WOX4 Promotes Procambial Development

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Plant shoot organs arise from initial cells that are recruited from meristematic tissues. Previous studies have shown that members of the WUSCHEL-related HOMEobox (WOX) gene family function to organize various initial cell populations during plant development. The function of the WOX4 gene is previously undescribed in any plant species. Comparative analyses of WOX4 transcription and function are presented in Arabidopsis (Arabidopsis thaliana), a simple-leafed plant with collateral vasculature, and in tomato (Solanum lycopersicum), a dissected-leafed species with bicollateral venation. WOX4 is transcribed in the developing vascular bundles of root and shoot lateral organs in both Arabidopsis and tomato. RNA interference-induced down-regulation of WOX4 in Arabidopsis generated small plants whose vascular bundles accumulated undifferentiated ground tissue and exhibited severe reductions in differentiated xylem and phloem. In situ hybridization analyses of Atwox4-RNA interference plants revealed delayed and reduced expression of both the phloem developmental marker ALTERED PHLOEM1 and HOMEobox GENES, a marker of the vascular procambium. Overexpression of SlWOX4 correlated with overproliferation of xylem and phloem in transgenic tomato seedlings. The cumulative data suggest that the conserved WOX4 function is to promote differentiation and/or maintenance of the vascular procambium, the initial cells of the developing vasculature.

The plant vasculature provides mechanical support and transports water, nutrients, and signaling molecules to plant tissues. Plant vasculature is organized as a network (Esau, 1965) of bundles containing water-conducting xylem and photosynthate-conducting phloem that interconnects the major organ and tissue systems of the plant (Scarpella and Meijer, 2004; Kim et al., 2005; Sieburth and Deyholos, 2006; Turner et al., 2007; Dettmer et al., 2009). The developmental mechanisms governing the cross talk between plant morphology and vascular patterning are the subject of intensive research (Scarpella et al., 2006; for review, see Dengler and Kang, 2001; Beerling and Fleming, 2007). Vascular patterning begins during embryogenesis and is specified by the position of the procambium, meristematic vascular progenitor cells that differentiate into xylem and phloem elements in the root, hypocotyl, and cotyledons (Sieburth and Deyholos, 2006). The ordered separation of the xylem and phloem requires PXY gene function, which encodes a receptor-like kinase that controls polarized cell divisions of the procambium (Fisher and Turner, 2007). Absent from the shoot apical meristem (SAM) and the youngest leaf primordia, procambial cells arise de novo from ground cells during growth and development of lateral organ primordia (Ye, 2002; Nieminen et al., 2004; Sieburth and Deyholos, 2006).

In leaves, a subset of these morphologically indistinct and isodiametric ground cells (Foster, 1952; Pray, 1955) are selected for procambial cell fate by the polar transport of auxin, as visualized by the activation of auxin response and the accumulation of the PIN1 auxin efflux transporter protein (Sachs, 1981, 1991; Mattsson et al., 1999, 2003; Scarpella et al., 2006; Scheres and Xu, 2006; Wenzel et al., 2007). PIN1 subcellular localization indicates that auxin is directed into developing vascular tissues, yet it remains unresolved whether auxin concentration or flux in the vascular tissues predominately signals specification (Scarpella et al., 2006; Bayer et al., 2009). Although PIN1 is the earliest known marker of vascular development, PIN expression in ground cells is not a committed step toward vascular fate. Cells that fail to maintain PIN1 accumulation revert back to ground cells, whereas persistent PIN1 expression marks cells stabilized toward procambial fate (Scarpella et al., 2006; Wenzel et al., 2007). Subsequent to PIN1 accumulation, expression of the HD-ZIPIII transcription factor HOMEbox GENES (AtHB8) defines the procambial cell, and is proposed to confer the committed step toward procambial cell fate (Baima et al., 1995, 2001; Kang and Dengler, 2004; Scarpella and Meijer, 2004; Sawchuk et al., 2007). Recent reports demonstrated that AtHB8 restricts procambial cell...
specification to a narrow zone and stabilizes prepro-
cambial cell fate during disruptions in auxin transport
(Donner et al., 2009). *AtHB8* expression in the prepro-
cambium is itself controlled by the auxin response
factor MONOPTEROS (ARF5; Donner et al., 2009).
Thus, specification of vascular fate in leaf primordia
is dependent upon appropriate auxin transport, lo-
calization, and signaling, implicating auxin as a pre-
dominant patterning factor during early vascular
development.

The WUSCHEL-related HOMEBOX (WOX) gene
family is comprised of 15 Arabidopsis (*Arabidopsis
thaliana*) paralogs that perform related functions during
initiation and/or maintenance of various embryonic,
meristematic, and organ initial cells (Haecker et al.,
2004). For example, *WUS1* organizes stem cells in shoot
meristems, while WOX5 and WOXII regulate stem cell
identity in root apical meristems and crown root mer-
istemS, respectively (Laux et al., 1996; Mayer et al., 1998;
Schoof et al., 2000; Sarkar et al., 2007; Zhao et al., 2009).
Likewise, *PRESSED FLOWER1* (*PRS1/ WOX3*) and
WOX1 recruit initial cells for lateral organs, whereas
WOX2 and WOX8/ WOX9 regulate cell fate in the api-
cal and basal poles of the early proembryo (Matsumoto
and Okada, 2001; Haecker et al., 2004; Nardmann et al.,
2004; Wu et al., 2007; Breuning et al., 2008; Shimizu
et al., 2009; Vandenbussche et al., 2009). Several WOX
genes (including *WUS1, PRS1/ WOX3, WOX5,* and
*WOX8*) are shown to function non-cell autonomously,
although there is no evidence that WOX proteins can
traffic intercellularly (Mayer et al., 1998; Scanlon, 2000;
Sarkar et al., 2007; Breuning et al., 2008; Shimizu
et al., 2009). Taken together, these data suggest that
WOX function proceeds via the activation of signaling
cascades that travel beyond the sites of WOX gene
expression. In keeping with predicted WOX functions
during transcriptional regulation of target genes con-
tributing to meristem cell fate, a growing body of
evidence points toward the subfunctionalization of
WOX gene paralogs to integrate auxin and cytokinin

![Figure 1. WOX4 gene structure, phylogeny, and constructs. A, WOX4 gene structure is similar in Arabidopsis (*AtWOX4*) and tomato (*SIWOX4*). B, Phylogenetic comparisons reveal that Arabidopsis WOX4 is more similar to tomato WOX4 than to other WOX genes in Arabidopsis. Bootstrap values were calculated from 1,000 replicates using both the neighbor joining and maximum likelihood parsimony (in parentheses) methods. C, The Arabidopsis WOX4-RNAi and tomato WOX4 overexpression constructs used in this study.](image-url)
responses in a variety of stem/initial cells throughout plant development (Leibfried et al., 2005; Dai et al., 2007; Zhang et al., 2007; Breuninger et al., 2008; Ikeda et al., 2009; Nardmann et al., 2009; Su et al., 2009; Zhao et al., 2009).

No prior analyses of WOX4 function are as yet described in any plant species. Here we present expression and genetic analyses of WOX4 orthologs from Arabidopsis and tomato (Solanum lycopersicum), plant species that differ markedly in leaf complexity and vasculature anatomy. WOX4 transcripts are detected in the procambium of developing vascular bundles in both Arabidopsis and tomato. Arabidopsis plants with down-regulated WOX4 expression conferred by RNA interference (RNAi) exhibit reduced vascular development and overaccumulate undifferentiated ground tissue, whereas expression of the vascular developmental marker genes AtHB8 and ALTERED PHLOEM1 (APL1) are, respectively, reduced and delayed in young primordia, and fail to accumulate in older leaf primordia. Overexpression of WOX4 in tomato confers a hypervascularization phenotype. These comparative analyses suggest a conserved WOX4 function during development of the procambium, the meristematic initial cells of the developing vasculature.

RESULTS

Identification of SIWOX4

Degenerate PCR was utilized to amplify WOX homeobox-containing genomic DNA from the tomato cv Ailsa Craig (see “Materials and Methods”). Comparison with the Arabidopsis WOX gene sequences (Haecker et al., 2004) identified a specific genomic fragment with close similarity to AtWOX4, a WOX gene that has not been genetically characterized in any plant species. Sequences upstream and downstream from the cloned tomato gene were PCR amplified (see “Materials and Methods”) to isolate the putative full-length SIWOX4 cDNA, which is predicted to encode a protein of 242 amino acids (Fig. 1A). Phylogenetic analyses revealed that the 251-amino acid AtWOX4 protein has higher sequence similarity to SIWOX4 than to any of the other 14 WOX proteins in Arabidopsis (Fig. 1B). DNA gel-blot analyses suggested that SIWOX4 is a single copy gene in tomato (Supplemental Fig. S1), and genetic mapping anchored SIWOX4 to chromosome arm 4 L, between markers TG182 and CP57. Homologies in genic structure and sequence conservation suggest that SIWOX4 and AtWOX4 are orthologous genes (Fig. 1). The AtWOX4 and SIWOX4 cDNA clones were utilized in comparative analyses of WOX4 expression and function.

WOX4 Is Transcribed in the Developing Vasculature of Multiple Tissues

Quantitative reverse transcription (qRT)-PCR analyses revealed that AtWOX4 is expressed in multiple tissues and is especially abundant in inflorescence stems, leaves, and young flowers (Fig. 2A). Analyses of SIWOX4 showed similar expression patterns, although transcripts from the tomato ortholog also predominate in the hypocotyl (Fig. 2B). More detailed analyses of the tissue-specific accumulation of WOX4 transcripts were obtained using in situ hybridization.

Analyses of Arabidopsis and tomato seedling shoots revealed WOX4 expression in the developing vascular traces of the stem and leaf primordia (Fig. 3). WOX4 transcripts are not detected in the SAM or in the newly initiated plastochron 1 (P1) leaf, tissues that are unvascularized in both Arabidopsis and tomato (Fig. 3, A, B, and D). WOX4 expression is first detected in the differentiating vascular bundle of the P2-staged leaf primordium in both species, and is discernible until the P5-staged leaf primordium. No transcripts are detected beyond the P6 primordium in tomato (Fig. 3B) or Arabidopsis (data not shown). Higher magnification of transverse sections of the tomato P2 primordium reveals WOX4 accumulation in the procambial tissue, located abaxial to the developing xylem. Longitudinal sections of tomato seedlings con-
firmed the accumulation of SlWOX4 transcripts in vascular traces of the stem and leaf primordia, although no expression was again noted in the unvascularized SAM or the P1 primordium (Fig. 3D).

### Analysis of Reduced WOX4 Function in Arabidopsis Using RNAi

No genetic analyses of WOX4 function have been described previously, and searches of The Arabidopsis Information Resource database identified no T-DNA insertion alleles of WOX4. Therefore, an RNAi strategy was utilized to generate transgenic Arabidopsis plants with reduced WOX4 function. Constructs comprised of an inverted repeat sequence of the 225 bp 3′ untranslated region (UTR) region of AtWOX4 that is unique to AtWOX4, and driven by the 35S constitutive promoter were transformed into the Columbia ecotype, and transformant progeny were selected for resistance to the antibiotic Kanamycin (see “Materials and Methods”). As controls for any phenotypic effects due to Kanamycin-based selection of transgenic plants, control plants were transformed with the same cloning vector as RNAi lines, but contained no WOX4 inverted repeats. Two RNAi lines, designated wox4-12 and wox4-4, were identified that displayed a small plant phenotype as compared to controls (Fig. 4, A–D), although the wox4-RNAi plants were fully fertile (Fig. 4E). Both lines wox4-12 and wox4-4 each harbored a single insertion of the wox4-RNAi construct (“Materials and Methods”), and qRT-PCR analyses of WOX4 expression in wox4-RNAi and control seedlings confirmed the variably reduced WOX4 expression in wox4-RNAi plants (Fig. 4F).

Histological analyses of transverse sections through the vascular bundles of rosette leaves, mature inflorescence stems, and mature primary roots all revealed reduced accumulation of differentiated xylem and phloem vessels, as well as an overaccumulation of undifferentiated ground tissue (Fig. 5). For example, the vascular bundles of Safranin-O and Fast Green-stained wox4-RNAi leaves (Fig. 5B) contained fewer xylem vessels and fibers (red-stained cells), and fewer phloem tissues (blue/green-stained cells) than nonmutant rosette leaves (Fig. 5A). In addition, the midveins of wox4-RNAi plants accumulated an abundance of isodiametric, red-stained ground cells as compared to nonmutant plants. Likewise, the vascular bundles in mature, control inflorescence stems were much larger than wox4-RNAi bundles, and develop abundant xylem and fiber cells in adaxial regions and phloem tissue abaxially (Fig. 5C). In contrast, the adaxial
domains of wox4-RNAi mutant stem vascular bundles accumulate far fewer xylem and fiber cells, whereas the abaxial domains contain numerous red-stained ground cells (Fig. 5, D and E) that are not observed in nonmutant control stems.

The vascular bundles of mature wox4-RNAi primary roots (Fig. 5, G and H) contain fewer xylem and phloem elements and accumulate far more isodiametric, red-stained cells than are observed in the steles of nonmutant control sibling roots (Fig. 5F). Nonmutant vascular bundles of the Arabidopsis root exhibit diarch arrangement, wherein the younger protoxylem is oriented at two poles and the older differentiated metaxylem is located in the center of the vascular cylinder (Fig. 5F). In contrast the arrangement of the wox4-RNAi vascular cylinder is disorganized and chaotic, such that metaxylem is sometimes seen at the edge of the central cylinder and protoxylem is not easily identified (Fig. 5, G and H). Lastly, the radial patterning of the wox4-RNAi root is also disrupted. Specifically, the innermost endodermal and pericycle layers, located closest to the mutant vascular bundle, are poorly differentiated and are difficult to identify.

Transcripts of Vascular Patterning Genes Are Delayed and Reduced in Atwox4-RNAi Mutant Plants

To investigate the developmental status of the differentially stained cells observed in wox4-RNAi vascular bundles (Fig. 5), in situ hybridizations were performed utilizing gene-specific probes for the procambial identity marker AtHB8 and the phloem developmental marker APL (Fig. 6). As described previously, transcripts of the HD-ZIPIII gene AtHB8 accumulate throughout the developing procambium of Arabidopsis seedling shoots (Baima et al., 1995; Kang and Dengler, 2004; Scarpella and Meijer, 2004; Sawchuk et al., 2007). Initiating as punctate spots in the preprocambium of the developing midvein in young leaf primordia, AtHB8 expression later expands mediolaterally to demarcate the procambium of lateral veins in the developing primordium (Fig. 6A). In slightly older leaf (P12-staged) primordia, AtHB8 expression is observed in multiple patches corresponding to the expanded procambial tissue in these larger vascular bundles (Fig. 6C). In contrast, AtHB8 transcript accumulation is markedly reduced in young wox4-RNAi primordia (Fig. 6B), and fails to persist in the vascular bundles of older (P12-staged) mutant leaf primordia (Fig. 6D).

Transcripts of the phloem-specific developmental marker APL accumulate later in shoot development than AtHB8 (Bonke et al., 2003). First observed in the vascular bundles of P5- to P6-staged control leaf primordia (Fig. 6E) and identified in multiple patches of older (P11-staged) vascular bundles (Fig. 6G), in situ hybridization analyses failed to detect APL accumula-

Figure 4. Phenotypes and qRT-PCR analyses of Atwox4-RNAi plants. wox4-RNAi seedlings (B–D) are smaller in comparison to control, nonmutant siblings (A) that are grown in identical selective media. E, At flowering, wox4-RNAi plants (right) are shorter than controls (left), although wox4-RNAi plants are fertile. F, qRT-PCR analyses of WOX4 transcript reveal reduced accumulation in wox4-4-RNAi seedlings as compared to the transcript levels among nonmutant siblings, which are set to 100%. Values represent three biological replicates of whole seedlings normalized to AtACTIN2. Bars = 3 cm.
tion in transverse sections of wox4-RNAi seedling shoots (Fig. 6F) or in older (P11-staged) leaf primordia (Fig. 6H).

Vascular Phenotypes of WOX4 Overexpression Mutants in Tomato

No overt mutant phenotypes were observed in screens of tomato transformants harboring RNAi constructs comprised of inverted repeats of SIWOX4, perhaps due to genetic redundancy in tomato with other factors controlling vascular development. Therefore, tomato transformants expressing SIWOX4 from the cauliflower mosaic virus 35S promoter were generated (Fig. 1; “Materials and Methods”) to investigate the phenotypic effects of SIWOX4 overexpression. Eight independent lines were recovered that contained the SIWOX4 overexpression construct; three of these lines were verified as WOX4 overexpressers by qRT-PCR, and accumulated 12 to 57 times more SIWOX4 transcript as compared to wild-type VF36 (Fig. 7A).

Unlike in Arabidopsis, the arrangement of the vascular bundles in tomato is bicollateral, wherein xylem is lined with phloem on both its inner and outer faces (Fig. 8A). Interestingly, increased specification of vascular cell fates remained within the general context of the vascular column, subsuming the normal intermitten mesophyll tissue. Transverse sections from stems and leaf petioles revealed the overaccumulation of phloem and xylem conductive elements in SIWOX4 overexpressing plants, which formed in dense clusters throughout the stele as opposed to the more sporadic and separated arrangement of elements seen in wild-type plants (Fig. 8). Although more numerous, many of the xylem and phloem conductive elements are smaller in plants overexpressing SIWOX4 compared with those
Figure 6. Transcript accumulation of the vascular developmental genes *AtHB8* and *APL* is altered in *Atwox4-RNAi* shoots. Accumulation of *AtHB8* (blue-black staining in A–D) and *APL* (blue-black staining in E–H) is more abundant in control shoots (A and E) and leaf midveins (C and G) than in *wox4-RNAi* shoots (B and F) and leaf midveins (D and H). *AtHB8* accumulates in the preprocambium and procambium of nonmutants young leaf primordia but not in the SAM (A). C, Higher magnification of the vascular bundle of a control P12 primordium reveals *AtHB8* accumulation in the procambium. *AtHB8* also accumulates in young primordia of *wox4-RNAi* plants, although staining is less conspicuous (B). *AtHB8* accumulation is not maintained in older wox4-RNAi leaf primordia; very little staining is observed the smaller vascular bundle of the mutant P12 primordium (D). *APL* accumulates in developing phloem of older nonmutant leaf primordia (E and G); no *APL* staining is observed in wox4-RNAi vascular bundles (F and H). Bars = 50 μm. Samples shown in B, D, F, and H were all obtained from the RNAi line *wox4-4*.

in wild-type VF36. In addition to these vascular phenotypes, the size, shape, and alignment of nonvascular cells (i.e., the epidermis, parenchyma, and cortex cells) was distorted and overall plant growth was severely inhibited by *SlWOX4* overexpression (Fig. 8, B and D).

Finally, we investigated whether *SlWOX4* overexpression is correlated with abnormal expression of *SlREV*, a tomato ortholog of an Arabidopsis HD-ZIPIII gene implicated during vascular patterning (Zhong and Ye, 1999). In all three independent 35S::*SlWOX4* transformants analyzed, *SlREV* expression was up-regulated from 1.4 to 4.1 times as compared to wild type (Fig. 7B).

DISCUSSION

A Conserved WOX4 Function in the Vascular Procambium

Analyses of *WOX4* transcript accumulation in Arabidopsis and tomato seedlings reveal a conserved pattern of expression in developing vascular tissues that is consistent with a function within developing vascular bundles (Figs. 2 and 3). Arabidopsis seedlings in which *WOX4* transcript accumulation is down-regulated by RNAi exhibit a pronounced reduction in differentiated xylem and phloem vessels that is correlated with an overaccumulation of undifferentiated ground tissue in the vascular bundles of the leaf, mature stem, and mature root (Fig. 4). Moreover, transcripts of the phloem developmental marker *APL* are not detectable by in situ hybridization of *wox4-RNAi* shoots, and accumulation of the class III HD-ZIP gene *AtHB8*, a developmental marker of procambial cell fate, is also disrupted in wox4-RNAi plants (Fig. 6). Specifically, although in situ hybridization reveals that the developmental timing of initial *AtHB8* transcript accumulation in the young wox4-RNAi leaf primordia is equivalent to that in nonmutant siblings, transcript levels are markedly reduced in young primordia (Fig. 6, A and B). Significantly, no *AtHB8* transcripts are detected in older wox4 primordia.

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Arabidopsis roots exhibit radial patterning of concentric tissue layers surrounding the vascular bundle, as well as bilateral symmetry in the diarch arrangement of tissues within the stele (Dolan et al., 1993; Fig. 5F). In addition to defective patterning of vascular bundle, wox4-RNAi roots also exhibit irregularities in the endodermis and adjacent pericycle that suggest a role for WOX4 during radial patterning of the root (Fig. 5, G and H). A single layer of cells comprising the outermost layer of the stele, the pericycle has been recently shown to contain two distinct cell types whose development is controlled by the same genetic pathways that regulate patterning of diarch vasculature within the root (Parizot et al., 2008). In light of these data implicating the coordinated differentiation of the pericycle and the vasculature, we speculate that the irregular patterning of the pericycle, and perhaps the endodermis as well, observed in wox4-RNAi roots may result from knockdown of WOX4 function within the vascular procambium.

An Ancestral WOX Function in Meristematic Initial Cells

The genetic and transcriptional analyses presented herein suggest that WOX4 function is localized to the procambium, the meristematic initial cells of the developing vasculature. These findings correlate with previous analyses of paralogous WOX family gene functions, which demonstrated the subfunctionalization of WOX gene activity among the wide variety of meristematic cell populations that are interspersed throughout the developing plant (Haeker et al., 2004). Our data lend additional support for a proposed ancestral WOX gene function during the regulation of stem cell fate in plants (Haeker et al., 2004; Breuninger et al., 2008; Deveaux et al., 2008; Nardmann et al., 2009; Shimizu et al., 2009).

MATERIALS AND METHODS

Plant Growth Conditions

Tomato (Solanum lycopersicum) plants were grown in a greenhouse at 25°C ± 2°C under natural daylight. Arabidopsis (Arabidopsis thaliana) plants were sown in solid (0.7% agar) medium containing one-half-strength Murashige and Skoog basal medium (Sigma-Aldrich), 1% Suc (pH 5.7), and 50 mg/L Kanamycin, stratified at 4°C for 3 d, and grown in a laboratory at 23°C under continuous illumination. Otherwise, Arabidopsis plants were cultivated in RediEarth medium in the greenhouse of the Plant Biology Department, University of Georgia.

Genomic DNA Cloning

Genomic DNA was isolated from tomato leaves or Arabidopsis whole seedlings as described (Dellaporta et al., 1983), or from young leaves with Extract-N-Amp plant PCR kits (Sigma-Aldrich). A degenerate primer was designed from the most conserved C terminus of the homeodomain region of WOX proteins in Arabidopsis and from WUS proteins from different species including tomato, petunia (Petunia hybrida), and Antirrhinum (see Supplemental Table S1). Degenerate Genome Walker (CLONTECH) was then employed to clone the 5’ end of the SIWOX4 gene from tomato. The promoters of SIWOX4 and AtWOX4 were PCR amplified from, respectively, diluted Ailsa Craig or Columbia genomic DNA preparations. All PCR products were

(P12), whereas control primordia exhibit widespread AtHB8 accumulation throughout the procambium of the P12 vascular bundle (Fig. 6, C and D). Taken together, these genetic and expression data strongly suggest that WOX4 functions during differentiation and/or maintenance of procambial cell fate in Arabidopsis.

Likewise, the hypervascularized phenotypes and up-regulated expression of the vascular developmental gene SIREV that is observed in SIWOX4 overexpression lines suggest that the functions of SIWOX4 and AtWOX4 during procambial development within the disparately patterned vascular bundles of tomato and Arabidopsis are also conserved, despite their divergence from a common ancestor approximately 150 million years ago (Van der Hoeven et al., 2002). Interestingly, the increased development of xylem and phloem conductive elements induced by SIWOX4 overexpression was restricted to the vascular column, suggesting that SIWOX4 requires additional factors that are present within the vascular bundle to promote procambial fate specification.

Figure 7. qRT-PCR analyses of SIWOX4 (A) and SIREV (B) transcript accumulation in 35S::SIWOX4 overexpression plants. Three independent SIWOX4 overexpression lines (LT2-2, LT2-9, and LT2-18) are presented; transcript levels are shown relative to that detected in wild-type VF36 samples, which are set to 100%. All samples were assayed in three biological replicates and normalized to expression of SIACTIN as described in “Materials and Methods.”
Figure 8. Vasculature phenotypes of 35S::SlWOX4 overexpression plants. Transverse sections of leaf petiole (A and B) and higher magnification images of stem sections (C and D) of wild-type VF36 (A and C) and WOX4 overexpressing tomato plants (B and D) reveal increased accumulation of xylem and phloem conductive elements in WOX4 overexpressing plants. Note that although the xylem elements in the SlWOX4 overexpressing plants (B and D) are more numerous and clustered together than in wild type (A and C), many of the overly abundant xylem elements are of smaller diameter than wild-type elements. Note that phloem accumulates on both the inner and outer sides of the xylem elements in the bicollateral vasculature of tomato. Bars = 50 µm.

Table S1. The following materials are available in the online version of this article. Supplemental Figure S1. DNA gel-blot hybridization of SlWOX4. Supplemental Table S1. Summary of oligonucleotide primers used in this work.
LITERATURE CITED


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