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Opinion

Recruitment of IC-*WOX* Genes in Root Evolution

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Root evolution has resulted in the extant bifurcating roots in lycophytes, adventitious/lateral roots in euphyllophytes (ferns and seed plants), and primary roots in seed plants. Here, we hypothesize a role for intermediate-clade-*WUSCHEL-RELATED HOMEOBOX* (IC-*WOX*) genes in root evolution. IC-*WOX* might not be specifically involved in lycophyte bifurcation rooting. In the fern *Ceratopteris richardii*, IC-*WOX* is expressed in adventitious/lateral root founder cells. In the seed plant *Arabidopsis thaliana*, there are two IC-*WOX* subclades, *AtWOX11/12* and *AtWOX8/9*, in adventitious and primary root founder cells, respectively. Thus, IC-*WOX* was recruited in the common ancestor of ferns and seed plants for adventitious/lateral root organogenesis and evolved into two subclades in seed plants: one was retained in adventitious root organogenesis, while the other was recruited for primary root organogenesis.

Highlights

Three root-evolution events were proposed during vascular plant evolution, giving rise to the extant bifurcating roots in lycophytes, adventitious/lateral roots in euphyllophytes (ferns and seed plants), and primary roots in seed plants.

IC-*WOX* was recruited to the adventitious/lateral root founder cells in ferns.

IC-*WOX* evolved into two subclades in seed plants: one subclade was retained in the adventitious root founder cell and the other was recruited to the primary root founder cell.

Introduction to Root Evolution in Vascular Plants

In evolution, the appearance of vascular plants was a great step during the colonization of land by plants [1–3]. Then, vascular plants evolved into several lineages, and two of them survive today: lycophytes and euphyllophytes [3–11] (Figure 1A). The two lineages diverged more than 400 million years ago [3]. Lycophytes, such as *Selaginella* (spike mosses), have bifurcating branches (also called dichotomous branches or dichopodial branches) and microphylls [3]. Euphyllophytes include ferns and seed plants. The evolution of roots in vascular plants was a step toward the successful adaptation to different environments, because they anchor plants and allow for retrieving soil nutrients [5,12–14]. In this opinion article, we summarize recent studies on *WUSCHEL-RELATED HOMEOBOX* (*WOX*) genes and provide our view on the relationship between *WOX* evolution and root evolutionary events.

Three Root-Evolution Events

Based on the fossil evidence and the root anatomy of extant vascular plants, there were separate root-evolution events [12,13]. The first root-evolution event was in the lycophytes, which had evolved roots by the Early Devonian [12–14] (Figure 1A). At that time roots were not observed in other vascular plants. The extant lycophytes usually produce bifurcating roots, in which the division of the root apical meristem gives rise to independent autonomous twin root meristems [3,15] (Figure 1B). The second root-evolution event was in the ancestor of the euphyllophytic lineage, which evolved roots until the Middle Devonian [12,13] (Figure 1A). Thus, root organogenetic events might be independent in lycophytes and euphyllophytes [12,13]. The extant ferns usually have adventitious roots and lateral roots, but do not have a primary root [16,17] (Figure 1C). The third root-evolution event might have occurred during the appearance of seed plants (Figure 1A), as indicated by the fundamental evolutionary distinction in which extant seed plants undergo allorhizic rooting behavior (i.e., embryonic root formation from a bipolar embryo). This is different from homorhizic rooting behavior (i.e., all roots originating from the shoot system) found in other vascular plants. Therefore, seed plants have

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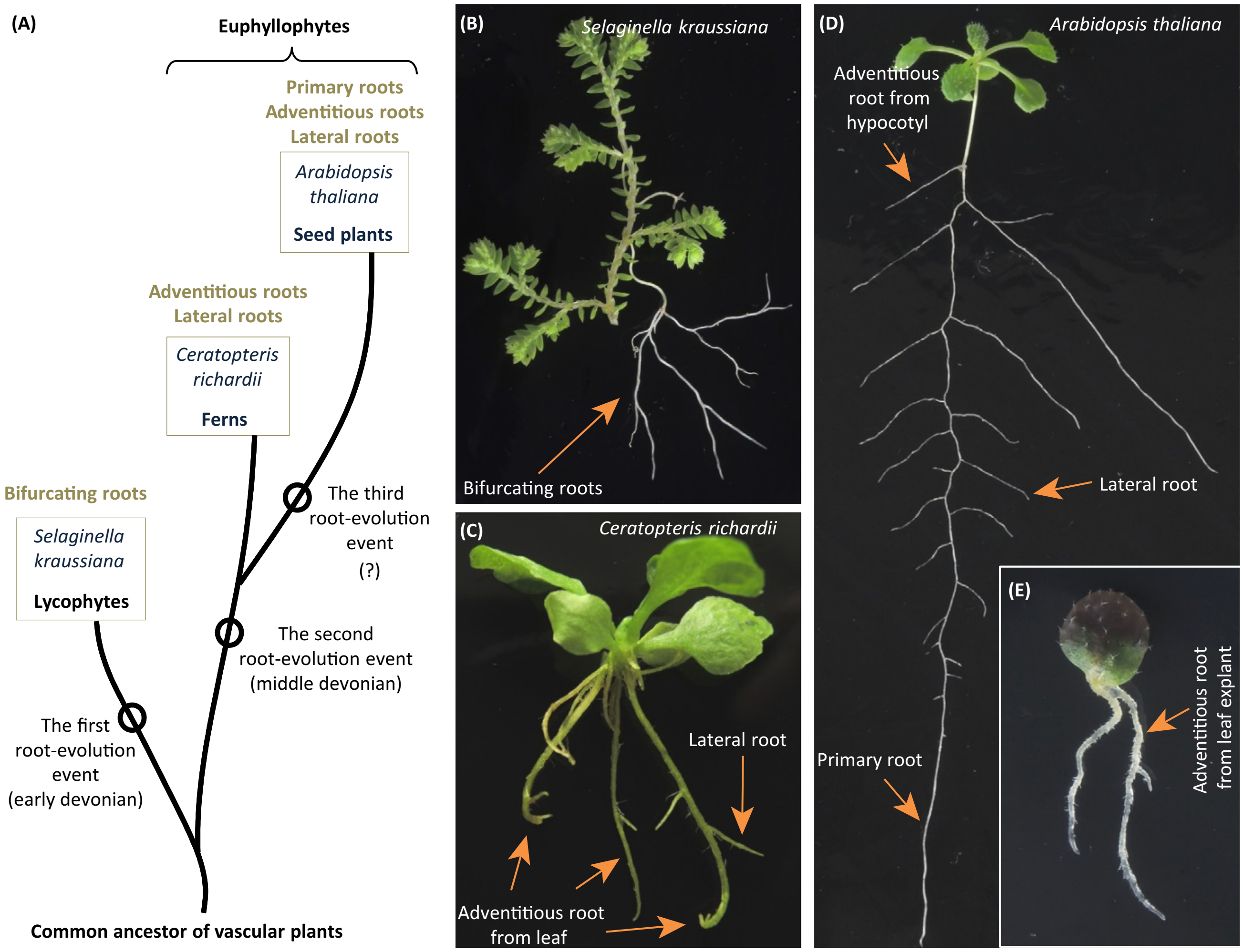
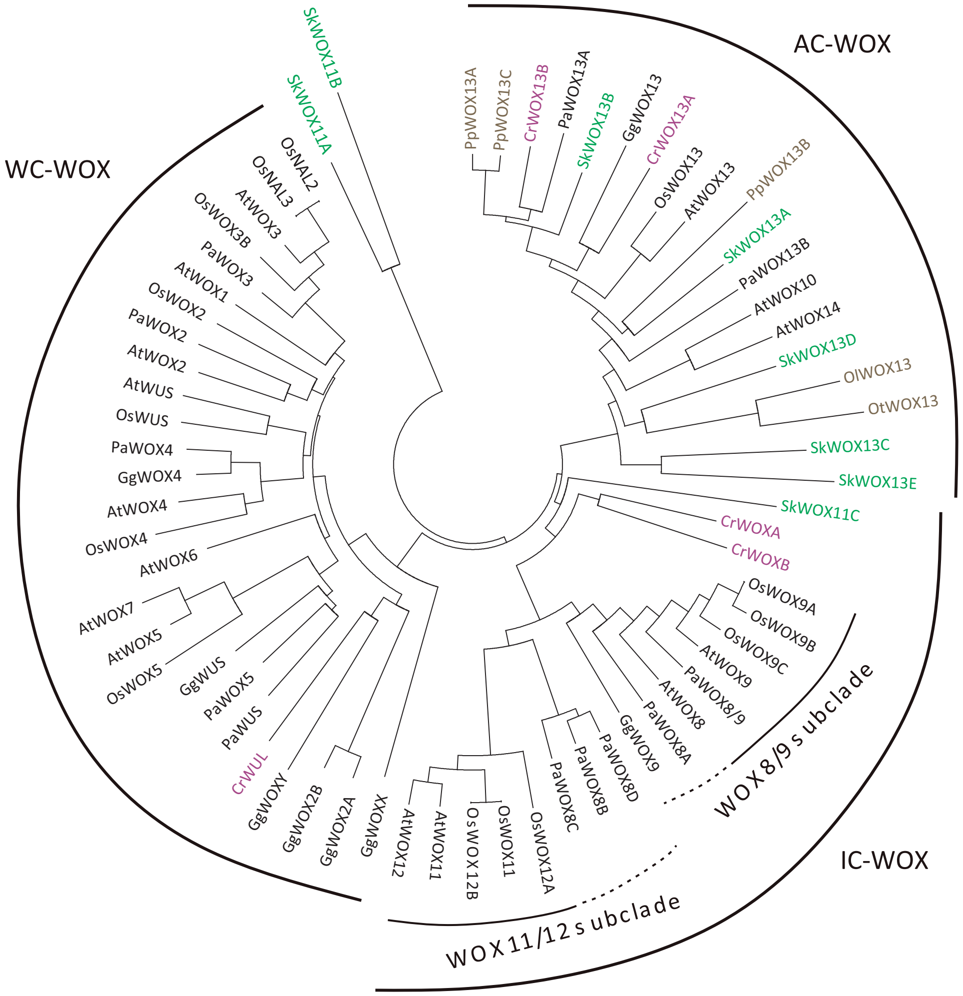


Figure 1. Root Evolution in Vascular Plants. (A) Simplified evolutionary route of vascular plants, showing three root-evolution events in lycophyte and euphyllophyte lineages (black circles). (B–D) Root systems of *Selaginella kraussiana* (B), *Ceratopteris richardii* (C), and *Arabidopsis thaliana* (D and E).

primary roots in addition to adventitious and lateral roots [12,13,18] (Figure 1D,E). Overall, the three root-evolution events gave rise to bifurcating roots in extant lycophytes, adventitious/lateral roots in extant euphyllophytes, and primary roots in extant seed plants, respectively (Figure 1).

IC-*WOX* Expression in Root Founder Cells

In vascular plants, all of the organs are initiated from stem cells. Because *WOX* genes are associated with stem cell functions, the evolution of the WOX family may provide insights into the evolution of plant organs. WOX family genes are largely classified into three clades [19–24] (Figure 2): the ancient-clade-*WOX* (AC-*WOX*) genes, which are present in all green plants; the intermediate-clade-*WOX* (IC-*WOX*) genes, which are found only in vascular plants, including lycophytes and euphyllophytes, but not in non-vascular plants; and the WUS-clade-*WOX* (WC-*WOX*) genes, which are present in euphyllophytes, including ferns and seed plants, but not in non-vascular plants and lycophytes (Figure 2). The evolution and complexity of the WOX family may be associated with the diversification of stem cell types during plant evolution.



Trends in Plant Science

Figure 2. Phylogeny of *WOX* Genes in Plants. Phylogenetic analysis of the homeodomain sequences of WOX proteins, using the neighbor-joining method of MEGA7.0 [40–42]. The sequences were obtained from published data [19–24,26,27,43,44]. *SkWOX11A* and *SkWOX11B* are not classified into the three clades. Subclade classification of the gymnosperm IC-WOX proteins is not clearly determined [27]. WOX proteins from non-vascular plants *Ostreococcus tauri* (OtWOX), *Ostreococcus lucimarinus* (OlWOX), and *Physcomitrella patens* (PpWOX) are labeled in brown. The lycophyte *Selaginella kraussiana* WOX proteins (SkWOX) are labeled in green. The fern *Ceratopteris richardii* WOX proteins (CrWOX) are labeled in purple. WOX proteins from seed plants *Gnetum gnemon* (GgWOX), *Picea abies* (PaWOX), *Oryza sativa* (OsWOX), and *Arabidopsis thaliana* (AtWOX) are labeled in black. MEGA7.0, Molecular Evolutionary Genetics Analysis Version 7.0.

Recent studies suggested that the IC-*WOX* genes could have evolutionarily conserved functions in root organogenesis, which raises the possibility of IC-*WOX* gene recruitment in root evolutionary events. The first IC-*WOX* gene might have evolved in the common ancestor of lycophytes and euphyllophytes [19,22].

In the lycophyte *Selaginella kraussiana*, there is only one IC-*WOX* gene, *SkWOX11C* [19] (Figure 2). However, *SkWOX11C* is expressed ubiquitously in many tissues, including microphylls, rhizophores, shoots, and stems [19]. Thus, *SkWOX11C* might not be a specific gene controlling root organogenesis (Figure 3A). However, whether other *WOX* genes in *S. kraussiana*

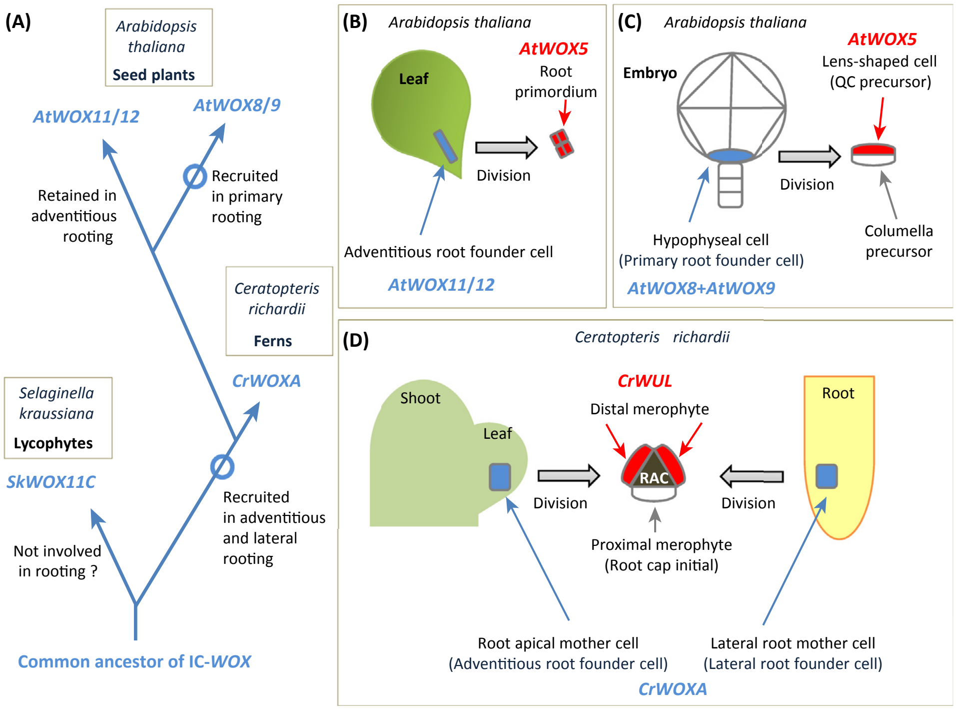


Figure 3. Hypothesis on the Recruitment of IC-*WOX* Genes in Root Evolution. (A) Possible evolutionary route of IC-*WOX* genes showing their hypothesized recruitments in root-evolution events (blue circles). (B and C) Adventitious root (B) and primary root (C) organogenesis in the *Arabidopsis thaliana* leaf explant and embryo, respectively [20,28,29,31,34]. (D) Adventitious and lateral root organogenesis in *Ceratopteris richardii* [26]. Blue cells are IC-*WOX*-mediated root founder cells, and red cells are WC-*WOX*-mediated cells in the root apical meristems (B–D). QC, quiescent center; RAC, root apical cell.

function in root organogenesis is not clear. Expression of *SkWOX13B*, an AC-*WOX* gene, might be closely related to root organogenesis [19]. In addition, diverse root structures and developmental patterns were observed in lycophytes [25], suggesting that root evolution in lycophytes could be complex. Further analysis of the *WOX* genes together with other root-related genes at the molecular and cellular levels will be helpful to analyze their roles in root organogenesis in lycophytes.

In the fern *Ceratopteris richardii*, there are at least two IC-*WOX* genes, *CrWOXA* and *CrWOXB* [26] (Figure 2). *CrWOXB* is widely expressed in many tissues [26]. Interestingly, *CrWOXA* is specifically expressed in root mother cells, also called root founder cells, during lateral and adventitious root initiation [16,17,26] (Figure 3A,D). Specifically, during lateral rooting, an endodermal cell changes its fate to become the lateral root mother cell that expresses *CrWOXA* (Figure 3D). During adventitious rooting from the leaf, a hypodermal cell becomes the root apical mother cell that expresses *CrWOXA* (Figure 3D). *CrWOXA*'s expression is no longer detected when the root mother cell changes its fate, through division, to become the root apical cell [26] (Figure 3D). Therefore, *CrWOXA* appears to specifically define the root mother (founder) cell.

In seed plants, the IC-*WOX* genes evolved into two subclades: the *WOX8/9* subclade and the *WOX11/12* subclade [20,22,24,27] (Figures 2 and 3A). In the seed plant *Arabidopsis thaliana*, the *WOX11/12* subclade genes *AtWOX11* and *AtWOX12* are specifically expressed in the adventitious root founder cell during rooting from detached leaf explants [28–30] (Figure 3B). The expression levels of *AtWOX11* and *AtWOX12* decrease after the division of the adventitious root founder cell to become the root primordium [28,29,31]. The *WOX11/12* subclade was also reported to be involved in adventitious rooting in rice (*Oryza sativa*) and poplar (*Populus deltoides* × *Populus euramericana*) [32,33]. However, *AtWOX11* and *AtWOX12* are not expressed in the lateral root founder cell when plants are vertically grown on medium [30]. The *A. thaliana WOX8/9* subclade genes *AtWOX8* and *AtWOX9* are coexpressed in the hypophyseal cell (hypophysis) of the embryo [20,34] (Figure 3C). The hypophyseal cell's division gives rise to the quiescent center precursor (i.e., lens-shaped cell) and the columella precursor (Figure 3C). Therefore, the hypophyseal cell might be regarded as the founder cell of the primary root. The expression levels of *AtWOX8* and *AtWOX9* are no longer detected after the hypophyseal cell's division [20,34].

Overall, IC-*WOX* genes have specific expression patterns in different types of root founder cells of euphyllophytes, suggesting that their molecular functions in root organogenesis might be conserved during the evolution of euphyllophytes. However, there are only expression patterns but no functional evidence of *WOX* genes in either lycophytes or ferns. Further investigation of the *WOX* family at the genetic and molecular levels will improve our understanding of how *WOX* genes are involved in root development in lycophytes and ferns.

Hypothesis on the Recruitment of IC-*WOX* in Root Evolution

Here, we present our hypothesis on the molecular bases of root-evolution events (Figure 3A). IC-*WOX* might not be specifically involved in the first root-evolution event, which resulted in bifurcating roots in lycophytes. The second root-evolution event, which resulted in adventitious and lateral roots in ferns, recruited IC-*WOX* to establish the root founder cells. In ferns, adventitious and lateral root organogenesis might share a very similar process that requires IC-*WOX*. The third root-evolution event, which resulted in primary roots in seed plants, was based on the separation of the two subclades of IC-*WOX*. The *WOX8/9* subclade was recruited to the founder cells of primary roots, and the *WOX11/12* subclade was retained in the founder cells of adventitious roots. Overall, the IC-*WOX* genes were successively recruited to root founder cells during the second and third root-evolution events in euphyllophytes.

An interesting molecular event that follows the root founder cell division is the expression of the WC-*WOX* genes in the daughter cells. In *C. richardii*, the division of the root apical mother cell or lateral root mother cell results in the formation of the tetrahedral root apical cell, which has four division planes that form three proximal merophytes and a distal merophyte (the root cap initial cell), and the *C. richardii* WC-*WOX* gene *WUS lineage* (*CrWUL*) is specifically expressed in the proximal merophytes [26] (Figure 3D). During adventitious rooting from *A. thaliana* leaf explants, the division of the adventitious root founder cell results in the root primordium that expresses the WC-*WOX* gene *AtWOX5* [28,29,31] (Figure 3B). In the *A. thaliana* embryo, the quiescent center precursor, one of the daughter cells of the hypophyseal cell, expresses *AtWOX5* [20] (Figure 3C). Therefore, the formation of daughter cells that express WC-*WOX* after the root founder cell's division might be a conserved cellular mechanism of root organogenesis in euphyllophytes.

Currently, it is not clear why IC-*WOX* genes are not expressed in the lateral root founder cells in *A. thaliana* [30]. It is possible that the lateral root formation mechanisms are not conserved between seed plants and ferns. In *C. richardii*, the lateral root is initiated from the endodermal cell, while in *A. thaliana*, the lateral root is initiated from pericycle cells. In addition, a single endodermal cell may serve as the founder cell to initiate lateral rooting in *C. richardii*, while the asymmetric division of several pairs of founder cells is required for lateral root initiation in *A.*

thaliana [35–37]. Therefore, one possibility is that lateral rooting in ferns and seed plants might be independent evolutionary events.

After initiation, the formation and maintenance of the root apical meristems might share some similar mechanisms in lycophytes and euphyllophytes [38]. Transcriptome analysis indicated that conserved gene expression programs might be adopted in developing roots of lycophytes and euphyllophytes [38]. This suggests that the molecular network of root growth could be conserved in evolution, although the molecular mechanism for root initiation could be different between lycophytes and euphyllophytes.

Concluding Remarks and Future Perspectives

The function of the evolutionarily conserved IC-*WOX* genes in euphyllophytes could be the establishment of the root founder cells, which is the first step during root organogenesis. IC-*WOX* was recruited into root founder cells in the ancestor of euphyllophytes to produce adventitious and lateral roots, and then IC-*WOX* was further developed during the evolution of embryonic root formation in seed plants to produce primary roots. Therefore, the complexity of root types in euphyllophyte evolution could be based on the diversification of IC-*WOX* genes.

There are many questions that remain to be answered regarding IC-*WOX* genes in root evolution (see Outstanding Questions). In addition, many IC-*WOX* genes might not function only in root founder cells but also in other developmental processes [26,33,39]. Thus, it will be interesting to analyze the non-root-related functions of IC-*WOX* in evolution. Furthermore, we have limited information on the expression patterns of *WOX* genes in diverse species. For example, the phylogenetic classification and the developmental role of IC-*WOX* genes in gymnosperms have not been clearly characterized [27], and some of the *WOX* genes are not easily classified into the three clades [19,27] (Figure 2). The investigation of more genes and their functions in the organogenesis of different root types in diverse species, including lycophytes, ferns, gymnosperms, and angiosperms, will help us to test and modify this hypothesis and improve our understanding of the molecular bases of root evolution.

Outstanding Questions

How did the protein structure change lead to the recruitment of IC-*WOX* in the rooting of euphyllophytes?

What is the function of the C-terminal domain, which is present in IC-*WOX* genes from seed plants but not in IC-*WOX* genes from ferns and lycophytes?

How did the changes in promoters lead to the recruitment of IC-*WOX* to diverse types of root founder cells?

What are the common target genes of IC-*WOX* in root founder cells of diverse species?

Why are WC-*WOX* genes expressed in daughter cells after the division of IC-*WOX*-mediated root founder cells?

Were the lateral root-evolution events in ferns and seed plants independent?

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