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 maintenance 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-binding 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 functionally 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 TOPELESS (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 branches 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].
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 Phytozone 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

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| Gene  | Expression pattern                                      | Function                                                                 | Reference                        |
|-------|--------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------|
| ArWUS | Shoot apical meristem; anther; ovule                    | Stem cell maintenance; leaf, anther and ovule development                | [8, 9, 18, 32–34]                |
| ArWOX1| Leaf primordium; procambial tissue                     | Leaf, sepal, and petal development                                       | [34, 35]                         |
| ArWOX2| Eggs; zygote; apical embryo domain                     | Embryo patterning                                                        | [36]                             |
| ArWOX3| Leaf primordium; floral primordium; sepal and petal primordia | Leaf, sepal, and petal development                                       | [34, 35, 37]                     |
| ArWOX4| Inflorescence stem; leaf primordium; flower            | Procambial development                                                   | [38, 39]                         |
| ArWOX5| Root apical meristem                                   | Stem cell maintenance                                                    | [21]                             |
| ArWOX6| Seedling; ovule primordium                             | Ovule development; freezing tolerance                                   | [40, 41]                         |
| ArWOX7| Root apical meristem                                   | Stem cell maintenance; sugar response                                   | [42]                             |
| ArWOX8| Zygote; basal embryo domain                            | Embryo patterning                                                        | [36, 43]                         |
| ArWOX9| Zygote; basal embryo domain                            | Embryo patterning                                                        | [36, 43]                         |
| ArWOX10| Unknown                                                | Unknown                                                                  | [44]                             |
| ArWOX11| Root apical meristem                                   | Root organogenesis                                                       | [44]                             |
| ArWOX12| Root apical meristem                                   | Root organogenesis                                                       | [44]                             |
| ArWOX13| Root; inflorescence                                    | Root development; floral transition                                     | [45]                             |
| ArWOX14| Root; inflorescence                                    | Root development; floral transition                                     | [45]                             |

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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. Moellendorfii, A. trichopoda, A. coerulea, and Arabidopsis were retrieved from Phytozone 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 moellendorfii, Marchantia polymorpha, Physcomitrella patens, and M. pusilla) from Phytozone 12.1 by using the BLAST + package with tBLASTn algorithm. The retrieved 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 Trebouxiaria 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 WOX11/12 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
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 WOX3 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) syntenic 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, the \( \text{z} \) WGD 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 WOX11/12 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 lyco- phytes and ferns, including a paleopolyploidization in the most basal euphyllophytic lineage to
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 intercellular 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 eukaryotes 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-diverging 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 cytokinin 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
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design to decipher how and when 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.

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.

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.

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.

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.

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
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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.

S1 Table. Proteins included in the dataset WOXaa.

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

S3 Table. Synteny analysis of Arabidopsis WOX genes.

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

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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. Haecher 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):657–666. 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 A, Henrichs 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, Haecher A, Mayer KFX, Juergens G, Laux T. The stem cell population 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 predated 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 interactions 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: 11163165

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.109.080162 PMID: 19897670

20. Kamiya N, Naganishi 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, Lujten 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: 17769306

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, Billou 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-dependent states. Plant Molecular Biology. 2005; 57(5):613–628. https://doi.org/10.1007/s11103-005-0955-6 PMID: 15988859

26. Galinha C, Hofhuis H, Luijten M, Willemsen V, Billou 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. AID 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

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 AINTEGUMEN TA 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. 2008; 320:154–159. 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. Breuning 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 meristic 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/10.1105/tpc.109.149461 PMID: 20044540

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, Stirn 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 homeodomain 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-Nico C, Claissé G, Tharreau 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/msi125 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. Sympleiomorphies 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(1793):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, Sohliberg 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.plaph.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. https://doi.org/10.1007/s11434-016-0581-0

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 signaling. BMC Evolutionary Biology. 2009; 9:126.

59. 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

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.plants.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, Dharmawardana 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, Jouanneau 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/s41467-018-02356-2 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 K, Tsuge T, Aoyama T, Oka A. ARR1 and ARR2 response regulators specify the shoot stem cell niche by dual regulation of the Arabidopsis response Regulators. The Plant Journal. 2000; 24(6):703–711. https://doi.org/10.1046/j.1365-313x.2000.00909.x PMID: 11135105
76. Meng WJ, Cheng ZJ, Sang YL, Zhang MM, Rong XF, Wang ZW, et al. Type-B ARABIDO PSIS RESPONSE REGULATORS specify the shoot stem cell niche by dual regulation of the WUSCHEL. The Plant Cell. 2017; 29(6):1357–1372. https://doi.org/10.1105/tpc.16.00579 PMID: 28576845
77. Zubo YO, Blakney 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.106.00579 PMID: 28576845
79. Xie M, Chen H, Huang L, O’Neill 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, Pfeiffer 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. 2016; 7(12):3286–3298. https://doi.org/10.1093/gbe/evv1035 PMID: 26606340

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, Burglin 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: 19734299

96. Jiao YN, Wickett NJ, Ayyampalayam S, Chandrabal 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. Mukherjee K, Brocchieri L, Burglin 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: 21478875

98. 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.

99. 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.

100. Sessa EB, Der JP. Chapter Seven—Evolutionary Genomic Analyses 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. Chennathoo M, Christenhusz MJM, Forster K, Soltis DE, Soltis PS, Wu GR. The angiosperm phylogeny. Systematic Botany. 2016; 41(4):967–1010. https://doi.org/10.1600/0363-6445-41.4.967

103. Yadav RK, Perales M, Grue J, Girke T, Jonsson 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/ssu118 PMID: 23939433

106. Han P, Li Q, Zhu Y-X. Mutation of Arabidopsis BARD1 causes eristem 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 renamedROW1 because it functions mainly as a REPRESSOR OF WUSCHEL. 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. Magallo n 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. Magallo n 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. Sultikov Z, Medzhidovski A, Wenzl C, Emakova O, et al. WUSCHEL acts as a rheostat on the auxin pathway to maintain apical stem cells in Arabidopsis. bioRxiv. 2018:468421.

119. 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