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Large-scale phylogenomic analysis suggests three ancient superclades of the WUSCHEL-RELATED HOMEOBOX transcription factor family in plants

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Abstract

The adaptation of plants to land required multiple morphological innovations. Among these include a variety of lateral organs that are initiated from apical meristems, in which the mantainance of undifferentiated stem cells is regulated by the homeodomain WUSCHEL-RELATED (WOX) transcription factors. Expansion of the *WOX* gene family has been associated with whole genome duplication (WGD) events and postulated to have been pivotal to the evolution of morphological complexity in land plants. Previous studies have classified the *WOX* gene family into three superclades (e.g., the ancient clade, the intermediate clade, and the modern clade). In order to improve our understanding of the evolution of the *WOX* gene family, we surveyed the *WOX* gene sequences from 38 genomes and 440 transcriptomes spanning the Viridiplantae and Rhodophyta. The WOX phylogeny inferred from 1039 WOX proteins drawn from 267 species with improved support along the backbone of the phylogeny suggests that the plant-specific WOX family contains three ancient superclades, which we term Type 1 (T1WOX, the WOX10/13/14 clade), Type 2 (T2WOX, the WOX8/9 and WOX11/12 clades), and Type 3 (T3WOX, the WUS, WOX1/6, WOX2, WOX3, WOX4 and WOX5/7 clades). Divergence of the T1WOX and T2WOX superclades may predate the diversification of vascular plants. Synteny analysis suggests contribution of WGD to expansion of the *WOX* family. Promoter analysis finds that the capacity of the *WOX* genes to be regulated by the auxin and cytokinin signaling pathways may be deeply conserved in the Viridiplantae. This study improves our phylogenetic context for elucidating functional evolution of the *WOX* gene family, which has likely contributed to the morphological complexity of land plants.

Introduction

The radiation of plants in their quest for land was accompanied by morphological innovations, such as 3D growth, roots, leaves, and flowers [1–4]. These morphological novelties are initiated from apical or axillary meristems that contain undifferentiated stem cells [5]. Meristem development is controlled by the WUSCHEL-RELATED HOMEMOBOX (WOX) transcription factors. The WOX proteins share a DNA-biding homeodomain (HD) of 60–66 amino acids [6, 7], while other regions of the *WOX* coding regions are highly divergent in sequence. In vascular plants, the maintenance of the stem cell niche in a shoot apical meristem (SAM) is regulated by the *WUSCHEL* (*WUS*) gene of the *WOX* family and its partners of the WUS-CLAVATA (WUS-CLV) signaling pathway [8–15]. *WUS* also promotes stem cell proliferation in floral meristems, and helps activate the Type 2 MADS-box gene *AGAMOUS* (*AG*), which specifies reproductive floral organ identity and determinate growth of floral meristems, in collaboration with another transcription factor, *LEAFY* (*LFY*) [16–19]. Another *WOX* gene, *WOX5*, maintains the stem cell niche in the root apical meristem (RAM) [12, 20–22]. *WOX5* expression in the quiescent center (QC) of root [23] is regulated by the protein complexes of (1) the double APETALA2 (AP2)-domain transcription factors PLETHORAs (PLTs) [24–26], (2) the GRAS family transcription factor SCARECROW (SCR)[27, 28], and (3) the TEOSINTE-BRANCHED CYCLOIDEA PCNA (TCP) transcription factors [29, 30], which bind to the PLT-binding site in the *WOX5* promoter [31]. Besides *WUS* and *WOX5*, the other 13 members of the *WOX* gene family in Arabidopsis (*Arabidopsis thaliana*), except *WOX10*, have been funcitonally characterized to regulate meristem development in embryos, as well as vegetative and reproductive organs (Table 1).

Given the pivotal roles of the *WOX* gene family in meristem development, the evolution of the *WOX* gene family has been associated with morphological innovations [12, 13, 46–51]. Previous phylogenetic studies of the *WOX* gene family based on the homeodomain or full-length sequences identified three *WOX* superclades, termed the ancient, intermediate and modern clades [12, 13, 47, 52–55]. Three characteristic peptide motifs in the HD were suggested as signatures of these superclades: NVYNWFQNR of the ancient clade, NVFYWFQNR of the intermediate clade, and NVFYWFQNH of the modern clade [13, 56]. Proteins of the modern clade (WUS, WOX1/6, WOX2, WOX3, WOX4, and WOX5/7 subclades, named after their Arabidopsis members) share a WUS-box motif [7], and the WUS, WOX5 and WOX7 proteins additionally contain an ERF-associated amphiphilic repression (EAR) motif in their carboxy (C)-termini [57, 58]. The EAR motif interacts with the TOPLESS (TPL)/TPL-Related (TPR) co-repressors to repress the transcription of auxin response genes [59]. The intermediate clade includes the WOX8/9 and WOX11/12 subclades, which share the VFIN WOX8 MOG and LQxG WOX8 MOG motifs in their C-termini, while the WOX10/13/14 proteins of the ancient clade contain the WOX13 MOG motif of 39 amino acids upstream of the HD [45]. However, these phylogenetic analyses have been restricted by (1) the nature of the *WOX* sequences, (2) limited available sampling spanning the Viridiplantae, and (3) a lack of robust sequence alignment method; meaning that the origin and relationship among clades and subclades remain unclear. For instance, the "ancient" clade has been frequently inferred as paraphyletic and lacking support [12, 13, 52, 53, 55]. In addition, a polytomy has been commonly reconstructed along the backbone of the modern clade [12, 13, 47, 49, 52, 53, 55, 60]. For example, a recent phylogenetic reconstruction based on an alignment of 350 WOX proteins compiled with MUSCLE [61] and manual adjustment showed that the braches leading to all three superclades, as well as to all clades in the modern clade, have bootstrap support (BS) below 50 [53]. Consequently, the interpretation of experimental data on the functional divergence of the *WOX* genes is challenging [13, 15, 62].

Table 1. Summary of Arabidopsis *WOX* gene expression patterns and functions.

Gene	Expression pattern	Function	Reference
AtWUS	Shoot apical meristem; anther; ovule	Stem cell maintenance; leaf, anther and ovule development	[8, 9, 18, 32–34]
AtWOX1	Leaf primordium; procambial tissue	Leaf, sepal, and petal development	[34, 35]
AtWOX2	Eggs; zygote; apical embryo domain	Embryo patterning	[36]
AtWOX3	Leaf primordium; floral primordium; sepal and petal primordia	Leaf, sepal, and petal development	[34, 35, 37]
AtWOX4	Inflorescence stem; leaf primordium; flower	Procambial development	[38, 39]
AtWOX5	Root apical meristem	Stem cell maintenance	[21]
AtWOX6	Seedling; ovule primordium	Ovule development; freezing tolerance	[40, 41]
AtWOX7	Root apical meristem	Stem cell maintenance; sugar response	[42]
AtWOX8	Zygote; basal embryo domain	Embryo patterning	[36, 43]
AtWOX9	Zygote; basal embryo domain	Embryo patterning	[36, 43]
AtWOX10	Unknown	Unknown	
AtWOX11	Root apical meristem	Root organogenesis	[44]
AtWOX12	Root apical meristem	Root organogenesis	[44]
AtWOX13	Root; inflorescence	Root development; floral transition	[45]
AtWOX14	Root; inflorescence	Root development; floral transition	[45]

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The *WOX* function is regulated in part by the auxin and cytokinin signaling pathways [63]. The AUXIN RESPONSE FACTOR (ARF) transcription factors of the canonical TRANSPORT INHIBITOR-RESPONSE 1 (TIR1)-AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA)-ARF pathway [36] activate or repress the *WOX* genes by binding to Auxin Response Elements (AuxREs) [64–67] in the *WOX* promoters [44, 68–72]. On the other hand, cytokinin activates *WUS* expression through direct binding of the type-B ARABIDOPSIS RESPONSE REGULATORs (ARR-Bs) to the B-ARR motif [73–75] in the *WUS* promoter [76–79]. ARR10, which is one of the ARR-Bs, also binds to the B-ARR motif in the promoters of *WOX1* and *WOX12* [77]. However, it is unclear how evolutionarily conserved the regulation of the *WOX* function by these two major phytohormone signaling pathways is.

In order to explore the origin and evolution of the *WOX* gene family, we developed a robust bioinformatics pipeline to compile the most comprehensive sampling of the WOX protein sequences to date for phylogenetic reconstruction without manual adjustment, including 38 genomes and 440 transcriptomes covering most extant Viridiplantae (i.e., chlorophytes, charophytes, bryophytes, lycophytes, ferns, gymnosperms, and angiosperms) orders and Rhodophyta (i.e., red algae). Conserved protein motifs of the WOX clades, as well as AuxREs and B-ARR motifs in target *WOX* promoters, were identified. The reconstructed WOX phylogeny inferred three ancient superclades of this pivotal family of transcription factors, and provides the phylogenetic context for research in its genetic and biochemical evolution, which underpins the evolution of morphological complexity in plants.

Materials and methods

Phylogenetic reconstruction

Sequences of 15 Arabidopsis WOX proteins were used as queries to BLAST against the coding sequences (CDSs) of 360 transcriptomes from the 1KP database by using the Python pipeline BlueDevil with E-value cutoff of 1e-5 [80], 29 published genomes of land plants from Phytozome 10 and CoGe [81, 82], and the fern genomes of *Azolla filiculoides* and *Salvinia cucullata* [83] by using the BLAST+ package with tBLASTn algorithm and E-value cutoff of 1e-5 [84]. Another 79 transcriptomes of red and green algae from 1KP and 7 genomes (*Chlamydomonas*

reinhardtii, *Coccomyxa subellipsoidea*, *Klebsormidium nitens*, *Micromonas pusilla CCMP1545*, *Micromonas sp*. *RCC299*, *Ostreococcus lucimarinus* and *Volvox carteri*) from Phytozome 10 and *Klebsormidium flaccidum* genome project[85] were also BLASTed against for *WOX* homologs. The retrieved sequences and an additional six *WOX* coding sequences from the *Gunnera manicata* transcriptome (Chiu and Elhai, unpublished) were translated into protein sequences. 1098 WOX protein sequences with lengths between 120 and 971 amino acids were aligned using PASTA [86] and then filtered by the two sequential criteria: (1) aligned columns with more than 50% missing data were removed, and (2) sequences filtered from (1) with less than half of the total alignment length were removed. This procedure of alignment and filtering was taken six times until no sequences were filtered out. Phylogenetic reconstruction of the compiled protein alignment, WOXaa (S1 File), of 1039 sequences with 145 sites in length (S1 Table) was performed by RAxML 8.2.4 [87] under the JTT model with gamma-distribution of rate variation among sites, which was selected by ProtTest 3.4.2 [88], with 1,000 BS replicates on the CIPRES Science Gateway [89]. Chlorophyte WOX proteins from *Bathycoccus prasinos*, *Ostreococcus lucimarinus*, *Micromonas pusilla CCMP1545* and *Micromonas sp*. *RCC299* were used as outgroup for rooting the WOX phylogeny. In order to test whether the reconstructed topology would be consistent between analysis of the WOXaa dataset and analysis of WOX sequences retrieved only from candidate genomes (i.e., not from transcriptomes), we prepared another dataset with 446 WOX proteins from candidate genomes. This smaller dataset was aligned and filtered following the aforementioned criteria to generate an alignment (WOXaa_g) of 442 sequences with 150 sites in length (S2 File) for phylogenetic reconstruction. The phylogenetic reconstruction based on WOXaa_g was conducted following the aforementioned approach.

Search of WOX motifs

Motifs shared by clades of the WOX proteins were discovered by using MEME 4.11.2 [90] with E-value cutoff of 1e-5 and minimum length of five amino acids on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University.

Synteny analysis

In order to examine whether clades with weak phylogenetic resolution were derived from genome duplication, we performed synteny analysis by using the SynFind program [91] with default setting on CoGe [81, 82]. A syntenic score is defined as the number of homologous genes shared within the vicinity of a total of 41 genes (the anchor gene and its 20 upstream and 20 downstream). A retrieved genomic region is determined syntenic with a syntenic score of at least 4. A syntenic proxy is referred if the gene in the query is lost in the syntenic region.

Search of AuxRE and B-ARR-6-BA motifs, and the *ARF* and *ARR-B* genes

Genomic sequences 1.5kb upstream of the transcription start sites (TSS) of all *WOX* genes from *O*. *lucimarinus*, *M*. *pusilla CCMP1545*, *Micromonas sp*. *RCC299*, *M*. *polymorpha*, *P*. *patens*, *S*. *Moellendorffii*, *A*. *trichopoda*, *A*. *coerulea*, and Arabidopsis were retrieved from Phytozome 12.1 for prediction of AuxRE and B-ARR-6-BA motifs by PlantPAN 2.0[92]. Overlapping sites of each motif were counted only once. To investigate the presence of the *ARF* and *ARR-B* genes, all Arabidopsis ARF and ARR-B protein sequences (S2 Table) were used as queries to BLAST against a CDS database of eight genomes (e.g., *A*. *thaliana*, *Solanum lycopersicum*, *Aquilegia coerulea*, *Amborella trichopoda*, *Selaginella moellendorffii*, *Marchantia polymorpha*, *Physcomitrella patens*, and *M*. *pusilla*) from Phytozome 12.1 by using the BLAST + package with tBLASTn algorithm. The retrived CDS sequences were translated into protein

sequences using EMBOSS Transeq (https://www.ebi.ac.uk/Tools/st/emboss_transeq/). The protein sequences were run through InterPro 71.0 (https://www.ebi.ac.uk/interpro/) for search of conserved domains as verification.

Results

Major clades of the *WOX* gene family are ancient

In order to elucidate the origin and deep divergence of major WOX clades, we compiled an alignment (WOXaa) of 1039 WOX proteins from 267 species covering all divisions of the Viridiplantae after trimming columns of low occupancy and short sequences. The alignment has a length of 145 amino acids with 21.01% of characters as gaps. No *WOX* gene was found in any of the 26 rhodophyte transcriptomes sampled by 1KP. Among the 45 transcriptomes and 3 genomes of sampled chlorophytes, the WOX proteins were retrieved from 9 species (*Bathycoccus prasinos*, *Codium fragile*, *M. pusilla CCMP1545*, *M. RCC299*, *Ostreococcus lucimarinus*, *Picocystis salinarum*, *Scherffelia dubia*, *Scourfieldia sp*. and *Trebouxia arboricola*). Rooted with a sampling of chlorophyte WOX sequences, the Maximum-likelihood (ML) phylogeny inferred from WOXaa is divided into three superclades, which we term Type 1 (T1WOX), Type 2 (T2WOX) and Type 3 (T3WOX). A sequence from *Selaginella moellendorffii* 417553 is sister to the T2WOX + T3WOX clade (Fig 1). The *T1WOX* superclade comprises of the WOX10/13/14 proteins from all divisions of the Viridiplantae. The T1WOX superclade has low support (BS 19), which is consistent with previous studies [12, 52–54, 60]. The well-supported T2WOX superclade includes the WOX8/9 and WOX 11/12 clades, which were previously referred to as the intermediate clade. The T2WOX superclade is sister to the T3WOX superclade with BS support of 78. The T3WOX superclade (BS 75) contains the WUS, WOX5/7, WOX3, WOX1/6, WOX4, and WOX2 clades, as well as some lycophyte, fern and gymnosperm WOX proteins that are sister to the aforementioned angiosperm clades.

In order to test the topological consistency of the inferred WOX phylogeny, we applied the same alignment and reconstruction approach to a smaller dataset WOXaa_g in which the WOX proteins were drawn from candidate genomes only. The phylogeny inferred from WOXaa_g recovers three superclades and conserved topology among the T3WOX clades (S1 Fig). However, there are several major differences. For instance, the WOX8/9 proteins, as well as several fern T3WOX proteins, are paraphyletic to the WOX11/12 clade in the WOXaa_g phylogeny. In addition, there is no lycophyte WOX sequence nested within the T3WOX superclade. Generally BS supports at the backbone of the WOXaa_g phylogeny are lower than those of the WOXaa phylogeny.

In the N-terminal domain of the T1WOX proteins, the T1WOX motif, which was previously referred to as WOX13 MOG [45], is highly conserved but is absent from the other types of WOX proteins (Fig 2). SynFind analysis (S3 Table) shows synteny among *AtWOX10*, *AtWOX13*, and *AtWOX14*.

The monophyly of the T2WOX superclade, which comprises the WOX8/9 and WOX11/12 clades, is recovered with BS support of 88 (S2 Fig). Downstream of the HD, the T2WOX proteins share the superclade-specific 60-amino-acid T2WOX motif (Fig 2), which comprises motifs previously named VFIN WOX8 MOG and LQxG WOX8 MOG [45]. This superclade comprises only proteins from seed plants. This result differs from previous studies in which some lycophyte and fern WOX homologs were clustered with the WOX8/9 and WOX11/12 clades [13, 15, 47, 52–54, 93]. However, those lycophyte and fern WOX proteins, including CrWOXA and CrWOXB of *Ceratopteris richardii*, do not contain the T2WOX motif, consistent with our result (see also below). These non-seed-plant WOX proteins contain the NVFYWFQNR motif in the HD, however, the NVFYWFQNR motif is also present is several

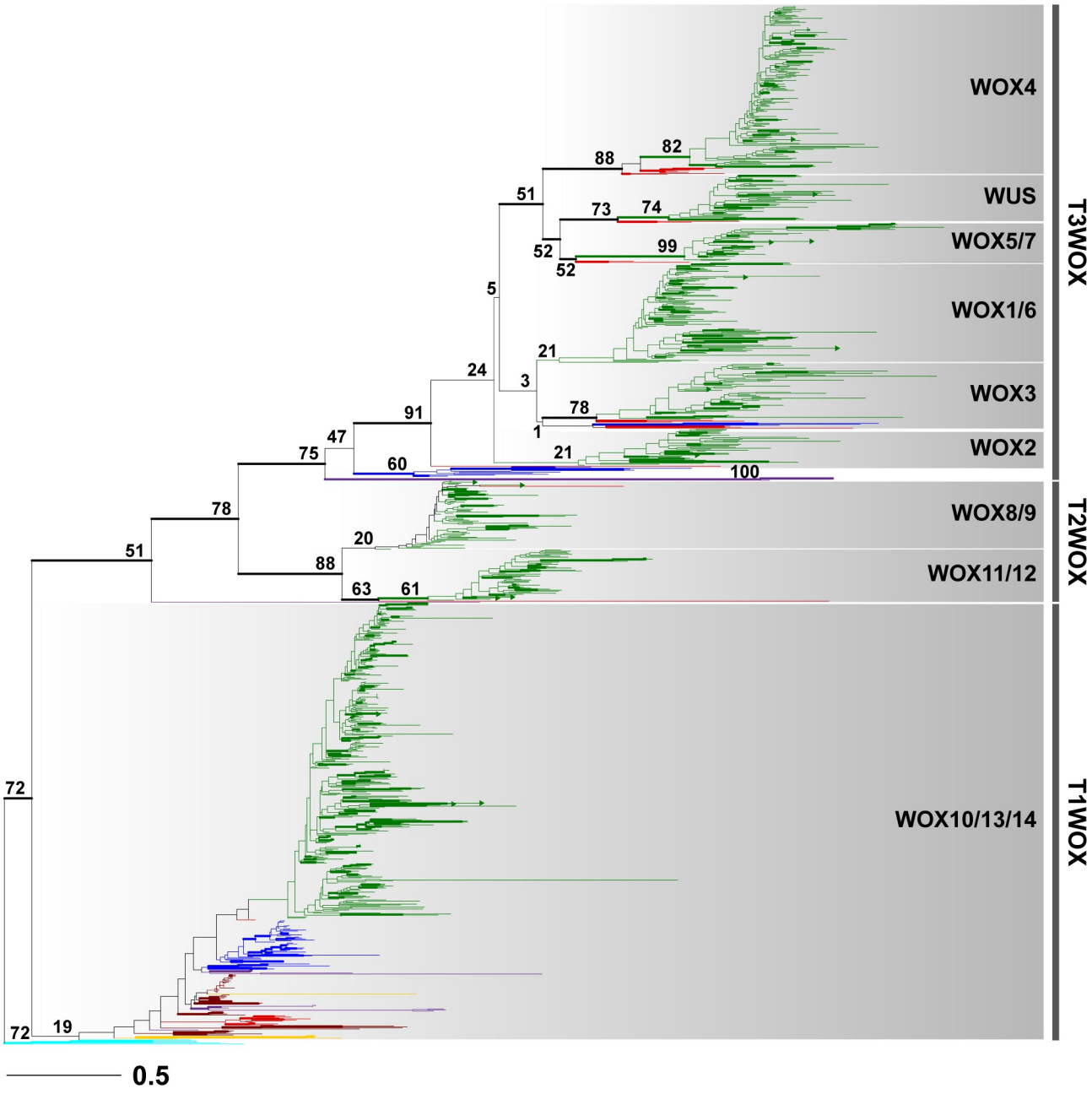

Fig 1. Maximum-likelihood (ML) phylogeny of the WOX protein family. The ML phylogeny was reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Thicker branch width indicates BS support equal or greater than 50. BS values are shown at key branches. Branches are colored according to taxonomic affiliation: green for angiosperms; red, gymnosperms; blue, ferns; purple, lycophytes; brown, bryophytes; yellow, charophytes; cyan, chlorophytes. Clades are shaded in a gradient of gray. Major superclades are marked with vertical lines. The scale is amino acid substitution rate of 0.5.

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T3WOX proteins (e.g., GbWOX3A of *Ginkgo biloba*, GgWOX2A and GgWOX2B of *Gnetum gnemon*, Migut.N02641 of *Mimulus gutatus*, cassava4.1 021403m of *Manihot esculenta*). On the other hand, we found no syntenic region of the T2WOX genes in the genomes of *A. filiculoides* and *S. moellendorffii*. These results suggest that the *T2WOX* genes may be lost in lycophytes and ferns. Synteny analysis also finds synteny between *AtWOX8* and *AtWOX9*, as well as that between *AtWOX11* and *AtWOX12*.

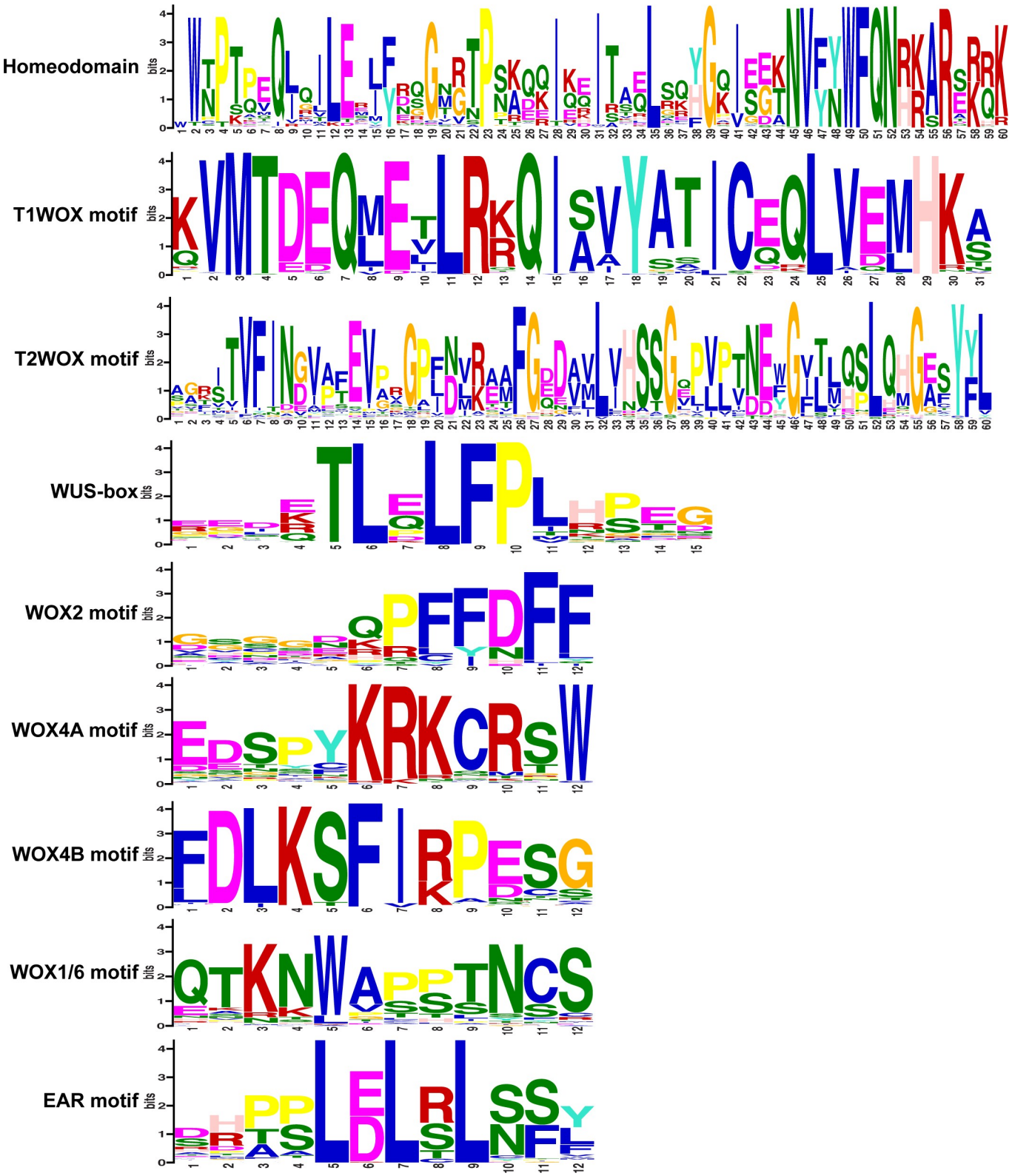

Fig 2. Conserved motifs in the WOX proteins. Amino acid motifs identified using MEME, with E-value cutoff smaller than 1e-5. The relative font size for each residue indicates sequence conservation, with a larger font representing higher conservation.

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The moderately supported (BS 75) T3WOX proteins constitute a monophyletic superclade of (1) a well-supported (BS 100) clade of four lycophyte T3WOX proteins, (2) a weakly supported (BS 60) clade of 10 fern T3WOX proteins, and (3) a strongly-supported (BS 91) clade that includes major T3WOX clades of seed plant proteins and three fern homologs (S3 Fig). The phylogeny confirms that the T3WOX at least predates vascular plants (i.e., lycophytes, ferns, and seed plants). The T3WOX proteins generally possess a common sequence signature termed the WUS-box (Fig 2). However, the lycophyte T3WOX proteins, the weakly supported fern T3WOX clade members, AtWOX7, and XTZP-0091187 of *Araucaria rulei* lack this motif. The WOX2 clade has low support (BS 21), but shares the WOX2 motif in the C-terminal (Fig 2). The angiosperm WOX3 proteins form a monophyletic group with a WOX3 homolog from *G. gnemon* and two *G. biloba* WOX3 members with moderate BS support of 78 (S4 Fig). It is worth noting that GgWOX2A and GgWOX2B of *G. gnemon*, which were previously nested within the WOX2 clade [12, 13], are in the WOX3 clade, although with low support (BS 1). Neither GgWOX2A or GgWOX2B contains the WOX2 motif. *CrWUL* of *C. richardii* has previously been reconstructed as falling among polytomous lineages of the modern clade [13, 15, 49, 54, 55], along with two Azolla T3WOX proteins (Azfi_s0343.g065738 and Azfi_s0051.g031311), are also clustered with GgWOX2A and GgWOX2B. Despite little BS support for the WOX1/6 monophyletic clade (S5 Fig), most WOX1/6 proteins maintain the conserved WOX1/6 motif between the HD and the WUS box (Fig 2). Consistent with previous literature [12, 22, 49], no *WOX1/6* gene was found in the monocot genomes or transcriptomes sampled in this study. No syntelog of *AtWOX1* or *AtWOX6* was discovered by SynFind in the monocot genomes of *Anana comosus*, *Musa acuminata*, *Oryza sativa*, *Phalaenopsis equestris*, *Phoenix dactylifera*, *Triticum aestivum* and *Zea mays*. However, one genomic region (pos 29212359 on contig CM000126) sytenic to *AtWOX6* was found in rice. These lines of evidence suggest that the MRCA of monocots may have had a *WOX6* coding sequence but lost it before the divergence of extant monocot orders. Within the weakly supported the WOX5/7 clade (BS 52; S6 Fig), the monophyly of the angiosperm WOX5/7 proteins was recovered with strong support (BS 99). As sister to the WOX5/7 clade, the WUS proteins from seed plants constitute a monophyletic lineage with moderate support (BS 73). Among the seed plant WUS proteins, the angiosperm members comprise a monophyletic group with also moderate support (BS 74). The EAR motif [57] is recovered in the C-termini of all members of both WOX5/7 and WUS clades, except in AtWOX7 [47]. The EAR motif is encoded as L[DE]LRLS in the WOX5/7 clade members, while in the WUS clade proteins it is L[DE]L[ST]LN. The WOX4 clade has modest support (BS 88; S7 Fig). The gymnosperm WOX proteins are paraphyletic with the angiosperm WOX4 proteins nested in it (BS 82). Among the angiosperm WOX4 proteins, monocot members form a monophyletic clade, which is sister to all the other angiosperm WOX4 homologs. Most WOX4 proteins share the conserved WOX4 motif, which is upstream of the HD (Fig 2).

Auxin Response Elements and B-ARR-6-BA motifs in the *WOX* promoters are deeply conserved in plants

In order to elucidate how ancient the regulation of *WOX* genes by auxin and cytokinin may be in land plants, 1.5kb of sequence upstream of the transcriptional start sites of select *WOX* loci were obtained from Phytozome v12 for identification of known AuxRE and B-ARR-6-BA sequences (S4 Table). AuxREs and B-ARR-6-BA were found in the *WOX* promoters of all selected taxa. We also identified *ARR-B* homologs in all selected plant genomes but *ARF* homologs were restricted to land plant genomes (Fig 3).

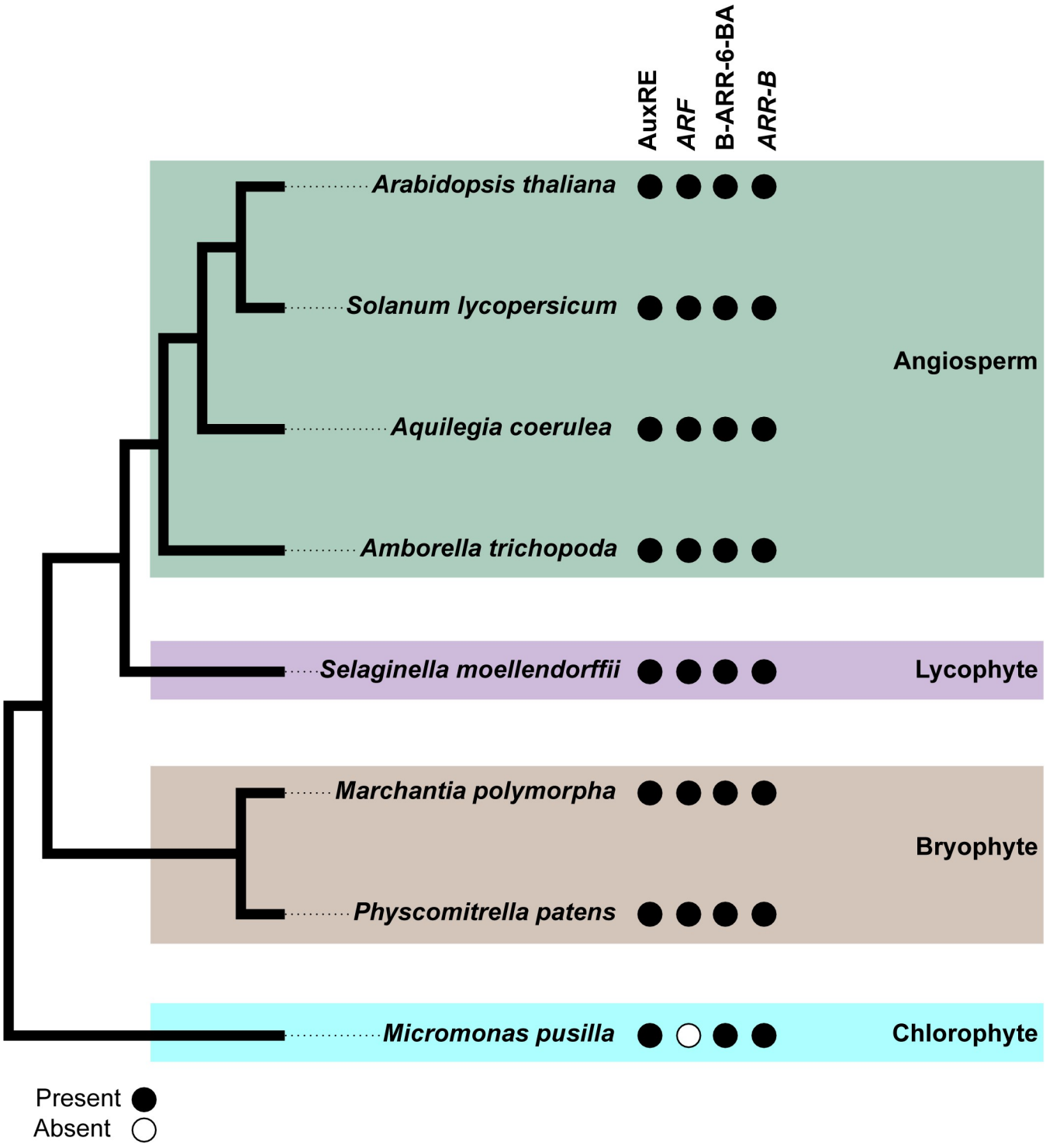

Fig 3. Conservation of the *WOX* regulation by the *ARF* and *ARR-B* genes in plants. Sequences 1.5kp upstream of the *WOX* genes in representative taxa were scanned using PlantPAN 2.0 for the presence of the AuxRE and B-ARR-6-BA motifs. Coding sequences of Arabidopsis *ARF* and *ARR-B* genes were used as queries to BLAST against genomes of representative taxa for the presence of their homologous genes. Closed circles depict the presence of the AuxRE or B-ARR-6-BA motif in the 1.5kp upstream of the *WOX* genes or that of the *ARF* or *ARR-B* genes in the representative genomes. Branches are boxed according to taxonomic affiliation: green for angiosperms; purple, lycophytes; brown, bryophytes; cyan, chlorophytes.

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Discussion

Advantages and remaining challenges of the current pipeline

To better understand the evolution of the developmentally critical *WOX* gene family, a large number of sequences spanning the majority of plant orders was employed for phylogenetic reconstruction and comparative analyses. Previous phylogenetic analyses of the *WOX* gene family were often inferred from datasets using only the HD, as sequences outside the HD are highly divergent, or using full-length sequences but with manual adjustment. Without efficient automatized alignment using robust software, it was difficult for the limited characters in those datasets to provide high phylogenetic resolution. By using PASTA, which performs better than other alignment softwares [94], the alignment strategy adopted in this study allowed the inclusion of additional 85 amino acids outside of the HD and improved the accuracy of the WOX protein phylogeny. Nevertheless, addressing the following challenges would likely contribute to finer resolution within the T1WOX and T3WOX superclades. First, most sequenced transcriptomes of the 1KP project were extracted from shoots or leaves, so the *WOX* genes expressed in other organs could have been missed. Second, transcripts from transcriptomes are often fragmentary. Third, the stringent filtering strategy for our alignment may have removed diagnostic characters for specific protein lineages. Development of an alignment pipelines that can better handle fragmentary sequences, as well as the addition of more sequenced genomes, will improve the robustness of phylogenetic inference.

All WOX superclades are ancient

The WOX phylogeny inferred here reveals deep duplications that gave rise to three distinct superclades. The absence of the *WOX* genes in Rhodophyta supports the hypothesis that the *WOX* gene family is Viridiplantae-specific [95]. A gene duplication prior to the common ancestor of the Viridiplantae may have contributed to the establishment of two clades of chlorophytic WOX proteins: the T1WOX superclade, and the ancestor of the T2WOX + T3WOX superclades. No *T2WOX* or *T3WOX* loci are found in the genomes or transcriptomes of the sampled bryophytes, nor was their syntelog or syntenic proxy in the genomes of *Marchantia* or *Physcomitrella*. Syntenic analysis with sequenced genomes of hornworts could elucidate whether the MRCA of all non-vascular land plants lost the ancestor of the T2WOX + *T3WOX* genes. Most importantly, the *T1WOX* and *T2WOX* + *T3WOX* gene lineages are equally ancient and it is not evolutionarily accurate to consider the *T1WOX* lineage more "ancient" than the other two, or the functions of its members to be necessarily more ancestral. For this reason, we have used a different nomenclature than previous publications.

Whole-genome duplications may contribute to divergence and expansion of the WOX proteins

The WOX phylogeny inferred here reveal several gene duplications coinciding ancient WGD events (Fig 4). For instance, theζWGD event, which occurred before the divergence of seed plants [96, 97], appears to correspond with the gene duplication and subsequent diversification of the WOX8/9 and WOX 11/12 clades. The respective synteny between *AtWOX8* and *AtWOX9*, as well as *AtWOX11* and *AtWOX12* suggests gene duplication of these loci by another WGD. The WOX phylogeny also presents a major radiation of the T3WOX clades at the base of euphyllophytes (i.e., ferns and seed plants). Polyploidy is prevalent in extant lycophytes and ferns, including a paleopolyploidization in the most basal euphyllophytic lineage to

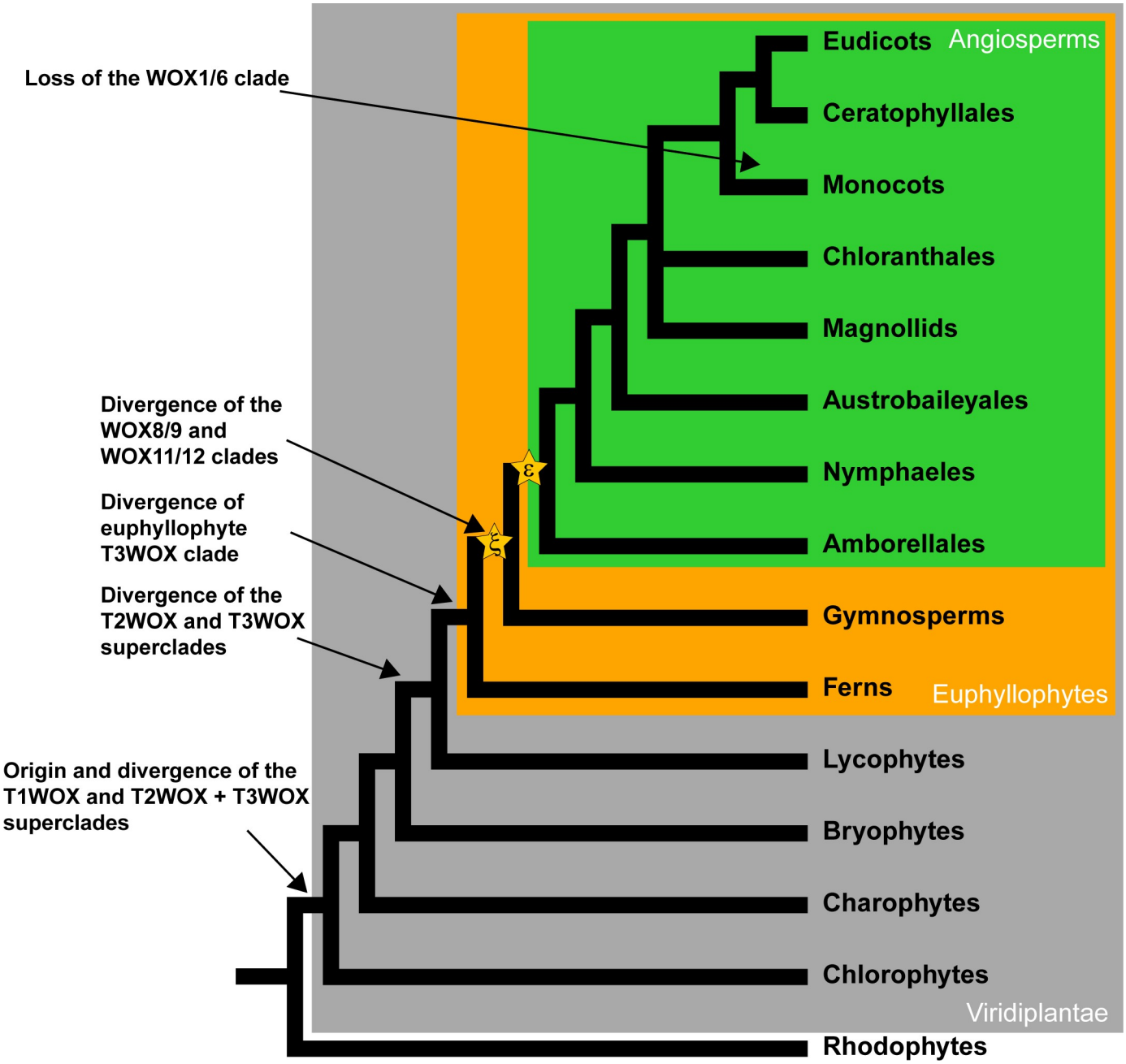

Fig 4. Major diversification events of the *WOX* family in plants. The simplified land plant phylogeny is plotted according to Ruhfel et al. [102] and Chase et al. [103]. The ε andζWGD events are marked in yellow stars. Angiosperms are boxed in green, euphyllophytes in orange, and the Viridiplantae in grey.

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Equisetidae (approximately 92.42 MYA) and a WGD near the base of the Polypodiidae (approximately 178 MYA) [98, 99]. However, no WGD has been discovered in the stem group of vascular plants to date [100]. In order to decipher whether WGD contributes to the divergence of the WOX clades at the base of vascular plants, additional sequenced genomes from lycophytes (e.g., Lycopodiaceae and Isoetaceae) and eusporangiate ferns (e.g., Equisetidae, Ophioglossidae, Marattiidae, etc.) are necessary [101].

Divergence of the WUS and WOX5/7 clades may predate the emergence of euphyllophytes

In this study, our result recovers the sister relationship between the WUS and WOX5/7 clades and suggests that the divergence of the WUS and WOX5/7 clades may predate the emergence of euphyllophytes. We also show strong support for the monophyly of the angiosperm WOX5/7 proteins, better support for the monophyletic group of angiosperm WUS proteins than previous studies [13, 15, 55], and moderate support for the monophyly of the seed plant WUS proteins, which is lower than that of some previous studies inferred from smaller WOX datasets [12, 54, 60]. The WUS and WOX5/7 clades are the best-studied clades among the WOX clades. *AtWUS* mediates the stem-cell niche in the organizing center (OC) of the SAM through the WUS-CLAVATA3 (CLV3) feedback circuit, which requires the intercellular movement of AtWUS [9–11, 104]. Similarly, AtWOX5 regulates the stem-cell niche of the QC in the RAM via a feedback loop with auxin-related response factors [105]. AtWUS and AtWOX5 function non-cell-autonomously and are biochemically interchangeable for stem cell maintenance of the SAM and RAM [21]. AtWUS and AtWOX5 interact with the BREAST CANCER ASSOCIATED RING 1 (BARD1)/REPRESSOR OF WUSCHEL1 (ROW1) and HAIRY MERISTEM (HAM) proteins in the SAM and RAM, respectively, to regulate the stem-cell niche [106–109].

The evolutionary trajectory underlying the functional divergence of the WUS and WOX5/7 clades remains unknown. Previous phylogenetic studies suggested that the WUS and WOX5/7 clades diverged prior the crown group of seed plants and after emergence of euphyllophytes [12, 13, 15, 47, 49, 53–55, 93]. Gene expression patterns of the *WUS* and *WOX5/7* genes have been characterized in a limited number of euphyllophyte taxa. Expression of *Ginkgo GbWUS*, *Gnetum GgWUS*, *Pinus PsWOX5* and *Picea PaWOX5* were detected in both shoot and root, while *GbWUS* was also expressed during reproductive organ development [12, 52]. In *Pinus pinaster*, *PpWUS* expression was detected in the shoot only, while high *PpWOX5* expression was observed in the root with low expression levels in the shoot [55]. In the basal angiosperm *Nymphea*, *NjWUS* was also shown to be expressed in the shoot, but not in the root. Heterologous expression of *GgWUS* driven by the promoter of *AtWUS* in Arabidopsis increased stem cell population in the SAM, inflorescence meristem, and floral meristem [12]. Employing a similar transgenic approach, another study showed that GbWUS, PaWUS, PsWUS, PaWOX5, and PsWOX5 can all complement AtWUS and AtWOX5 function in Arabidopsis, including the capability to regulate stem-cell maintenance and to move from cell to cell [15]. In the same analysis, *CrWUL* of *C. richardii*, which was thought to be sister to the WUS and WOX5/7 clades with gene expression in root tips and gametophytes but not in the shoot apical cell [13], could not rescue the *AtWUS* or *AtWOX5* mutants when driven by the *AtWUS* or *AtWOX5* promoter. However, when driven by *AtCLV* promoter, *CrWUL* was shown to have the capability to maintain stem-cell niche in SAM, demonstrating a lack of intercellular mobility in Arabidopsis. One possible explanation for this result is that CrWUL may not have the capacity of intercellular movement in its endogenous context. Alternatively, it is possible that the inteceullar mobility of CrWUL was prohibited in Arabidopsis because of a mismatch in the protein transport machinery.

The genomic context and gene function may have diversified since the MRCA of these taxa, and thus heterologous expression of a gene may not reflect actual gene function in the endogenous genomic context [110]. Ancestors of Arabidopsis and *Ceratopteris* diverged approximately 411 million years ago (MYA), while that of Arabidopsis and gymnosperms (e.g., *Ginkgo*, *Gnetum*, and *Pinus*) diverged approximately 330 MYA, and that of Arabidopsis and *Nymphea* diverged approximately 139 MYA [111, 112]. These lines of evidence, as well as the WOX phylogeny inferred here, suggests that the biochemical capacity to maintain stem-cell

niche may be synapomorphic for the crown group of euphyllophytes and that the subfunctionalization of either the *AtWUS* homologs in shoots and flowers or the *AtWOX5/7* homologs in roots may have occurred after the duplication in the lineage leading to seed plants.

In addition, AtWOX11 and AtWOX12 proteins directly bind to the promoters of *AtWOX5* and *AtWOX7* to activate their expression [113], but the evolutionary conservation of the regulation of the T3WOX genes by the T2WOX genes remains elusive. Functional analyses of the T3WOX genes in ferns and lycophytes, as well as *Selaginella* SmWOXII, which is positioned as sister to all the other T3WOX clades are critical to understanding (1) whether the cell-to-cell mobility occurs in the fern lineage, (2) the origin and functional divergence of the WUS and WOX5/7 clades, and (3) evolution of the regulation of the T3WOX genes by the T2WOX genes.

Regulation of the *WOX* genes by auxin and cytokinin signaling pathways may exist early in plant evolution

Auxin and cytokinin are prominent phytohormones that regulate various plant developmental processes [114]. In particular, the auxin and cytokinin signaling pathways control meristem development, in which the *WOX* genes also play critical roles [115–117]. Our survey of the *WOX* gene promoters from representative plants reveals the prevalence of the AuxREs across the Viridiplantae. This result is consistent with previous studies [118–121]. Although AUX/IAA family members are absent from green algae [122] and *ARF* genes are not found in chlorophytes [121], our result suggests that the capability of *WOX* genes to be regulated by auxin signaling pathway was established early in Viridiplantae, although most components of the pathway did not evolve until the emergence of the land plants [121–123]. Similarly, the presence of both the *ARR-B* genes and the B-ARR-6-BA motif in the *WOX* promoters across plant lineages is consistent with deep conservation of *WOX* regulation by the cytokinin signaling pathway. Alternatively, it could be possible that the early-divering plant lineages may use another B-ARR motif or AuxRE that is different from what is included in the PlantPAN matrix based on angiosperm collections. We can not rule out the possibility that the detection of these binding sites is not functionally relevant and just due to the likelihood of finding a given short sequence in a large DNA string. Genetic analysis is necessary to confirm whether the ARF and ARR-B proteins actually regulate the *WOX* genes in nonvascular land plants.

The *WOX* genes are indeed both up- and down-stream of the auxin and cytokinine pathways in meristem development [63]. For instance, WUS protects apical stem cells from differentiation by restricting the auxin signaling pathway via regulation of histone de-acetylation [124]. In addition, *WUS* directly represses the type-A *ARR5*, *ARR6*, *ARR7*, and *ARR15* genes, which function in the negative feedback loop of cytokinin signaling [125]. However, functional analysis is necessary to determine whether the other *WOX* genes reciprocally regulate the auxin or cytokinin signaling pathways.

Conclusions

In conclusion, our phylogenetic reconstruction based on 1039 protein sequences from 267 species across the Viridiplantae suggests three ancient WOX superclades: T1WOX, T2WOX, and T3WOX. Divergence of the T1WOX and T2WOX superclades may predate diversification of vascular plants. Our analysis of the *WOX* promoters also suggests that the capacity of the WOX genes to be regulated by the auxin and cytokinin signaling pathways could be deeply conserved in the Viridiplantae. As expansion of the *WOX* gene family and the gene families involved in the auxin and cytokinin signaling pathways have been correlated with morphological innovations during plant radiation [12, 46, 47, 50, 54, 120], robust phylogenies of the *WOX* genes and the auxin and cytokinin pathway components may provide insight for experimental

design to decipher how and when the these genes were recruited into the gene regulatory network underlying developmental and morphological complexity during plant evolution.

Supporting information

S1 Fig. Maximum-likelihood (ML) phylogeny of the WOX protein family based on WOX-aa_g. The ML phylogeny was reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Quadruple branch width indicates BS support equal or greater than 50. BS values are shown at key branches. Branches are colored according to taxonomic affiliation: green for angiosperms; red, gymnosperms; blue, ferns; purple, lycophytes; brown, bryophytes; yellow, charophytes; cyan, chlorophytes. Clades are shaded in a gradient of gray. Major superclades are marked with vertical lines. The scale is amino acid substitution rate of 0.5.
(TIF)

S2 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the T2WOX proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis T2WOX proteins are indicated by arrowheads. Branches are colored according to taxonomic affiliation: green for angiosperms and red for gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S3 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the basal T3WOX proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis WOX2 is indicated by an arrowhead. Branches are colored according to taxonomic affiliation: green for angiosperms and red for gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S4 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the WOX3 proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis WOX4 is indicated by an arrowhead. Branches are colored according to taxonomic affiliation: green for angiosperms and red for gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S5 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the WOX1/6 proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis WOX1 and WOX6 are indicated by arrowheads. Branches are colored according to taxonomic affiliation: green for angiosperms and red for gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S6 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the WUS and WOX5/7 proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis WOX proteins are indicated by arrowheads. Branches are colored according to taxonomic affiliation: blue for ferns, green for angiosperms and red for

gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S7 Fig. Maximum likelihood phylogeny of the WOX protein family. Portion of tree showing the WOX4 proteins. The ML phylogeny is reconstructed by RAxML 8.2.4 with 1,000 bootstrap replicates. Internal nodes with bootstrap values equal to and more than 50 are marked. Arabidopsis WOX proteins are indicated by arrowheads. Branches are colored according to taxonomic affiliation: blue for ferns, green for angiosperms and red for gymnosperms. The scale is an amino acid substitution rate of 0.5.
(TIF)

S1 Table. Proteins included in the dataset WOXaa.
(DOCX)

S2 Table. Arabidopsis *ARF* and *B-ARR* genes used as queries for BLAST search.
(DOCX)

S3 Table. Synteny analysis of Arabidopsis *WOX* genes.
(XLSX)

S4 Table. Predicted Auxin Response Elements and B-ARR-6-BA motif in promoters of plant *WOX* genes.
(DOCX)

S1 File. WOXaa.
(PHY)

S2 File. WOXaa_g.
(PHY)

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References

1. Graham LE, Cook ME, Busse JS. The origin of plants: Body plan changes contributing to a major evolutionary radiation. Proc Natl Acad Sci USA. 2000; 97(9):4535–4540. https://doi.org/10.1073/pnas.97.9.4535 PMID: 10781058

2. Langdale JA. Evolution of developmental mechanisms in plants. Current Opinion in Genetics & Development. 2008; 18(4):368–373.
3. Soltis PS, Soltis DE. Ancient WGD events as drivers of key innovations in angiosperms. Current Opinion in Plant Biology. 2016; 30:159–165. https://doi.org/10.1016/j.pbi.2016.03.015 PMID: 27064530
4. Jill Harrison C. Development and genetics in the evolution of land plant body plans. Philosophical Transactions of the Royal Society B: Biological Sciences. 2017; 372(1713):20150490. https://doi.org/10.1098/rstb.2015.0490 PMID: 27994131
5. Sussex IM, Kerk NM. The evolution of plant architecture. Current Opinion in Plant Biology. 2001; 4:33–37. PMID: 11163165
6. Gehring W, Muller M, Affolter M, Percival-Smith A, Billeter M, Qian Y, et al. The structure of the homeodomain and its functional implications. Trends in Genetics. 1990; 6:323–329. https://doi.org/10.1016/0168-9525(90)90253-3 PMID: 1980756
7. Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, et al. Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development. 2004; 131(3):657–668. https://doi.org/10.1242/dev.00963 PMID: 14711878
8. Laux T, Mayer KFX, Berger J, Juergens G. The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development. 1996; 122:87–96. PMID: 8565856
9. Mayer K, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T. Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell. 1998; 95(6):805–815. https://doi.org/10.1016/s0092-8674(00)81703-1 PMID: 9865698
10. Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R. Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. Science. 2000; 299:617–619.
11. Schoof H, Lenhard M, Haecker A, Mayer KFX, Juergens G, Laux T. The stem cell polulation of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell. 2000; 100:635–644. https://doi.org/10.1016/s0092-8674(00)80700-x PMID: 10761929
12. Nardmann J, Reisewitz P, Werr W. Discrete shoot and root stem cell-promoting *WUS/WOX5* functions are an evolutionary innovation of angiosperms. Molecular Biology and Evolution. 2009; 26:1745–1755. https://doi.org/10.1093/molbev/msp084 PMID: 19387013
13. Nardmann J, Werr W. The invention of WUS-like stem cell-promoting functions in plants predates leptosporangiate ferns. Plant Molecular Biology. 2012; 78(1):123–134.
14. Somssich M, Je BI, Simon R, Jackson D. CLAVATA-WUSCHEL signaling in the shoot meristem. Development. 2016; 143(18):3238–3248. https://doi.org/10.1242/dev.133645 PMID: 27624829
15. Zhang Y, Jiao Y, Jiao H, Zhao H, Zhu Y-X. Two-step functional innovation of the stem-cell factors WUS/WOX5 during plant evolution. Molecular Biology and Evolution. 2017; 34(3):640–653. https://doi.org/10.1093/molbev/msw263 PMID: 28053005
16. Lenhard M, Bohnert A, Juergens G, Laux T. Termination of stem cell maintenance in *Arabidopsis* floral meristems by interacitons between *WUSCHEL* and *AGAMOUS*. Cell. 2001; 105:805–814. https://doi.org/10.1016/s0092-8674(01)00390-7 PMID: 11440722
17. Lohmann JU, Hong RL, Hobe M, Busch MA, Parcy F, Simon R, et al. A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell. 2001; 105:793–803. https://doi.org/10.1016/s0092-8674(01)00384-1 PMID: 11440721
18. Gross-Hardt R, Lenhard M, Laux T. *WUSCHEL* signaling functions in interregional communication during *Arabidopsis* ovule development. Genes & Development. 2002; 16(9):1129–1138.
19. Ikeda M, Mitsuda N, Ohme-Takagi M. *Arabidopsis* WUSCHEL is a bifunctional transcription factor that acts as a repressor in stem cell regulation and as an activator in floral patterning. The Plant Cell. 2009; 21(11):3493–3505. https://doi.org/10.1105/tpc.109.069997 PMID: 19897670
20. Kamiya N, Nagasaki H, Morikami A, Sato Y, Matsuoka M. Isolation and characterization of a rice *WUSCHEL*-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. The Plant Journal. 2003; 35:429–441. https://doi.org/10.1046/j.1365-313x.2003.01816.x PMID: 12904206
21. Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, et al. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. Nature. 2007; 446(7137):811–814. https://doi.org/10.1038/nature05703 PMID: 17429400
22. Nardmann J, Zimmermann R, Durantini D, Kranz E, Werr W. *WOX* gene phylogeny in Poaceae: A comparative approach addressing leaf and embryo development. Molecular Biology and Evolution. 2007; 24(11):2474–2484. https://doi.org/10.1093/molbev/msm182 PMID: 17768306
23. Zhao S, Jiang Q-T, Ma J, Zhang X-W, Zhao Q-Z, Wang X-Y, et al. Characterization and expression analysis of *WOX5* genes from wheat and its relatives. Gene. 2014; 537(1):63–69. https://doi.org/10.1016/j.gene.2013.12.022 PMID: 24368329

24. Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, et al. The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell. 2004; 119(1):109–120. https://doi.org/10.1016/j.cell.2004.09.018 PMID: 15454085

25. Nole-Wilson S, Tranby TL, Krizek BA. *AINTEGUMENTA*-like (*AIL*) genes are expressed in young tissues and may specify meristematic or division-competent states. Plant Molecular Biology. 2005; 57 (5):613–628. https://doi.org/10.1007/s11103-005-0955-6 PMID: 15988559

26. Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, et al. PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature. 2007; 449:1053–1057. https://doi.org/10.1038/nature06206 PMID: 17960244

27. Wysocka-Diller JW, Helariutta Y, Fukaki H, Malamy JE, Benfey PN. Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. Development. 2000; 127(3):595–603. PMID: 10631180

28. Sabatini S, Heidstra R, Wildwater M, Scheres B. SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes & Development. 2003; 17(3):354–358.

29. Li C, Potuschak T, Colón-Carmona A, Gutiérrez RA, Doerner P. *Arabidopsis* TCP20 links regulation of growth and cell division control pathways. Proc Natl Acad Sci USA. 2005; 102(36):12978–12983. https://doi.org/10.1073/pnas.0504039102 PMID: 16123132

30. Hervé C, Dabos P, Bardet C, Jauneau A, Auriac MC, Ramboer A, et al. In vivo interference with AtTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development. Plant Physiology. 2009; 149(3):1462–1477. https://doi.org/10.1104/pp.108.126136 PMID: 19091878

31. Shimotohno A, Heidstra R, Blilou I, Scheres B. Root stem cell niche organizer specification by molecular convergence of PLETHORA and SCARECROW transcription factor modules. Genes & Development. 2018; 32(15–16):1085–1100.

32. Sieber P, Gheyselinck J, Gross-Hardt R, Laux T, Grossniklaus U, Schneitz K. Pattern formation during early ovule development in *Arabidopsis thaliana*. Developmental Biology. 2004; 273(2):321–334. https://doi.org/10.1016/j.ydbio.2004.05.037 PMID: 15328016

33. Deyhle F, Sarkar A, Tucker E, Laux T. WUSCHEL regulates cell differentiation during anther development. Developmental Biology. 2007; 302:154–159. https://doi.org/10.1016/j.ydbio.2006.09.013 PMID: 17027956

34. Zhang F, Tadege M. Repression of *AS2* by WOX family transcription factors is required for leaf development in *Medicago* and *Arabidopsis*. Plant Signaling & Behavior. 2015; 10(7):e993291.

35. Vandenbussche M, Horstman A, Zethof J, Koes R, Rijpkema A, Gerats T. Differential recruitment of WOX transcription factors for lateral development and organ fusion in Petunia and *Arabidopsis*. The Plant Cell. 2009; 21:2269–2283. https://doi.org/10.1105/tpc.109.065862 PMID: 19717616

36. Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T. Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. Developmental Cell. 2008; 14:867–876. https://doi.org/10.1016/j.devcel.2008.03.008 PMID: 18539115

37. Shimizu R, Ji J, Kelsey E, Ohtsu K, Schnable P, Scanlon M. Tissue specificity and evolution of meristematic WOX3 function. Plant Physiology. 2009; 149:841–850. https://doi.org/10.1104/pp.108.130765 PMID: 19073779

38. Ji J, Strable J, Shimizu R, Koenig D, Sinha N, Scanlon MJ. WOX4 promotes procambial development. Plant Physiology. 2010; 152(3):1346–1356. https://doi.org/10.1104/pp.109.149641 PMID: 20044450

39. Etchells JP, Provost CM, Mishra L, Turner SR. *WOX4* and *WOX14* act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. Development. 2013; 140(10):2224–2234. https://doi.org/10.1242/dev.091314 PMID: 23578929

40. Zhu J, Shi H, Lee B, Damsz B, Cheng S, Stirm V, et al. An *Arabidopsis* homeodomain transcription factor gene, *HOS9*, mediates cold tolerance through a CBF-independent pathway. Proc Natl Acad Sci USA. 2004; 101:9873–9878. https://doi.org/10.1073/pnas.0403166101 PMID: 15205481

41. Park S, Zheng Z, Oppenheimer D, Hauser B. The *PRETTY FEW SEEDS2* gene encodes an *Arabidopsis* homeo domain protein that regulates ovule development. Development. 2005; 132:841–849. https://doi.org/10.1242/dev.01654 PMID: 15659481

42. Kong D, Hao Y, Cui H. The WUSCHEL Related Homeobox protein WOX7 regulates the sugar response of lateral root development in *Arabidopsis thaliana*. Molecular Plant. 2016; 9(2):261–270. https://doi.org/10.1016/j.molp.2015.11.006 PMID: 26621542

43. Ueda M, Zhang Z, Laux T. Transcriptional activation of *Arabidopsis* axis patterning genes *WOX8*/*9* links zygote polarity to embryo development. Developmental Cell. 2011; 20(2):264–270. https://doi.org/10.1016/j.devcel.2011.01.009 PMID: 21316593

44. Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, et al. *WOX11* and *12* are involved in the first-step cell fate transition during de novo root organogenesis in *Arabidopsis*. The Plant Cell. 2014; 26(3):1081–1093. https://doi.org/10.1105/tpc.114.122887 PMID: 24642937

45. Deveaux Y, Toffano-Nioche C, Claisse G, Thareau V, Morin H, Laufs P, et al. Genes of the most conserved WOX clade in plants affect root and flower development in Arabidopsis. BMC Evolutionary Biology. 2008; 8:291. https://doi.org/10.1186/1471-2148-8-291 PMID: 18950478

46. Nardmann J, Werr W. The shoot stem cell niche in angiosperms: expression patterns of WUS orthologues in rice and maize imply major modifications in the course of mono- and dicot evolution. Mol Biol Evol. 2006; 23:2492–2504. https://doi.org/10.1093/molbev/msl125 PMID: 16987950

47. van der Graaff E, Laux T, Rensing S. The WUS homeobox-containing (WOX) protein family. Genome Biology. 2009; 10(12):248. https://doi.org/10.1186/gb-2009-10-12-248 PMID: 20067590

48. Katayama N, Koi S, Kato M. Expression of *SHOOT MERISTEMLESS*, *WUSCHEL*, and *ASYMMETRIC LEAVES1* homologs in the shoots of Podostemaceae: implications for the evolution of novel shoot organogenesis. The Plant Cell. 2010; 22(7):2131–2140. https://doi.org/10.1105/tpc.109.073189 PMID: 20647344

49. Nardmann J, Werr W. Symplesiomorphies in the WUSCHEL clade suggest that the last common ancestor of seed plants contained at least four independent stem cell niches. New Phytologist. 2013; 199(4):1081–1092. https://doi.org/10.1111/nph.12343 PMID: 23721178

50. Harrison CJ, Morris JL. The origin and early evolution of vascular plant shoots and leaves. Philosophical Transactions of the Royal Society B: Biological Sciences. 2018; 373(1739):20160496.

51. Chandler JW, Werr W. Histology versus phylogeny: Viewing plant embryogenesis from an evo-devo perspective. In: Grossniklaus U, editor. Current Topics in Developmental Biology. 131: Academic Press; 2019. p. 545–564. https://doi.org/10.1016/bs.ctdb.2018.11.009 PMID: 30612629

52. Hedman H, Zhu T, von Arnold S, Sohlberg J. Analysis of the *WUSCHEL-RELATED HOMEOBOX* gene family in the conifer *Picea abies* reveals extensive conservation as well as dynamic patterns. BMC Plant Biology. 2013; 13(1):89.

53. Lian G, Ding Z, Wang Q, Zhang D, Xu J. Origins and evolution of WUSCHEL-Related Homeobox protein family in plant kingdom. The Scientific World Journal. 2014; 2014:12.

54. Segatto ALA, Thompson CE, Freitas LB. Molecular evolution analysis of *WUSCHEL-related homeobox* transcription factor family reveals functional divergence among clades in the homeobox region. Development Genes and Evolution. 2016; 226(4):259–268. https://doi.org/10.1007/s00427-016-0545-4 PMID: 27150824

55. Alvarez JM, Bueno N, Cañas RA, Avila C, Cánovas FM, Ordás RJ. Analysis of the WUSCHEL-RELATED HOMEOBOX gene family in Pinus pinaster: New insights into the gene family evolution. Plant Physiology and Biochemistry. 2018; 123:304–318. https://doi.org/10.1016/j.plaphy.2017.12.031 PMID: 29278847

56. Zeng M, Hu B, Li J, Zhang G, Ruan Y, Huang H, et al. Stem cell lineage in body layer specialization and vascular patterning of rice root and leaf. Science Bulletin. 2016; 61(11):847–858.

57. Ohta M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M. Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. The Plant Cell. 2001; 13(8):1959–1968. https://doi.org/10.1105/TPC.010127 PMID: 11487705

58. Paponov I, Teale W, Lang D, Paponov M, Reski R, Rensing S, et al. The evolution of nuclear auxin signalling. BMC Evoltionary Biology. 2009; 9:126.

59. Szemenyei H, Hannon M, Long JA. TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis. Science. 2008; 319(5868):1384–1386. https://doi.org/10.1126/science.1151461 PMID: 18258861

60. Zhang X, Zong J, Liu J, Yin J, Zhang D. Genome-wide analysis of *WOX* gene family in rice, sorghum, maize, Arabidopsis and poplar. Journal of Integrative Plant Biology. 2010; 52(11):1016–1026. https://doi.org/10.1111/j.1744-7909.2010.00982.x PMID: 20977659

61. Edgar R. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 2004; 32(5):1792–1797. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

62. Liu W, Xu L. Recruitment of IC-*WOX* genes in root evolution. Trends in Plant Science. 2018; 23(6):490–496. https://doi.org/10.1016/j.tplants.2018.03.011 PMID: 29680635

63. Su Y-H, Liu Y-B, Zhang X-S. Auxin–cytokinin interaction regulates meristem development. Molecular Plant. 2011; 4(4):616–625. https://doi.org/10.1093/mp/ssr007 PMID: 21357646

64. Ulmasov T, Hagen G, Guilfoyle TJ. ARF1, a transcription factor that binds to auxin response elements. Science. 1997; 276(5320):1865–1868. https://doi.org/10.1126/science.276.5320.1865 PMID: 9188533

65. Xu N, Hagen G, Guilfoyle T. Multiple auxin response modules in the soybean *SAUR 15A* promoter. Plant Science. 1997; 126(2):193–201.

66. Tiwari SB, Hagen G, Guilfoyle T. The roles of auxin response factor domains in auxin-responsive transcription. The Plant Cell. 2003; 15(2):533–543. https://doi.org/10.1105/tpc.008417 PMID: 12566590

67. Weiste C, Dröge-Laser W. The *Arabidopsis* transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. Nature Communications. 2014; 5:3883. https://doi.org/10.1038/ncomms4883 PMID: 24861440

68. Bao Y, Dharmawardhana P, Arias R, Allen MB, Ma C, Strauss SH. *WUS* and *STM*-based reporter genes for studying meristem development in poplar. Plant Cell Rep. 2009; 28(6):947–962. https://doi.org/10.1007/s00299-009-0685-3 PMID: 19280192

69. Zhao Y, Hu Y, Dai M, Huang L, Zhou D. The *WUSCHEL*-related homeobox gene *WOX11* is required to activate shoot-borne crown root development in rice. The Plant Cell. 2009; 21:736–748. https://doi.org/10.1105/tpc.108.061655 PMID: 19258439

70. Cheng S, Huang Y, Zhu N, Zhao Y. The rice *WUSCHEL*-related homeobox genes are involved in reproductive organ development, hormone signaling and abiotic stress response. Gene. 2014; 549 (2):266–274. https://doi.org/10.1016/j.gene.2014.08.003 PMID: 25106855

71. Guan C, Wu B, Yu T, Wang Q, Krogan NT, Liu X, et al. Spatial auxin signaling controls leaf flattening in *Arabidopsis*. Current Biology. 2017; 27(19):2940–2950. https://doi.org/10.1016/j.cub.2017.08.042 PMID: 28943086

72. Brackmann K, Qi J, Gebert M, Jouannet V, Schlamp T, Grünwald K, et al. Spatial specificity of auxin responses coordinates wood formation. Nature Communications. 2018; 9(1):875. https://doi.org/10.1038/s41467-018-03256-2 PMID: 29491423

73. Sakai H, Aoyama T, Oka A. *Arabidopsis* ARR1 and ARR2 response regulators operate as transcriptional activators. The Plant Journal. 2000; 24(6):703–711. https://doi.org/10.1046/j.1365-313x.2000.00909.x PMID: 11135105

74. Hosoda K, Imamura A, Katoh E, Hatta T, Tachiki M, Yamada H, et al. Molecular structure of the GARP family of plant Myb-related DNA binding motifs of the Arabidopsis response Regulators. The Plant Cell. 2002; 14(9):2015–2029. https://doi.org/10.1105/tpc.002733 PMID: 12215502

75. Taniguchi M, Sasaki N, Tsuge T, Aoyama T, Oka A. ARR1 directly activates cytokinin response genes that encode proteins with diverse regulatory functions. Plant and Cell Physiology. 2007; 48(2):263–277. https://doi.org/10.1093/pcp/pcl063 PMID: 17202182

76. Meng WJ, Cheng ZJ, Sang YL, Zhang MM, Rong XF, Wang ZW, et al. Type-B ARABIDOPSIS RESPONSE REGULATORs specify the shoot stem cell niche by dual regulation of *WUSCHEL*. The Plant Cell. 2017; 29(6):1357–1372. https://doi.org/10.1105/tpc.16.00640 PMID: 28576846

77. Zubo YO, Blakley IC, Yamburenko MV, Worthen JM, Street IH, Franco-Zorrilla JM, et al. Cytokinin induces genome-wide binding of the type-B response regulator ARR10 to regulate growth and development in *Arabidopsis*. Proc Natl Acad Sci USA. 2017; 114(29):E5995–E6004. https://doi.org/10.1073/pnas.1620749114 PMID: 28673986

78. Wang J, Tian C, Zhang C, Shi B, Cao X, Zhang T-Q, et al. Cytokinin signaling activates *WUSCHEL* expression during axillary meristem initiation. The Plant Cell. 2017; 29(6):1373–1387. https://doi.org/10.1105/tpc.16.00579 PMID: 28576845

79. Xie M, Chen H, Huang L, O'Neil RC, Shokhirev MN, Ecker JR. A B-ARR-mediated cytokinin transcriptional network directs hormone cross-regulation and shoot development. Nature Communications. 2018; 9(1):1604. https://doi.org/10.1038/s41467-018-03921-6 PMID: 29686312

80. Li F-W, Villarreal JC, Kelly S, Rothfels CJ, Melkonian M, Frangedakis E, et al. Horizontal transfer of an adaptive chimeric photoreceptor from bryophytes to ferns. Proc Natl Acad Sci USA. 2014; 111 (18):6672–6677. https://doi.org/10.1073/pnas.1319929111 PMID: 24733898

81. Lyons E, Freeling M. How to usefully compare homologous plant genes and chromosomes as DNA sequences. The Plant Journal. 2008; 53(4):661–673. https://doi.org/10.1111/j.1365-313X.2007.03326.x PMID: 18269575

82. Lyons E, Pedersen B, Kane J, Alam M, Ming R, Tang HB, et al. Finding and comparing syntenic regions among *Arabidopsis* and the outgroups papaya, poplar, and grape: CoGe with rosids. Plant Physiology. 2008; 148(4):1772–1781. https://doi.org/10.1104/pp.108.124867 PMID: 18952863

83. Li F-W, Brouwer P, Carretero-Paulet L, Cheng S, de Vries J, Delaux P-M, et al. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. Nature Plants. 2018; 4(7):460–472. https://doi.org/10.1038/s41477-018-0188-8 PMID: 29967517

84. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009; 10(1):1–9.

85. Hori K, Maruyama F, Fujisawa T, Togashi T, Yamamoto N, Seo M, et al. *Klebsormidium flaccidum* genome reveals primary factors for plant terrestrial adaptation. Nature Communications. 2014; 5:3978. https://doi.org/10.1038/ncomms4978 PMID: 24865297

86. Mirarab S, Nguyen N, Warnow T. PASTA: Ultra-Large Multiple Sequence Alignment. In: Sharan R, editor. Research in Computational Molecular Biology: 18th Annual International Conference, RECOMB 2014, Pittsburgh, PA, USA, April 2–5, 2014, Proceedings. Cham: Springer International Publishing; 2014. p. 177–191.

87. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014; 30(9):1312–1313. https://doi.org/10.1093/bioinformatics/btu033 PMID: 24451623

88. Darriba D, Taboada GL, Doallo R, Posada D. ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics. 2011; 27(8):1164–1165. https://doi.org/10.1093/bioinformatics/btr088 PMID: 21335321

89. Miller MA, Pfeitter W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE). 2010:1–8.

90. Bailey TL, Elkan C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proceedings International Conference on Intelligent Systems for Molecular Biology. 1994;2:28–36.

91. Tang H, Bomhoff MD, Briones E, Zhang L, Schnable JC, Lyons E. SynFind: compiling syntenic regions across any set of genomes on demand. Genome Biology and Evolution. 2015; 7(12):3286–3298. https://doi.org/10.1093/gbe/evv219 PMID: 26560340

92. Chow C-N, Zheng H-Q, Wu N-Y, Chien C-H, Huang H-D, Lee T-Y, et al. PlantPAN 2.0: an update of plant promoter analysis navigator for reconstructing transcriptional regulatory networks in plants. Nucleic Acids Research. 2016; 44(D1):D1154–D1160. https://doi.org/10.1093/nar/gkv1035 PMID: 26476450

93. Ge Y, Liu J, Zeng M, He J, Qin P, Huang H, et al. Identification of *WOX* family genes in *Selaginella kraussiana* for studies on stem cells and regeneration in lycophytes. Frontiers in Plant Science. 2016; 7:93. https://doi.org/10.3389/fpls.2016.00093 PMID: 26904063

94. Mirarab S, Nguyen N, Guo S, Wang L-S, Kim J, Warnow T. PASTA: ultra-large multiple sequence alignment for nucleotide and amino-acid sequences. Journal of Computational Biology. 2015; 22 (5):377–386. https://doi.org/10.1089/cmb.2014.0156 PMID: 25549288

95. Mukherjee K, Brocchieri L, Bürglin TR. A comprehensive classification and evolutionary analysis of plant homeobox genes. Molecular Biology and Evolution. 2009; 26(12):2775–2794. https://doi.org/10.1093/molbev/msp201 PMID: 19734295

96. Jiao YN, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, et al. Ancestral polyploidy in seed plants and angiosperms. Nature. 2011; 473(7345):97–100. https://doi.org/10.1038/nature09916 PMID: 21478875

97. Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, et al. Early genome duplications in conifers and other seed plants. Science Advances. 2015; 1(10):e1501084. https://doi.org/10.1126/sciadv.1501084 PMID: 26702445

98. Barker MS. Evolutionary genomic analyses of ferns reveal that high chromosome numbers are a product of high retention and fewer rounds of polyploidy relative to angiosperms. American Fern Journal. 2009; 99(2):136–141.

99. Vanneste K, Sterck L, Myburg Z, Van de Peer Y, Mizrachi E. Horsetails are ancient polyploids: evidence from *Equisetum giganteum*. The Plant Cell. 2015; 27:1567–1578. https://doi.org/10.1105/tpc.15.00157 PMID: 26002871

100. Sessa EB, Der JP. Chapter Seven—Evolutionary Genomics of Ferns and Lycophytes. In: Stefan AR, editor. Advances in Botanical Research. 78: Academic Press; 2016. p. 215–254.

101. PPG_I. A community-derived classification for extant lycophytes and ferns. J Syst Evol. 2016; 54 (6):563–603.

102. Ruhfel BR, Gitzendanner MA, Soltis PS, Soltis DE, Burleigh JG. From algae to angiosperms–inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. Bmc Evolutionary Biology. 2014; 14(1):1–27.

103. Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, Soltis DE, et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society. 2016; 181(1):1–20.

104. Yadav RK, Perales M, Gruel J, Girke T, Jönsson H, Reddy GV. WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. Genes & Development. 2011; 25 (19):2025–2030.

105. Tian H, Wabnik K, Niu T, Li H, Yu Q, Pollmann S, et al. WOX5-IAA17 feedback circuit-mediated cellular auxin response is crucial for the patterning of root stem cell niches in Arabidopsis. Molecular Plant. 2014; 7(2):277–289. https://doi.org/10.1093/mp/sst118 PMID: 23939433

106. Han P, Li Q, Zhu Y-X. Mutation of *Arabidopsis BARD1* causes meristem defects by failing to confine *WUSCHEL* expression to the organizing center. The Plant Cell. 2008; 20(6):1482–1493. https://doi.org/10.1105/tpc.108.058867 PMID: 18591352

107. Han P, Zhu Y-X. *BARD1* may be renamed *ROW1* because it functions mainly as a *REPRESSOR OF WUSCHEL1*. Plant Signaling & Behavior. 2009; 4(1):52–54.

108. Zhang Y, Jiao Y, Liu Z, Zhu Y-X. ROW1 maintains quiescent centre identity by confining *WOX5* expression to specific cells. Nature Communications. 2015; 6:6003. https://doi.org/10.1038/ncomms7003 PMID: 25631790

109. Zhou Y, Liu X, Engstrom EM, Nimchuk ZL, Pruneda-Paz JL, Tarr PT, et al. Control of plant stem cell function by conserved interacting transcriptional regulators. Nature. 2015; 517(7534):377–380. https://doi.org/10.1038/nature13853 PMID: 25363783

110. Kramer E. A stranger in a strange land: the utility and interpretation of heterologous expression. Frontiers in Plant Science. 2015; 6:734. https://doi.org/10.3389/fpls.2015.00734 PMID: 26442047

111. Magallón S, Hilu KW, Quandt D. Land plant evolutionary timeline: Gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. American Journal of Botany. 2013; 100(3):556–573. https://doi.org/10.3732/ajb.1200416 PMID: 23445823

112. Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, Hernández-Hernández T. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. New Phytologist. 2015; 207(2):437–453. https://doi.org/10.1111/nph.13264 PMID: 25615647

113. Hu X, Xu L. Transcription factors WOX11/12 directly activate *WOX5/7* to promote root primordia initiation and organogenesis. Plant Physiology. 2016; 172(4):2363–2373. https://doi.org/10.1104/pp.16.01067 PMID: 27784768

114. Santner A, Estelle M. Recent advances and emerging trends in plant hormone signalling. Nature. 2009; 459(7250):1071–1078. https://doi.org/10.1038/nature08122 PMID: 19553990

115. Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM. Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. Proc Natl Acad Sci USA. 2009; 106(38):16529–16234. https://doi.org/10.1073/pnas.0908122106 PMID: 19717465

116. Buechel S, Leibfried A, To JPC, Zhao Z, Andersen SU, Kieber JJ, et al. Role of A-type ARABIDOPSIS RESPONSE REGULATORS in meristem maintenance and regeneration. European Journal of Cell Biology. 2010; 89(2):279–284.

117. Zhao Z, Andersen SU, Ljung K, Dolezal K, Miotk A, Schultheiss SJ, et al. Hormonal control of the shoot stem-cell niche. Nature. 2010; 465:1089–1092. https://doi.org/10.1038/nature09126 PMID: 20577215

118. Pils B, Heyl A. Unraveling the evolution of cytokinin signaling. Plant Physiology. 2009; 151(2):782–791. https://doi.org/10.1104/pp.109.139188 PMID: 19675156

119. Gruhn N, Halawa M, Snel B, Seidl MF, Heyl A. A subfamily of putative cytokinin receptors Is revealed by an analysis of the evolution of the two-component signaling system of plants. Plant Physiology. 2014; 165(1):227–237. https://doi.org/10.1104/pp.113.228080 PMID: 24520157

120. Wang C, Liu Y, Li S-S, Han G-Z. Insights into the origin and evolution of the plant hormone signaling machinery. Plant Physiology. 2015; 167(3):872–886. https://doi.org/10.1104/pp.114.247403 PMID: 25560880

121. Mutte SK, Kato H, Rothfels C, Melkonian M, Wong GK-S, Weijers D. Origin and evolution of the nuclear auxin response system. eLife. 2018; 7:e33399. https://doi.org/10.7554/eLife.33399 PMID: 29580381

122. De Smet I, Voß U, Lau S, Wilson M, Shao N, Timme RE, et al. Unraveling the evolution of auxin signaling. Plant Physiology. 2011; 155(1):209–221. https://doi.org/10.1104/pp.110.168161 PMID: 21081694

123. Rensing S, Lang D, Zimmer A, Terry A, Salamov A, Shapiro H, et al. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science. 2008; 319(5859):64–69. https://doi.org/10.1126/science.1150646 PMID: 18079367

124. Ma Y, Miotk A, Sutikovic Z, Medzihradszky A, Wenzl C, Ermakova O, et al. WUSCHEL acts as a rheostat on the auxin pathway to maintain apical stem cells in Arabidopsis. bioRxiv. 2018:468421.

125. Leibfried A, To J, Busch W, Stehling S, Kehle A, Demar M, et al. WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature. 2005; 438:1172–1175. https://doi.org/10.1038/nature04270 PMID: 16372013