Plant Biotechnology 33, 245–253 (2016)
DOI: 10.5511/plantbiotechnology.16.0202a

Original Paper

WUSCHEL-RELATED HOMEOBOX 2 is a transcriptional repressor involved in lateral organ formation and separation in *Arabidopsis*

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Received December 18, 2015; accepted February 2, 2016 (Edited by T. Mizoguchi)

Abstract In this study, we characterized the function of WUSCHEL-RELATED HOMEOBOX 2 (WOX2) using overexpression, CRES-T, and VP16 fusion techniques. Although the function of WOX2 has been described mainly in embryogenesis, it was unclear whether it also plays a role in the post-embryogenic developmental stage. We found that WOX2 has transcriptional repression activity and that either overexpression of *WOX2* or expression of its chimeric repressor causes severe growth defects and other morphological phenotypes by impairing plant organ formation and separation. By contrast, VP16-fused *WOX2*-expressing plants did not display such severe phenotypic defects. In addition, some of them displayed phenotypic defects such as fusion of organs and induction of undifferentiated cells in the boundary regions of organs where GUS staining was clearly observed in the pro*WOX2:GUS* transgenic plants. We suggest that WOX2 is involved in regulation of lateral organ formation and separation during the post-embryogenic development processes.

Key words: *Arabidopsis thaliana*, CRES-T, transcription factor, VP16, WOX2.

Plant growth and development are regulated through balanced cooperation among diverse transcriptional activators and repressors. To date, a variety of transcription factors have been precisely characterized in diverse developmental aspects of *Arabidopsis*. However, many transcription factors with functional redundancy remain uncharacterized because of the lack of clear phenotypes of their single loss-of-function mutants.

The WOX (WUSCHEL-RELATED HOMEOBOX) protein is a part of the homeobox transcription factor family, which are mainly involved in development by regulation of cell division and differentiation. Among the WOX-family transcription factors, WUSCHEL (WUS), WOX5, and WOX4 are well studied and are involved in the maintenance of stem cells in shoot apical, root, and vascular meristems, respectively (Chu et al. 2013; Hirakawa et al. 2010; Laux et al. 1996; Mayer et al. 1998; Tian et al. 2014). In contrast, only a few reports have described the function of WOX2, and these have focused only on its function during embryogenesis. WOX2 is involved in the determination of apical cell fate after the asymmetric division of the zygote during early embryogenesis (Haecker et al. 2004). It is also involved in cotyledon development together with other redundant *WOX* genes including *WOX8* (Breuninger et al. 2008; Lie et al. 2012; Wu et al. 2007). However, it was unknown whether WOX2 continued to play roles after embryogenic development. We suspected that the currently limited information on WOX2 function was a consequence of its gene redundancy, given that the function of WOX2 could be masked by those of other multiple factors with functional and genetic redundancy in its single loss-of-function mutant and even in the *wox2 wox8* double loss-of-function mutant which don't show defects in the post embryonic development (Wu et al. 2007).

Two simple and useful techniques have been proposed for functional analysis of transcription factors with gene redundancy. The first technique, the Chimeric Repressor Gene-Silencing Technology (CRES-T) system, in which a plant-specific repression domain named SRDX-fused transcriptional activator is expressed, is useful

Abbreviations: CRES-T, Chimeric Repressor Gene-Silencing Technology; pro35S, Cauliflower mosaic virus 35S promoter; GUS, β-glucuronidase; WOX, WUSCHEL-related homeobox; GAL4DB, GAL4 DNA Binding domain; LOF, LATERAL ORGAN FUSION; LOB1, LATERAL ORGAN BOUNDARIES 1; CUC, CUP-SHAPED COTYLEDON; dpg, day post germination.

This article can be found at http://www.jspcmb.jp/
Published online March 19, 2016

for studying the function of transcriptional activators, because the SRDX-fused transcriptional activators can dominantly repress the expression of target genes even in the presence of its redundant transcription factors (Hiratsu et al. 2003; Mitsuda et al. 2011). Thus, the CRES-T lines can exhibit similar phenotypes shown in the multiple knockout mutants of the redundant factors. The second technique, in which the transcriptional repressor fused to an activation domain called VP16 (which originated in the herpes simplex virus) is expressed (Triezenberg et al. 1988), is also considered to be useful, as several reports have shown that fusion with VP16 can convert transcriptional repressors into activators (Fujiwara et al. 2014a; b; Hanano and Goto 2011; Shih et al. 2014).

In the current study, we investigated the functions of WOX2 using overexpression, CRES-T, and VP16 fusion. Our results suggest that WOX2 is a transcriptional repressor and it also functions during post-embryogenic development processes. In addition, WOX2 function is involved in the regulation of lateral organ formation and separation.

Materials and methods

Plant materials and growth conditions

Arabidopsis Columbia-0 was used in all experiments. Wild-type and transgenic plants were grown at 22°C under a 16/8-h light/dark cycle. The seeds of transgenic plants were surface-sterilized and plated on Murashige and Skoog medium (Murashige and Skoog 1962) containing 0.8% agar, 30 mg l^{-1} hygromycin or 25 mg l^{-1} kanamycin with 250 μg l^{-1} vancomycin. After incubation in the dark at 4°C for at least 2 days, the plates were placed in a growth chamber. Transgenic seedlings on each antibiotic-medium plate were randomly selected and transplanted to the soil at 16 days post-germination (dpg) for further phenotypic observation.

GUS staining

GUS staining was performed as previously described (Chung et al. 2011). Samples were treated with 90% acetone on ice for 15 min and rinsed with water prior to staining with the following solution: 1.9 mM X-gluc, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 0.3% Triton X-100 in 100 mM sodium phosphate buffer, pH 7.2. The samples were vacuum-filtered for 15 min at room temperature and then incubated overnight at 37°C. Stained seedlings were dehydrated in a graded ethanol series (30, 50, 70, 90, and 100%).

Plasmid construction

All primers used in this study are shown in Supplemental Table 1.

The coding region of the *WOX2* (At5g59340) gene was amplified through PCR from an *Arabidopsis thaliana* cDNA using appropriate primers. The plasmids expressing *WOX2*, *WOX2-SRDX*, and *WOX2-VP16* under a 35S promoter were constructed using pro35SG (Oshima et al. 2011), pro35SSRDXG (Mitsuda et al. 2006), and a modified pro35SVP16-1 vector, respectively. The modified pro35SVP16-1 vector was made by insertion of a KpnI recognition site before the VP16 region. After confirmation of the insert sequences, each transgene cassette region was transferred into the T-DNA destination vector pBCKH (Mitsuda et al. 2006), which contains a hygromycin resistance gene, by Gateway LR reaction (Thermo Fisher Scientific, MA, USA).

To prepare a plasmid expressing a *WOX2* promoter driven *GUS* gene, 3076 bp of the region 5′ upstream from the start codon was amplified through PCR from *Arabidopsis* genomic DNA. The amplified PCR product was digested with HindIII and SmaI and inserted into the same enzyme sites of the pGUS_entry vector (Mitsuda et al. 2007). After confirmation of the insert sequences, the cassette region was transferred into the T-DNA destination vector, pBCKK (Mitsuda et al. 2006), containing a kanamycin resistance gene, by Gateway LR reaction (Thermo Fisher Scientific).

A plasmid expressing the GAL4 DNA binding domain (*GAL4DB*) driven by pro35S was prepared by insertion of the *GAL4DB* region into a p35SHSPG vector (Oshima et al. 2011). For effector plasmids, the amplified regions of *WOX2*, and *WOX2-VP16* from the plasmids named pro35S:*WOX2* and pro*35S:WOX2-VP16*, respectively, were transferred into the same enzyme sites of the pro35S:GAL4DB vector, generating pro35S:GAL4DB-WOX2 and pro35S:GAL4DB-WOX2-VP16 plasmids, respectively.

Plant transformation

The pro35S:*WOX2*, pro35S:*WOX2-SRDX*, pro35S:*WOX2-VP16* in pBCKH and pro*WOX2:GUS* in pBCKK were introduced into *Agrobacterium tumefaciens* GV3101 (Koncz and Schell 1986). The wild-type *Arabidopsis thaliana* was transformed by the floral dip method (Clough and Bent 1998).

Transient luciferase assay

Protoplasts were isolated from *Arabidopsis* rosette leaves grown for 3–4 weeks using the Tape-Arabidopsis Sandwich method (Wu et al. 2009) and prepared as previously described (Yoshida et al. 2013). Each effector plasmid was co-transfected with a reporter plasmid containing 5×*GAL4-TATA-Luciferase* (Hiratsu et al. 2004) and an internal control plasmid (phRLHSP) (Oshima et al. 2013) expressing modified *Renilla* luciferase. The reporter activity was normalized to the activity of *Renilla* luciferase and expressed as relative luciferase activity.

Results

Ectopic expression of WOX2 *and its chimeric repressor* WOX2-SRDX *disturb normal organogenesis*

To identify novel transcription factors that function

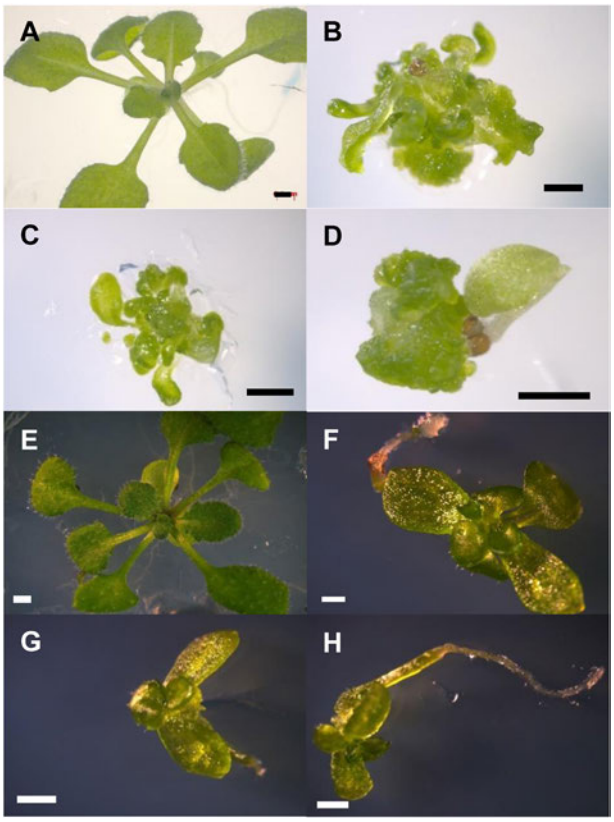

Figure 1. Ectopic expression of *WOX2* and its chimeric repressor *WOX2-SRDX* disturb normal organogenesis. (A) and (E) are the pictures of a wild-type plant which was grown with *WOX2-SRDX* and *WOX2-ox* plants, respectively, as each control. Representative phenotypes of (B–D) *WOX2-SRDX* and (F–H) *WOX2-ox* plants at 14 dpg. Bar=1 mm.

mainly in growth and development of *Arabidopsis*, we randomly chose and observed lines from an *Arabidopsis* CRES-T library, which is a pool of transgenic plants expressing SRDX-fused transcription factors driven by the 35S promoter. Finally, we selected the WOX2 CRES-T lines (*WOX2-SRDX*), because they displayed extreme morphological phenotypes. We also produced *WOX2*-overexpressing plants (*WOX2-ox*) for further analysis. Most *WOX2-SRDX* T1 transgenic lines (26 of 37) showed abnormal morphological phenotypes having incompletely differentiated and fused multiple leafy organs and were unable to maintain growth (Figure 1B–D). The remaining 11 *WOX-SRDX* lines and all *WOX2-ox* plants (82 transformants) had similar phenotypes exhibiting hyperhydric symptoms, which are among the major problems causing the failure of plant regeneration (Fauguel et al. 2008; Yu et al. 2011). Moreover, although *WOX2-ox* plants could form a pair of cotyledons and leaves which most of *WOX2-SRDX* could not, they were still abnormal and displayed severe growth defects (Figure 1F–H). These results suggest that the ectopic expression of *WOX2-SRDX* or *WOX2* exerts negative effects on normal organogenesis regardless of the transgene expression levels, probably by strongly impairing organ differentiation and separation.

WOX2 has transcriptional repression activity that is partially attenuated by VP16 fusion

SRDX fusion can convert the transcriptional activator into a repressor, thus causing the CRES-T lines and overexpressing plants without SRDX fusion to display contrasting phenotypes (Hiratsu et al. 2003; Mitsuda et al. 2011). However, SRDX fusion does not affect or enhances the effects of a native repressor in transgenic plants, leading to phenotypes similar to or stronger than those of its overexpressing plants (Ikeda and Ohme-Takagi 2009; Matsui et al. 2008; Nakata et al. 2013). As described above, given that *WOX2-SRDX* lines and *WOX2-ox* plants commonly exhibited similar phenotypes and some *WOX2-SRDX* lines displayed more severe defects in organogenesis and growth than *WOX2-ox* plants (Figure 1), we hypothesized that WOX2 acts as a transcriptional repressor. To confirm this hypothesis, we tested the transcriptional activity of WOX2 by transient luciferase assays. As shown in Figure 2, the reporter gene activity in GAL4DB-WOX2 was significantly lower than that in GAL4DB as a control, suggesting that WOX2 is a transcriptional repressor.

Fusion of transcriptional repressors to VP16 activation domain is a useful technique for characterizing transcriptional repressors, as VP16 fusion can convert the repressors into activators (Fujiwara et al. 2014a, b; Hanano and Goto 2011; Shih et al. 2014). Moreover, transgenic plants expressing a VP16-fused transcriptional repressor can display the phenotypes resembling those of multiple knockout mutants of the factor and its redundant factors caused by the upregulation of their target gene transcriptions that are repressed by those transcriptional repressors in wild-type plants (Fujiwara et al. 2014b). However, there is also a report showing the case that VP16 fusion cannot convert the transcriptional repressor into the activator (Ohta et al. 2001). Having found that WOX2 has strong transcriptional repression activity and that the *WOX2-SRDX* and *WOX2-ox* transgenic plants exhibit severe growth retardation, we used the VP16 fusion technique for further analysis. First we tested the effect of VP16 fusion on the transcriptional repression activity of WOX2 using transient luciferase assays (Figure 2). Reporter gene expression by GAL4DB-WOX2-VP16 was stronger than that by GAL4DB-WOX2, but was still lower than the basal level shown for the GAL4DB vector control (Figure 2B). On the other hand, strong activation of reporter gene expression by GAL4DB-VP16 was detected. These results suggest that the fusion with VP16 partially attenuates the repression activity of WOX2 but is not strong enough to convert the strong repression activity of WOX2 into activation activity.

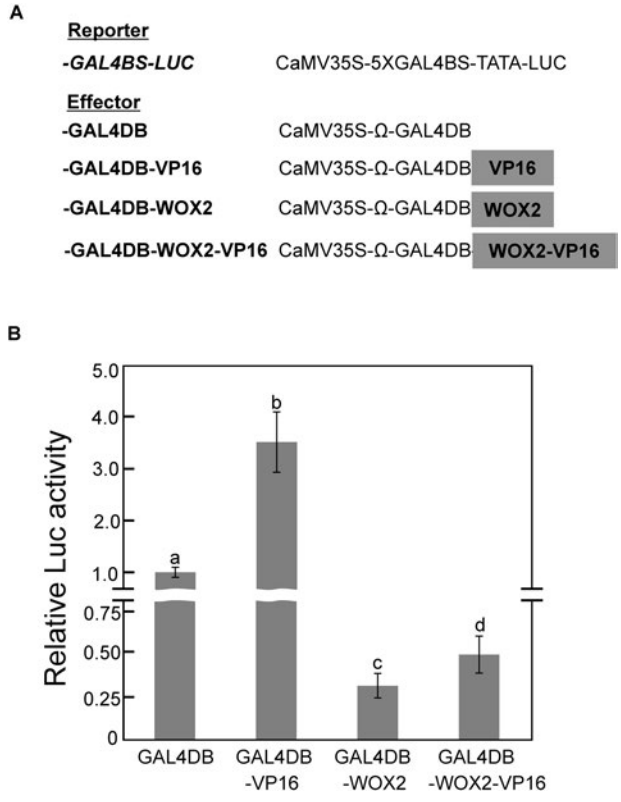

Figure 2. WOX2 has transcriptional repression activity and fusion with VP16 domain partially attenuates the transcriptional repression activity. (A) Schematic representation of the constructs used for transient luciferase assay. The reporter constructs consist of Cauliflower mosaic virus 35S promoter (CaMV35S), fifth repeated GAL4 binding sequence (5×GAL4BS) and firefly luciferase (LUC) coding sequence. (B) Transient expression assays of GAL4 DNA Binding domain (GAL4DB)-fused VP16, WOX2 and WOX2-VP16. Constructs for the assays are shown in (A). The mean of values for the vector control GAL4DB was set to 1, and relative values were calculated. Error bars represent standard deviation of the results of five or six technical replicates. Different letters indicate significant differences with Welch's *t*-test followed by Holm-Bonferroni correction for multiple comparisons.

WOX2 *promoter activity is high in cotyledons and boundary regions of organs in aerial parts during the post-embryogenic developmental stage*

Previous reports on WOX2 have mainly focused on its function during early embryogenesis (Haecker et al. 2004; Lie et al. 2012; Ueda et al. 2011). However, it has been unclear whether WOX2 also plays a role in post-embryogenic developmental stages. To investigate this possibility, we generated transgenic plants harboring a 3-kb *WOX2* promoter-*β*-glucuronidase (*GUS*) fusion construct (pro*WOX2:GUS*). In the seedlings, the GUS staining was detected in the cotyledon and early developing leaves (Figure 3A). In mature plants, GUS staining was detected mainly in junction regions, particularly vascular regions connecting inflorescence stems and pedicels (Figure 3B, C), between the stem and axillary stems or cauline leaves (Figure 3D), and between the hypocotyl and each rosette leaf (Figure 3E, F, G). These results suggest that WOX2 is involved in developmental regulation not only in the early embryogenic stage but also during post-embryogenic processes. They also suggest that WOX2 functions in the boundary regions of organs in plants.

Fusion with VP16 attenuates the negative effect of WOX2 *overexpression on plant organogenesis*

To assess the effect of VP16 fusion on WOX2 in plants, we produced transgenic plants expressing *VP16*-fused *WOX2* (*WOX2-VP16* plants) and analyzed a total of 70 independent T1 lines. The *WOX2-VP16* plants did not exhibit the severe growth defects shown in *WOX2-SRDX* lines or *WOX2-ox* plants (Figure 4B). However, most plants showed slightly irregular phyllotaxis and 24 (30%) showed distinct phenotypic alteration. For example, parts within each single inflorescence stem were fused by rolling (Figure 4C, D), and basal regions, which are considered to induce lateral stems, induced undifferentiated cells instead (Figure 4E, F). Moreover, several organs such as inflorescence stems (Figure 4H, I, K), pedicels (Figure 4M) or carpels (Figure 4N, O) were fused each other. Interestingly, the regions showing the phenotypic alterations were almost identical to those displaying clear GUS staining in the pro*WOX2:GUS*-expressing plants. Given that the results of transient luciferase assay suggested that VP16 fusion attenuated the repression activity of WOX2, it is likely that the negative effects of *WOX2* overexpression on plant growth were mitigated by VP16 fusion and that then phenotypic alterations mainly occurred in the regions where endogenous WOX2 originally functioned. Taken together, we suggest that WOX2 negatively regulates lateral organ formation and separation.

Discussion

In this study, we focused on the function of WOX2, because its CRES-T line expressing *WOX2-SRDX* displayed the most severe defects in both morphology and growth among the CRES-T lines which we observed. Previous reports have mainly focused on the function of WOX2 during the early embryogenic developmental stage, and it was unknown whether WOX2 remains functional after embryogenic development (Haecker et al. 2004; Lie et al. 2012; Ueda et al. 2011). Moreover, public databases including the Arabidopsis eFP Browser (Winter et al. 2007) also show that *WOX2* is mainly expressed in the seeds and embryos. Here we showed that WOX2 plays a role during the post-embryogenic developmental stages. We found that the activity of *WOX2* promoter is high not only in the cotyledon but also in developing leaves and the boundary regions connecting two independent organs including leaves, lateral stems, and petioles in inflorescence stems

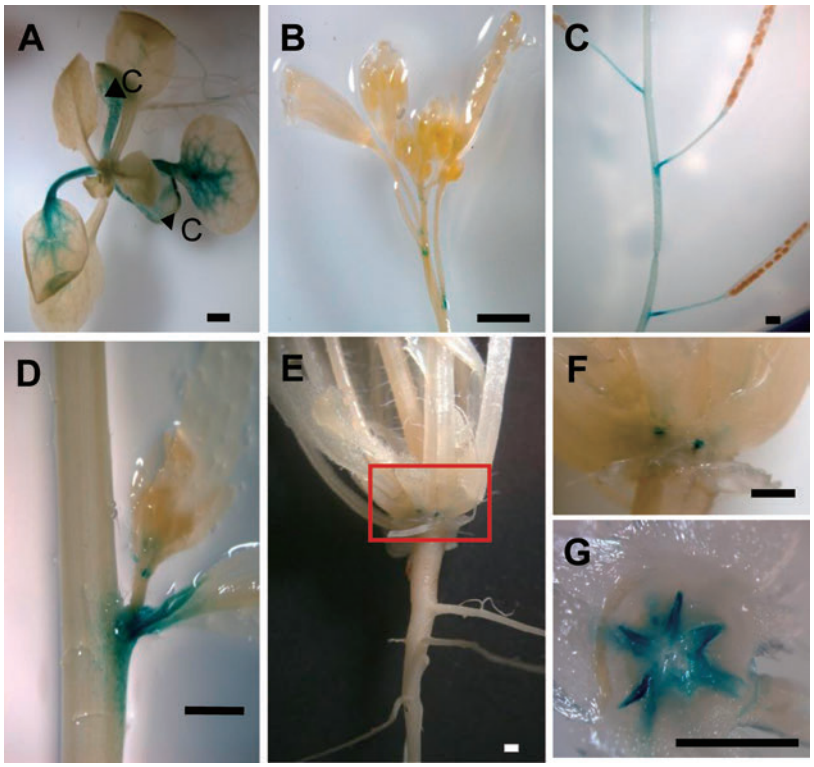

Figure 3. *WOX2* promoter activity is high in the lateral organ boundary regions. GUS staining in pro*WOX2:GUS*-expressing plants. (A) Cotyledons and 1st and 2nd leaves in seedlings at 14 dpg. Both black arrows and C indicate cotyledons. (B) Inflorescence apex. (C) Mature inflorescence with siliques. (D) Inflorescence stem inducing a cauline leaf and an axillary shoot. (E to G) The region at the crown of the rosette leaves. (F) Magnified view of the boxed region shown in (E). (G) Cross section of the boxed region shown in (E). Bar=1 mm.

(Figure 3). As shown in Figure 1, *WOX2-ox* and *WOX2-SRDX* plants commonly displayed marked phenotypic alterations including severe defects in growth and organogenesis suggesting the involvement of WOX2 in regulation of those processes. Several transgenic plants overexpressing other *WOX* family genes have been already reported. For example, overexpression of *WUS* is known to induce severe growth defects including ectopic somatic embryo formation (Gallois et al. 2004; Ikeda et al. 2009; Zuo et al. 2002). Overexpression effects of *WOX1* shown in its activation-tagging mutant is known to cause pleiotropic growth defects including low fertility and reduced organ growth although they keep growing (Zhang et al. 2011). Additionally, the transgenic plants ectopically expressing *WOX4*, which is involved in development of vascular stem cells, were reported to mainly display impaired root growth inducing abnormal cell proliferation (Hirakawa et al. 2010). Based on these reports, ectopically expressed *WOX* genes as well as *WOX2* can affect the plant growth and development, suggesting the growth defects shown in *WOX2-ox* plants may not be caused only by *WOX2*-specific effects. However, since the phenotypes of transgenic plants overexpressing each *WOX* gene differ in their types, severity and positions, most phenotypes shown in *WOX2-ox* plants probably reflect the WOX2-specific effects by its overexpression.

The common phenotypes shown in *WOX2-ox* and *WOX2-SRDX* plants led us to the hypothesis that WOX2 has transcriptional repression activity. This hypothesis was confirmed by the result of the transient expression assay (Figure 2). Given that WOX2 is known to have the conserved WUS-box motif containing the TLPLFP, which contributes to the transcriptional repression activity in WUS and other WOX proteins (Ikeda et al. 2009; Lin et al. 2013), it is possible that the transcriptional repression activity of WOX2 depends on the motif. We also found that VP16 fusion can attenuate the transcriptional repression activity of WOX2, but not sufficiently convert repression into activation activity (Figure 2). Given that the *WOX2-ox* and *WOX2-SRDX* transgenic plants could not maintain growth, it was difficult to analyze WOX2-induced phenotypic changes in further stages of development. We found that *WOX2-VP16* plants do not exhibit such severe growth defects as *WOX2-ox* and *WOX2-SRDX* plants, suggesting that VP16 fusion can mitigate the negative effect of *WOX2* overexpression on plant growth, probably by attenuation of the transcriptional repression activity of WOX2 (Figures 1, 2, 4). These results also suggest that the VP16 fusion technique may be useful for studying strong transcriptional repressors that cause severe phenotypic defects or lethality.

The *wox2 wox8* double loss-of-function mutant is

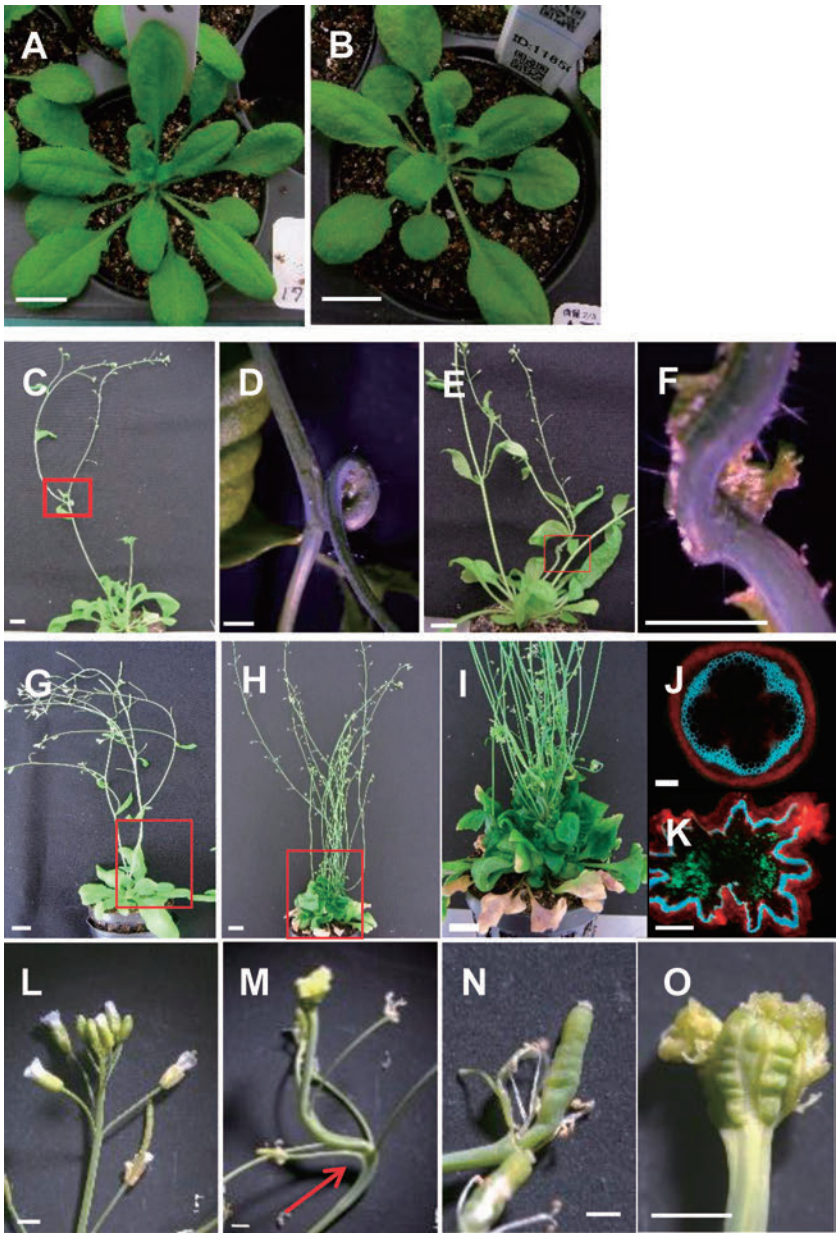

Figure 4. VP16 fusion attenuates the negative effect of *WOX2* overexpression on plant growth. The phenotypes of (A) wild-type and (B) *WOX2-VP16* plants at 30 dpg. The phenotypes of *WOX2-VP16* transgenic plants displaying phenotypic alteration (C, E, H) and wild-type (G) at 45 dpg. Bar=1 cm. (D) and (F) Magnified views of the regions in the boxes of panels (C) and (E), respectively. Bar=1 mm. (I) Magnified view of the region in the box of panel (H). Bar=1 cm. (J) and (K) Cross sections of the stems in the boxes of panels (G) and (H) under UV illumination. Bar=500 μm. Inflorescence apex of (L) wild-type and (M) *WOX2-VP16* plants. Red arrow indicates the fused pedicels in (M). (N) and (O) Fused siliques in *WOX2-VP16* plants. Bar=1 mm.

reported not to show phenotype in the post embryonic development (Wu et al. 2007). This suggests that other factors including WOX family members have redundant functions with WOX2 in the post embryonic development. However, in the multiple mutants of *WOX2* and other more than two *WOX* genes, the function of WOX2 and other WOX during later developmental stages was not shown because their loss-of-function-induced effects on early embryogenesis were too strong to persist their growing (Breuninger et al. 2008; Haecker et al. 2004). On the other hand, VP16-fused WOX2 has effects only on post-embryogenic developmental stages probably because it was expressed by 35S promoter whose activity is undetectable during early embryogenesis (Sunikumar et al. 2002). Therefore, combination of VP16 fusion and usage of the 35S promoter provided us new insights into the possible functions of WOX2 in the post-embryogenic developmental stage.

WOX2-VP16 plants exhibited morphological

alteration such as induction of fused or undifferentiated lateral organs mainly in boundary regions (Figure 4). Some of these phenotypic alterations are also shown in the 35S:*WUS-VP16* transgenic plants (Fujiwara et al. 2014a; Ikeda et al. 2009). It could be caused by the ectopic expression of genes with similar structures and fusion of VP16. It is also possible that WUS and WOX2 have redundant functions in these boundary regions. Further analyses would be required to see whether native *WUS* is also expressed in the same regions where WOX2 functions and whether WUS can redundantly function with WOX2 for the lateral organ formation and separation.

Although we confirmed that the total expression level of *WOX2* region from the transgene and the endogenous gene was much higher in the *WOX2-VP16* transgenic seedlings compared to that of WT (Supplementary Figure 1), these transgenic plants seemed not to always consistently induce strong and constant phenotypic defects. In addition, consistent correlation between the strength of phenotype and the expression of transgene was not observed (data not shown). Moreover, most of morphological alterations were mainly shown in the regions where *WOX2* promoter activity was high (Figures 3, 4), although *WOX2-VP16* was ectopically expressed by the 35S promoter. An explanation could be that some unknown specific working partners are required for the function of WOX2 and WOX2-VP16 which are located only in the regions where endogenous *WOX2* is expressed in the post embryonic stages. WOX2 and such partners may function together to appropriately repress target gene expressions for proper lateral organ formation and separation in wild-type plants. In the case of *WOX2-VP16* plants, WOX2-VP16 and the partners may also function together but have weakened repression activity to the target gene expressions which causes the defects in lateral organ formation and separation.

In conclusion, the present results suggest that WOX2 is a transcriptional repressor and plays a role in post-embryogenic as well as embryogenic development processes. Furthermore, WOX2 may be an important hidden factor in the regulation of lateral organ formation and separation. Several factors including LOF (LATERAL ORGAN FUSION) 1 and 2, LOB1 (LATERAL ORGAN BOUNDARIES 1), CUC (CUP-SHAPED COTYLEDON) 1, 2 and 3 are involved in boundary formation and act in junction regions during both embryogenic and post embryogenic developmental stages (Aida et al. 1997, 1999; Bell et al. 2012; Burian et al. 2015; Hibara et al. 2006; Lee et al. 2009; Shuai et al. 2002). All of these factors may play a redundant role with WOX2. It is also possible that other WOX family proteins also function in junction regions together with WOX2. Further analysis of WOX2 including identification of its downstream genes and its working partners will shed light on WOX2-mediated novel transcriptional networks in lateral organ formation and separation.

Acknowledgements

We thank Ms. Yoshimi Sugimoto, Ms. Miyoko Yamada, Ms. Yukie Kimura, Ms. Naomi Ujiie, and Ms. Sumiko Takahashi for their technical assistance. We are also grateful to our laboratory members for their help and encouragement. This study was partially supported by the Funding Program for Next Generation World-Leading Researchers Program (GS030) to S.F.

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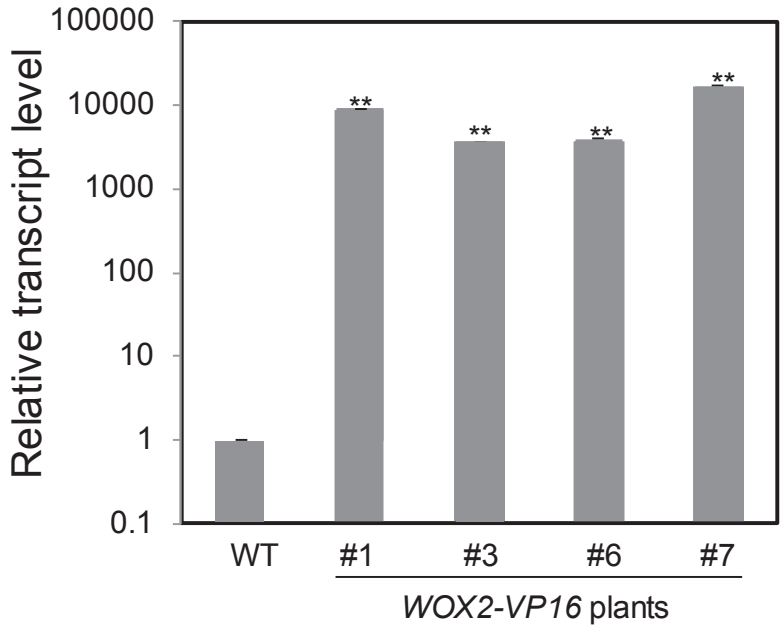
Supplemental method

RNA extraction and qRT-PCR

Total RNA was extracted from 14 day-old wild type and *WOX2-VP16* plants using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RNA (1 μg) was reverse transcribed using the PrimeScript RT reagent kit (Takara Holdings Inc, Japan) according to the manufacturer's instructions. Real time qRT-PCR was performed using the ABI7300 real-time PCR system (Thermo Fisher Scientific, MA, USA). Gene specific primers for amplifying both endogenous and transgene transcripts of *WOX2* and endogenous *PP2AA3* (At1g13320) transcripts as an internal control are shown in Supplemental Table 2.

Supplemental figure 1. Expression level of total *WOX2* transcripts in WT and *WOX2-VP16* plants

Total *WOX2* transcription level was determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) in wild-type (WT) and *WOX2-VP16* plants. Total gene expression was calculated relative to that of the reference gene *PP2AA3* (AT1g13320). All values were normalized to the WT value, which was set to 1. Data bars represent the average of three technical replicates. Error bars represent the standard deviation of the mean. Asterisks above the bars represent the significant differences of the gene expression level between wild-type and *WOX2-VP16* plants (Welch's t test, **: $p<0.01$).



Chung et al. Supplemental Figure 1

Supplemental Table 1. Primer pairs used in this study

Amplified region	The name of primer	Sequences
WOX2 for expressing *WOX2-SRDX*	AT5G59340N	5`-GATGGAAAACGAAGTAAACGCAGGAACAGC-3`
	AT5G59340C	5`-CAACCCATTACCATTACTATCGAAA-3`
WOX2 for overexpressing *WOX2* (SmaI-WOX2 (with Stop codon)-KpnI)	SmaI WOX2 cDNA F	5`-CCCCCCGGGATGGAAAACGAAGTAAACGC-3`
	KpnI WOX2 stop R	5`-GGGGGTACCTTACAACCCATTACCATTAC-3`
WOX2 for expressing *WOX2-VP16* (SmaI-WOX2 (without stop codon)-KpnI)	SmaI WOX2 cDNA F	5`-CCCCCCGGGATGGAAAACGAAGTAAACGC-3`
	KpnI WOX2 nonstop R	5`-GGGGGTACCCAACCCATTACCATTAC-3`
The promoter region of *WOX2*	HindIII pWOX2 3076 F	5`-TTTAAGCTTACGTGCAAACAGGTTACGAC-3`
	SmaI pWOX2 R	5`-CCCCCCGGGTTATTTGTGTTCAAGGATATTTTTTTT-3`
WOX2 for fusing to GAL4DB (SmaI-WOX2 with stop codon-SalI)	SmaI WOX2 cDNA F	5`-CCCCCCGGGATGGAAAACGAAGTAAACGC-3`
	SalI WOX2 CDS stop R	5`-GGGGTCGACTTACAACCCATTACCATTAC-3`
WOX2-VP16 for fusing to GAL4DB (SmaI-WOX2-VP16-SalI)	SmaI WOX2 cDNA F	5`-CCCCCCGGGTTATTTGTGTTCAAGGATATTTTTTTT-3`
	SalVP16 R	5`-AAAAATTTGTCGACCTACCCACCGTACTCGTCAATTC-3`

Supplemental Table 2. Primer pairs used for qRT-PCR in this study

Amplified region	The name of primer	Sequences
WOX2 for qRT-PCR (At5g59340)	WOX2 qRT-F	5`-CTTCAATCGCCTCCTCCACAA-3
	WOX2 qRT-R	5`-GACTGACACAACCCACGTTTGA-3`
PP2AA3 for qRT-PCR (At1g13320)	1G13320Rf2	5`-CCCCCCGGGATGGAAAACGAAGTAAACGC-3`
	1G13320Rr2	5`-GGGGGTACCTTACAACCCATTACCATTAC-3`