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Corresponding Author: Woo Taek Kim

Authors: Mi Young Byun[1], Li Hua Cui[2], Hyoungseok Lee[1], Woo Taek Kim[2,*]

Institution: [1]Unit of Polar Genomics, Korea Polar Research Institute, Incheon 21990, Korea,
[2]Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Korea,

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Running Title: Roles of OsTRFL1 in plant telomeres

Telomere association of *Oryza sativa* Telomere Repeat-binding Factor Like 1 and its roles in telomere maintenance and development in rice, *Oryza sativa* L.

Mi Young Byun[1], *Li Hua Cui*[2], *Hyoungseok Lee*[1] & *Woo Taek Kim*[2,*]

[1]Unit of Polar Genomics, Korea Polar Research Institute, Incheon 21990, Korea

[2]Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Korea

*Corresponding author. Tel: +82-2-2123-2661; Fax: 82-2-312-5657; E-mail: wtkim@yonsei.ac.kr

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ABSTRACT

Telomeres are specialized nucleoprotein complexes that function to protect eukaryotic chromosomes from recombination and erosion. Several telomere binding proteins (TBPs) have been characterized in higher plants, but their detailed *in vivo* functions at the plant level are largely unknown. In this study, we identified and characterized OsTRFL1 (*Oryza sativa* Telomere Repeat-binding Factor Like 1) in rice, a monocot model crop. Although OsTRFL1 did not directly bind to telomere repeats $(TTTAGGG)_4$ *in vitro*, it was associated with telomeric sequences *in planta*. OsTRFL1 interacted with rice TBPs, such as OsTRBF1 and RTBP1, in yeast and plant cells as well as *in vitro*. Thus, it seems likely that the association of OsTRFL1 with other TBPs enables OsTRFL1 to bind to telomeres indirectly. T-DNA inserted *OsTRFL1* knock-out mutant rice plants displayed significantly longer telomeres (6–25 kb) than those (5–12 kb) in wild-type plants, indicating that OsTRFL1 is a negative factor for telomere lengthening. The reduced levels of *OsTRFL1* caused serious developmental defects in both vegetative and reproductive organs of rice plants. These results suggest that OsTRFL1 is an essential factor for the proper maintenance of telomeres and normal development of rice.

INTRODUCTION

Telomeres, which are present at the ends of linear eukaryotic chromosomes, are essential for the preservation of genome integrity (1). Telomeres are tandem arrays of G/C-rich repeats and are associated with several telomere binding proteins (TBPs), which play critical roles in the protection and stabilization of chromosomal ends (2, 3). There are two distinct types of TBPs. One type is a group of single-stranded DNA-binding proteins that bind to the 3′ overhang sequences at telomere termini and are required for chromosome capping and telomerase regulation (4). The other group of TBPs, which includes human TRF1/PIN2 and TRF2 and yeast Rap1 and Taz1, bind to duplex telomeric DNA and regulate telomere length and structure (5). In human, shelterin is a telomere-protein complex composed of six proteins, POT1, TRF1, TRF2, TIN2, TPP1, and Rap1 (6). This complex forms a defined structure that prevents telomeres from being recognized as damaged DNA (7, 8).

In plants, double-stranded telomere binding proteins (DS-TBPs) are classified according to their structural characteristics (3, 9). Three distinct classes of DS-TBPs have been identified and characterized in higher plants, all of which contain a single telobox Myb domain (10). The first class harbors a Myb domain at the N-terminal end and a linker histone H1/H5 motif in the central region (11). *Arabidopsis* AtTRB1, AtTRB2, and AtTRB3, rice OsTRBFs, and maize ZmSMH belong to this group of proteins, which specifically binds to plant double-stranded telomeric repeats $(TTTAGGG)_n$ (12-16). The second group is typified by a single Myb-like DNA-binding domain and an additional Myb-extension in the C-terminal region. Structural analysis of the telomere-binding properties of tobacco NgTRF1 and rice RTBP1 suggested that this plant-specific C-terminal Myb extension is required for the interaction of NgTRF1 and RTBP1 with telomeric sequences (17, 18). The expression level of *NgTRF1* is closely linked to cell viability and the maintenance of telomere length and

stability of tobacco BY-2 suspension cultured cells (19). Loss-of-function *rtbp1* knockout mutant allele exhibited developmental defects, accompanied by genome instability in rice (20). These results suggested that NgTRF1 and RTBP1 DS-TBPs are functional components that have crucial roles in the maintenance of genome stability in tobacco and rice plants.

The third group of DS-TBPs contains a Myb DNA-binding domain in the C-terminus, without the C-terminal extension, resulting in different binding capacities to telomeric repeats *in vitro* (21). *Arabidopsis* contains six telomere repeat binding factor-like (TRFL) proteins, which belong to this third group. A gel retardation assay showed that the *Arabidopsis* TRFLs were unable to bind to telomeric repeats *in vitro*; thus, their biochemical and physiological roles remain to be elucidated (21).

In this study, we identified and characterized OsTRFL1, a rice TRFL protein. OsTRFL1 failed to directly interact with plant telomeric repeats $(TTTAGGG)_4$ *in vitro*. However, a chromatin immuno-precipitation (ChIP) assay showed that OsTRFL1 is associated with telomeric repeats *in planta*. Yeast-two hybrid, *in vitro* pull-down, and bimolecular fluorescence complementation (BiFC) assays showed that OsTRFL1 interacted with the rice DS-TBPs OsTRBF1 and RTBP1. T-DNA inserted *ostrfl1* knockout mutant rice plants displayed severe developmental abnormalities and failed to produce functional seeds. These results suggest that OsTRFL1 functions in telomere maintenance by interacting with other DS-TBPs in rice.

RESULTS and DISCUSSION

Identification and characterization of OsTRFL1

Although *Arabidopsis* TRFL proteins were previously identified, their *in vivo* roles are

unknown (21). A database search revealed a putative *TRFL* gene in rice, which we named *Oryza sativa* Telomere Repeat-binding Factor Like 1 (*OsTRFL1*; XP_015625825.1). Total RNA was prepared from rice leaves, and full-length *OsTRFL1* cDNA was obtained by RT-PCR. The coding region of *OsTRFL1* is 1869 bp, encoding 623 amino acids (molecular mass, 69 kDa; Fig. 1A). OsTRFL1 possesses a single DNA-binding Myb domain at its C-terminal end. The Myb-extension was absent in OsTRFL1. Amino acid sequence alignment showed that the Myb domain of OsTRFL1 is highly conserved with those of *Arabidopsis* and rice DS-TBPs (Supplementary Fig. S1). As OsTRFL1 contains the conserved DNA-binding Myb motif, the binding affinity of OsTRFL1 to telomeric repeats was examined. A bacterially-expressed Myc-$(His)_6$-$OsTRFL1^{486-623}$ recombinant protein, which contains the Myb DNA-binding motif (Supplementary Fig. S2), was incubated with plant telomere repeats $(TTTAGGG)_4$, and its binding affinity was examined by a gel-retardation assay. As shown in Fig. 1B, Myc-$(His)_6$-$OsTRFL1^{486-623}$ failed to interact with the telomere repeat sequence *in vitro*. In contrast, $(His)_6$-$OsTRBF1^{1-128}$ and Myc-$(His)_6$-$RTBP1^{506-620}$ showed high binding affinities for the telomeric repeats, as reported previously (14, 18).

Next, a ChIP assay was conducted to investigate the possible *in vivo* association of OsTRFL1 with telomeric DNA. OsTRFL1-sGFP, OsTRBF1-sGFP, OsRAD51D-sGFP, and RTBP1-sGFP fusion proteins were expressed in tobacco leaf epidermal cells using an *Agrobacterium*-mediated infiltration method. The nuclear genomic DNA-protein complex was extracted and subjected to immunoprecipitation with an anti-GFP antibody. The immunoprecipitated DNA was then hybridized with a ^{32}P-labeled $(TTTAGGG)_{70}$ telomere repeat probe. The results showed that OsTRFL1 is associated with telomeric sequences *in planta* (Fig. 1C). However, the signal for OsTRFL1 was not as strong as the signals for OsTRBF1 and RTBP1, indicating that OsTRFL1 binds to telomeres indirectly. OsRAD51D was used as a negative control in the ChIP assay. As previously reported (22), OsRAD51D

showed no binding to telomere repeats. Thus, it seems likely that OsTRFL1 is not able to directly bind to telomeric repeats, but it interacts with telomeres indirectly in plant cells.

OsTRFL1 interacts with rice the DS-TBPs OsTRBF1 and RTBP1 in yeast cells and *in vitro*

Numerous TBPs associate with telomeres to maintain the proper function and structure of chromosomal ends (23-25). Although OsTRFL1 failed to bind directly to the telomeric sequences *in vitro*, it was associated with telomere repeats *in vivo* (Fig. 1). These results raised the possibility that OsTRFL1 interacts with telomeres indirectly in the presence of other TBPs. Therefore, we tested the interaction of OsTRFL1 with two rice DS-TBPs, OsTRBF1 and RTBP1 (Fig. 2A). A yeast-two hybrid assay showed that OsTRFL1 interacts with both OsTRBF1 and RTBP1 (Fig. 2B). OsTRFL1 also formed a homo-dimeric complex in yeast cells. In contrast, OsTRFL1 did not interact with OsKu70, which does not bind to telomeres (26). To investigate the direct interactions of these DS-TBPs, we conducted an *in vitro* pull-down assay. As shown in Figure 2C, OsTRFL1 formed hetero-dimeric complexes with OsTRBF1 and RTBP1 as well as homo-dimer *in vitro*.

***In vivo* interactions of OsTRFL1 with OsTRBF1 and RTBP1**

Because RTBP1 and OsTRBF1 have high affinities for telomeric sequences (Fig. 1B), these DS-TBPs are expected to be localized to the nucleus. A subcellular localization assay using tobacco infiltration system revealed that both RTBP1-sGFP and OsTRBF1-sGFP do not display uniform distributions, but instead show speckle-like localization patterns in the nucleus (Fig. 3A). OsTRFL1-sGFP also showed a similar localization pattern that is speckle-like structures inside the nuclei. In addition, mRFP-OsTRFL1 was co-localized with RTBP1-sGFP and OsTRBF1-sGFP to the nuclear speckle-like bodies (Fig. 3B).

The *in planta* interactions of OsTRFL1 with RTBP1 and OsTRBF1 were tested by BiFC assay. For this assay, OsTRFL1 was fused to the 155 N-terminal amino acids of yellow fluorescent protein (YFP^N), and OsTRBF1 and RTBP1 were fused to the 86 C-terminal amino acids of YFP (YFP^C). The fusion constructs were co-expressed in tobacco leaf cells by *Agrobacterium*-mediated infiltration. Mesophyll cells of the infiltrated leaves were visualized by fluorescence microscopy. Strong YFP fluorescent signals were detected in nuclear speckle-like structures when OsTRFL1-YFP^N + RTBP1-YFP^C and OsTRFL1-YFP^N + OsTRBF1-YFP^C fusion constructs were co-expressed, suggesting *in planta* interaction of OsTRFL1 with RTBP1 and OsTRBF1 (Fig. 3C). In addition, homo-dimeric formation of OsTRFL1 was detected as a speckle-like pattern within the nuclei. Again, there was no detectable signal when OsKu70-YFP^N and OsTRFL1-YFP^C were co-expressed, suggesting that OsKu70 and OsTRFL1 do not interact with each other *in vivo*. Taken together, the results presented in Figures 1–3 suggest that OsTRFL1 interacts with RTBP1 and OsTRBF1 and that these interactions result in the indirect association of OsTRFL1 with telomere repeats *in planta*.

Abnormal telomere lengthening and developmental defects of *ostrfl1*

RT-PCR analysis demonstrated the presence of *OsTRFL1* transcripts in most developing tissues examined, including callus, shoots, leaves, panicles, and seeds, except for roots (Fig. 4A). However, the expression of *OsTRFL1* was very low in mature stems and seeds, suggesting that OsTRFL1 may play a major role in developing organs in rice. To determine the detailed cellular functions of OsTRFL1, a loss-of-function *ostrfl1* mutant line that contained a T-DNA insertion in the fifth exon of the *OsTRFL1* gene was isolated (Fig. 4B). A homozygous allele for the T-DNA insertion was identified by genomic PCR with the LP/RP and LB/RP primer sets (Fig. 4C). Genomic Southern blot analysis indicated that the *ostrfl1*

mutant progeny contained a single T-DNA integration into the *OsTRFL1* gene (Fig. 4D). Figure 4E shows that homozygous *ostrfl1* mutant leaves contained markedly reduced, but still detectable, amounts of *OsTRFL1* transcript, while the heterozygous *ostrfl1* line contained slightly lower transcript levels (Fig. 4E). These results indicated that *ostrfl1* is not a null mutant.

To assess whether the reduced level of *OsTRFL1* affected telomere maintenance, telomere lengths in wild-type and *ostrfl1* plants were measured by the terminal restriction fragments assay (19). The telomere length of wild-type rice was 5–12 kb (Fig. 4F), which is consistent with our previous results (20). In contrast, the telomere lengths of heterozygous and homozygous *ostrfl1* mutant plants were 6–25 kb (Fig. 4F). Thus, it appears that OsTRFL1 is involved in the negative regulation of telomere lengthening in rice plants.

To explore the effects of decreased levels of *OsTRFL1* and telomere lengthening on the development of rice plants, the phenotypic properties of wild-type and *ostrfl1* plants were compared. As presented in Figure 4G, the homozygous *ostrfl1* line exhibited serious growth retardation at the vegetative stage. The mutant plants were also defective in the development of reproductive organs, resulting in sterile flowers without functional seeds. The abnormal phenotypes of the heterozygous *ostrfl1* mutant were intermediate between the wild-type and homozygous mutant plant phenotypes (Fig. 4G).

In conclusion, OsTRFL1, which harbors a single DNA-binding Myb motif in its C-terminus, is associated with plant telomeric repeats *in vivo* possibly via its interactions with the DS-TBPs RTBP1 and OsTRBF1 in rice. Suppression of *OsTRFL1* resulted in abnormal telomere lengthening and developmental anomalies in both vegetative and reproductive growth. Overall, these results indicate that OsTRFL1 plays a critical role in telomere length homeostasis and the normal development of rice plants.

MATERIALS AND METHODS

Recombinant protein expression and gel retardation assay

The Myc-$(His)_6$-OsTRFL1$^{486-623}$, $(His)_6$-OsTRBF1$^{1-128}$, and Myc-$(His)_6$-RTBP1$^{506-620}$ recombinant proteins, all of which possess the Myb DNA-binding domain (Supplementary Fig. S2), were expressed in *E. coli* BL21 (DE3) cells and purified by affinity chromatography using Ni-NTA agarose (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The gel retardation assay was performed as described by Byun et al. (22).

Yeast two-hybrid and *in vitro* pull-down assays

The yeast two-hybrid assay was performed according to the method of Byun et al. (14), with slight modifications. To investigate the interactions between OsTRFL1 and rice DS-TBPs, full-length *OsTRFL1* was ligated into the pGBK T7 vector (Clontech, Mountain View, CA, USA). The full-length coding regions of *OsTRBF1*, *RTBP1*, and *OsTRFL1* were inserted into the pGAD T7 vector. As negative controls, *OsKu70* and *T-antigen* were also inserted into the pGAD T7 vector. These constructs were co-transformed into AH109 yeast cells. The transformed yeast cells were plated onto three-minus (–Leu/–Trp/–His) medium supplemented with 10 mM 3-amino-1,2,4,-triazole and grown at 30°C for 4 days.

Bacterially expressed MBP-OsTRFL1, MBP-OsTRBF1, $(His)_6$-OsTRFL1, $(His)_6$-OsTRBF1, and $(His)_6$-RTBP1 recombinant proteins were purified by affinity chromatography using Ni-NTA agarose (Qiagen) for $(His)_6$-tagged proteins and amylose resin (New England Biolabs) for MBP-tagged proteins. *In vitro* pull-down and immunoblot analyses were performed as described by Byun et al. (26) with anti-His and anti-MBP antibodies (Applied Biological Materials, Richmond, BC, Canada).

***In vivo* ChIP assay**

The ChIP assay was carried out as described by Byun and Kim (22), with slight modifications. *Agrobacterium* containing the *35S:OsTRFL1-sGFP*, *35S:OsTRBF1-sGFP*, *35S:OsRAD51D-sGFP*, and *35S:RTBP1-sGFP* constructs were infiltrated into tobacco (*Nicotiana benthamiana* L.) leaves. After 48 h of infection, the infiltrated leaves were crosslinked with 1% formaldehyde and then quenched with 125 mM glycine. Chromatin was extracted from the leaves and incubated without (negative control) or with an anti-GFP antibody (1:1000 dilution; Applied Biological Materials). The samples were precipitated with protein G sepharose resin (GE Healthcare, Little Chalfont, United Kingdom), blotted onto a Hybond-N nylon membrane, and hybridized to ^{32}P-labeled $(TTTAGGG)_{70}$ telomeric repeats under high stringency conditions.

Subcellular localization and BiFC assays

The 3′ end of the *OsTRFL1* coding region was tagged with synthetic green fluorescent protein (*sGFP*) in-frame and inserted into the pEarleyGate (pEG) 100 binary vector. The vector was then transformed into *Agrobacterium* strain LBA4404 by electroporation. The *35S:OsTRFL1-sGFP*, *35S:RTBP1-sGFP*, *35S:OsTRBF1-sGFP*, *35S:NLS-mRFP*, and *35S:mRFP-OsTRFL1* constructs were expressed in tobacco leaves using *Agrobacterium*-mediated infiltration. After two days of infection, protoplasts were extracted from the tobacco leaves, and fluorescent protein signals were visualized by fluorescence microscopy (BX51; Olympus, Japan). NLS-mRFP was used as a nuclear marker.

For the BiFC assay, the 3′ end of the *OsTRFL1* coding region was tagged with the 155 N-terminal amino acids of YFP (YFP^N), and the 3′ ends of *OsTRBF1* and *RTBP1* were fused to the 86 C-terminal amino acids of YFP (YFP^C). The fusion constructs were co-expressed in tobacco leaves by *Agrobacterium*-mediated infiltration. Mesophyll cells of the infiltrated

leaves were visualized by fluorescence microscopy.

Molecular and phenotypic analysis of the *ostrfl1* T-DNA mutant line

Identification and analysis of the *ostrfl1* mutant were performed according to the method of Byun and Kim (22). For the terminal restriction fragments assay, leaf genomic DNA was isolated from wild-type and *ostrfl1* mutant rice plants and then digested with *Taq*I restriction enzyme. The obtained DNA fragments were separated on a 0.7% agarose gel by pulse-field gel electrophoresis using a CHEFDR III system (Bio-Rad, Hercules, CA, USA) at 6 V m^{-1} for 12 h at an angle of 120° with switching times ramped from 1 to 10 s at 14°C. The DNA gel blot was hybridized to a ^{32}P-labeled $(TTTAGGG)_{70}$ telomere repeat probe. Hybridization signals were visualized by autoradiography at -80°C.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Fig. 1. OsTRFL1 is associated with double-stranded telomeric repeats *in planta*. (A) Schematic representation of rice OsTRFL1. The C-terminal Myb DNA-binding motif is indicated. (B) Gel retardation assay. Different amounts (0, 0.5, and 1.0 μg) of bacterially-expressed Myc-$(His)_6$-$OsTRFL1^{486-623}$, $(His)_6$-$OsTRBF1^{1-128}$, and Myc-$(His)_6$-$RTBP1^{506-620}$ fusion proteins, all of which possess the Myb DNA-binding domain, were incubated with ^{32}P-labeled $(TTTAGGG)_4$ telomere repeats. After incubating for 10 min on ice, the reaction mixtures were loaded on an 8% non-denaturing polyacrylamide gel. (C) ChIP assay of OsTRFL1. The *35S:OsTRFL1-sGFP*, *35S:OsTRBF1-sGFP*, *35S:RTBP1-sGFP*, and *35S:OsRAD51D-sGFP* constructs were introduced into tobacco leaves by *Agrobacterium*-mediated infiltration. After 2 days of infiltration, nuclear genomic DNA-protein complexes were isolated and subjected to immunoprecipitation with an anti-GFP antibody. The pull-downed DNA was hybridized with a ^{32}P-labeled $(TTTAGGG)_{70}$ telomere repeat probe. IP, immunoprecipitation.

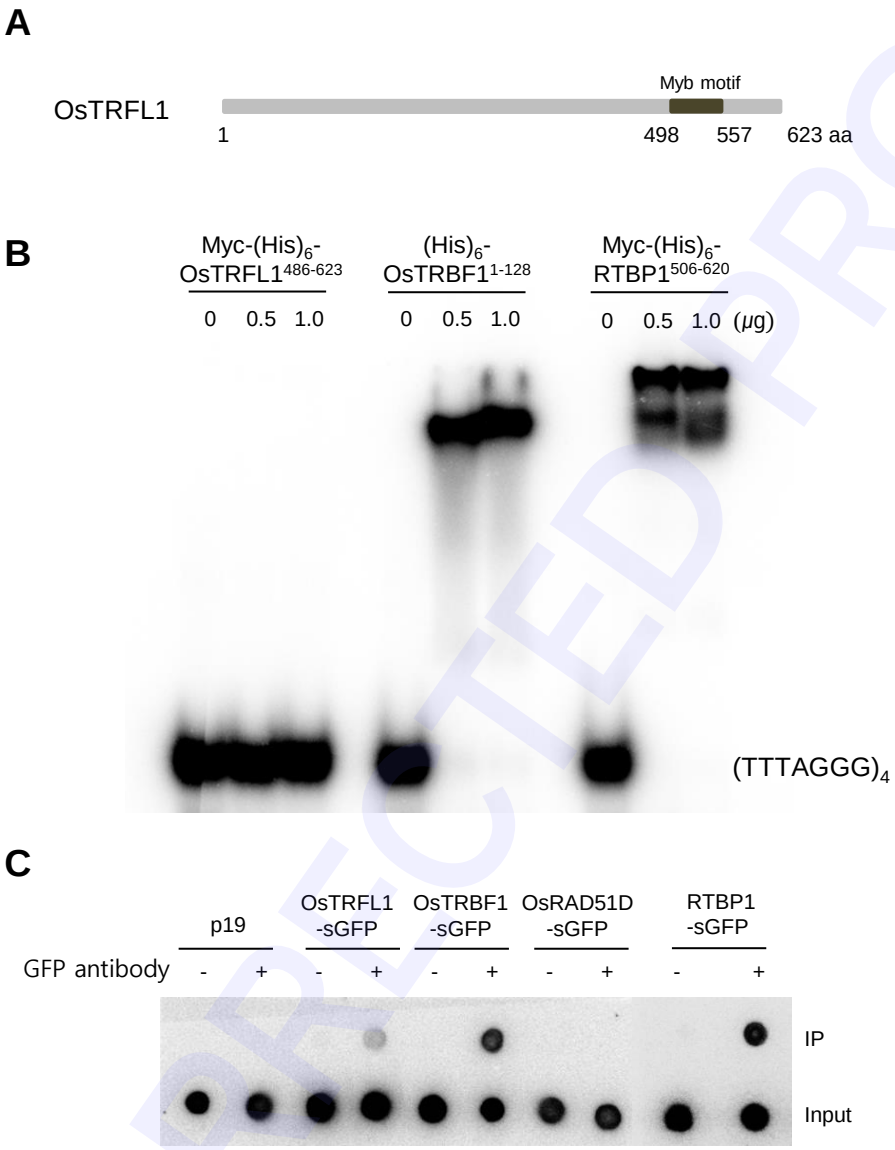
Fig. 2. OsTRFL1 interacts with rice DS-TBPs in yeast cells and *in vitro*. (A) Structural comparison of OsTRFL1, RTBP1, and OsTRBF1. The UBL domain, Myb DNA-binding motif, and SMH domain are indicated. (B) Yeast-two hybrid assay. *OsTRFL1* was cloned into the pGBK T7 vector, and *OsTRBF1*, *RTBP1*, *OsTRFL1*, *OsKu70*, and *T-antigen* were cloned separately into the pGAD T7 vector. Each combination of the indicated plasmids was co-transformed into yeast AH109 cells. To test for protein-protein interactions, yeast cells were plated onto two-minus (-Leu/-Trp; left panel) or three-minus (-Leu/-Trp/-His) medium containing 10 mM 3-AT (right panel) and grown at 30°C for 4 days. T-antigen was used as a

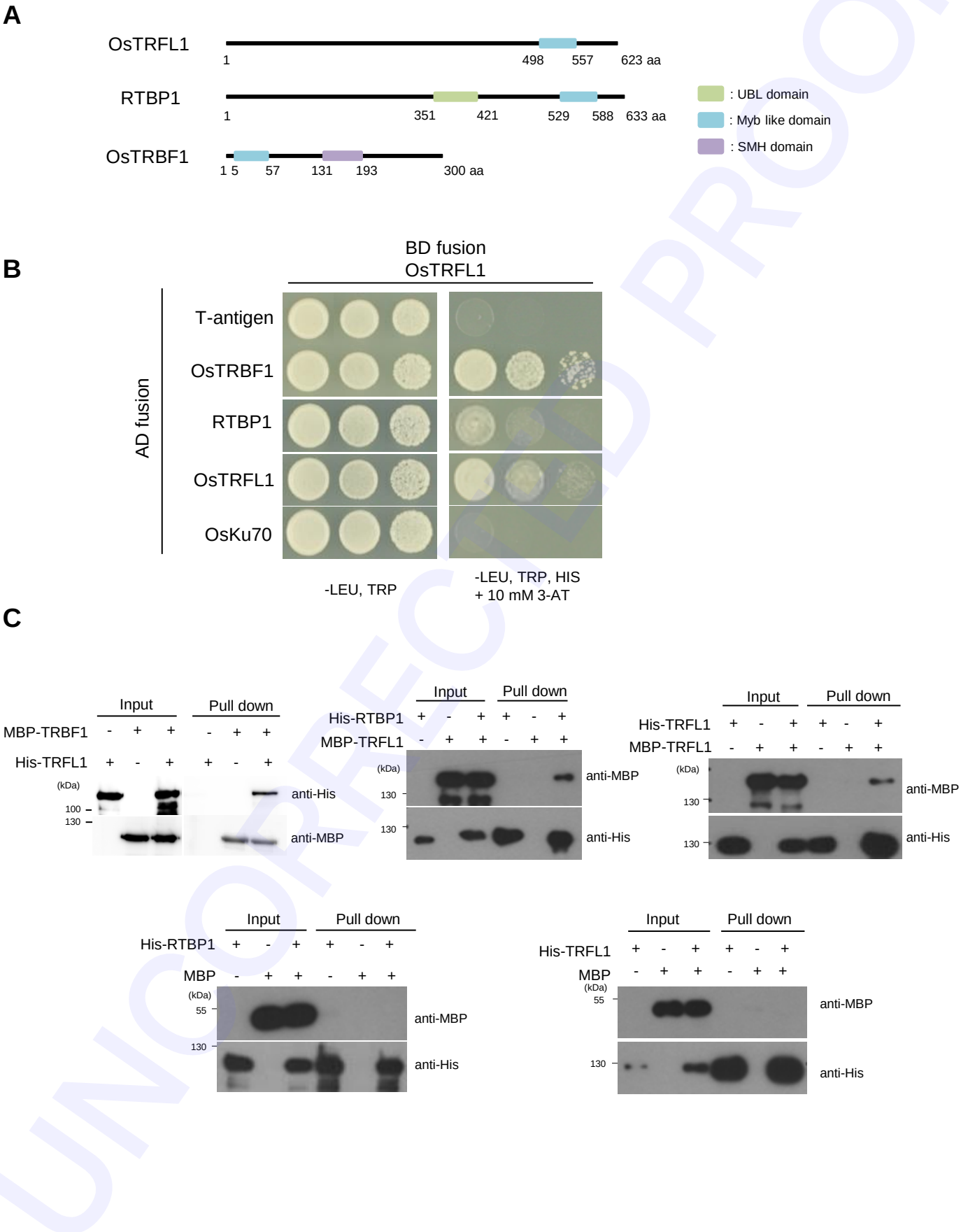
negative control. (C) *In vitro* pull-down assay. OsTRFL1, OsTRBF1, and RTBP1 DS-TBPs were expressed as MBP- or $(His)_6$-fusion proteins in *E. coli*. The purified fusion proteins were co-incubated as indicated in the presence of His-affinity matrix. The bound protein was then eluted from the resin and immunoblotted with either anti-MBP or anti-His antibody.

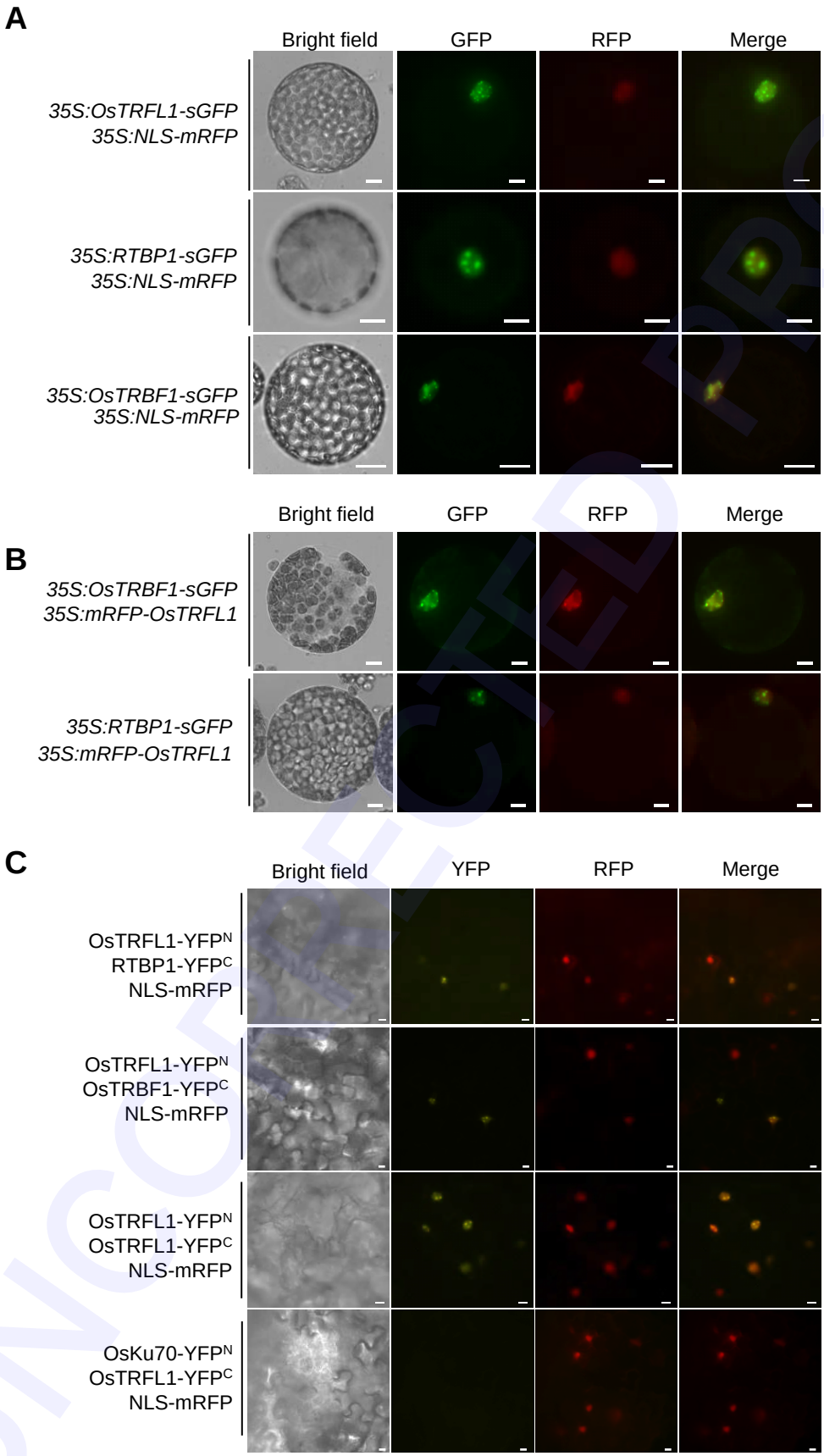
Fig. 3. OsTRFL1 interacts with rice DB-TBPs in nuclear speckle-like structures. (A) The *35S:OsTRFL1-sGFP*, *35S:RTBP1-sGFP*, and *35S:OsTRBF1-sGFP* constructs were co-expressed with 35:*NLS-mRFP* in tobacco leaves by *Agrobacterium*-mediated infiltration. After two days of infection, protoplasts were prepared from the infected leaves, and fluorescent protein signals were visualized by fluorescence microscopy. NLS-mRFP was used as a nuclear marker protein. Scale bars = 5 μm. (B) *35S:OsTRBF1-sGFP* or *35S:RTBP1-sGFP* was co-infiltrated with *35S:mRFP-OsTRFL1* into tobacco leaves. Fluorescent signals in the protoplasts were visualized by fluorescence microscopy. Scale bars = 5 μm. (C) BiFC assay. OsTRFL1-YFP^N + RTBP1-YFP^C, OsTRFL1-YFP^N + OsTRBF1-YFP^C, and OsTRFL1-YFP^N + OsTRFL1-YFP^C proteins were transiently expressed in three-week-old tobacco leaf cells. After 3 days of infiltration, the fluorescent signals were detected by fluorescence microscopy. OsKu70-YFP^N + OsTRFL1-YFP^C was included as a negative control. NLS-mRFP was used as a nuclei-localized marker protein. Scale bars = 5 μm.

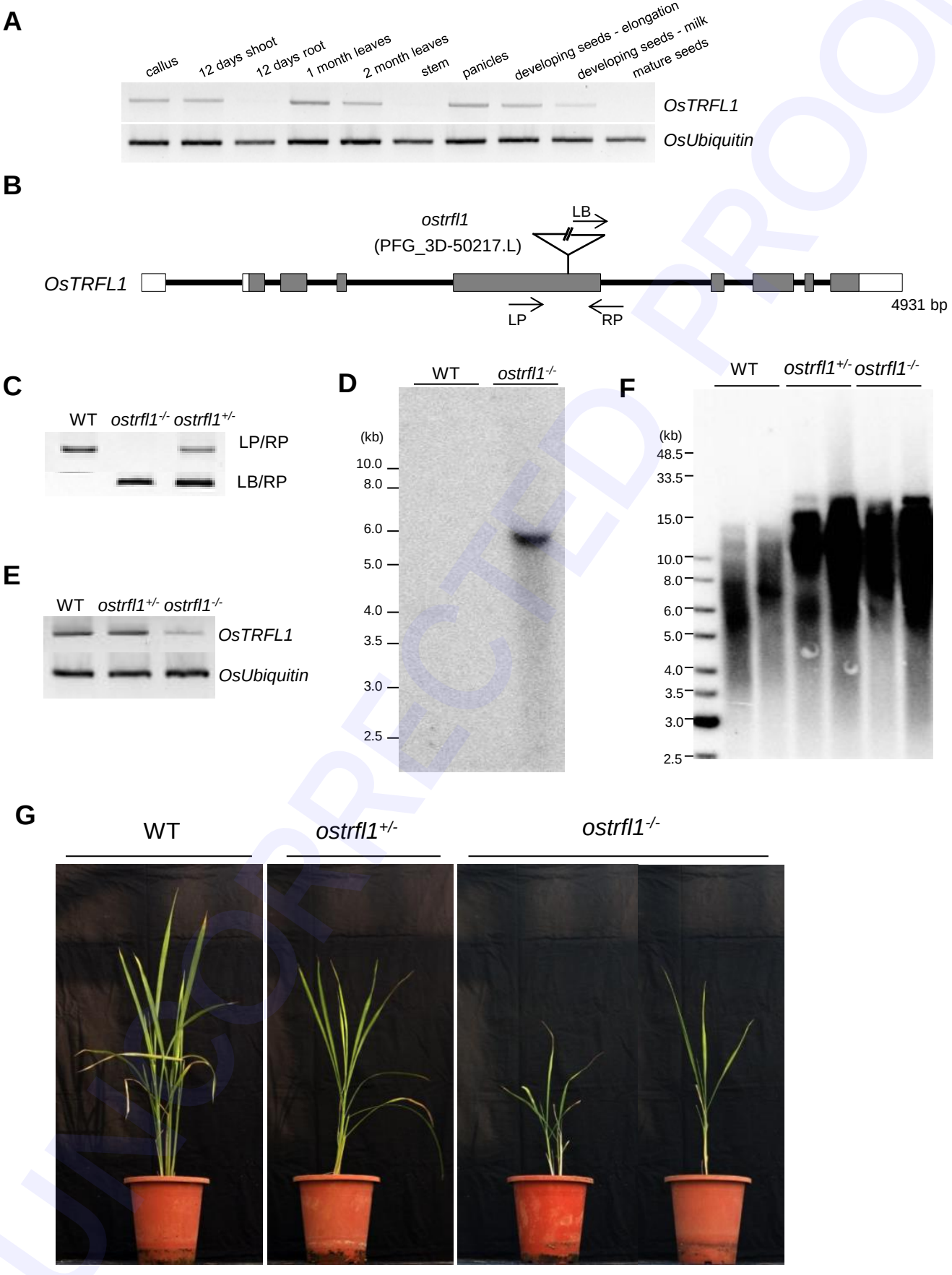
Fig. 4. Phenotypic analysis of a T-DNA-inserted *ostrfl1* mutant line. (A) RT-PCR analysis of *OsTRFL1*. Total RNA was isolated from various rice tissues as indicated and analyzed by RT-PCR. *OsUbiquitin* was included as a loading control. (B) Schematic structure of the rice *ostrfl1* mutant (line PFG_3D-50217.L) with the inserted T-DNA. The inverted triangle indicates the location of the integrated T-DNA in the fifth exon of *OsTRFL1* located on chromosome 2. The white bars indicate the 5′- and 3′-untranslated regions. The grey bars

represent the coding regions, and the black lines indicate the introns. The T-DNA-specific LB primer and gene-specific primers (LP and RP) used for genotyping PCR are indicated with arrows. (C) Genotyping PCR for *ostrfl1*. The primer sets used for genomic PCR are shown on the right side of the agarose gel. WT, wild-type; *ostrfl1*$^{-/-}$, homozygous allele; *ostrfl1*$^{+/-}$, heterozygous allele. (D) Genomic Southern blot analysis. Total leaf genomic DNA was isolated from wild-type (WT) and homozygous *ostrfl1* mutant rice plants. The isolated DNA was digested with *Eco*RV and hybridized to ^{32}P-labeled hygromycin B phosphotransferase (Hph) probes under high stringency conditions. (E) Expression levels of *OsTRFL1* in wild-type (WT) and homozygous (*ostrfl*$^{-/-}$) and heterozygous (*ostrfl*$^{+/-}$) mutant plants. (F) Telomere lengths in wild-type (WT) and *ostrfl1* mutant plants. Rice leaf genomic DNA was digested with *Taq*I restriction enzyme, subjected to pulse-field gel electrophoresis, and analyzed by Southern blotting using ^{32}P-labeled $(TTTAGGG)_{70}$ telomere repeat probes. WT, wild-type; *ostrfl1*$^{-/-}$, homozygote; *ostrfl1*$^{+/-}$, heterozygote. (G) The overall morphology of 2-month-old wild-type, heterozygous (*ostrfl*$^{+/-}$), and homozygous (*ostrfl*$^{-/-}$) mutant rice plants grown under greenhouse conditions.

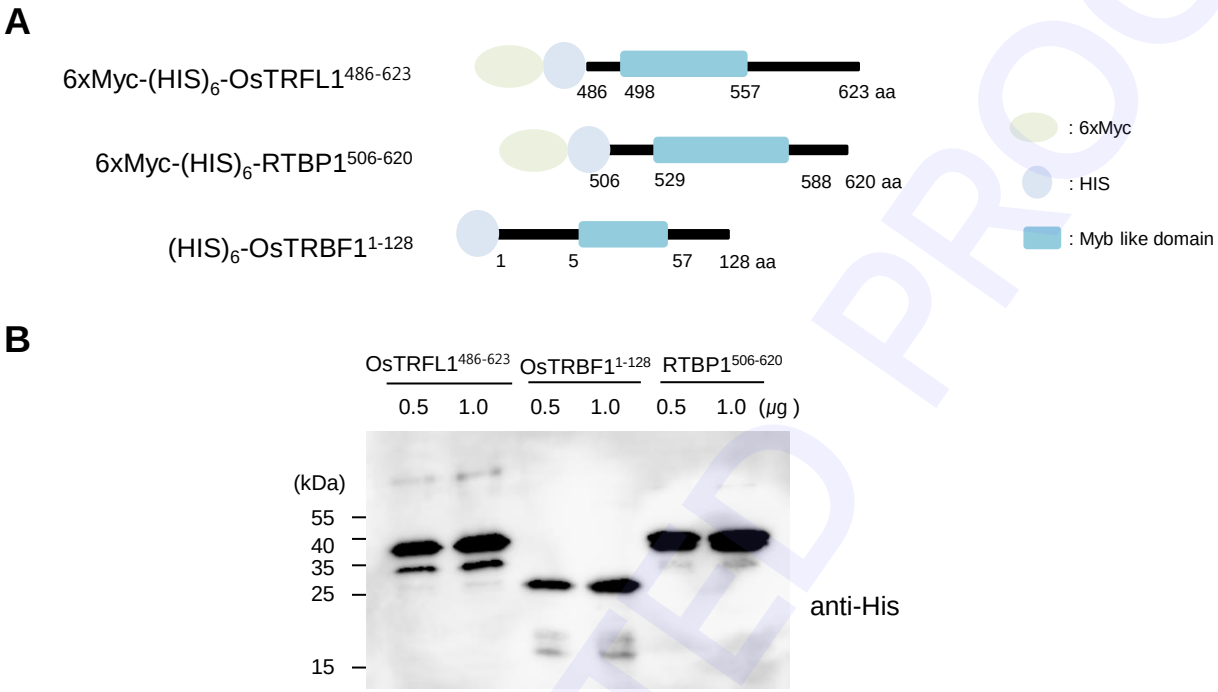








Supplementary Figure S1. Sequence alignment of OsTRFL1 and DS-TBPs of rice and *Arabidopsis*. The red lines denote the Myb DNA binding domain and the blue box indicates the Myb extension motif. Amino acid residues conserved in at least four of the six sequences are shaded. Amino acids identical in all six proteins are highlighted in black.



Supplementary Figure S2. Schematic structure of the recombinant protein used in gel retardation assays. (A) The Myb domain-containing regions of OsTRFL1, RTBP1, and OsTRBF1 were expressed in *E.coli* (DE3) cells and purified by affinity chromatography. (B) Immuno-blot analysis was performed with anti-His antibody.