induction was higher in the roots than in the leaves. In contrast, the low-temperature (4 °C) and ABA (100 μM) treatments enhanced the transcript levels of *OsERF83*, specifically in the leaves and roots, respectively. Under drought treatments, the transcript levels of *OsERF83* were increased in both the roots and leaves (Figure 1a). The regulation of *OsERF83* expression by various stresses shows that it might have multiple roles in response to environmental stresses.

To investigate the spatiotemporal expression patterns of *OsERF83*, we conducted qRT-PCR with various tissues of rice. The results revealed that *OsERF83* was expressed at all developmental stages, and at particularly high levels in the roots (Figure 1b).

One study reported that *OsERF83* was localized to the nucleus in onion epidermal cells (Tezuka *et al.*, 2019). To confirm the subcellular localization of *OsERF83* in rice, we transformed the full-length *OsERF83*, fused to green fluorescent

protein (GFP), into rice protoplasts (Figure 2a). We also used OsNF-YA7-mCherry as a positive control for nucleus localization (Lee *et al.*, 2015). Both constructs were transiently co-expressed in rice protoplasts, and the signals of both green fluorescence and mCherry fluorescence were observed in the nucleus of the rice protoplasts (Figure 1c). This indicated that the OsERF83 was localized at the nucleus. Collectively, OsERF83 is a transcription factor responsive to multiple stresses.

2. Overexpression of *OsERF83* in rice confers drought tolerance at the vegetative stage

To investigate the biological role(s) of *OsERF83* in rice development and the drought-stress response, we generated over 30 independent *OsERF83* overexpression (*OsERF83*^{OX}) transgenic rice plants using the *GOS2* (rice eukaryotic translation initiation factor 1-like gene) promoter (*GOS2::OsERF83*), which drives expression in the whole plant body (de Pater *et al.*, 1992) (Figure 2b). Initially, we selected single-copy homozygous lines of *OsERF83*^{OX} using TaqMan PCR, and then somaclonal variations were eliminated by paddy field selection for three generations. Finally, we chose three homozygous elite lines of *OsERF83*^{OX} (#1, 2, 11) for further study. The transcript levels of *OsERF83* were greatly enhanced in both the leaves and roots of all *OsERF83*^{OX} (#1, 2, 11) plants, compared to non-transgenic (NT) plants. We checked the expression of each transgenic sister line and confirmed their overexpression (Figure 4).

We next tested the performance of *OsERF83*^{OX} under drought conditions at the vegetative stage. *OsERF83*^{OX} and NT plants were grown in a greenhouse for 5 weeks and then exposed to drought conditions for 2 days by withholding water. After 1 day of drought treatment, the NT plants started to display slightly rolled leaves.

After 2 days of drought treatment, drought-induced visual symptoms, such as leaf rolling, wilting, and chlorosis, appeared more severely in NT plants than *OsERF83*^{OX} (#1, 2, 11) plants (Figure 5a). Soil moisture consistently decreased during the drought treatment, indicating the drought stress was uniformly applied to both the NT and *OsERF83*^{OX} (Figure 5b) plants. After 8 days of rewatering, most of the *OsERF83*^{OX} plants had recovered and showed an 83 to 95% survival rate, while NT plants showed an approximately 20% survival rate (Figure 5c). We repeated the testing three times through T3–T4 and obtained the same results (Figure 6).

To further confirm the tolerance of *OsERF83*^{OX} plants to drought stress, we measured the activity of photosynthetic machinery damaged by drought stress using the JIP test. The JIP test showed the *Fv/Fm* (Fv: variable fluorescence; Fm: maximum fluorescence) value for the photochemical efficiency of photosystem (PS) II and the PI_{total} (performance index) value for the photochemical efficiency of PS I and PS II (Bussotti *et al.*, 2010). *OsERF83*^{OX} and NT plants were exposed to drought conditions for 7 days after transplanting into big pots, by withholding water for 7 days (Figure 5f). The *Fv/Fm* values of the NT plants started to decrease at 5 days after drought treatment, whereas the *Fv/Fm* values of *OsERF83*^{OX} plants did not decrease (Figure 5d). Additionally, the PI_{total} values sharply decreased in NT plants at 5 days after drought treatment, whereas the PI_{total} values of *OsERF83*^{OX} were maintained during the drought treatment (Figure 5e). Consistent with the photochemical efficiency, after 5 days of drought treatment, the NT plants started to display drought-induced visual symptoms (Figure 5f). These results indicated that *OsERF83* overexpression in rice plants protects PS I and PS II from drought stress, thereby enhancing drought tolerance; in this way, *OsERF83* positively regulates drought tolerance. We concluded that overexpression of *OsERF83* enhanced drought tolerance at the vegetative stage.

3. Overexpression of *OsERF83* in rice shows growth retardation and affects grain yield at normal condition

The *OsERF83*^{OX} plants showed smaller plant height, including culm length and panicle length at all stages (Figure 7a, b). At the maturity stage, the average plant height of NT, *OsERF83*^{OX} #1, *OsERF83*^{OX} #2, and *OsERF83*^{OX} #11 was 98.86 cm (100%), 75.50 cm (76.4%), 87.63 cm (87.6%), and 81.50 cm (81.5%), respectively (Figure 7b, Table 1). The yield component values of the *OsERF83*^{OX} plants, such as the number of spikelets per panicle (NSP), the number of total spikelets (NTS), the number of filled grains (NFG), the grain-filling rate (FR), and total grain weight (TGW), were reduced compared to the NT plants (Figure 8, Table 1). Moreover, the *OsERF83*^{OX} plants showed shorter grain width than the NT plants (Figure 7c, d). Collectively, these results indicate that overexpression of *OsERF83* affects plant growth and grain yield negatively in rice.

4. CRISPR/Cas9-mediated loss-of-function study analysis of *OsERF83*

For the loss-of-function study analysis, knock-out (*OsERF83*^{KO}) mutants were generated using a clustered regularly interspaced short palindromic repeats/recombinant codon-optimized Cas9 (CRISPR/Cas9) genome editing system (Figure 2c) (Chung *et al.*, 2020), with which various types of induced mutations were found. The single-guide RNA (sgRNA) target sites were designed at the upstream region of the AP2/ERF domain for generating mutants edited in *OsERF83* specific regions (Figure 9a). Four types of mutants containing 1 bp insertions were generated at the same upstream position of the PAM sequence (Figure 9a, b). Sequencing analysis revealed mutation patterns in transgenic plants, including frameshifts and premature stop codons (Figure 9c). Among 30 independent transgenic plants, 23.3%

were null, and 20% were biallelic heterozygous mutant plants. A further 50% were biallelic homozygous, and we selected four independent *OsERF83*^{KO} mutants for further study (Figure 9b).

We expected that the phenotype of *OsERF83*^{KO} mutants would show more sensitivity to drought stress compared with NT plants. However, *OsERF83*^{KO} mutants showed similar phenotypes under drought conditions (Figure 9d). We speculated that the redundancy of other AP2/ERF transcription factor family genes caused such no significant phenotypes to occur in *OsERF83*^{KO} compared to NT plants.

5. *OsERF83* is expressed predominantly in the vascular tissue

As shown in Figure 1b, *OsERF83* was expressed ubiquitously in all the examined tissues, with high expression levels in roots. To further explore the function of *OsERF83* in drought tolerance, we investigated the tissue expression pattern of *OsERF83* using histochemical GUS staining with Paraplast section. We generated transgenic rice plants with a β-glucuronidase (*GUS*) reporter gene, driven by the native *OsERF83* promoter (*OsERF83::GUS*) (Figure 2d). We used a coleoptile, stem, leaves, and roots of plants, as exhibited in Figure 10a-k. Interestingly, the strong GUS signals were consistently detected in vascular tissues of the coleoptile (Figure 10a), stem (Figure 10b, c), leaves (Figure 10d-g), and roots (Figure 10h-k), as shown in the longitudinal section of the roots (Figure 10i), despite the difference in developmental stage and organs. These data indicated that *OsERF83* was a constitutively expressed gene and was involved in the vascular tissues.

To investigate possible morphological changes in the vascular tissue caused by *OsERF83* expression, we observed the vascular tissue in the roots, stems, and

leaves in *OsERF83*^{OX}, *OsERF83*^{KO}, and NT plants. However, there were no significant differences in morphology among NT, *OsERF83* overexpressing, and knock-out plants (Figure 11).

6. Identification of genes involved in the *OsERF83*-mediated drought tolerance pathway

To perform further functional analysis of *OsERF83*, we generated *OsERF83*-6myc overexpressing transgenic (*OsERF83-MYC*^{OX}) plants (Figure 2e). We confirmed protein expression levels of *OsERF83* in *OsERF83-MYC*^{OX} using Western blot analysis (Figure 12a, f) and transcript levels using qRT-PCR (Figure 12b). Both levels were higher in *OsERF83-MYC*^{OX} than in the NT plants. *OsERF83-MYC*^{OX} plants also showed drought tolerance in the vegetative stage (Figure 12c-e). To identify downstream genes regulated by *OsERF83*, we performed an RNA-sequencing (RNA-seq) analysis using a 3-week-old *OsERF83-MYC*^{OX}. We used whole seedlings of *OsERF83-MYC*^{OX} and NT, with two independent biological replicates, and calculated the reads per transcript kilobase per million fragments mapped reads (FPKM) values for each sample with count data. The heatmap indicated that the biological replicates showed a similar expression pattern (Figure 13a). A total of 33,221 differentially expressed genes (DEGs) were identified, of which 540 were up-regulated (B) and 1109 were down-regulated (A) in *OsERF83* overexpressing plants (Figure 13b).

To identify the putative functions of these DEGs, we conducted gene ontology analysis using gProfiler (https://biit.cs.ut.ee/gprofiler/orth). The DEGs were categorized into three categories: biological process, molecular function, and cellular component. As a result, the significantly enriched GO terms were mainly associated with the oxidation–reduction process and stress response under the biological process

category, plasma membrane and the extracellular region under the cellular component category, and catalytic activity and ion binding under the molecular function category (Figure 13c). In addition, KEGG pathway analysis showed that DEGs were related to 'diterpenoid biosynthesis', 'phenylpropanoid biosynthesis', and 'plant hormone signal transduction' (Figure 13d). These results are consistent with our proposed role of *OsERF83* in the regulation of drought stress tolerance.

Then, we identified that 289 drought-inducible genes were up-regulated in the *OsERF83-MYC*^{OX} plants by filtering with a public database containing drought-treated RNA-seq data in shoots and roots (Figure 13e) (Kawahara *et al.*, 2016). These drought-inducible genes were classified into several groups: transporters, transcription factors, abiotic stress-related genes, the cytochrome P450 family, terpenoid-associated genes, disease resistance-related genes, hormone-associated genes, lignin biosynthesis-associated genes, and F-box proteins (Table 2), as well as others (Table 2). Some of the genes in each group were analyzed using qRT-PCR analysis for validation (Figure 14). The results confirmed that *OsERF83-MYC*^{OX} induces a class of transporter genes (*OsNPF8.10*, *OsNPF8.17*, and *OsLHT1*) (Figure 14a), MYB or Myb/SANT transcription factors (Figure 14b), terpenoid-associated genes (*OsTPS3* and *OsCPS4*) (Figure 14c), and lignin biosynthesis-associated genes (*OsLAC17*, *OsLAC10*, and *CAD8D*) (Figure 14d). We drew the co-expression matrix using up-regulated genes in *OsERF83-MYC*^{OX} using RiceFREND to identify coregulatory networks with a high co-expression frequency (mutual rank (MR): ~20) (Figure 15) (Sato *et al.*, 2013). There were many drought-inducible genes in the co-expression matrix (Figure 15).

A previous study reported that *OsERF83* interacts with the GCC box (Tezuka *et al.*, 2019). Therefore, we analyzed the presence of the GCC box within 3 kb of the 5′ upstream region of up-regulated drought-inducible genes in *OsERF83-*

MYC^{OX} (Table 2). We found that most of them have the GCC box, which indicates that *OsERF83* might directly regulate them.

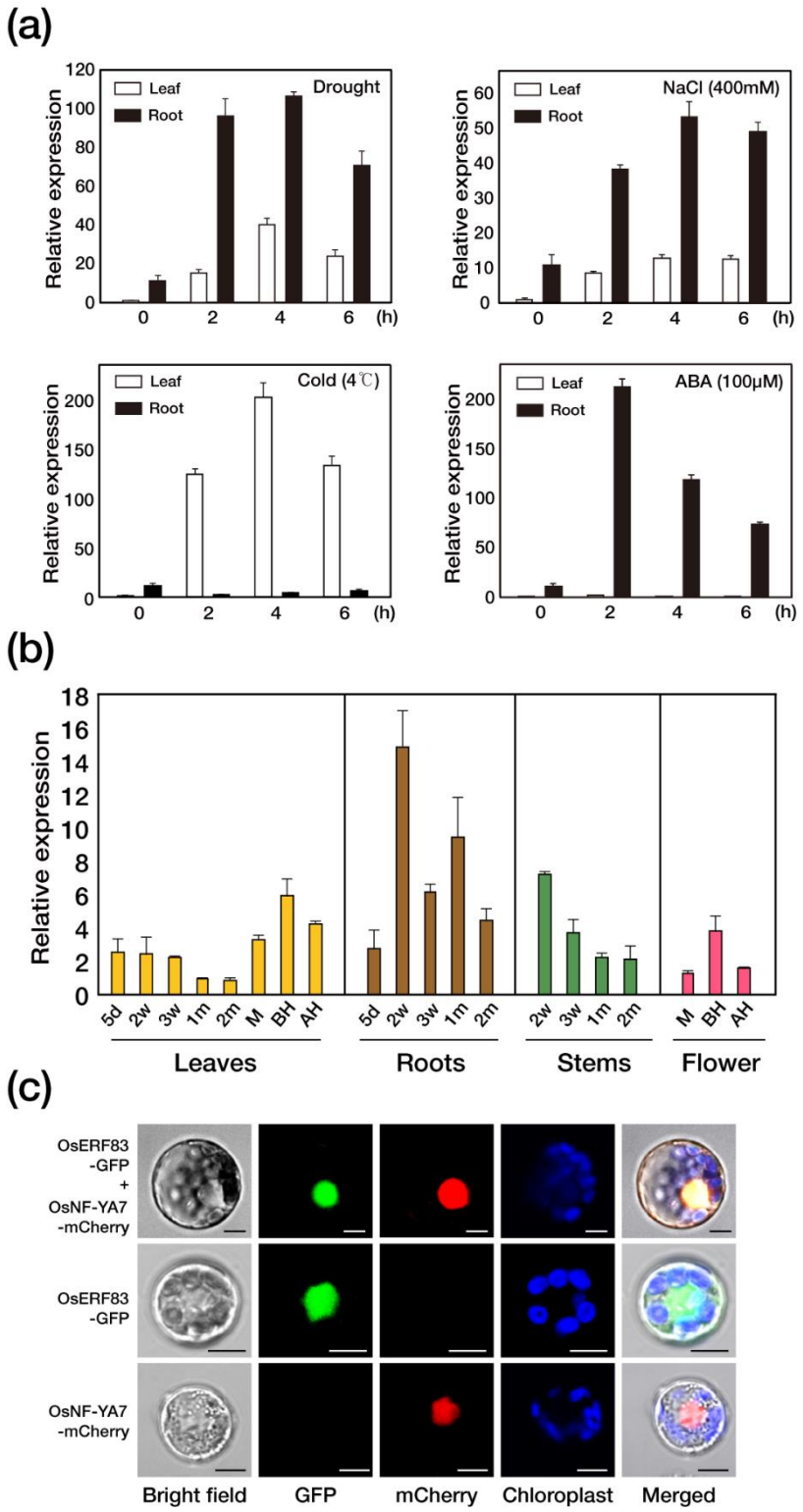

(a)
Relative expression
Leaf
Root
Drought
NaCl (400mM)
Cold (4℃)
ABA (100μM)
0
2
4
6
(h)
(b)
Relative expression
5d
2w
3w
1m
2m
M
BH
AH
Leaves
Roots
Stems
Flower
(c)
OsERF83
-GFP
+
OsNF-YA7
-mCherry
OsERF83
-GFP
OsNF-YA7
-mCherry
Bright field
GFP
mCherry
Chloroplast
Merged

Figure 1. Expression pattern of *OsERF83* and subcellular localization of OsERF83 in rice protoplasts

(**a**) The relative expression level of *OsERF83* by quantitative RT-PCR in the roots and shoots of 2-week-old seedlings treated with air-drying (drought), 400 mM NaCl (high salinity), 4 °C (cold), and 100 μM abscisic acid (ABA). *OsUbi* (*Ubiquitin1*; Os06g0681400) expression was used as an internal control for normalization. Error bars indicate the standard deviation based on three technical replicates. (h: hour) (**b**) The expression level of *OsERF83* in various tissues and at different growth stages. (d, day; w, week; m, month; M, meiosis; BH, before heading; AH, after heading). *OsUbi1* expression was used as an internal control for normalization. Error bars indicate the standard deviation based on three technical replicates. (**c**) Subcellular localization of the OsERF83 protein in rice protoplasts. *35S::OsERF83-GFP* and *35S::OsNF-YA7-mCherry* as a control vector were transiently expressed in rice leaf protoplasts. Scale bar, 10 μm.

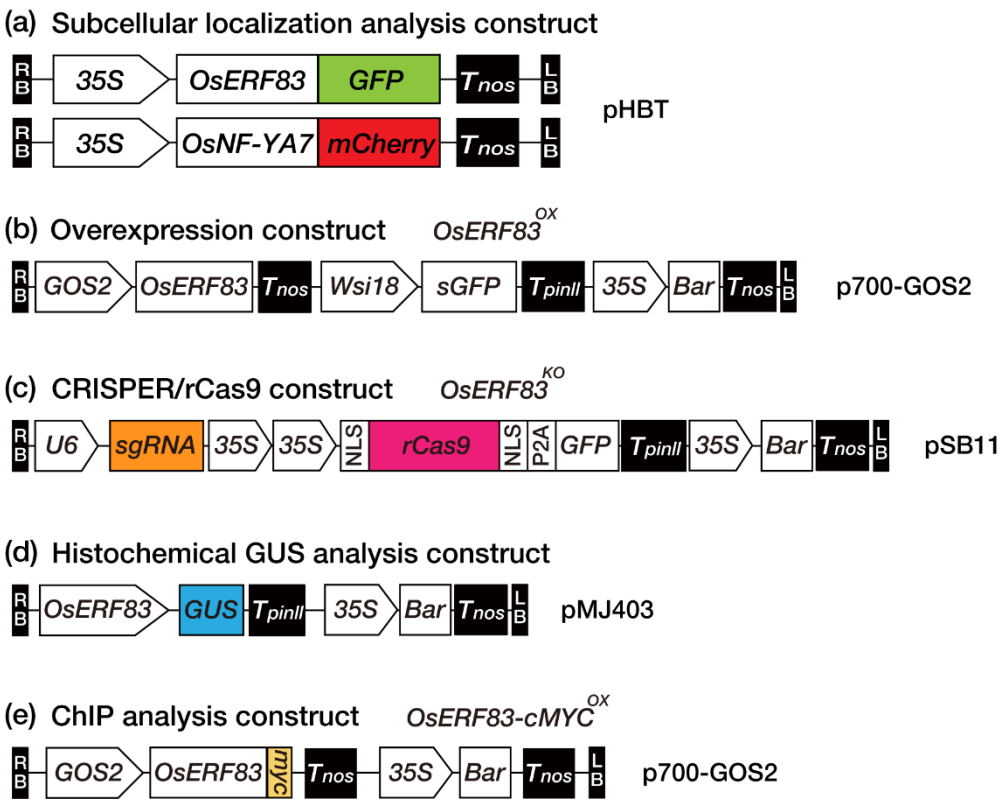
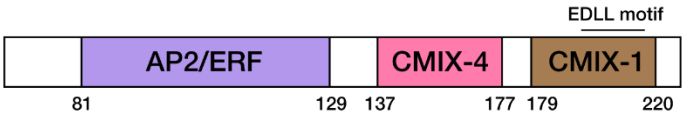


Figure 2. Schematic diagram of the vector constructs used in this study

GOS2, promoter of rice *eukaryotic translation initiation factor 1-like gene* (Os07g0529800); *Tnos*, the 3' region of nopaline synthase gene; *TpinII*, the 3' region of the potato (Solanum tuberosum) proteinase inhibitor II gene; *35S*, 35S promoter of Cauliflower mosaic virus; *Bar*, the bacterial phosphinothricin acetyltransferase gene; *Wsi18*, promoter of *a stress-inducible gene*; LB, left border; RB, right border; T, terminator.

(a)



(b)

```
1    ATGCATTGCTGCATGTCGCTTCATCCTCACCGCCGCCACGGCGACGGCGACGTCGACGGATCAGCATCAGGA
1    M  H  C  C  M  S  L  H  P  H  R  R  H  G  D  G  D  V  D  G  S  A  S  G

73   TCAGGATCAGCGCGCCTCACCGCCGGCCTCATCAACTTCCTCGAATCGCGTCGCGCCGGCGCCATGAGCACC
25   S  G  S  A  R  L  T  A  G  L  I  N  F  L  E  S  R  R  A  G  A  M  S  T

142  ACCAACAGCTCATCCTCTGTCTCTGTCCCAGCCATGGACGCCCATGGACAGGAGGAGGAGGAGGAGCCGATG
49   T  N  S  S  S  S  V  S  V  P  A  M  D  A  H  G  Q  E  E  E  E  E  P  M

211  CAGGTGCAGCAACAGCAGGCGTTCCGCGGGGTGCGCAAGCGGCCATGGGGCAAGTTTGCGGCGGAGATC
73   Q  V  Q  Q  Q  Q  A  F  R  G  V  R  K  R  P  W  G  K  F  A  A  E  I
                                AP2/ERF domain
280  CGCGACTCGACGCGCAACGGCGTGCGCGTGTGGCTGGGCACGTTCGACAGCGCGGAGGAGGCGGCGCTG
96   R  D  S  T  R  N  G  V  R  V  W  L  G  T  F  D  S  A  E  E  A  A  L

355  GCCTACGACCAGGCGGCGTTCGCCATGCGCGGGTCGGCGGCGGTGCTCAACTTCCCCATGGAGCAGGTG
119  A  Y  D  Q  A  A  F  A  M  R  G  S  A  A  V  L  N  F  P  M  E  Q  V

423  AGGCGTTCCATGGACATGTCCCTCCTGCAGGAAGGGGCGTCGCCGGTGGTGGCGCTGAAGCGGCGGCAC
142  R  R  S  M  D  M  S  L  L  Q  E  G  A  S  P  V  V  A  L  K  R  R  H
                          CMIX-4
492  TCCATGCGAGCGGCAGCAGCGGGGCGGCGGCGCAAGAGCGCTGCACCTGCACCGGCGGATCAGGAAGGC
165  S  M  R  A  A  A  A  G  R  R  R  K  S  A  A  P  A  P  A  D  Q  E  G
                                            CMIX-1

561  GGAGGAGGGGTGATGGAGCTGGAGGACCTGGGACCTGACTACCTGGAGGAGCTGCTAGCCGCCTCTCAGCCC
188  G  G  G  V  M  E  L  E  D  L  G  P  D  Y  L  E  E  L  L  A  A  S  Q  P
                                      EDLL motif
633  ATCGATATCACCTGCTGCACAAGCCCAAGCCACCACTCCATCTGA
222  I  D  I  T  C  C  T  S  P  S  H  H  S  I  *
```

Figure 3. The structure of domains and motif of *OsERF83*

(a) A schematic representation of domains and motif based on (Nakano *et al.*, 2006) is shown. The box indicates the CDS of *OsERF83.* The functional domains are colored and the motif is represented as a bar. (b) The amino acid and nucleotide sequences of the OsERF83 are aligned. Gray small circle-shaped box indicates the set of the codon. The purple box indicates AP2/ERF domain, Pink and brown boxes represent CMIX-4, -1 domain, respectively. EDLL motif is indicated in CMIX-1 domain at the end of C-terminal.

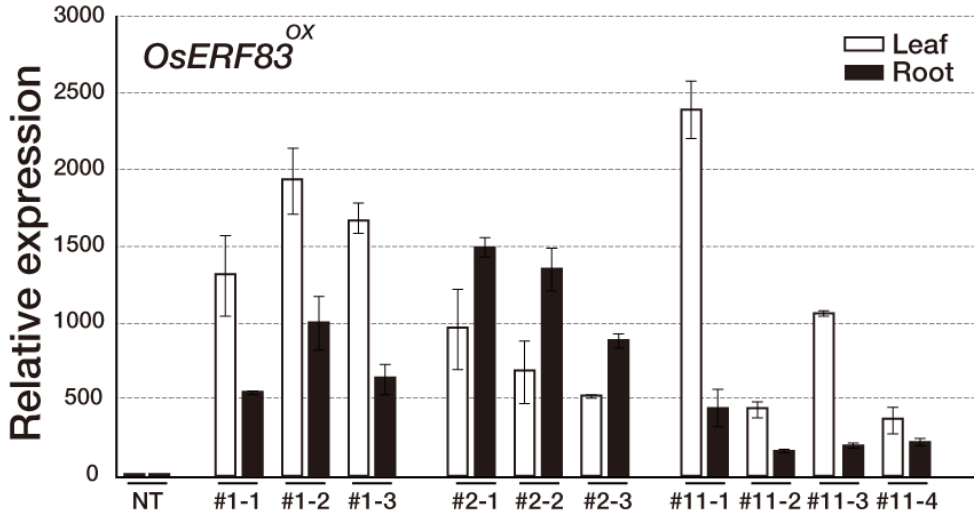


Figure 4. Relative expression levels of *OsERF83* in non-transgenic (NT) and three independent *OsERF83* overexpressed (*OsERF83*^{OX}) transgenic rice

Total RNAs extracted from leaves and roots of each sister line. *OsUbi1* was used as the internal control for normalization. Data represent mean value ± *SD (n=3).*

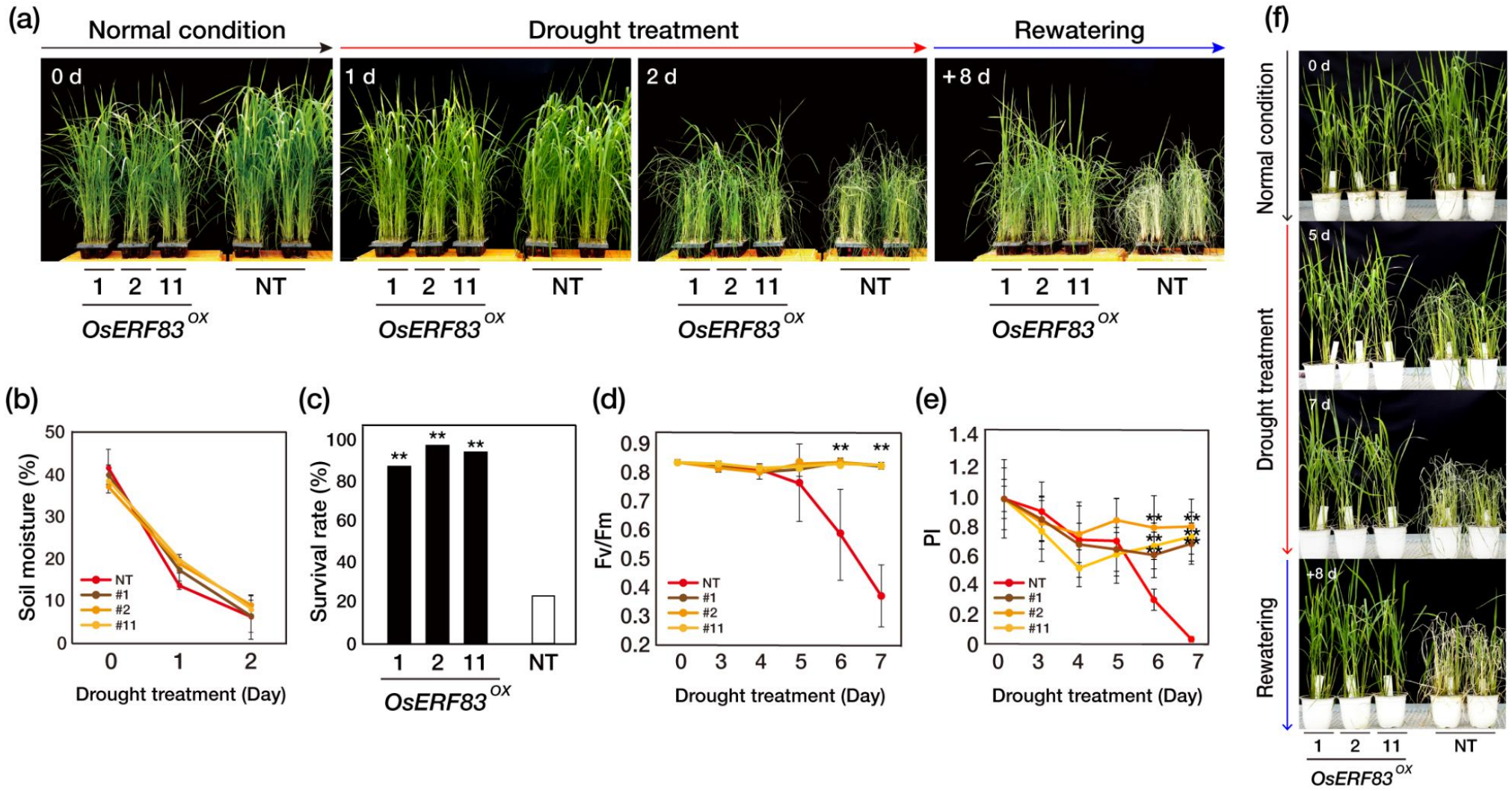

(a)
Normal condition
Drought treatment
Rewatering
0 d
1 d
2 d
+8 d
1 2 11 NT
OsERF83OX
(b)
Soil moisture (%)
Drought treatment (Day)
NT
#1
#2
#11
(c)
Survival rate (%)
**
1 2 11 NT
OsERF83OX
(d)
Fv/Fm
Drought treatment (Day)
(e)
PI
Drought treatment (Day)
(f)
Normal condition
Drought treatment
Rewatering
0 d
5 d
7 d
+8 d
1 2 11 NT
OsERF83OX

Figure 5. Overexpression of Os*ERF83* in rice enhances drought tolerance

(**a**) Drought tolerance phenotypes of *OsERF83* overexpression ($OsERF83^{OX}$) transgenic plants (lines 1, 2, 11). The 5-week-old plants (0 d) were exposed to drought by withholding water for 2 days and rewatering for 8 days. (**b**) Measurement of soil moisture content (%). Error bars represent the means ± SD (n = 10). (**c**) The survival rate of the transgenic plants, scored 8 days after rewatering. (**d**, **e**) Determination of the photosynthetic viability of transgenic and NT plants under drought conditions. At the indicated time point after exposure to drought stresses, the chlorophyll fluorescence (Fv/Fm) (**d**) and performance index (PI) (**e**) of overexpression plants and NT plants were measured. The data represent the mean ± SD (n = 10 points per independent line of each genotype). (**f**) Drought tolerance phenotypes of $OsERF83^{OX}$ transgenic plants (lines 1, 2, 11) in big pots. The 6-week-old plants (0 d) were exposed to drought by withholding water for 7 days and rewatering for 8 days. Asterisks (**) indicate statistically significant differences compared with non-transgenic (NT) plants according to a Student's t-test ** $p < 0.001$).

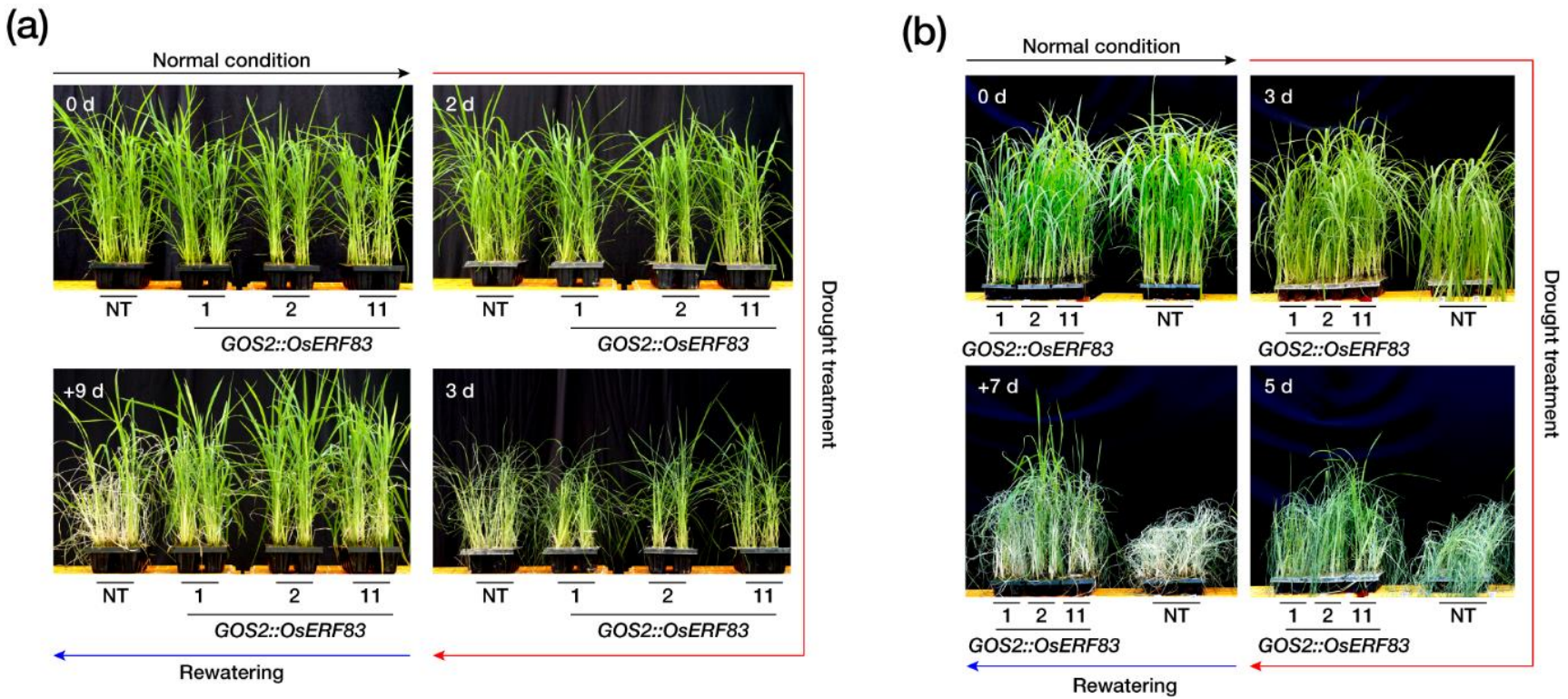


Figure 6. Phenotypes of T3 (left panel), T4 (right panel) generation of *OsERF83*^{OX} transgenic rice and NT plants under drought treatments

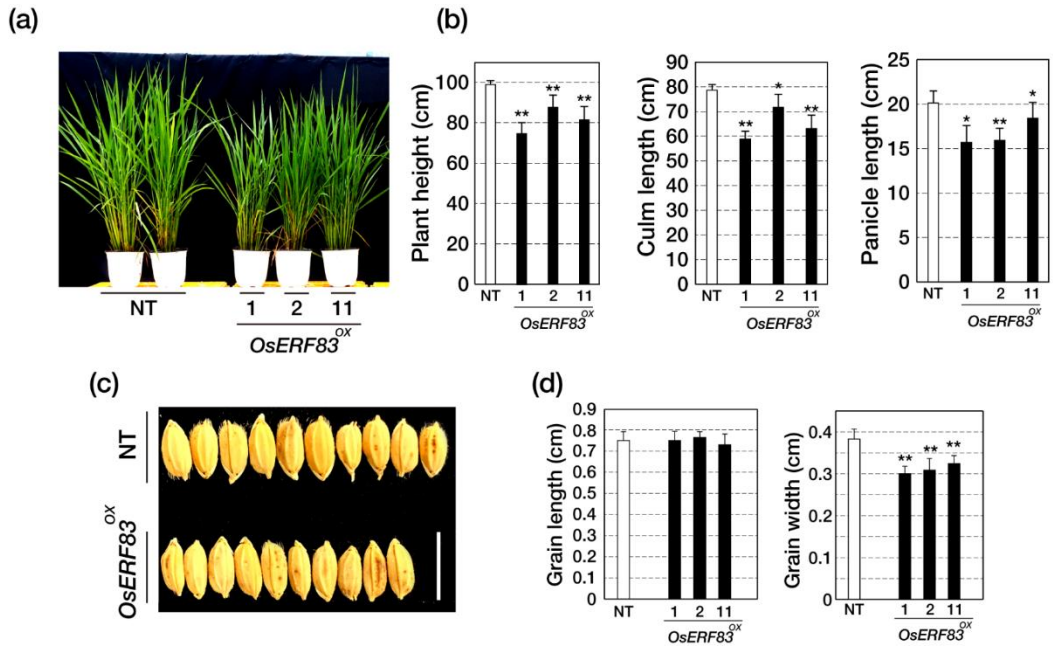


Figure 7. Morphological analysis of *OsERF83*^{OX} transgenic plants

(**a**) The whole plant height of 2-month-old *OsERF83*^{OX} and NT plants grown in paddy fields. (**b**) The plant height was measured after the heading stage of the rice. A total of 18 plants for each line were measured. Data represent the mean value ± SD (n = 10). (**c**, **d**) The grain size of *OsERF83*^{OX} line and NT plants. A total of 25 grains from each line were measured. Bar = 1 cm. Two asterisks (**) indicate statistically significant differences at $p < 0.01$, and one asterisk (*) indicates significant differences at $p < 0.05$ compared with NT based on the Student's t-test. Data are shown as the mean ± SD (n = 25).

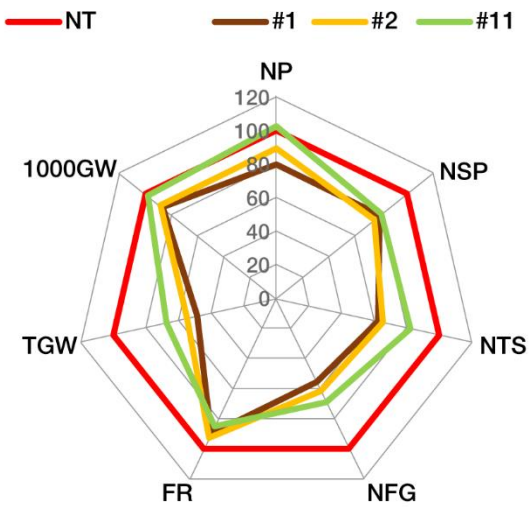


Figure 8. Agronomic traits of three independent T4 homozygous *OsERF83*^{OX} transgenic rice (lines 1, 2, 11) compared with NT in the paddy field (2020)

Each data point represents the percentage of the mean values (n=18) listed in Table S2. Mean values from NT plants were assigned a reference value of 100%. NP, number of panicles per hill; NSP, number of spikelets per panicle; NTS, number of total spikelets; NFG, number of filled grains; FR, filling rate; TGW. Total grain weight; 1000 GW, 1000 grain weight.

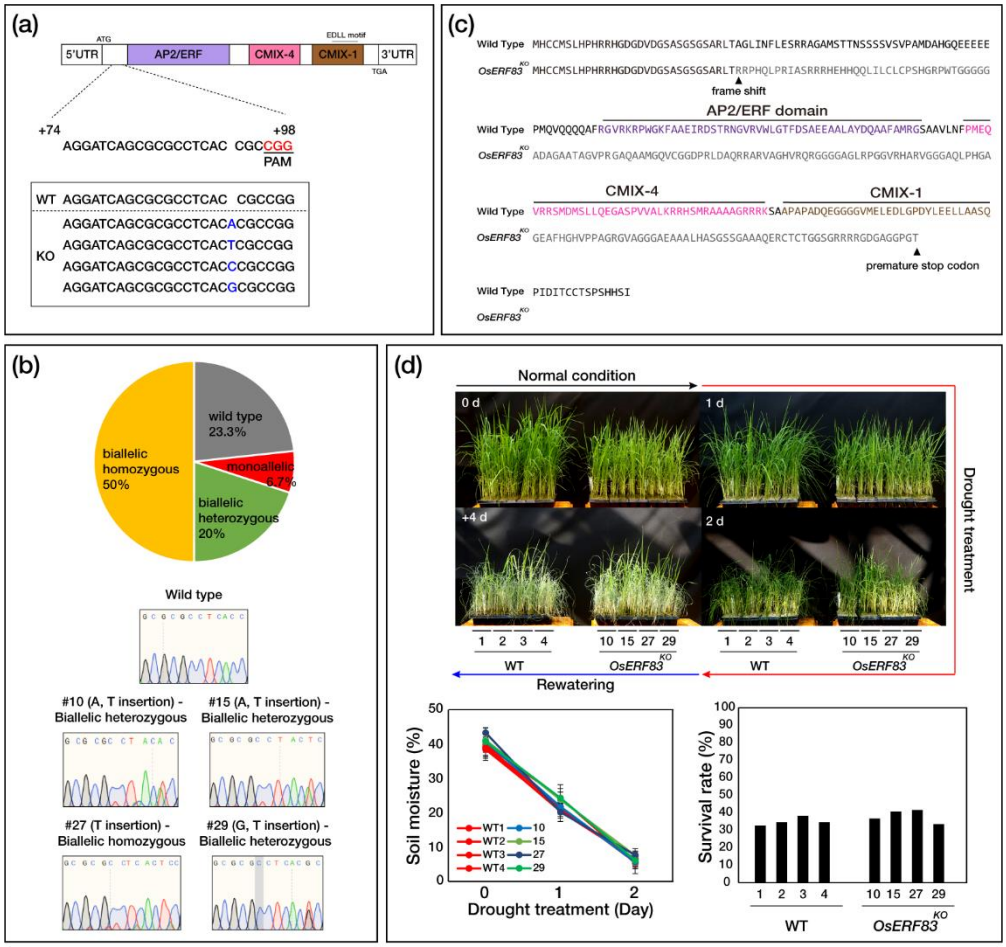

(a)
ATG
EDLL motif
5'UTR
AP2/ERF
CMIX-4
CMIX-1
3'UTR
TGA
+74
+98
AGGATCAGCGCGCCTCAC CGCCGG
PAM
WT AGGATCAGCGCGCCTCAC CGCCGG
KO
AGGATCAGCGCGCCTCACACGCCGG
AGGATCAGCGCGCCTCACTCGCCGG
AGGATCAGCGCGCCTCACCCGCCGG
AGGATCAGCGCGCCTCACGCGCCGG
(c)
Wild Type MHCCMSLHPHRRHGDGDVDGSASGSGSARLTAGLINFLESRRAGAMSTTNSSSSVSVPAMDAHGQEEEEE
OsERF83KO MHCCMSLHPHRRHGDGDVDGSASGSGSARLTRRPHQLPRIASRRRHEHHQQLILCLCPSHGRPWTGGGGG
frame shift
AP2/ERF domain
Wild Type PMQVQQQQAFRGVRKRPWGKFAAEIRDSTRNGVRVWLGTFDSAEEAALAYDQAAFAMRGSAAVLNFPMEQ
OsERF83KO ADAGAATAGVPRGAQAAMGQVCGGDPRLDAQRRARVAGHVRQRGGGGAGLRPGGVRHARVGGGAQLPHGA
CMIX-4
CMIX-1
Wild Type VRRSMDMSLLQEGASPVVALKRRHSMRAAAAGRRRKSAAPAPADQEGGGGVMELEDLGPDYLEELLAASQ
OsERF83KO GEAFHGHVPPAGRGVAGGGAEAAALHASGSSGAAAQERCTCTGGSGRRRRGDGAGGPGT
premature stop codon
Wild Type PIDITCCTSPSHHSI
OsERF83KO
(b)
wild type 23.3%
biallelic homozygous 50%
monoallelic 6.7%
biallelic heterozygous 20%
Wild type
#10 (A, T insertion) - Biallelic heterozygous
#15 (A, T insertion) - Biallelic heterozygous
#27 (T insertion) - Biallelic homozygous
#29 (G, T insertion) - Biallelic heterozygous
(d)
Normal condition
0 d
1 d
+4 d
2 d
Drought treatment
1 2 3 4
10 15 27 29
WT
OsERF83KO
Rewatering
Soil moisture (%)
WT1 10
WT2 15
WT3 27
WT4 29
Drought treatment (Day)
Survival rate (%)

Figure 9. Construction of knock-out (*OsERF83^{KO}*) mutants and their phenotypes

(**a**) Schematic illustration of the target sites in the OsERF83 genomic sequence. The protospacer adjacent motif (PAM) sequence is marked in red. Mutation sequences are marked in blue. (**b**) Mutation pattern on *OsERF83^{KO}* #10, #15, #27, and #29 plants. (**c**) Amino acid sequence alignment of *OsERF83^{KO}* plants revealed a frameshift and premature stop codon. (**d**) Phenotypes of *OsERF83^{KO}* (#10, #15, #27, #29) and WT plants under drought stress. All plants were grown in soil for 5 weeks under well-watered conditions, exposed to drought stress for 2 days, and then rewatered for 4 days, in the greenhouse. Measurement of the soil moisture contents (%). The data represent the mean value ± SD of five measurements performed at different locations in the soil. The survival rate of transgenic plants was scored 4 days after rewatering.

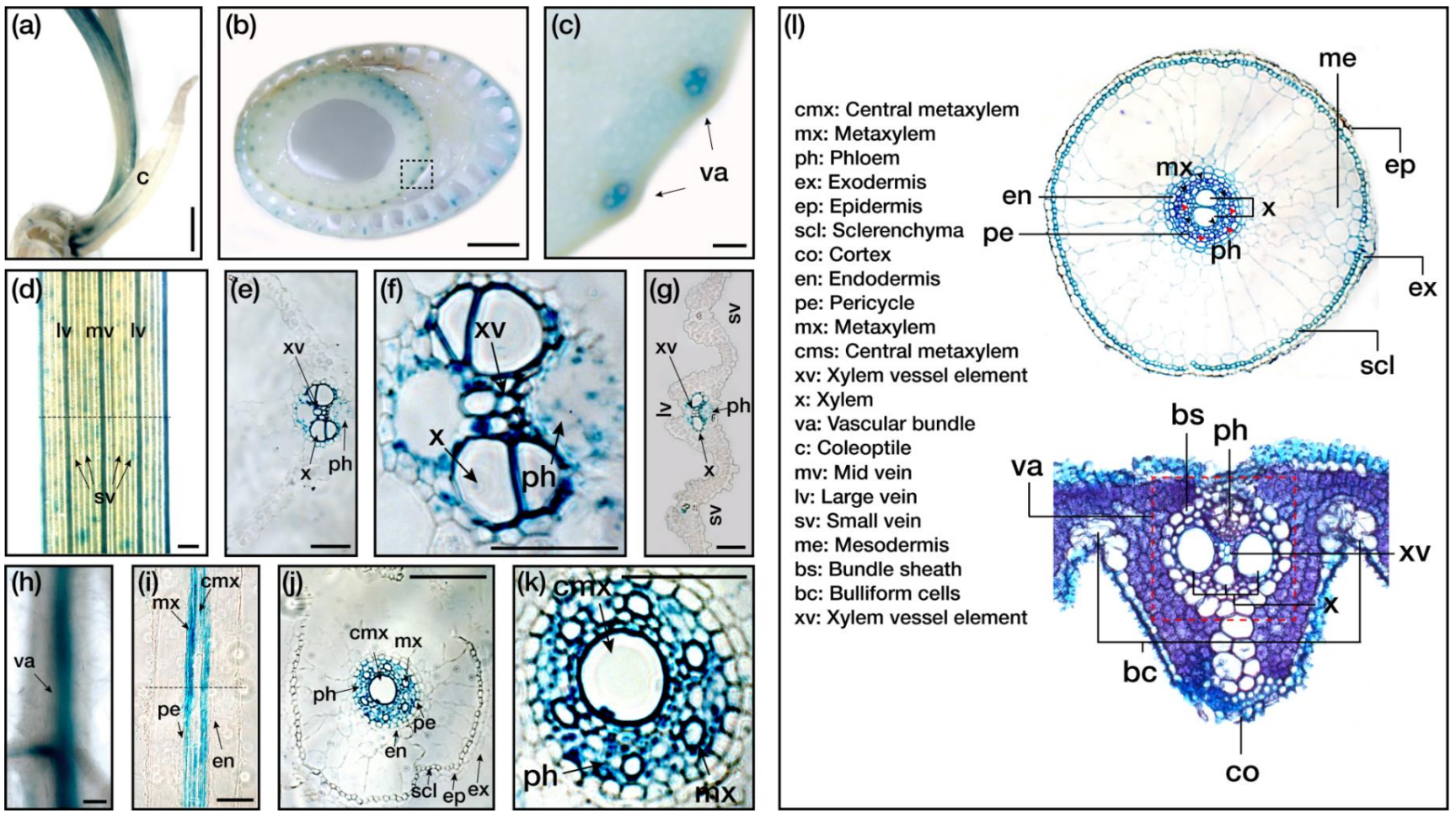
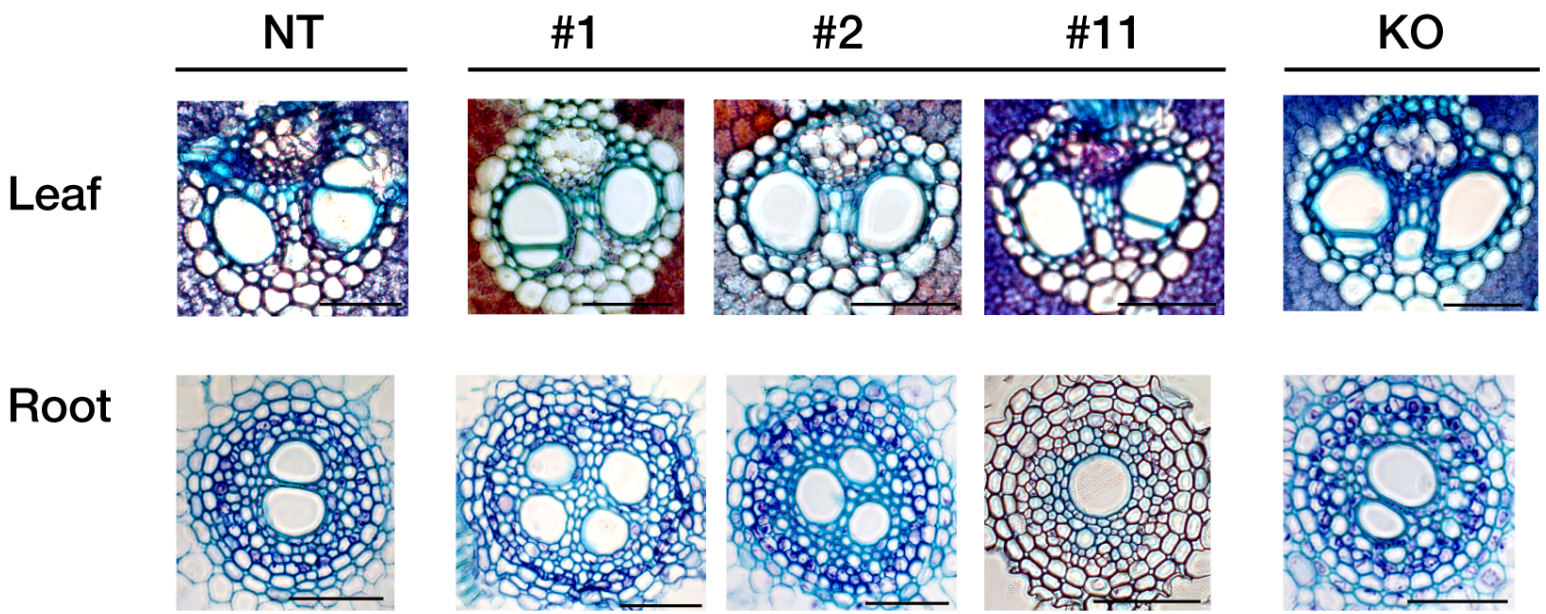

(a)
c
(b)
(c)
va
(d)
lv mv lv
sv
(e)
xv
x
ph
(f)
xv
x
ph
(g)
sv
xv
lv
ph
x
sv
(h)
va
(i)
cmx
mx
pe
en
(j)
cmx
mx
ph
pe
en
scl
ep
ex
(k)
cmx
ph
mx
(l)
cmx: Central metaxylem
mx: Metaxylem
ph: Phloem
ex: Exodermis
ep: Epidermis
scl: Sclerenchyma
co: Cortex
en: Endodermis
pe: Pericycle
mx: Metaxylem
cms: Central metaxylem
xv: Xylem vessel element
x: Xylem
va: Vascular bundle
c: Coleoptile
mv: Mid vein
lv: Large vein
sv: Small vein
me: Mesodermis
bs: Bundle sheath
bc: Bulliform cells
xv: Xylem vessel element
me
ep
mx
en
x
pe
ph
ex
scl
bs
ph
va
xv
x
bc
co

Figure 10. **Histochemical analysis of *OsERF83***

(**a**) The *OsERF83* expression in different tissues of the *OsERF83::GUS* transgenic rice plants by GUS staining analysis, in the coleoptile of 5-day-old seedlings (**a**), stem cross-sections of 2-month-old plants (**b**, **c**), leaves of 2-week-old plants (**d**), leaf cross-sections of 2-week-old plants (**e**, **g**), the root of a 5-day-old seedling (**h**), longitudinal root section of 2-week-old plants (I), and root cross-sections of 2-week-old plants (**j**, **k**). Scale bars represent 1 mm in (**a**) and (**b**), 100 μm in (**c**, **d**) and (**h**), and 50 μm in (**e**–**k**). (**i**) Transverse section images represent structures of the roots and leaves.



scale bar: 50um

Figure 11. Cross-section of NT, *OsERF83*^{OX} transgenic rice, and *OsERF83*^{KO} mutants

The images showing 2-month old mature leaves (upper panels) and roots (bottom panels) of NT, *OsERF83*^{OX} transgenic rice, and knock-out (*OsERF83*^{KO}) mutants. Scale bar represents 50μm

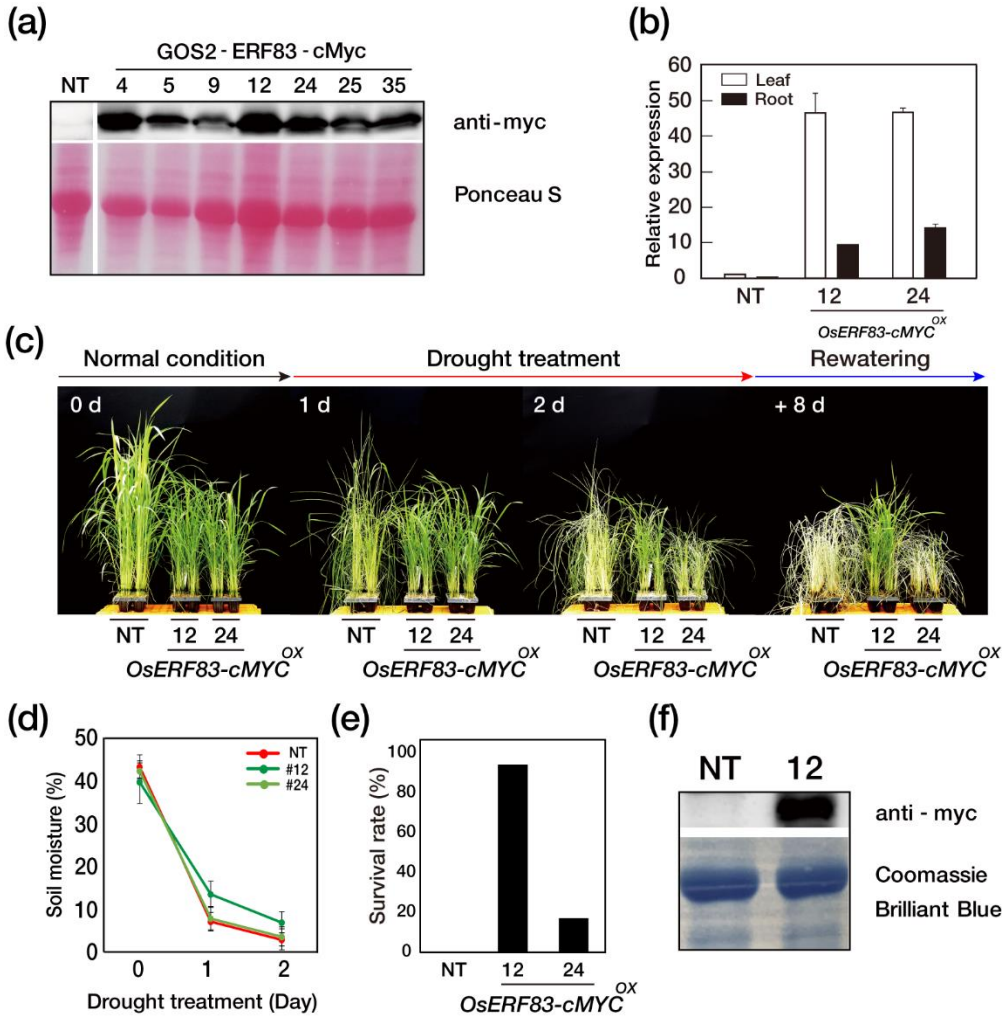

(a)
GOS2 - ERF83 - cMyc
NT 4 5 9 12 24 25 35
anti-myc
Ponceau S
(b)
Relative expression
60
50
40
30
20
10
0
Leaf
Root
NT 12 24
OsERF83-cMYC OX
(c)
Normal condition
Drought treatment
Rewatering
0 d
1 d
2 d
+ 8 d
NT 12 24
OsERF83-cMYC OX
(d)
Soil moisture (%)
50
40
30
20
10
0
NT
#12
#24
0 1 2
Drought treatment (Day)
(e)
Survival rate (%)
100
80
60
40
20
0
NT 12 24
OsERF83-cMYC OX
(f)
NT 12
anti - myc
Coomassie
Brilliant Blue

Figure 12. The protein expression of *OsERF83* overexpression myc tagging transgenic (*OsERF83-MYC^{OX})* and the phenotype under drought treatment

(a) Western blot analysis of *OsERF83-MYC^{OX}* (lines 4, 5, 9, 12, 24, 25, 35) plants leaves with anti-myc antibody. The upper panel showed OsERF83-myc recombinant proteins in blot and the lower panel showed Ponceau S staining as a loading control of each line. (b) Relative expression levels of *OsERF83-MYC^{OX}* (lines 12, 24). *OsUbi1* (*Ubiquitin1*; Os06g0681400) was used as the internal control for normalization. Data represent mean value + *SD (n=3)* (c) Drought tolerance phenotypes of *OsERF83-MYC^{OX}* (lines 12, 24) transgenic plants. All plants were grown in soil 5 weeks under a well-watered condition and exposed to drought stress for 2 days, followed by re-watering for 8 days in the greenhouse. (d) Measurement of soil moisture contents (%). Data represent mean value ± SD of 15 measurements performed at different pots. (e) The survival rate of *OsERF83-MYC^{OX}* (lines 12, 24) transgenic plants scored 8 days after re-watering. (f) Western blot analysis of *OsERF83-MYC^{OX}* (lines 12, 24) plants from (c) leaves with anti-myc antibody.

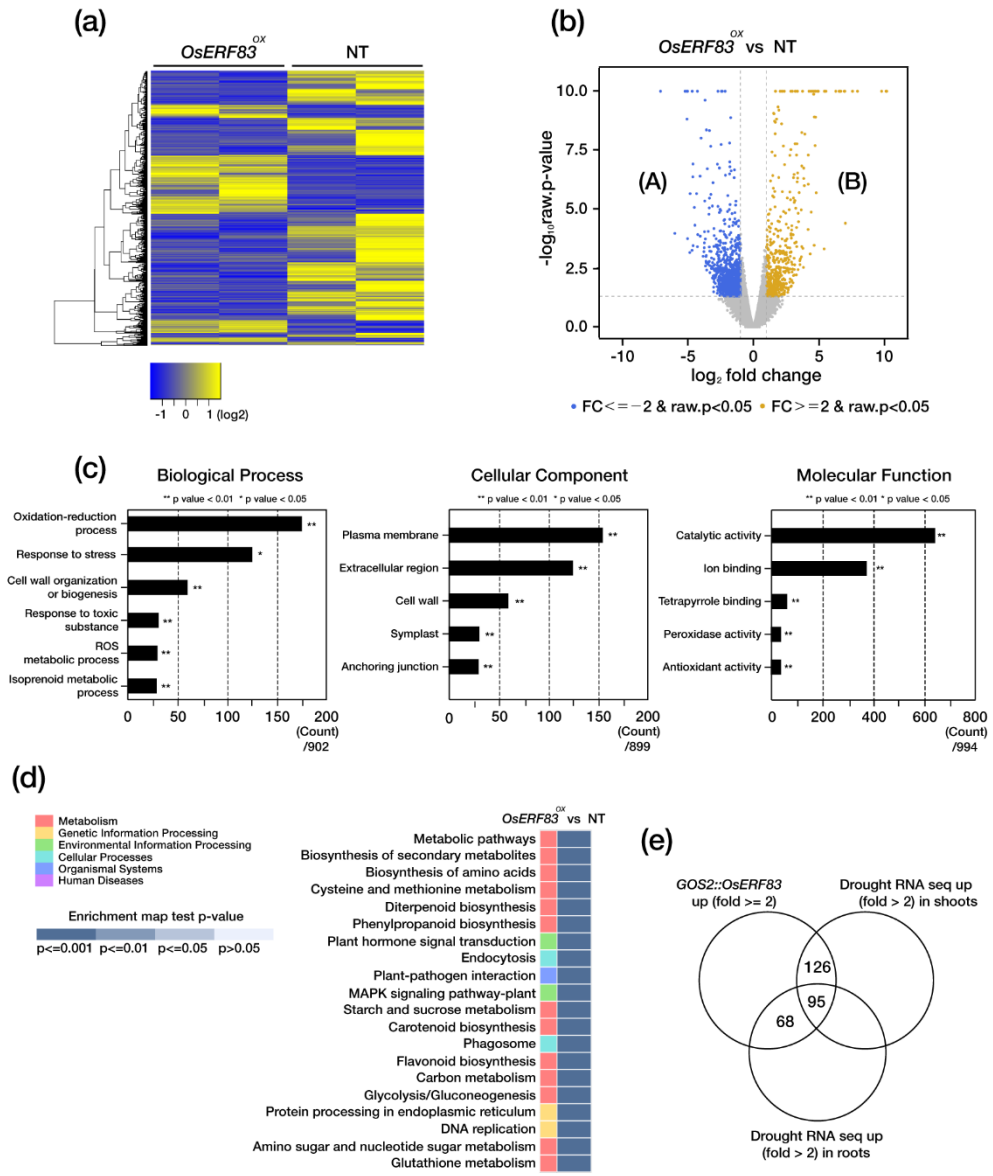

(a)
OsERF83ox
NT
-1 0 1 (log2)
(b)
OsERF83ox vs NT
-log10raw.p-value
log2 fold change
(A)
(B)
FC<=−2 & raw.p<0.05
FC>=2 & raw.p<0.05
(c)
Biological Process
Cellular Component
Molecular Function
** p value < 0.01 * p value < 0.05
Oxidation-reduction process
Response to stress
Cell wall organization or biogenesis
Response to toxic substance
ROS metabolic process
Isoprenoid metabolic process
Plasma membrane
Extracellular region
Cell wall
Symplast
Anchoring junction
Catalytic activity
Ion binding
Tetrapyrrole binding
Peroxidase activity
Antioxidant activity
(Count)
/902
/899
/994
(d)
Metabolism
Genetic Information Processing
Environmental Information Processing
Cellular Processes
Organismal Systems
Human Diseases
Enrichment map test p-value
p<=0.001 p<=0.01 p<=0.05 p>0.05
OsERF83ox vs NT
Metabolic pathways
Biosynthesis of secondary metabolites
Biosynthesis of amino acids
Cysteine and methionine metabolism
Diterpenoid biosynthesis
Phenylpropanoid biosynthesis
Plant hormone signal transduction
Endocytosis
Plant-pathogen interaction
MAPK signaling pathway-plant
Starch and sucrose metabolism
Carotenoid biosynthesis
Phagosome
Flavonoid biosynthesis
Carbon metabolism
Glycolysis/Gluconeogenesis
Protein processing in endoplasmic reticulum
DNA replication
Amino sugar and nucleotide sugar metabolism
Glutathione metabolism
(e)
GOS2::OsERF83 up (fold >= 2)
Drought RNA seq up (fold > 2) in shoots
Drought RNA seq up (fold > 2) in roots
126
95
68

Figure 13. RNA-seq analysis using leaves of *OsERF83-MYC*^{OX} plants

(**a**) Heat map analysis of differentially expressed genes (DEGs) between *OsERF83-MYC*^{OX} and NT plants. (**b**) Venn diagram of the numbers of up- and down-regulated DEGs in *OsERF83-MYC*^{OX} plants compared to NT plants. (**c**) Gene ontology (GO) enrichment analysis of DEGs between *OsERF83-MYC*^{OX} and NT plants. The most enriched GO term is shown in 'biological process', 'molecular function', and 'cellular component'. Asterisks indicate significant enriched GO terms (** $p < 0.01$, * $p < 0.05$). (**d**) KEGG pathway enrichment analysis of DEGs. (**e**) Venn diagram of up-regulated genes amongst the DEGs identified in *OsERF83-MYC*^{OX} and DEGs identified in drought-treated NT shoots and roots from public data (TENOR: http://tenor.dna.affrc.go.jp/, accessed on 30 November 2020).

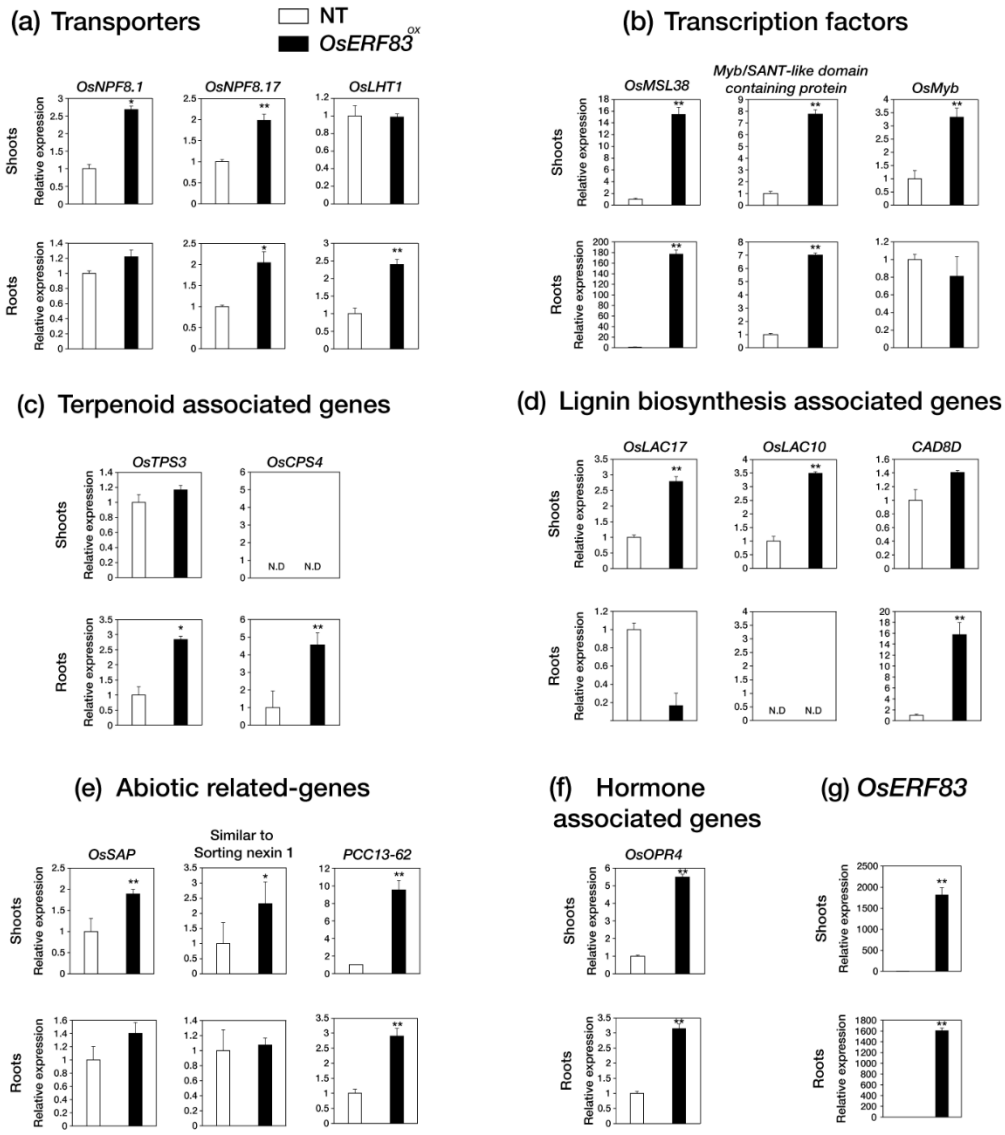

(a) Transporters
NT
OsERF83ox
OsNPF8.1
OsNPF8.17
OsLHT1
Shoots
Roots
Relative expression
(b) Transcription factors
OsMSL38
Myb/SANT-like domain containing protein
OsMyb
(c) Terpenoid associated genes
OsTPS3
OsCPS4
N.D
(d) Lignin biosynthesis associated genes
OsLAC17
OsLAC10
CAD8D
(e) Abiotic related-genes
OsSAP
Similar to Sorting nexin 1
PCC13-62
(f) Hormone associated genes
OsOPR4
(g) OsERF83

Figure 14. Expression analysis of up-regulated genes in both *OsERF83-MYC*^{OX} plants and drought-treated samples

(**a**) Relative expression levels of transporter genes (**b**), transcription factors (**c**), terpenoid-associated genes (**d**), lignin biosynthesis-associated genes (**e**), abiotic stress-related genes, (f) and hormone associated genes (g) qRT-PCR analysis of *OsERF83* expression in 3-week-old *OsERF83*^{OX} (line 1). The white bar is the expression value in NT plants, and the black bar is the expression value in *OsERF83*^{OX} plants. *OsUbi* (*Ubiquitin1*; Os06g0681400) expression was used as an internal control for normalization. Error bars indicated the standard deviation based on three technical replicates. Asterisks indicate significant enriched GO terms (** $p < 0.01$, * $p < 0.05$).

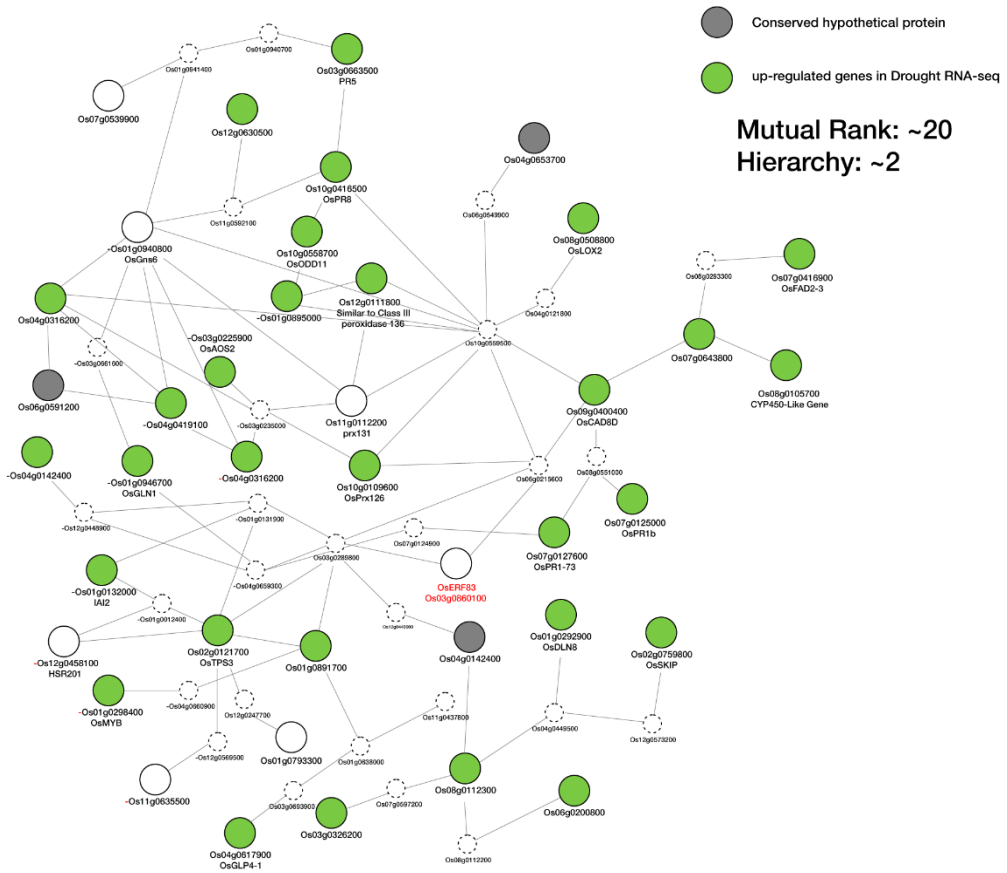


Figure 15. *OsERF83* transcriptional co-regulatory network

Co-regulatory genes were selected from differentially expressed genes (DEGs) in *OsERF83-MYC*^{OX}. The dashed-line circle represents genes that not belongs to DEGs

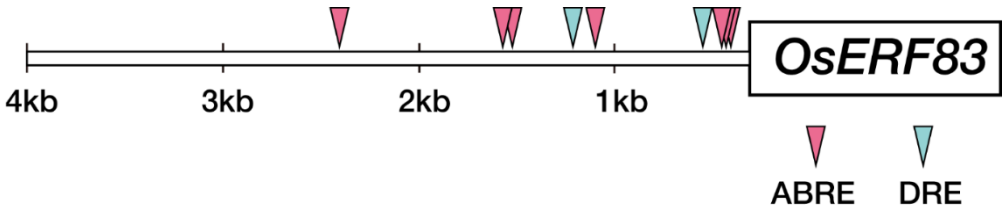


Figure 16. Cis-elements in a promoter of *OsERF83*

A dehydration-responsive element (DRE; TACCGACAT) and ABA-responsive element (ABRE; ACGTGG/TC) cis-elements were represented.

.

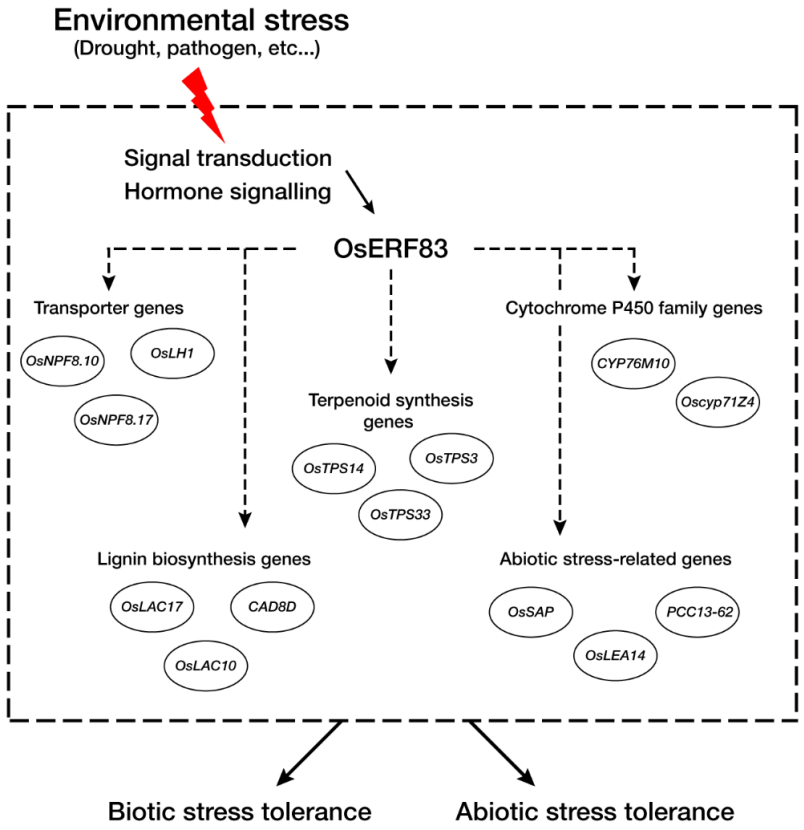


Figure 17. Schematic representation of *OsERF83*-mediated stress tolerance

The rice recognizes the stress signal through a plant hormone signaling pathway, and *OsERF83* is up-regulated in response to stresses. Transporter genes (*OsNPF.10, OsLH1, OsNPF8.17)*, terpenoid synthesis genes (*OsTPS14, OsTPS33, OsTPS3*), cytochrome P450 genes (*Oscyp71Z4*, *CYP76M10)*, lignin biosynthesis genes (*OsLAC17, OsLAC10, CAD8D*), and abiotic stress-related genes (*OsSAP, OsLEA14, PCC13-62)* were induced by *OsERF83.*

Table 1. Agronomic traits of *OsERF83* overexpression transgenic rice plants grown under normal conditions.

Geno Type	Plant height (cm)	Culm length (cm)	Panicle length (cm)	No. of panicle /hill	No. of spikelet /panicle	No. of total spikelet/hill	Number of filled grains (NFG)	Filling rate (%)	Total grain weight (g)	1000 grain weight (g)
NT	98.86	78.71	20.14	17.55	104.52	1783.90	1507.00	84.95	35.75	23.76
OX-#1	75.50	59.67	15.7	14.00	82.91	1116.50	841.55	76.56	17.35	20.65
% △	-23.6	-24.19	-22.05	-20.23	-20.68	-37.41	-44.16	-9.88	-51.49	-13.10
p-value	0.000	0.000	0.001	0.006	0.011	0.000	0.000	0.000	0.000	0.000
	**	**	**	**	*	**	**	**	**	**
OX-#2	87.63	71.69	15.94	15.67	78.33	1168.50	928.83	78.71	19.57	21.09
% △	-11.4	-8.92	-20.86	-10.73	-25.06	-34.50	-38.37	-7.34	-45.25	-11.24
p-value	0.000	0.006	0.000	0.242	0.000	0.000	0.000	0.000	0.000	0.000
	**	**	**		**	**	**	**	**	**
OX-#11	81.50	63.06	18.44	18.00	84.08	1468.74	1040.74	72.36	24.07	23.31
% △	-17.6	-19.88	-8.45	2.56	-19.56	-17.67	-30.94	-14.82	-32.68	-1.89
p-value	0.000	0.000	0.020	0.743	0.014	0.029	0.000	0.000	0.000	0.344
	**	**	*		*	*	**	**	**	

Table 2. List of genes up-regulated (> 2 fold) in *OsERF83-MYC*^{OX} and drought treatment in shoots and roots.

Descriptions	Gene ID	OX/NT fc.	OX/NT pval.	GCC box
Transporter				
Similar to sugar transport protein 14	Os09g0322000	12.80	0.000	-1858, -1702, -902
OsLTP2.7	Os10g0504650	4.78	0.002	
OsNPF8.10	Os01g0142800	4.27	0.006	
OsATL11	Os02g0101000	3.95	0.003	
ABC transporter-like domain containing protein	Os08g0564300	3.14	0.021	
NPF8.17	Os10g0112500	2.53	0.040	
OsACA1	Os03g0203700	2.34	0.044	-1439, -1052, -1049
NPF5.1	Os05g0336200	2.33	0.040	
OsLHT1	Os08g0127100	2.21	0.008	-1281
Transcription factor				
OsMSL38	Os11g0282700	25.08	0.000	-2906
Zinc finger, RING-type domain containing protein	Os06g0534800	11.73	0.002	-2989, -2975
OsC3H32	Os04g0671800	10.06	0.000	-2976, -2918, -2879, -2876, -2835, -1283, -1144

Myb/SANT-like domain containing protein	Os08g0496700	3.51	0.005	-1455, -1191, -900
OsC3H17, OsWD40-49	Os02g0677700	3.44	0.015	
OsbZIP89	Os12g0634500	3.27	0.028	-483
OsABI5, OsbZIP10	Os01g0859300	3.04	0.010	-1554
Zinc finger, RING/FYVE/PHD-type domain containing protein	Os09g0504700	2.67	0.030	-1835, -1803, -1740
ONAC79	Os04g0437000	2.63	0.038	-2800, -506
OsERF86	Os07g0410700	2.62	0.029	-2589, -2495, -657
OsSPL1, OsDLN8	Os01g0292900	2.47	0.000	-2338, -2204, -1991
OsMyb	Os01g0298400	2.45	0.011	-1348, -1325, -1089, -889
OsbHLH025	Os01g0196300	2.41	0.046	-1581, -1578
Zinc finger, RING/FYVE/PHD-type domain containing protein	Os07g0484300	2.16	0.045	-2923, -2920, -888
Abiotic stress-related genes				
OsDjA1	Os02g0656500	26.99	0.000	-2460, -2354, -2351
OsSAP, OsTET13	Os09g0425900	12.49	0.000	-908
OsTHIC	Os03g0679700	10.84	0.000	-1881, -1878, -1875
OsLEA14, wsi18	Os01g0705200	9.95	0.000	-2526
Similar to Sorting nexin 1	Os01g0862300	6.86	0.000	-2778, -1217, -982, -979, -690, -544, -487, -481, -470, -359, -329, -298,

				-278, -188, -29
OsMPK14	Os05g0143500	6.85	0.013	
PCC13-62, OsEnS-64	Os04g0404400	5.60	0.000	-2980, -2977, -2974, -2223
OsHSP23.7	Os12g0569700	3.68	0.015	-1065, -1018, -988, -957, -444, -441, -379, -174
OsFAD2-3	Os07g0416900	3.43	0.005	-1476
Similar to Class III peroxidase 136	Os12g0111800	3.29	0.001	
OsBBX28	Os09g0509700	2.65	0.009	-2975
OsNTP3	Os01g0846500	2.63	0.040	-1783, -1780, -1685, -1653
OsPEX11-5	Os06g0127000	2.49	0.019	-2371, -2368, -2054, -2051, -2048
Glycine-rich RNA-binding protein	Os04g0653700	2.37	0.032	-2957, -2901, -2895, -2892, -2307, -2684, -2680, -1677, -1610, -1607, -1604, -1577, -1478, -1475, -1442, -1415, -1322, -1319, -1316, -1313, -710, -678
OsSKIP	Os02g0759800	2.23	0.001	-2986, -2831, -1142, -1139, -1136, -990, -906, -840, -837, -800
OsRZFP34, OsRFP1	Os01g0719100	2.21	0.027	-1128, -1114, -346, -120

OsVPE3	Os02g0644000	2.20	0.000	-2797, -2751
OsASMT1	Os09g0344500	2.10	0.007	
Cytochrome P450 family				
Oscyp71Z4	Os10g0439800	5.79	0.001	-2642, -2625
Cytochrome P450 family protein	Os12g0582666	4.86	0.001	
CYP76M10	Os08g0507100	4.09	0.007	-1515, -1512, -1354, -1297, -1008, -572
CYP450-Like Gene	Os08g0105700	4.09	0.000	-2870, -2337, -2065. -1006, -998, -995, -974
Similar to Cytochrome P450 family protein, expressed	Os12g0640200	3.53	0.010	-1064, -1061, -1050, -989, -940, -240, -190, -187
OsCYP71AA2	Os01g0957800	3.50	0.022	-2440, -2437, -2434, -2410, -2224, -2029
Cytochrome P450 family protein	Os02g0184700	3.01	0.047	-1217
Cytochrome P450 family protein	Os03g0594100	2.54	0.022	-2178
CYP76M8	Os02g0569400	2.28	0.039	
CYP99A2	Os04g0180400	2.26	0.046	-2113, -2010, -1980, -1959
CYP76M2	Os08g0508000	2.15	0.012	-2928, -937
Terpenoid associated genes				
OsTPS33	Os12g0491800	4.05	0.001	-608
OsTPS14	Os03g0428200	2.83	0.024	-1604
OsTPS3	Os02g0121700	2.80	0.027	

OsCPS4	Os04g0178300	2.68	0.000	-1983, -1933, -1926, -1897, -1825, -1797, -1794, -1786
OsTPS16	Os04g0179700	2.61	0.036	
Disease resistance related genes				
OsCSN5A	Os04g0654700	25.76	0.000	
Similar to blight-associated protein p12	Os09g0472700	23.77	0.000	-2580, -1500, -1353, -1308, -1305, -1302, -1069, -1066, -1063, -995, -992, -927, -924, -130, -98, -37
OsLysM-RLK8	Os11g0549300	13.44	0.000	-2808, -2279, -2093, -2021, -1995, -1992, -1972
OsCslE1	Os09g0478100	10.69	0.000	-2802
eIF4Gtsv1	Os07g0555200	9.14	0.000	-1520
OsAGP29	Os01g0607100	7.42	0.000	-2606
OsWAK89b	Os09g0561450	6.55	0.005	
OsTHI7	Os06g0514100	5.48	0.004	-1693, -995
OsGLP4-1	Os04g0617900	5.22	0.000	-738
Similar to Blight-associated protein p12 precursor	Os09g0472900	4.91	0.000	-2469, -2376, -178
Similar to Receptor-like kinase Xa21-binding protein 3.	Os05g0112000	4.86	0.016	-2973
Similar to Herbicide safener binding protein	Os11g0306400	4.67	0.042	

Leucine-rich repeat domain containing protein	Os04g0122000	4.57	0.010	-1725
OsPR8	Os10g0416500	4.50	0.000	-2994, -2939, -871, -726
OsGSL3	Os01g0754200	4.45	0.018	-2773, -2770, -584, 581, -578, -575, 572, -569
OsEXO70B3	Os05g0473500	4.31	0.003	-2788, -2764, -2748, -508, -468, -465, -438, -413, -264, -159
Leucine-rich repeat, typical subtype domain containing protein	Os11g0568800	3.83	0.009	-2429, -2426, -1239, -1210, -1185, -1130
Leucine-rich repeat, N-terminal domain containing protein.	Os12g0211500	3.80	0.017	
Similar to Pathogen-related protein.	Os01g0248300	3.80	0.000	-2698, -2680, -2644, -2637, -1700, -1620, -1560, -1557, -1534, -1317
OsDXS3	Os07g0190000	3.28	0.000	
Multi antimicrobial extrusion protein MatE family protein	Os09g0524300	3.27	0.026	
OsLOX9	Os08g0508800	3.20	0.000	-1626
OsWAKL21.2	Os12g0595800	2.93	0.016	-959, -842, -839, -836, -833, -308,

				-305, -302, -299, -296, -183, -167, -164, -161, -92
Similar to Resistance protein candidate (Fragment).	Os03g0258000	2.84	0.031	-577, -478, -391
IAI2	Os01g0132000	2.78	0.000	-1174, -1167
OsTHI9	Os06g0514800	2.74	0.025	-1718, -1715, -1697
Leucine-rich repeat, N-terminal domain containing protein.	Os01g0891700	2.70	0.031	
OsPR4d	Os11g0591800	2.26	0.001	-2134, -993
Allergen V5/Tpx-1 related family protein	Os07g0126401	2.24	0.014	
Allergen V5/Tpx-1 related family protein	Os07g0125600	2.23	0.015	-553, -386, -336, -333, -215
OsPR1b	Os07g0125000	2.22	0.015	
OsPR1-73	Os07g0127600	2.15	0.018	
Similar to Thaumatin-like protein.	Os12g0630500	2.15	0.000	-2453
PR5	Os03g0663500	2.12	0.006	
OsGLN1	Os01g0946700	2.06	0.000	-2549, -1263, -760, -669, -616, -457, -374, -323
OsAOS2	Os03g0225900	2.01	0.007	-2843, -2202, -349
Hormone associated gene				

OsLUGL, OsWD40-17	Os01g0607400	11.97	0.001	-2980, -2906, -2903, -2900, -2897, -2836, -2833
OsARF12	Os04g0671900	10.33	0.000	
OsJAZ8	Os09g0401300	6.01	0.000	-2541, -2538
OsGA20ox4	Os05g0421900	4.85	0.015	
OsARF13, OsARF12	Os04g0690600	2.74	0.030	
OsOPR4	Os06g0215900	2.46	0.000	-2863, -1540, -1481, -1478, -1459, -1431, -1428, -1279, -1276, -1238, -1206, -1133, -1129, -1126, -1070, -1040, -1016, -1013
Lignin biosynthesis associated genes				
OsUGT707A5	Os07g0503300	4.53	0.000	-1544, -1507, -1470
OsUGT93B	Os04g0556400	3.99	0.003	-2364
UDP-glucuronosyl/UDP-glucosyltransferase family protein	Os03g0804900	3.23	0.027	-2977, -805, -777, -774, -740, -737, -690, -473, -470, -407, -404, -376
OsLAC17	Os10g0346300	3.02	0.010	-1525, -1481, -1468, -151
OsLAC10	Os02g0749700	2.95	0.026	-1963, -1907
OsCAD8D	Os09g0400400	2.94	0.000	-2137
F-box protein				
OsFbox331	Os07g0118900	8.11	0.013	
OsFbox228	Os04g0571300	4.07	0.003	-1080
OsFbox552	Os10g0396400	3.34	0.036	

Others				
OsSNDP6	Os05g0545000	93.56	0.000	-2547
OsCDC48, PSD128	Os03g0151800	23.44	0.000	
OsASL1	Os03g0305500	8.59	0.001	-2950, -1607, -1491, -1488, -1365, -1362, -1302, -1158, -941, -328, -317, -234, -231, -214, -181, -178, -175, -27, -24
OsNABP	Os06g0215200	7.42	0.018	
OsSCP65, CBP1	Os12g0257000	6.16	0.034	-1760, -1493, -1421, -1306, -1260, -1176, -1086, -1083, -1039, -1036, -1002, -975
OsRab5B1	Os03g0666500	6.12	0.000	
OsVLN4	Os04g0604000	5.38	0.000	-1293, -843, -840
OsalphaCA3	Os08g0423500	4.72	0.025	-506, -472, -464, -434
OsPP2C14, OsPP24	Os02g0471500	4.42	0.028	-2409, -156
Glua1, OsEnS-16	Os01g0762500	4.26	0.042	
OSK28	Os07g0625400	3.98	0.001	-2892, -2823, -470, -467, -364, -129
OsRFP	Os03g0326300	3.82	0.050	
OsEnS-40	Os02g0586900	3.74	0.003	
OsTHI3, OsTHION3	Os06g0513050	3.59	0.006	
OsEnS-119	Os08g0286500	3.31	0.013	
OsPGK1	Os01g0800266	3.29	0.037	-201, -192
OsPUP2	Os09g0467300	3.17	0.040	
OsGSTF5	Os01g0369700	3.15	0.001	

OsTBL33	Os12g0516800	3.14	0.007	-1155, -1017, -1013
Osppc2a, OSPPC	Os08g0366000	3.09	0.017	
Tryptophan synthase	Os08g0135900	3.07	0.005	
OsCatB	Os05g0310500	2.98	0.000	-2294
Similar to Stearoyl-acyl carrier protein desaturse	Os03g0423300	2.91	0.031	
Similar to Oxidoreductase, 2OG-Fe oxygenase family protein, expressed	Os10g0558750	2.64	0.000	
OsPAP27a, OsNPP6	Os09g0506000	2.60	0.046	-2870, -2830, -2827, -1219, -1156, -1153, -1138, -1090, -1087, -1084, -1081
OsISC3	Os09g0270900	2.57	0.005	-2951, -2926, -2867, -2864, -2861
Similar to ACX4 (ACYL-COA OXIDASE 4); acyl-CoA oxidase/oxidoreductase	Os06g0346300	2.56	0.026	-280, -277, -213, -179, -161, -108, -90
OsSTA16	Os01g0548000	2.49	0.035	-2323
OsRePRP2.1	Os07g0418700	2.49	0.001	
Arf GTPase activating protein family protein	Os07g0563800	2.48	0.000	-552, -530, -358, -334, -327, -320, -295
OsPP2A-B"	Os10g0476600	2.46	0.011	-2887, -2836, -2802, -2781, - 856, -114, -111
OsTHI5, OsTHION4	Os06g0513862	2.33	0.043	-1718, -1715, -1697, -1002, -999

Zinc finger, RING/FYVE/PHD-type domain containing protein	Os06g0717600	2.11	0.006	-2908
OsCBSCBS3	Os04g0382300	2.07	0.000	-2575, -2572, -2390
OsaHMT4	Os12g0607000	2.04	0.005	
OsODD11, OsM3H	Os10g0558700	2.01	0.000	

Table 3. List of primers used in this study

Gene	Primer Sequence		
	Purpose	**Forward**	**Reverse**
OsERF83 (Os03g0860100)	qRT-PCR	5'-GACGGATCAGCATCAGGATCA-3'	5'-TCCATGGCTGGGACAGAGAC-3'
Ubi (Os06g0681400)	qRT-PCR	5'-GCCAAGATCCAGGACAAGGA-3'	5'-GCCATCCTCCAGCTGCTT-3'
OsNPF8.10 (Os01g0142800)	qRT-PCR	5'-TGCTGCCTGTTCAGTTTCTC -3'	5'-CCAAGCATTCCGTCCCTGAT-3'
OsNPF8.17 (Os10g0112500)	qRT-PCR	5'-GGGATGATCACGCTCACAGT-3'	5'-AGGTAGAGCCCCAGGAACAC-3'
OsLHT1 (Os08g0127100)	qRT-PCR	5'-AAGAAGTTCCACGACGTGCT-3'	5'-GTTGAAGTTTGGGAGCTGCG-3'
OsMSL38 (Os11g0282700)	qRT-PCR	5'-CCTAAAACTAAGGTATCGAAGATGC-3'	5'-ATTCATAGGCCATAACAGTGACC-3'
Myb/SANT-like domain domain containing protein. (Os08g0496700)	qRT-PCR	5'-GGGCTAAATTCATTAACGTCCCC-3'	5'-TCAACATCACCAGGACTGCC-3'
OsMYB (Os01g0298400)	qRT-PCR	5'-ACGAACCACCACCTGATGAC-3'	5'-AACTGATCCCAACGCTCGTG-3'
OsTPS3 (Os02g0121700)	qRT-PCR	5'-ATGGCGAATTCAGGCTCGAT-3'	5'-TGCAAGATTTGGCTCGAGGT-3'
OsCPS4 (Os04g0178300)	qRT-PCR	5'-GGGTTCTACAATTAATGTTCCGGT-3'	5'-ACAGACAGCATGCACTGTCA-3'
OsLAC17 (Os10g0346300)	qRT-PCR	5'-ACCCAGGGGCATGGTTAATG-3'	5'-TCATGGCCTTCCGTCCTAGA-3'
OsLAC10 (Os02g0749700)	qRT-PCR	5'-CAGCTTACGGCACTAGTTAGC-3'	5'-CCACGCCTGCTAGTAACCAA-3'
CAD8D (Os09g0400400)	qRT-PCR	5'-GCTTAGCAAGGAGTGGTCGAT-3'	5'-TGATGATGTGCAGGTCGGT-3'
OsSAP (Os09g0425900)	qRT-PCR	5'-GGGATCGGACCCAGTTCTTC-3'	5'-GACTGGCAGTTGTAGCACAG-3'

Similar to Sorting nexin 1 (Os01g0862300)	qRT-PCR	5'-AAAAATAATTCGGGCAGGATCTC-3'	5'-GGCTCAGTTGAACCTCCCAA-3'
PCC13-62 (Os04g0404400)	qRT-PCR	5'-TGCTACCAAGAAGTCGGCCA-3'	5'-GCTGTTCTCGTAGGGGTTGAA-3'
OsOPR4 (Os06g0215900)	qRT-PCR	5'-CCTACGGGAGGCACTTCTTG-3'	5'-CATAAGTAGCAGCGCCCTCG-3'
OsERF83 (Os03g0860100)	CDS	5'-GCATTGCTGCATGTCGCTTC-3'	5'-GCTGTTGGTGGTGCTCATGG-3'

Discussion

A number of studies have shown that APETALA2/ethylene-responsive factor (AP2/ERF) TFs are involved in the integration of signaling pathways in abiotic and biotic stress responses (Mizoi *et al.*, 2012; Muller and Munne-Bosch, 2015). AP2/ERF TFs are classified into several subfamilies, including the ERF family (Sakuma *et al.*, 2002). *OsERF83* is a member of group IXc, a subgroup of the ERF family reported to play an important role in abiotic stress (Berrocal-Lobo *et al.*, 2002; Liu *et al.*, 2012; Pre *et al.*, 2008). ERF TFs have been reported that regulate stress-inducible genes by binding *cis*-acting elements, such as GCC box (AGCCGCC) in the promoter (Fujimoto *et al.*, 2000). Previous studies have demonstrated that OsERF83 is a positive transcriptional regulator and interacts with the GCC box (Tezuka *et al.*, 2019), and we analyzed the cis-element GCC box in the promoter of DEGs in our search for putative direct targets (Table 2).

The expression of *OsERF83* was induced by ABA treatment (Figure 1a). In addition, *OsERF83* was also reported to be induced by exogenous SA, JA, and ET (Tezuka *et al.*, 2019). These results indicated that *OsERF83* is involved in multiple phytohormone signaling pathways during environmental stresses. It is well known that there are complex interactions between various hormones and stress signaling pathways in plants (Atkinson and Urwin, 2012). A single gene may play roles in various signaling pathways at the same time (Liu *et al.*, 2012). Therefore, our study suggested that *OsERF83* is a positive regulator in plant responses to abiotic and biotic stresses through various hormone signaling pathways.

OsERF83^{OX} plants resulted in growth retardation and low grain yields (Figure 7, 8, Table 1). Generally, plants containing stress tolerance have been reported to show growth retardation (Kim *et al.*, 2014; Ma *et al.*, 2020) because of

hypersensitivity in responding to stresses, directing resources to protection from stresses rather than growing or yielding, known as a trade-off. Thus, genes improving stress tolerance cause growth retardation even under normal growth conditions when they are constitutively overexpressed. In this study, we used the GOS2 promoter for constitutive overexpression (Figure 2b). However, if we used the stress-inducible promoter instead of the constitutive promoter, *OsERF83* could be a good candidate for crop biotechnology.

The essential functions of vascular tissue are transporting water and various nutrients and supporting structures (Lucas *et al.*, 2013). Consistent with this, there have been reports that vascular tissue development is involved in drought tolerance (Geng *et al.*, 2018; Zhang *et al.*, 2018). *OsERF83* is predominantly expressed in vascular tissue (Figure 10). However, there were no significant differences in phenotypes of vascular tissue in this study (Figure 11). On the contrary, several transcriptome studies have shown that *OsERF83* is induced under iron (Wu *et al.*, 2011; Zheng *et al.*, 2009) and nitrate deficiency (Hsieh *et al.*, 2018). Moreover, our study revealed that various transporters were up-regulated in *OsERF83-MYC*^{OX} (Table 2). Therefore, we speculated that *OsERF83* might also be involved in iron and nitrate absorption.

Previously, it was reported that *OsERF83* positively regulates disease resistance, and the constitutive overexpression of *OsERF83* in rice enhanced resistance to *Magnaporthe oryzae* (Tezuka *et al.*, 2019). However, no further report on the function of *OsERF83* in abiotic stress tolerance has been reported so far. In the present study, we analyzed the function of *OsERF83* as a regulator of responses to drought stress. *OsERF83* was strongly induced not only by drought stress, but also other abiotic stresses (Figure 1a). The *OsERF83* promoter also contains several stress-related *cis*-elements, including ABREs, DREs regulated by ABA and DREB

(Figure 16) (Agarwal and Jha, 2010). These results support *OsERF83* potentially playing an important role in abiotic stresses. *OsERF83*^{OX} plants showed drought tolerance under drought treatment in the vegetative stage (Figure 5), which suggested the positive role of *OsERF83* in drought tolerance. While *OsERF83*^{KO} mutants showed similar phenotypes compared with non-transgenic (NT) plants under drought treatment at the vegetative stage (Figure 9d), this could be explained by functional redundancy among a large number of the AP2/ERF TFs (Nakano *et al.*, 2006; Xie *et al.*, 2019).

RNA-seq analysis identified a large number of transporter genes that were up-regulated in *OsERF83* overexpression myc-tagged transgenic (*OsERF83-MYC*^{OX}) plants, consistent with the vascular-specific expression of *OsERF83*. In a recent report, *NRT1.2*/*NPF4.6* in *Arabidopsis* was found to affect ABA import activity (Zhang *et al.*, 2021). This suggested that a group of transporter genes up-regulated in *OsERF83-MYC*^{OX} (Table 2, Figure 14a), including NPFs (nitrate and peptide transporters), might affect the drought tolerance of rice through the ABA-dependent pathway. On the other hand, recent reports have shown that lignin plays an important role in protection against water losses (Bang *et al.*, 2019; Tu *et al.*, 2020). A group of lignin biosynthesis-associated genes up-regulated in *OsERF83-MYC*^{OX} (Table 2, Figure 14d), including *OsLAC17* (Li et al., 2017; Sun et al., 2020), *OsLAC10* (Liu *et al.*, 2017), and *OsCAD8D* (Barakat *et al.*, 2009), might be involved in protection against water losses and affect drought tolerance. A number of abiotic stress-related genes containing *SENESCENCE-ASSOCIATED PROTEIN* (*OsSAP*), *OsLEA14/Wsi18*, *PCC13-62*, and heat shock protein were up-regulated in *OsERF83-MYC*^{OX} (Table 2, Figure 14e). *OsSAP* is involved in drought tolerance by virtue of its antiapoptotic activity (Ubaidillah *et al.*, 2013). *OsLEA14/Wsi18* improves drought tolerance through higher proline and soluble sugar accumulation (Kaur *et al.*, 2018).

Desiccation-related protein (DRP)-encoding gene *PCC13-62* is involved in desiccation tolerance in *Linderniaceae* (Giarola *et al.*, 2018). Heat shock proteins play a role in membrane stability and regulating antioxidant enzymes and are produced under various abiotic and biotic stresses (Driedonks *et al.*, 2015; ul Haq *et al.*, 2019). It has been reported that *OsHSP23.7* enhances drought and salt tolerance in rice and overexpression transgenic plants show less membrane damage than NT plants (Zou *et al.*, 2012). It has also been shown that cytochrome P450 genes are involved in drought tolerance (Pandian *et al.*, 2020; Tamiru *et al.*, 2015). Many disease resistance-related genes, including *PATHOGENESIS-RELATED PROTEIN* (*PR*), *WALL-ASSOCIATED KINASE* (*WAK*) gene, *CELLULOSE SYNTHASE-LIKE PROTEIN E1* (*CslE1*), *LYSM RECEPTOR-LIKE KINASE* (*RLK*), and terpenoid-associated genes, were also up-regulated (Table 2). It has been reported that many stress-responsive genes are induced by both biotic and abiotic stress treatment. PRs correlate with drought tolerance (Liu *et al.*, 2013; Wu *et al.*, 2016). Cell wall-associated genes containing *WAK*, *CslE1*, and lignin biosynthesis genes are involved in both abiotic and biotic stresses (Houston *et al.*, 2016; Le Gall *et al.*, 2015). Accordingly, we speculated that *OsERF83* is involved in the core pathways in abiotic and biotic stress responses (Figure 17).

References

Agarwal PK, Jha B. 2010. Transcription factors in plants and ABA dependent and independent abiotic stress signalling. Biologia Plantarum **54**, 201-212.

Atkinson NJ, Urwin PE. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany **63**, 3523-3543.

Bang SW, Lee DK, Jung H, Chung PJ, Kim YS, Choi YD, Suh JW, Kim JK. 2019. Overexpression of OsTF1L, a rice HD-Zip transcription factor, promotes lignin biosynthesis and stomatal closure that improves drought tolerance. Plant Biotechnology Journal **17**, 118-131.

Bang SW, Park SH, Kim YS, Do Choi Y, Kim JK. 2015. The activities of four constitutively expressed promoters in single-copy transgenic rice plants for two homozygous generations. Planta **241**, 1529-1541.

Barakat A, Bagniewska-Zadworna A, Choi A, Plakkat U, DiLoreto DS, Yellanki P, Carlson JE. 2009. The cinnamyl alcohol dehydrogenase gene family in Populus: phylogeny, organization, and expression. Bmc Plant Biology **9**, 26.

Barnabas B, Jager K, Feher A. 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell and Environment **31**, 11-38.

Berrocal-Lobo M, Molina A, Solano R. 2002. Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant Journal **29**, 23-32.

Bussotti F, Desotgiu R, Pollastrini M, Cascio C. 2010. The JIP test: a tool to screen the capacity of plant adaptation to climate change. Scandinavian Journal of Forest Research **25**, 43-50.

Cheng MC, Liao PM, Kuo WW, Lin TP. 2013. The Arabidopsis ETHYLENE RESPONSE FACTOR1 Regulates Abiotic Stress-Responsive Gene Expression by Binding to Different cis-Acting Elements in Response to Different Stress Signals.

Plant Physiology **162**, 1566-1582.

Chung PJ, Chung H, Oh N, Choi J, Bang SW, Jung SE, Jung H, Shim JS, Kim JK. 2020. Efficiency of Recombinant CRISPR/rCas9-Mediated miRNA Gene Editing in Rice. Int J Mol Sci **21**.

de Pater BS, van der Mark F, Rueb S, Katagiri F, Chua NH, Schilperoort RA, Hensgens LA. 1992. The promoter of the rice gene GOS2 is active in various different monocot tissues and binds rice nuclear factor ASF-1. Plant J **2**, 837-844.

Driedonks N, Xu JM, Peters JL, Park S, Rieu I. 2015. Multi-Level Interactions Between Heat Shock Factors, Heat Shock Proteins, and the Redox System Regulate Acclimation to Heat. Frontiers in Plant Science **6**.

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M. 2000. Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell **12**, 393-404.

Geng DL, Chen PX, Shen XX, Zhang Y, Li XW, Jiang LJ, Xie YP, Niu CD, Zhang J, Huang XH, Ma FW, Guan QM. 2018. MdMYB88 and MdMYB124 Enhance Drought Tolerance by Modulating Root Vessels and Cell Walls in Apple. Plant Physiology **178**, 1296-1309.

Giarola V, Jung NU, Singh A, Satpathy P, Bartels D. 2018. Analysis of pcC13-62 promoters predicts a link between cis-element variations and desiccation tolerance in Linderniaceae. Journal of Experimental Botany **69**, 3773-3784.

Hirayama T, Shinozaki K. 2010. Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant Journal **61**, 1041-1052.

Houston K, Tucker MR, Chowdhury J, Shirley N, Little A. 2016. The Plant Cell Wall: A Complex and Dynamic Structure As Revealed by the Responses of Genes under Stress Conditions. Frontiers in Plant Science **7**, 984.

Hsieh PH, Kan CC, Wu HY, Yang HC, Hsieh MH. 2018. Early molecular events associated with nitrogen deficiency in rice seedling roots. Sci Rep **8**, 12207.

Jisha V, Dampanaboina L, Vadassery J, Mithofer A, Kappara S, Ramanan R. 2015. Overexpression of an AP2/ERF Type Transcription Factor OsEREBP1 Confers Biotic and Abiotic Stress Tolerance in Rice. Plos One **10**.

Jung H, Chung PJ, Park SH, Redillas MCFR, Kim YS, Suh JW, Kim JK. 2017. Overexpression of OsERF48 causes regulation of OsCML16, a calmodulin-like protein gene that enhances root growth and drought tolerance. Plant Biotechnology Journal **15**, 1295-1308.

Kaur R, Chakraborty A, Bhunia RK, Sen SK, Ghosh AK. 2018. Tolerance to soil water stress by Oryza sativa cv. IR20 was improved by expression of Wsi18 gene locus from Oryza nivara. Biologia Plantarum **62**, 129-139.

Kawahara Y, Oono Y, Wakimoto H, Ogata J, Kanamori H, Sasaki H, Mori S, Matsumoto T, Itoh T. 2016. TENOR: Database for Comprehensive mRNA-Seq Experiments in Rice. Plant and Cell Physiology **57**.

Kim H, Lee K, Hwang H, Bhatnagar N, Kim DY, Yoon IS, Byun MO, Kim ST, Jung KH, Kim BG. 2014. Overexpression of PYL5 in rice enhances drought tolerance, inhibits growth, and modulates gene expression. Journal of Experimental Botany **65**, 453-464.

Le Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C. 2015. Cell Wall Metabolism in Response to Abiotic Stress. Plants (Basel) **4**, 112-166.

Lee DK, Jung H, Jang G, Jeong JS, Kim YS, Ha SH, Do Choi Y, Kim JK. 2016. Overexpression of the OsERF71 Transcription Factor Alters Rice Root Structure and Drought Resistance. Plant Physiology **172**, 575-588.

Lee DK, Kim HI, Jang G, Chung PJ, Jeong JS, Kim YS, Bang SW, Jung H, Choi YD, Kim JK. 2015. The NF-YA transcription factor OsNF-YA7 confers drought stress tolerance of rice in an abscisic acid independent manner. Plant Science **241**, 199-210.

Lesk C, Rowhani P, Ramankutty N. 2016. Influence of extreme weather disasters

on global crop production. Nature **529**, 84-+.

Li WQ, Zhang MJ, Gan PF, Qiao L, Yang SQ, Miao H, Wang GF, Zhang MM, Liu WT, Li HF, Shi CH, Chen KM. 2017. CLD1/SRL1 modulates leaf rolling by affecting cell wall formation, epidermis integrity and water homeostasis in rice. Plant Journal **92**, 904-923.

Liu DF, Chen XJ, Liu JQ, Ye JC, Guo ZJ. 2012. The rice ERF transcription factor OsERF922 negatively regulates resistance to Magnaporthe oryzae and salt tolerance. Journal of Experimental Botany **63**, 3899-3911.

Liu Q, Luo L, Wang X, Shen Z, Zheng L. 2017. Comprehensive Analysis of Rice Laccase Gene (OsLAC) Family and Ectopic Expression of OsLAC10 Enhances Tolerance to Copper Stress in Arabidopsis. Int J Mol Sci **18**.

Liu WX, Zhang FC, Zhang WZ, Song LF, Wu WH, Chen YF. 2013. Arabidopsis Di19 Functions as a Transcription Factor and Modulates PR1, PR2, and PR5 Expression in Response to Drought Stress. Molecular Plant **6**, 1487-1502.

Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R. 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell **15**, 165-178.

Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav SR, Helariutta Y, He XQ, Fukuda H, Kang J, Brady SM, Patrick JW, Sperry J, Yoshida A, Lopez-Millan AF, Grusak MA, Kachroo P. 2013. The Plant Vascular System: Evolution, Development and Functions. Journal of Integrative Plant Biology **55**, 294-388.

Ma Z, Wu T, Huang K, Jin YM, Li Z, Chen M, Yun S, Zhang H, Yang X, Chen H, Bai H, Du L, Ju S, Guo L, Bian M, Hu L, Du X, Jiang W. 2020. A Novel AP2/ERF Transcription Factor, OsRPH1, Negatively Regulates Plant Height in Rice. Frontiers in Plant Science **11**, 709.

Mickelbart MV, Hasegawa PM, Bailey-Serres J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nature Reviews Genetics

16, 237-251.

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K. 2012. AP2/ERF family transcription factors in plant abiotic stress responses. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms **1819**, 86-96.

Muller M, Munne-Bosch S. 2015. Ethylene Response Factors: A Key Regulatory Hub in Hormone and Stress Signaling. Plant Physiology **169**, 32-41.

Nakano T, Suzuki K, Fujimura T, Shinshi H. 2006. Genome-Wide Analysis of the ERF Gene Family in Arabidopsis and Rice. Plant Physiology **140**, 411-432.

Oh SJ, Kim YS, Kwon CW, Park HK, Jeong JS, Kim JK. 2009. Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physiol **150**, 1368-1379.

Pandian BA, Sathishraj R, Djanaguiraman M, Prasad PVV, Jugulam M. 2020. Role of Cytochrome P450 Enzymes in Plant Stress Response. Antioxidants (Basel) **9**.

Pre M, Atallah M, Champion A, De Vos M, Pieterse CMJ, Memelink J. 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiology **147**, 1347-1357.

Redillas M, Bang SW, Lee DK, Kim YS, Jung H, Chung PJ, Suh JW, Kim JK. 2019. Allantoin accumulation through overexpression of ureide permease1 improves rice growth under limited nitrogen conditions. Plant Biotechnology Journal **17**, 1289-1301.

Redillas MCFR, Strasser RJ, Jeong JS, Kim YS, Kim JK. 2011. The use of JIP test to evaluate drought-tolerance of transgenic rice overexpressing OsNAC10. Plant Biotechnology Reports **5**, 169-175.

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. 2002. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression.

Biochemical and Biophysical Research Communications **290**, 998-1009.

Sato Y, Namiki N, Takehisa H, Kamatsuki K, Minami H, Ikawa H, Ohyanagi H, Sugimoto K, Itoh JI, Antonio BA, Nagamura Y. 2013. RiceFREND: a platform for retrieving coexpressed gene networks in rice. Nucleic Acids Research **41**, D1214-D1221.

Scarpeci TE, Frea VS, Zanor MI, Valle EM. 2017. Overexpression of AtERF019 delays plant growth and senescence, and improves drought tolerance in Arabidopsis. Journal of Experimental Botany **68**, 673-685.

Sun J, Cui X, Teng S, Kunnong Z, Wang Y, Chen Z, Sun X, Wu J, Ai P, Quick WP, Lu T, Zhang Z. 2020. HD-ZIP IV gene Roc8 regulates the size of bulliform cells and lignin content in rice. Plant Biotechnology Journal **18**, 2559-2572.

Tamiru M, Undan JR, Takagi H, Abe A, Yoshida K, Undan JQ, Natsume S, Uemura A, Saitoh H, Matsumura H, Urasaki N, Yokota T, Terauchi R. 2015. A cytochrome P450, OsDSS1, is involved in growth and drought stress responses in rice (Oryza sativa L.). Plant Mol Biol **88**, 85-99.

Tezuka D, Kawamata A, Kato H, Saburi W, Mori H, Imai R. 2019. The rice ethylene response factor OsERF83 positively regulates disease resistance to Magnaporthe oryzae. Plant Physiology and Biochemistry **135**, 263-271.

Tu M, Wang X, Yin W, Wang Y, Li Y, Zhang G, Li Z, Song J, Wang X. 2020. Grapevine VlbZIP30 improves drought resistance by directly activating VvNAC17 and promoting lignin biosynthesis through the regulation of three peroxidase genes. Hortic Res **7**, 150.

Ubaidillah M, Kim KA, Kim YH, Lee IJ, Yun BW, Kim DH, Loake GJ, Kim KM. 2013. Identification of a drought-induced rice gene, OsSAP, that suppresses Bax-induced cell death in yeast. Mol Biol Rep **40**, 6113-6121.

ul Haq S, Khan A, Ali M, Khattak AM, Gai WX, Zhang HX, Wei AM, Gong ZH. 2019. Heat Shock Proteins: Dynamic Biomolecules to Counter Plant Biotic and

Abiotic Stresses. International Journal of Molecular Sciences **20**.

Wu J, Kim SG, Kang KY, Kim JG, Park SR, Gupta R, Kim YH, Wang Y, Kim ST. 2016. Overexpression of a Pathogenesis-Related Protein 10 Enhances Biotic and Abiotic Stress Tolerance in Rice. Plant Pathology Journal **32**, 552-+.

Wu JJ, Wang CA, Zheng LQ, Wang L, Chen YL, Whelan J, Shou HX. 2011. Ethylene is involved in the regulation of iron homeostasis by regulating the expression of iron-acquisition-related genes in Oryza sativa. Journal of Experimental Botany **62**, 667-674.

Xie ZL, Nolan TM, Jiang H, Yin YH. 2019. AP2/ERF Transcription Factor Regulatory Networks in Hormone and Abiotic Stress Responses in Arabidopsis. Frontiers in Plant Science **10**.

Zandalinas SI, Mittler R, Balfagon D, Arbona V, Gomez-Cadenas A. 2018. Plant adaptations to the combination of drought and high temperatures. Physiologia Plantarum **162**, 2-12.

Zhang JS, Zhang H, Srivastava AK, Pan YJ, Bai JJ, Fang JJ, Shi HZ, Zhu JK. 2018. Knockdown of Rice MicroRNA166 Confers Drought Resistance by Causing Leaf Rolling and Altering Stem Xylem Development. Plant Physiology **176**, 2082-2094.

Zhang L, Yu Z, Xu Y, Yu M, Ren Y, Zhang S, Yang G, Huang J, Yan K, Zheng C, Wu C. 2021. Regulation of the stability and ABA import activity of NRT1.2/NPF4.6 by CEPR2-mediated phosphorylation in Arabidopsis. Molecular Plant **14**, 633-646.

Zhang Y, Su JB, Duan S, Ao Y, Dai JR, Liu J, Wang P, Li YG, Liu B, Feng DR, Wang JF, Wang HB. 2011. A highly efficient rice green tissue protoplast system for transient gene expression and studying light/chloroplast-related processes. Plant Methods **7**.

Zheng LQ, Huang FL, Narsai R, Wu JJ, Giraud E, He F, Cheng LJ, Wang F,

Wu P, Whelan J, Shou HX. 2009. Physiological and Transcriptome Analysis of Iron and Phosphorus Interaction in Rice Seedlings. Plant Physiology **151**, 262-274.

Zou J, Liu CF, Liu AL, Zou D, Chen XB. 2012. Overexpression of OsHsp17.0 and OsHsp23.7 enhances drought and salt tolerance in rice. Journal of Plant Physiology **169**, 628-635.

Abstract in Korean

벼의 가뭄 저항성 증진에 관여하며 관다발 특이적 발현패턴을 보이는 전사인자 유전자 OsERF83 의 분자적 특성 규명

정세은

서울대학교 국제농업기술대학원 국제농업기술학과

지도교수 김주곤

비생물학적 스트레스는 식물의 생장과 생산성에 심각한 영향을 끼친다. 이러한 비생물학적 스트레스에 대항하기 위하여 식물은 전자인자들을 고도로 조절함으로써 저항하는 매커니즘을 진화시켜 왔다. APETALA2/ethylene-responsive factor (AP2/ERF) 전자인자는 다양한 비생물학적 스트레스에 중요한 역할을 하는 것으로 알려져 있다. 그러나 이와 관련된 분자적 매커니즘에 대한 연구는 아직도 밝혀야 할 것들이 많이 남아있다. 본 연구에서 AP2/ERF 전사인자 그룹에 속하며 가뭄에 반응하는 유전자인 *OsERF83* 의 역할에 대하여 규명하였다. OsERF83

단백질은 핵에 위치하는 것으로 관찰되었으며, 가뭄과 ABA 와 같은 다양한 비생물학적 스트레스에 의해 발현이 유도되었다. *OsERF83* 를 과발현 시킨 형질전환 식물체는 대조구인 동진벼와 비교하였을 때, 광합성 효율 측정 결과를 포함하여 가뭄저항성이 향상되었다. 또한 *OsERF83* 과발현 형질전환 식물체에서는 일반 조건에서 생장이 저해되며, 생산성이 감소하는 표현형도 보여주었다. *OsERF83* 은 식물의 모든 부위의 관다발 조직에서 특이적으로 발현되었다. 전사체 분석을 통하여 *OsERF83* 은 *OsNPF8.10*, *OsNPF8.17*, *OsLH1* 을 포함하는 수송체, *OsLAC17*, *OsLAC10*, *CAD8D* 을 포함하는 리그닌 생합성 유전자, *OsTPS33*, *OsTPS14*, *OsTPS3* 을 포함하는 테르페노이스 합성유전자들, *Oscyp71Z4*, *CYP76M10* 을 포함하는 시토크롬 P450 패밀리, 그리고 비생물학적 스트레스와 관련이 있는 유전자 *OsSAP*, *OsLEA14*, *PCC13-62* 등 여러 가뭄에 반응하는 유전자들을 조절하는 것을 밝혔다. *OsERF83* 은 또한 생물학적 스트레스와 관련이 있는 *PATHOGENESIS-RELATED PROTEIN* (*PR*) 유전자들, *WALL-ASSOCIATED KINASE* (*WAK*) 유전자들, *LYSM RECEPTOR-LIKE KINASE* (*RLK*)들을 상향조절하는 것으로 밝혀졌다. 이러한 연구를 통하여 *OsERF83* 은 비생물학적과 생물학적 스트레스 사이의 상호작용측면에서 다중역할을 담당한다는 새로운 관점을 제시하였다.

주요어: 가뭄저항성, 식물생장, *OsERF83*, ERF 전자인자, 관다발 조직, 벼, 크리스퍼 유전자 가위

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