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Characterization of a *Tos*17 Insertion Mutant of Rice Auxin Signal Transcription Factor Gene, *OsARF*24

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ABSTRACT

Auxin signaling plays a key role in the regulation of various growth and developmental processes in higher plants. Auxin response factors (ARFs) are transcription factors that regulate the expression of auxin-response genes. The *osarf*24-1 mutant contains a truncation of domain IV in the C-terminal dimerization domain of a rice *ARF* protein, OsARF24. This mutant showed auxin-deficient phenotypes and reduced sensitivity to auxin. However, OsARF24 protein contains an SPL-rich repression domain in its middle region and acts as a transcriptional repressor. These results imply that the C-terminal dimerization domain, especially the C-terminal half of domain IV, is essential for the proper regulation of OsARF24 function as a transcriptional repressor in rice.

Keywords: Auxin; Auxin Response Factor (ARF); Mutant; Phyllotaxis; Retrotransposon *Tos*17; Rice

1. Introduction

Auxins are endogenous phytohormones that play important roles in regulating a wide variety of cellular and developmental processes. Analyses of auxin-insensitive mutants have provided solid evidence to support the models of auxin function proposed by conventional physiological experiments and have also provided new insights and ideas about auxin. Recent molecular genetic studies, mainly on *Arabidopsis* (*Arabidopsis thaliana* L.), have made significant progress in elucidating the auxin signaling pathway. The binding of a bioactive auxin such as indole-3-acetic acid (IAA) to members of the TIR1/AFB family of F-box proteins triggers the degradation of Aux/IAA transcriptional repressors, thereby allowing auxin response factor (ARF) transcription factors, which show either activator or repressor activity, to regulate the expression of auxin-response genes [1-5].

A typical *ARF* protein contains a conserved N-terminal DNA-binding domain, a non-conserved middle region, and a conserved C-terminal dimerization domain [3,6,7]. The DNA-binding domain of *ARF* protein binds with specificity to TGTCTC auxin response elements (AuxREs) in promoters of auxin-response genes to regulate their expression [8]. The C-terminal dimerization domain, which is related in amino acid sequence to domains III and IV in Aux/IAA protein, is involved in the homo- and hetero-dimerization of *ARF* proteins and the hetero-dimerization among *ARF* and Aux/IAA proteins [4,9,10]. The middle region, between the DNA-binding domain and the C-terminal dimerization domain, functions as either an activation domain or a repression domain [3,4,11,12].

The *ARF* proteins are encoded by a multigene family in plants, and in rice (*Oryza sativa* L.), 25 *OsARF* genes have been identified [13]. Among them, 9 *OsARF* genes encode transcriptional activators and the other 16 *OsARF* genes encode transcriptional repressors [14]. However, the biological function of most *OsARF*s is poorly understood. Here, we report the identification and characterization of the *osarf*24-1 mutant, a line containing a *Tos*17 retrotransposon insertion in the putative repressor *OsARF* gene *OsARF*24. Interestingly, this mutant showed auxin-deficient phenotypes and reduced sensitivity to auxin. Because OsARF24 contains an serine proline leucine (SPL)-rich repression domain and indeed acts as a repressor, we hypothesized that the C-terminal dimerization domain, especially the C-terminal half of domain IV, which is truncated in the *osarf*24-1 mutant, is essential for the regulation of OsARF24 function as a transcriptional repressor.

2. Materials and Methods

2.1. Plant Material

Seeds of wild-type rice (*Oryza sativa* L. “Nipponbare”),

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the *osarf*24-1 mutant, and transformants (described below) were sterilized in 1% NaClO for 30 min and sown on Murashige and Skoog agar medium. Seedlings were grown in a growth chamber at 28˚C under continuous light for 2 weeks. For morphological characterization, seedlings were transplanted and grown in the paddy field at the Experimental Farm of Ishikawa Prefectural University. For gene expression analyses, seedlings were selected for uniformity of growth and adapted to hydroponic culture for 2 days before treatment. IAA treatment (20 μM) was carried out by adding IAA to the culture medium.

2.2. Gene Expression Analysis

Total RNA was extracted from whole seedlings of wild-type and mutant rice and from mature leaves of transgenic rice by using an RNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands). Single-strand cDNAs were synthesized by using the Advantage RT-for-PCR Kit (Clontech, Palo Alto, CA, USA). Quantitative RT-PCR was performed with an iCycler iQ real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The primer sequences were 5'-CAGGAAGCTGGTGT GTTGTC-3' and 5'-CTTGATCAGGCGTGGCTGTG-3' for *OsARF*23, 5'-AATGACGCCTGACATCACAC-3' and 5'-GCTTG ATAAGACTCGATGAGG-3' for *OsARF*24, 5'-ACCA AGAGCCGCTCAATGAG-3' and 5'-ATCACACGTG GGCGAACATC-3' for *OsIAA*1, 5'-GATGAACAGGC GGTCGCTGC-3' and 5'-GGCTC CGGTAGTAGCTTG TG-3' for *OsGH*3-1, and 5'-CGCC AGTTTGGTCGCT CTCGATTTCG-3' and 5'-TCAGGA GCTCCGTGCTC TTCTGGTAC-3' for *Histone H*3. These primers specifically amplified the target gene sequences. Expression levels were normalized against the values obtained for *Histone H*3, which was used as an internal reference gene. For the gene expression experiments, we performed 3 biological repeats.

2.3. Electrophoresis Mobility Shift Assay

Full-length *OsARF*24 cDNA was inserted in the sense orientation into the pET-32a expression vector (Novagen, Madison, WI, USA) to generate a thioredoxin fusion protein when expressed in BL21(DE3) *E. coli* cells (Stratagene, La Jolla, CA, USA). The recombinant protein was purified by using Talon Metal Affinity Resin (Clontech Co., Palo Alto, CA). *OsIAA*1 promoter fragments containing WT or MT AuxRE were amplified by PCR with rice genomic DNA. The primer sequences were 5'-GGTTGAAATTGGAACGATGTG-3' and 5'-G GAACTTTCATCTACTACTAC-3' for *OsIAA*1 AuxRE (WT), and 5'-TTTGGATTCTCCATTATGAGAAAATC AAAACATGGTTTTTT-3' and 5'-TTAATAAAAAAC CATGTTTTGATTTTCTCATAATGGAGAATCC-3' for generating the AuxRE mutation (MT). The amplified fragments were cloned into pBluescript II SK (Stratagene) and their identities were confirmed by sequence analysis. The PCR-amplified fragments were excised with restriction endonucleases, purified by 10% PAGE, and labeled with biotin using a Biotin 3' End DNA Labeling Kit (Pierce, Rockford, IL, USA). The electrophoresis mobility shift assay was performed by using a LightShift Chemiluminescent EMSA Kit (Pierce).

2.4. Production of Transgenic Rice

The entire *OsARF*24 coding region was inserted between the rice *Actin* promoter and the *nopaline synthase* polyadenylation signal of the hygromycin-resistant binary vector pAct-Hm2. This vector was modified from pBI-H1 [15] to contain a rice *Actin* promoter. The resulting construct was introduced into *Agrobacterium tumefaciens* strain EHA105, and *Agrobacterium*-mediated transformation of rice (*O. sativa* L. "Nipponbare") was performed as described [16]. Transgenic plants were selected on Murashige and Skoog agar medium containing 50 $mg \cdot L^{-1}$ hygromycin, and then grown in a greenhouse at 28˚C under ambient light conditions.

3. Results and Discussion

3.1. Characterization of *osarf*24-1 Mutant Rice

*osarf*24-1 is a mutant of *OsARF*24 caused by insertion of the *Tos*17 retrotransposon. *osarf*24-1 showed a reduction in plant height (the height of *osarf*24-1 was 91% that of the wild-type, $n = 10$, $P < 0.001$; **Figure 1(a)** and **Table 1**) and a reduction in the leaf angle of flag leaves from the wild-type value of 22.3˚ to 13.0˚ ($n = 10$, $P < 0.001$). This mutant also showed the narrow leaf phenotype typical of auxin-deficient or auxin-insensitive rice mutants (**Figure 1(b)**) [17,18], and the ratio of blade width to blade length of the flag leaves was reduced (0.031, versus 0.050 in wild-type, $n = 10$, $P < 0.001$). Wild-type rice leaves form in a distichous alternate phyllotactic manner, and successive leaves develop on opposite sides of the shoot apical meristem with 180˚ of divergence (**Figure 1(c)**, left plant). However, disordered phyllotaxis was found in the *osarf*24-1 mutant. In these seedlings, leaf divergence was not 180˚, but instead twisted gradually (**Figure 1(c)**, right plant). Because similar twisted leaf development was also observed in 2,4-D-treated wild-type rice and constitutively active Aux/IAA repressor transgenic rice [19], an adequate auxin signal is important for normal rice leaf development. These phenotypes of *osarf*24-1 suggest that the *osarf*24-1 mutant has some defects in auxin response.

Sequence analysis revealed that *osarf*24-1 had an insertion of *Tos*17 in exon 13 (**Figure 1(d)**). In *osarf*24-1,

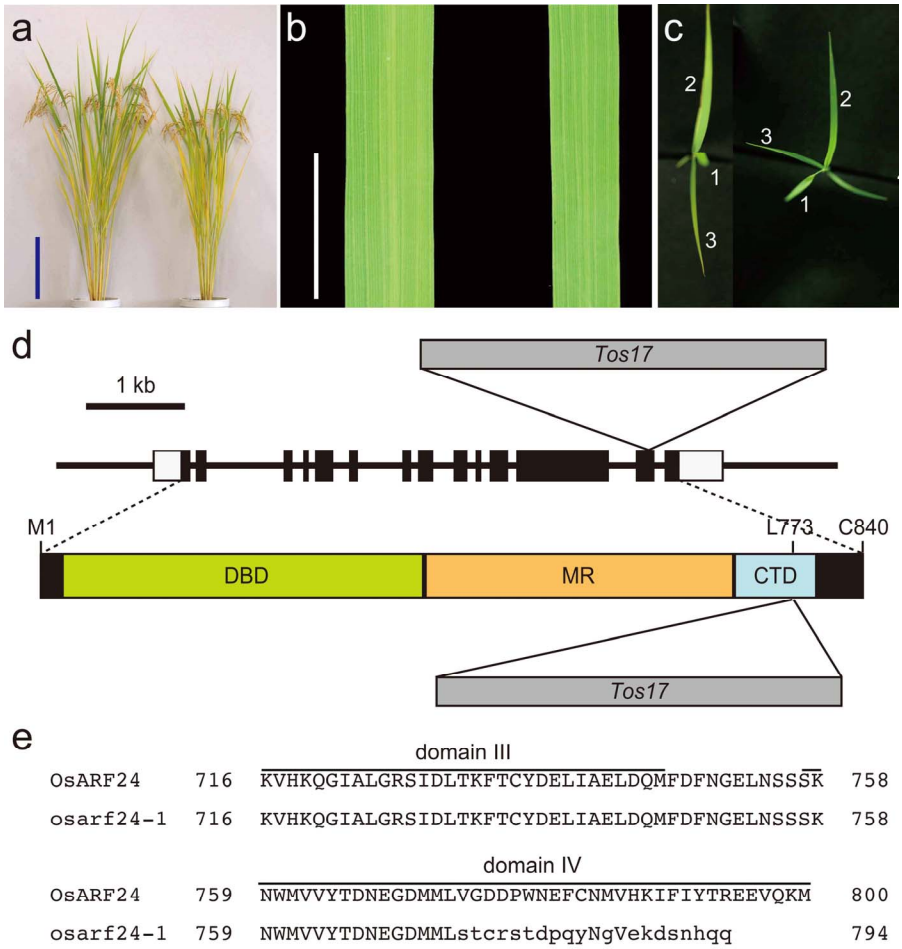

Figure 1. Phenotype of the *osarf*24-1 mutant. (a) Comparison of gross morphology between wild-type (left) and *osarf*24-1 mutant (right) rice. Bar, 20 cm. (b) Leaf morphology of wild-type (left) and *osarf*24-1 mutant (right) rice. Bar, 1 cm. (c) Phyllotaxis of wild-type (left) and *osarf*24-1 mutant (right) rice. Numbers indicate leaf number from top to bottom. (d) The upper panel indicates the genomic structure of the *OsARF*24 gene and the insertion site of *Tos*17. White and black boxes indicate non-coding and protein-coding regions, respectively. Lines between the boxes represent introns. In *osarf*24-1, *Tos*17 was inserted into exon 13 of *OsARF*24. The lower panel indicates the modular structure of the OsARF24 protein and the insertion site of *Tos*17. DBD, MR, and CTD indicate the DNA-binding domain, the middle region (which acts as repression domain), and the C-terminal dimerization domain, respectively. In *osarf*24-1, *Tos*17 was inserted into the part of the gene encoding the C-terminal dimerization of the OsARF24 protein at the 733 th leucine residue. (e) Sequence alignment of C-terminal dimerization domain of OsARF24 in wild-type (OsARF24) and *osarf*24-1 mutant (osarf24-1) rice. Lowercase letters indicate amino acid changes caused by the mutation. Bars above the sequences indicate domain III and domain IV, respectively.

Table 1. Phenotype of the *osarf*24-1 mutant.

	Plant height (cm)	Clum length (cm)	Panicle length (cm)	Number of rachis branch	Number of seeds per panicle	Number of panicle per plant
Nipponbare	106.70 ± 2.93[a]	98.14 ± 1.22	20.53 ± 1.45	11.6 ± 0.5	118.3 ± 8.1	12.1 ± 1.7
*osarf*24-1	97.13 ± 5.48	89.32 ± 3.99	18.97 ± 1.19	10.4 ± 0.8	96.8 ± 16.2	12.4 ± 2.0

[a]Each column represents mean ± s.d. of 10 independent plants.

*Tos*17 was inserted into the part of the gene encoding the C-terminal dimerization domain of the OsARF24 protein at the 773th leucine residue (**Figure 1(d)**). This insertion altered the amino acid sequence in the C-terminal dimerization domain of the OsARF24 protein; specifically, the C-terminal half of domain IV, which has a similar amino acid sequence to Aux/IAA proteins, was changed and truncated (**Figure 1(e)**). Therefore, *osarf*24-1 is considered to be a truncation mutant of the C-terminal dimerization domain in OsARF24 protein.

3.2. The *osarf*24-1 Mutant Is Less Sensitive to Auxin

The treatment of rice seedlings with IAA induced an increase in the expression of auxin-response genes *OsIAA*1 and *OsGH*3-1 [20,21]. After 10 min, IAA treatment of wild-type seedlings increased the expression of both *OsIAA*1 and *OsGH*3-1 to 1.5 times the levels in untreated seedlings (**Figure 2**). The expression levels gradually increased until 60 min after IAA treatment to 5.5 and 5.9 times those in untreated seedlings, respectively. In *osarf*24-1 seedlings, the steady-state levels of *OsIAA*1 and *OsGH*3-1 mRNA were 71% and 66%, respectively, of those in the wild-type seedlings (**Figure 2**). The kinetics of the increases in *OsIAA*1 and *OsGH*3-1 mRNA levels of *osarf*24-1 after IAA treatment were similar to those of wild-type rice. At 10 min after IAA treatment, the levels of *OsIAA*1 and *OsGH*3-1 mRNA in *osarf*24-1 seedlings were 1.0 and 0.9 times, respectively, those in the untreated wild-type control, and at 60 min after the treatment, had increased to 3.9 and 3.5 times, respectively. The levels of both *OsIAA*1 and *OsGH*3-1 mRNA in IAA-treated *osarf*24-1 were lower than those in the wild-type. However, the ratios of the relative expression levels of *OsIAA*1 and *OsGH*3-1 mRNA in IAA-treated *osarf*24-1 to untreated *osarf*24-1 were approximately 1.4 after 10 min and 5.3-5.4 after 60 min, demonstrating that *osarf*24-1 still has some ability to respond to IAA. Although multiple OsARF proteins are considered to regulate the expression of *OsIAA*1 and *OsGH*3-1, our results suggest that the *osarf*24-1 mutant is less sensitive to auxin than wild-type rice. Because the C-terminal dimerization domain in *ARF* proteins is considered to function in hetero-dimerization among *ARF* and Aux/IAA proteins [4,9,10], it is possible that the OsARF24 repressor protein with a C-terminal dimerization domain truncation in the *osarf*24-1 mutant cannot interact with Aux/IAA proteins in rice, and therefore that this mutant OsARF24 protein represses auxin-response genes constitutively.

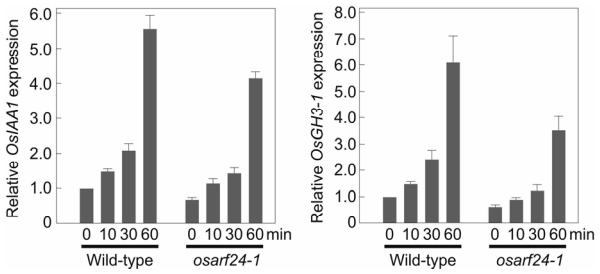

Figure 2. Effect of IAA treatment on the expression of *OsIAA*1 and *OsGH*3-1 in wild-type and *osarf*24-1 mutant rice seedlings. Expression levels were normalized against the values obtained for *Histone H*3. The value obtained from wild-type plants without IAA treatment was arbitrarily set at 1.0. Each column represents mean ± s.d. of 3 biological repeats.

3.3. Molecular Characterization of OsARF24

Among the 25 *ARF* genes in rice, the deduced amino acid sequence of OsARF24 is most closely related (71.4% identity) to OsARF23 (previously designated as OsARF1 [22]), and phylogenetic analysis grouped *OsARF*24 and OsARF23 with an *Arabidopsis* ARF, AtARF2 [13]. The predicted open reading frames (ORFs) of *OsARF*23 and *OsARF*24 encode proteins of 836 and 840 amino acids, respectively. The similarity of the deduced amino acid sequences of OsARF23 and OsARF24 is 71.4%, and the sequences are most closely related (55.1% and 52.7% similarity, respectively) to *Arabidopsis* AtARF2/HSS (At5g62010, 853 amino acids) [23]. The structures of OsARF23 and OsARF24 are similar to that of AtARF2/HSS throughout their lengths: three domains found in repressor ARFs-a DNA-binding domain, an SPL-rich repression domain, and a C-terminal dimerization domain that is related in amino acid sequence to domains III and IV in Aux/IAA proteins-are highly conserved. Because the SPL-rich repression domain is a characteristic of repressor *ARF* proteins [24], both OsARF23 and OsARF24 are considered to function as transcriptional repressors [13,14].

3.4. Expression of *OsARF*24 in Wild-Type Rice Plants

Quantitative reverse-transcription PCR analysis revealed that *OsARF*24 and *OsARF*23 were expressed at different levels in all the organs of wild-type rice that we tested, including the vegetative shoot apices, leaf sheaths, leaf blades, elongating internodes, roots, inflorescences (immature panicles), and panicles at flowering time (**Figure 3**). Both genes were expressed at the highest level in inflorescences. *OsARF*24 was also preferentially expressed in panicles at flowering time, elongating internodes, leaf sheaths, and leaf blades, and at low levels in vegetative shoot apices and roots. *OsARF*23 was also preferentially expressed in elongating internodes, leaf sheaths, leaf blades, roots, and panicles at flowering time, and at a low level in vegetative shoot apices.

Previous observations indicate that the expression levels of some *ARF* genes were not affected by IAA treatment, and those of the others were either increased or decreased [10,13]. Thus, we examined the effect of exogenously applied auxin on the expression of *OsARF*23 and *OsARF*24. IAA treatment increased the expression of both *OsARF*23 and *OsARF*24 to 1.5 times that in untreated plants within 10 min (**Figure 4**). However, the kinetics of the increases in *OsARF*23 and *OsARF*24 mRNA levels after IAA treatment were different. The

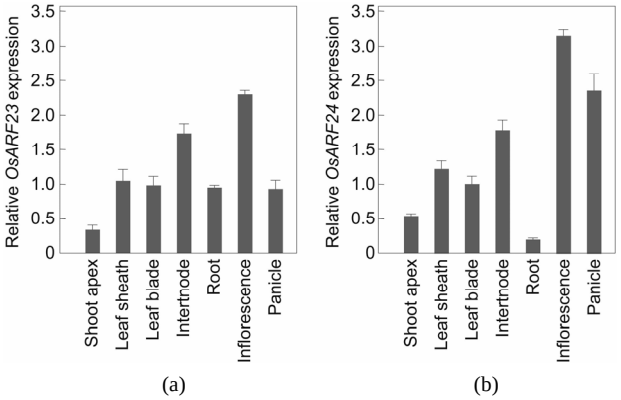

Figure 3. Relative mRNA levels of *OsARF*23 and *OsARF*24 in various organs of wild-type rice. Expression levels were normalized against the values obtained for *Histone H*3. Each column represents mean ± s.d. of 3 biological repeats.

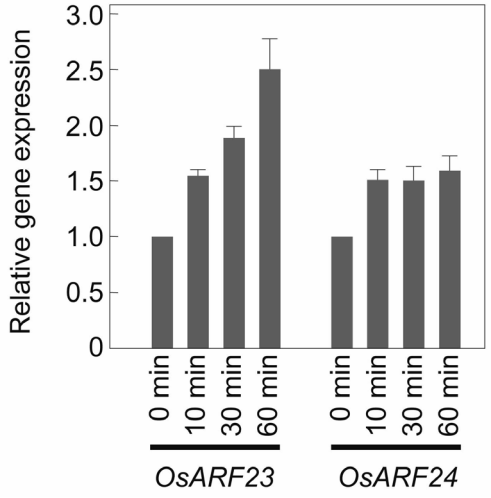

Figure 4. Effect of IAA treatment on the expression of *OsARF*23 and *OsARF*24 in wild-type rice seedlings. Expression levels were normalized against the values obtained for *Histone H*3. The values obtained without IAA treatment were arbitrarily set at 1.0. Each column represents mean ± s.d. of 3 biological repeats.

expression level of *OsARF*23 gradually increased until 60 min after IAA treatment to 2.5 times that in untreated plants, whereas the expression level of *OsARF*24 reached a maximum within 10 min and was maintained at that level until 60 min after IAA treatment. These results suggest that the expression of *OsARF*23 and *OsARF*24 is regulated by different mechanisms.

3.5. OsARF24 Binds to AuxRE in the *OsIAA*1 Promoter

The AuxRE has been identified in the promoters of some early auxin-response genes, and ARFs bind the AuxRE to regulate the transcription of these genes [24]. To examine whether the recombinant OsARF24 protein interacts with the AuxRE in the *OsIAA*1 promoter, we performed electrophoresis mobility shift assays (**Figure 5**). OsARF24 bound to a 377-bp *OsIAA*1 fragment containing the intact AuxRE (WT). The amount of retarded complex was reduced by the addition of increasing concentrations of unlabeled WT fragment as a competitor ("WT comp", in **Figure 5**). The binding of OsARF24 with the WT fragment was not affected by addition of the unlabeled *OsIAA*1 fragment containing the mutated AuxRE sequence as a competitor ("MT comp", in **Figure 5**). These results demonstrate that OsARF24 can bind to the promoter sequence of *OsIAA*1, and that this interaction depends only on the presence of an intact AuxRE sequence in the promoter fragment.

3.6. Overexpression of *OsARF*24 in Transgenic Rice

To assess the activity of the *OsARF*24 gene product *in vivo*, we fused the full-length *OsARF*24 cDNA to the rice *Actin* promoter in the sense orientation (*Act*::*OsARF*24) and introduced the construct into wild-type rice by *Agrobacterium*-mediated gene transfer (**Figure 6**). In this experiment, we selected 3 transgenic lines in which the *OsARF*24 expression level was doubled relative to wild-type (**Figure 7**). The *Act*::*OsARF*24 transformants showed a slight reduction in plant height (the average plant height of 5 plants from each of 3 different transgenic lines was 85% that of wild-type; **Figure 6(a)**). These transformants also showed the narrow leaf phenotype typical of auxin-deficient or auxin-insensitive rice mutants (**Figure 6(b)**), and the ratio of blade width to blade length of the flag leaves was reduced to 0.034 (the average of 5 flag leaves from each of 3 different transgenic lines), versus 0.050 in wild-type. Disordered phyllotaxis was also found in *Act*::*OsARF*24 transgenic seedlings (**Figure 6(c)**). These results suggest that the *Act*::*OsARF*24 transformants have some defects in auxin response, as was the case for the *osarf*24-1 mutant.

We also examined the expression of auxin-response genes in the *Act*::*OsARF*24 transformants. The steady-state levels of *OsIAA*1 and *OsGH*3-1 mRNA in the leaves of *Act*::*OsARF*24 transformants decreased to about 50% and 20%, respectively, of those in the leaves of wild-type plants (**Figure 7**), confirming that OsARF24, which has an SPL-rich middle region, acts as a transcriptional repressor of auxin signaling in rice.

3.7. The C-Terminal Dimerization Domain Is Essential for the Regulation of OsARF24 Function

As previously mentioned, OsARF24 protein, which contains the SPL-rich repression domain, is considered to be

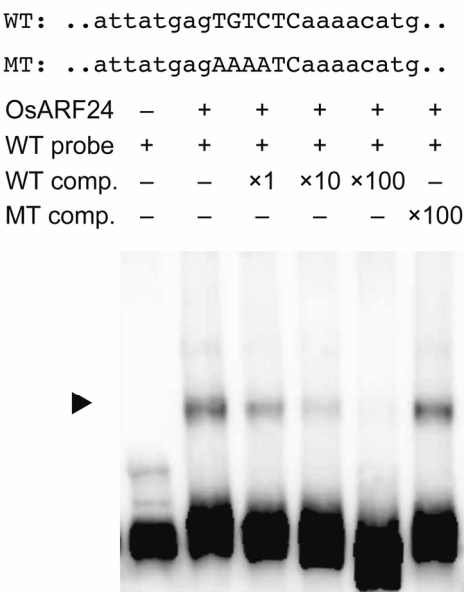

Figure 5. OsARF24 binds to the AuxRE in *OsIAA*1. Upper panel represents the core sequence of the AuxRE found in *OsIAA*1 (WT) and a mutated AuxRE sequence (MT). A labeled WT fragment was used as the probe. Binding affinity was estimated using non-labeled WT and MT fragments as competitors (comp.) at the relative concentrations indicated. OsARF24-DNA complexes are indicated by the arrowhead.

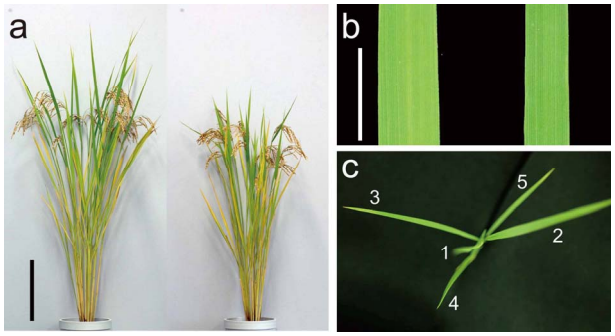

Figure 6. Phenotype of transgenic rice overexpressing the *OsARF*24 cDNA. (a) Comparison of gross morphology between wild-type (left) and *Act*::*OsARF*24 transgenic (right) rice. Bar, 20 cm. (b) Leaf morphology of wild-type (left) and *Act*::*OsARF*24 transgenic (right) rice. Bar, 1 cm. (c) Phyllotaxis of *Act*::*OsARF*24 transgenic rice. Numbers indicate leaf number from top to bottom.

an *ARF* that functions as a transcriptional repressor [13, 14]. In our experiments, transgenic rice overexpressing the *OsARF*24 cDNA showed auxin-deficient phenotypes including dwarf stature, narrow leaf, and aberrant phyllotaxis. In addition, the expression levels of auxin-response genes *OsIAA*1 and *OsGH*3-1 were decreased in these transformants. These results strongly support the hypothesis that OsARF24 acts as a repressor ARF. However, the *Tos*17 retrotransposon insertion mutant of *OsARF*24, *osarf*24-1, also showed both auxin-deficient

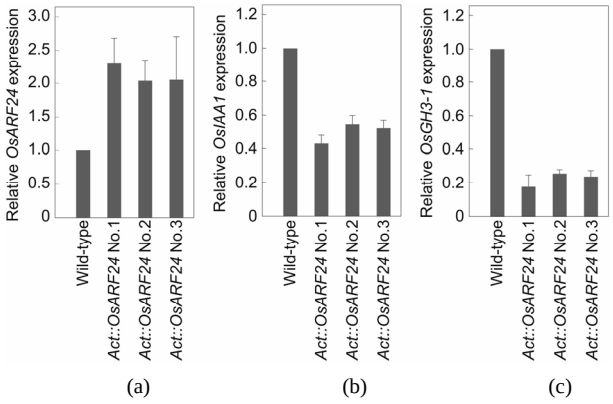

Figure 7. Relative mRNA levels of *OsARF*24, *OsIAA*1, and *OsGH*3-1 in the leaves of transgenic rice overexpressing the *OsARF*24 cDNA. Expression levels were normalized against the values obtained for *Histone H*3. The value obtained from wild-type rice leaves was arbitrarily set at 1.0. Each column represents mean ± s.d. of 3 biological repeats.

phenotypes and decreased levels and auxin responses of *OsIAA*1 and *OsGH*3-1 expression. Although another rice ARF, OsARF23, shows high amino acid sequence similarity with OsARF24, the expression of *OsARF*23 and *OsARF*24 is regulated by different mechanisms in various organs of wild-type rice plants and in response to IAA treatment. Based on these results, we consider that OsARF23 and OsARF24 do not function redundantly in rice.

Most *ARF* proteins contain a C-terminal dimerization domain related to domains III and IV in Aux/IAA proteins [4,7,10]. The C-terminal dimerization domains in both *ARF* and Aux/IAA proteins are protein-protein interaction domains that allow homo- and heterodimerization of *ARF* proteins and hetero-dimerization among *ARF* and Aux/IAA proteins [4,9,10]. Although *ARF* repressors can dimerize via their C-terminal dimerization domains, *ARF* repressor-Aux/IAA and *ARF* represssor-*ARF* activator interactions are much weaker than *ARF* activator-Aux/IAA and *ARF* activator-ARF activator interacttions, and it remains unclear whether Aux/IAAs interact with *ARF* repressors, or whether *ARF* repressors interact with *ARF* activators, to regulate target gene expression in plants [3,10,12,14].

In the *osarf*24-1 mutant, a *Tos*17 insertion in *OsARF*24 altered the amino acid sequence in the C-terminal dimerization domain of the OsARF24 protein, and the C-terminal half of domain IV was truncated. This mutation may reduce the formation of repressor *ARF* dimers; however, this does not explain why the *osarf*24-1 mutant showed reduced sensitivity to auxin because both monomers and dimers of *ARF* repressors can target and repress the expression of auxin-response genes [3]. Another hypothesis is that the C-terminal dimerization domain, especially the C-terminal half of domain IV, af-

fects the stability or the repressor activity of OsARF24. The *Arabidopsis* ARF1 repressor is targeted for proteasomal degradation via a different set of machinery than that used for Aux/IAA degradation [25]. In addition, we cannot exclude the possibility that the weak interactions found between *ARF* repressors and Aux/IAA proteins *in vitro* are enough to function *in vivo*, but that the truncated OsARF24 repressor protein in the *osarf*24-1 mutant is unable to interact with rice Aux/IAA proteins and thus constitutively represses auxin-response gene expression. We envisage that detailed analyses of the *osarf*24-1 mutant and OsARF24 protein will help to reveal the function of the C-terminal dimerization domain in *ARF* repressors.

4. Conclusion

The *osarf*24-1 mutant contains a truncation of domain IV in the C-terminal dimerization domain of OsARF24 protein. This mutant showed auxin-deficient phenotypes and reduced sensitivity to auxin. However, wild-type OsARF24 protein contains an SPL-rich repression domain and acts as a repressor ARF. These results imply that the C-terminal dimerization domain, especially the C-terminal half of domain IV, is essential for the proper regulation of OsARF24 function as a transcriptional repressor in rice.

5. Acknowledgements

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