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Coordination of growth in root and shoot apices by AIL/PLT transcription factors

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Growth at the root tip and organ generation at the shoot tip depend on the proper functioning of apical meristems and the transitioning of meristematic cell descendants from a proliferating state to cell elongation and differentiation. Members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family, a clade of two-AP2 domain proteins, specify both stem cell fate and control cellular progression of stem cell daughter cells toward differentiation. Here we highlight the importance of an AIL/PLT protein gradient in controlling distinct cellular behaviors in the root through the regulation of distinct targets in different parts of the root tip. Within the shoot, AIL/PLT proteins also promote organ growth and inhibit differentiation pointing to conserved roles in meristem function. However, they exhibit unequal genetic redundancy in these functions and do not always act in a purely additive manner. Differences in AIL/PLT regulation and perhaps transcriptional targets in roots and shoots suggest that these growth regulators have adapted to mediate growth control in distinct ways in these organ systems.

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Introduction

Three important separate processes involved in plant growth are cytoplasmic growth, oriented cell division and cell expansion. Coordination of these three processes and the concurrent delay of terminal differentiation are essential for the elaboration of plant architecture. Cytoplasmic growth involves the synthesis of macromolecules which is tightly coupled with cell division or endoreduplication to match DNA and cytoplasmic content. During cell expansion, controlled relaxation of the cell wall and turgor-driven water influx occur independently of cytoplasmic growth and drive enlargement of the vacuole to reach the target cell size. Terminally differentiating cells induce specialized biochemical pathways and elaborate cell wall structures which constrain cell expansion. The resulting conflict between growth and cell division on the one hand and cell differentiation on the other hand during modular growth of roots and shoots is resolved by compartmentalized growth regions, meristems, which continuously develop the 'differentiated' plant body. To ensure continuity of growth, cytoplasmic growth, cell division and cell expansion need to be coordinated while cell differentiation must be delayed. Over the last decade, several molecular networks contributing to the ordered transition to differentiation of plant cells in growth zones have been described. Here we focus on the AIL/PLT two-AP2 domain transcription factor clade [1]. Several other regulatory modules centered around transcription factors are operating in the growth regions of roots and shoots [2–8], and where appropriate we outline emerging connections of AIL/PLT function to these modules.

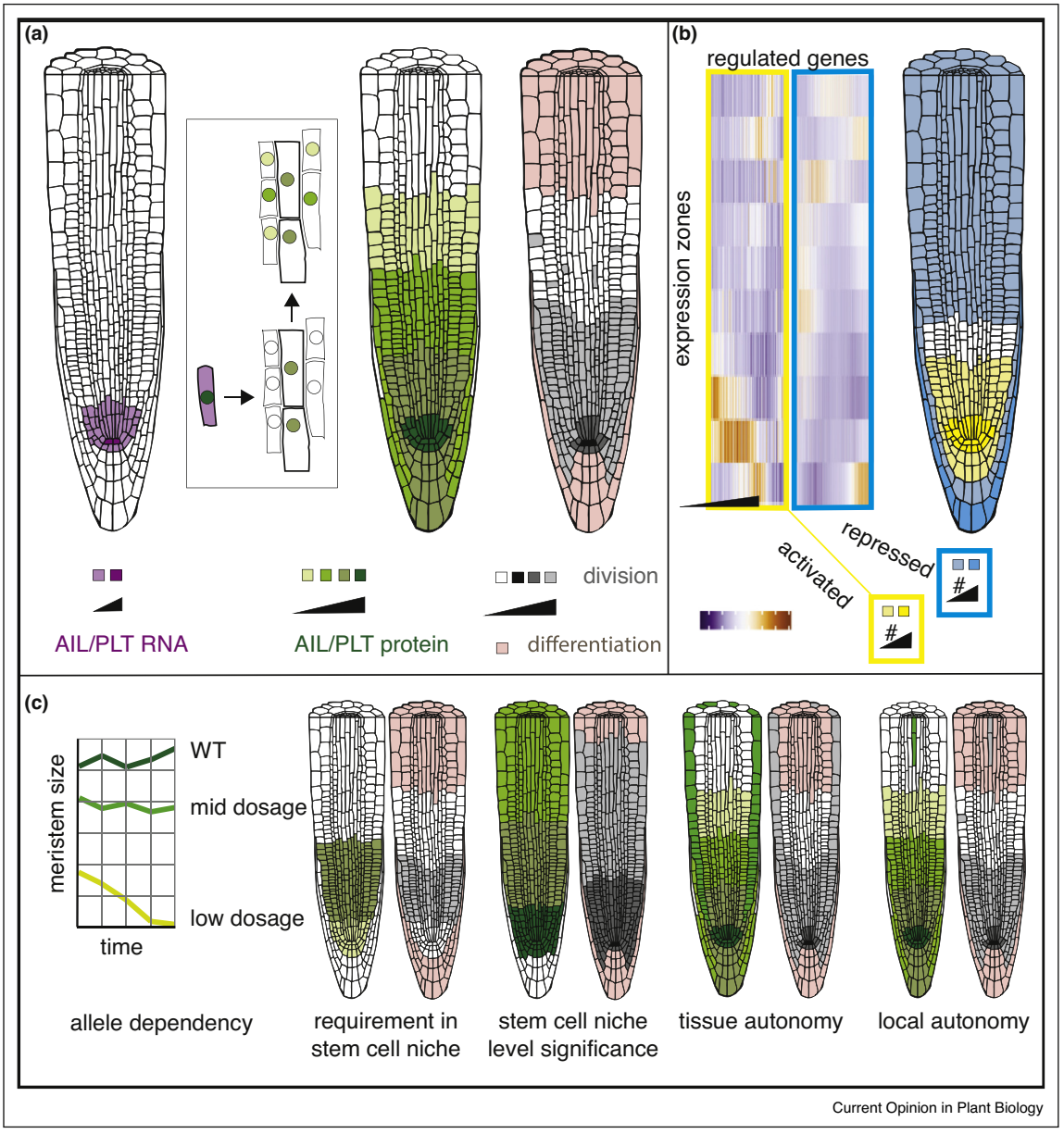
AIL/PLT proteins and coordination of developmental progression in roots

The PLETHORA1 (PLT1) and PLETHORA2 (PLT2) members of the AIL/PLT clade were associated to specific roles in stem cell maintenance because their RNA expression domain was associated with the root stem cell niche, the phenotype of *plt1 plt2* double mutants entailed a specific defect in stem cell maintenance and their overexpression led to ectopic induction of growth regions [9]. Subsequently, further redundancy between PLT1 and PLT2 and other AIL/PLT family members was discovered and complementing AIL/PLT-GFP fusions revealed a graded expression domain extending beyond the stem cells [10] (Figure 1a). Phenotypic analysis of mutant combinations and expression studies demonstrated that stem cell maintenance and cell division regulation were separable (Figure 1c). These data indicated that an AIL/PLT protein gradient in the root built up by joint expression of different AIL/PLT members, regulates stem cell maintenance and cell division in stem cell daughters.

Functional protein gradients

Induced AIL/PLT expression in specific tissue layers and in small cell clones prolonged the cell division state in the expansion and differentiation zone, and an extended AIL/PLT gradient enlarged the slow-division zone associated with the root stem cell niche [11] (Figure 1c). These data

Figure 1



AIL/PLT expression and function in roots. **(a)** Estimated sum of *PLT1*, *PLT2*, *AIL6*/*PLT3* and *BBM*/*PLT4* mRNA levels (purple) and estimated sum of the protein gradients (green) resulting from their translation. Box illustrates gradient formation by mitotic segregation and protein movement. Green scale depicts summed AIL/PLT protein levels. Greyscale depicts division frequency; pink cells depict differentiation. **(b)** Bound and activated targets (yellow) and genes repressed (blue) by root AIL/PLT proteins occupy distinct territories across the root tip [12••]. **(c)** Evidence for functional relevance of the root AIL/PLT gradient. From left to right, distinct *ail*/*plt* allele combinations separate meristem size and meristem maintenance [10]; transit amplifying cells but not stem cells are specified upon complementation by PLT2 in proximal meristem [10]; longer AIL/PLT gradient extends the stem-cell-like domain [11]; epidermal PLT2 expression maintains cell division and inhibits differentiation in that tissue [11]; PLT2 expressing clones maintain division and inhibit differentiation [11]. Green, grey and pink scales as in (a).

indicated that the collective AIL/PLT gradient defines at least two states in a dosage-dependent manner: the stem cell state at high dosage and a cell division state at lower dosage. AIL/PLT levels that promoted cell division were sufficient to inhibit differentiation (Figure 1c). The requirement of AIL/PLT proteins for organ development may be larger than suggested from published root phenotypes, as *plt2 plt4* double mutants display early embryo lethality [10]. Therefore, genetic and molecular epistasis experiments cannot decide whether other stem cell regulators are independent of the AIL/PLT pathway without complete removal of all AIL/PLT members.

How can a collective gradient of partially redundant AIL/PLT transcription factors control different cell states? Analysis of genes directly and indirectly regulated by AIL/PLT transcription factors in roots by ChIP-seq and transcriptome analysis [12••] revealed that upregulated AIL/PLT targets were strongly enriched for genes involved in growth and cell cycle, whereas downregulated targets were enriched for a number of specialized functions correlated with cell expansion and cell differentiation, such as cell wall remodeling enzymes and secondary metabolism pathways, respectively (Figure 1b). Many upregulated targets have a canonical ANT/AIL binding motif close to the transcription start, whereas downregulated targets showed no such enrichment. It is likely that many AIL/PLT bound genes are responsive to multiple AIL/PLT proteins as they contain a canonical binding motif, are activated by multiple AIL/PLT proteins and were identified in ChIP-seq data obtained with different AIL/PLT proteins (PLT2 and BBM/PLT4) and different tissues (roots, somatic embryos) [12••,13]. While ANT differs from other AIL/PLT proteins in a key residue of the DNA binding domain, these data indicate that redundancy in the AIL/PLT clade may in certain contexts encompass the entire clade. Consistent with this notion, in lateral root primordia expressing no AIL/PLT clade member, all AIL/PLT genes could complement the defective outgrowth and patterning of these primordia [14•].

The target gene analysis suggests that AIL/PLT proteins establish a graded cell differentiation trajectory by two mechanisms. For the stem cell and division state, they activate effector proteins for cell growth and division (Figure 1b). For the repression of differentiation, they may promote transcription or activation of repressive transcription factors, which delay the differentiation process. The total AIL/PLT protein level, rather than the mere presence of a subset of AIL/PLT proteins, may be important for cell states such as stem cell, transit-amplifying cell and differentiation. This precludes the use of individual AIL/PLT proteins as stem cell or meristem markers and instead warrants a quantitative assessment of most or all AIL/PLT proteins expressed in a given cell. Interestingly, the identified AIL/PLT targets lack significant overlap with reported targets of the SHORTROOT-SCARECROW-BIRD transcription factor module also involved in maintenance of root stem cells and the division zone [5,15]. Therefore, a combination of targets of diverse transcription factors required for stem cell and cell division potential may contribute to the fine-grained differentiation trajectory of cells traversing the root meristem.

Roles of auxin accumulation upstream of AIL/PLT protein gradients

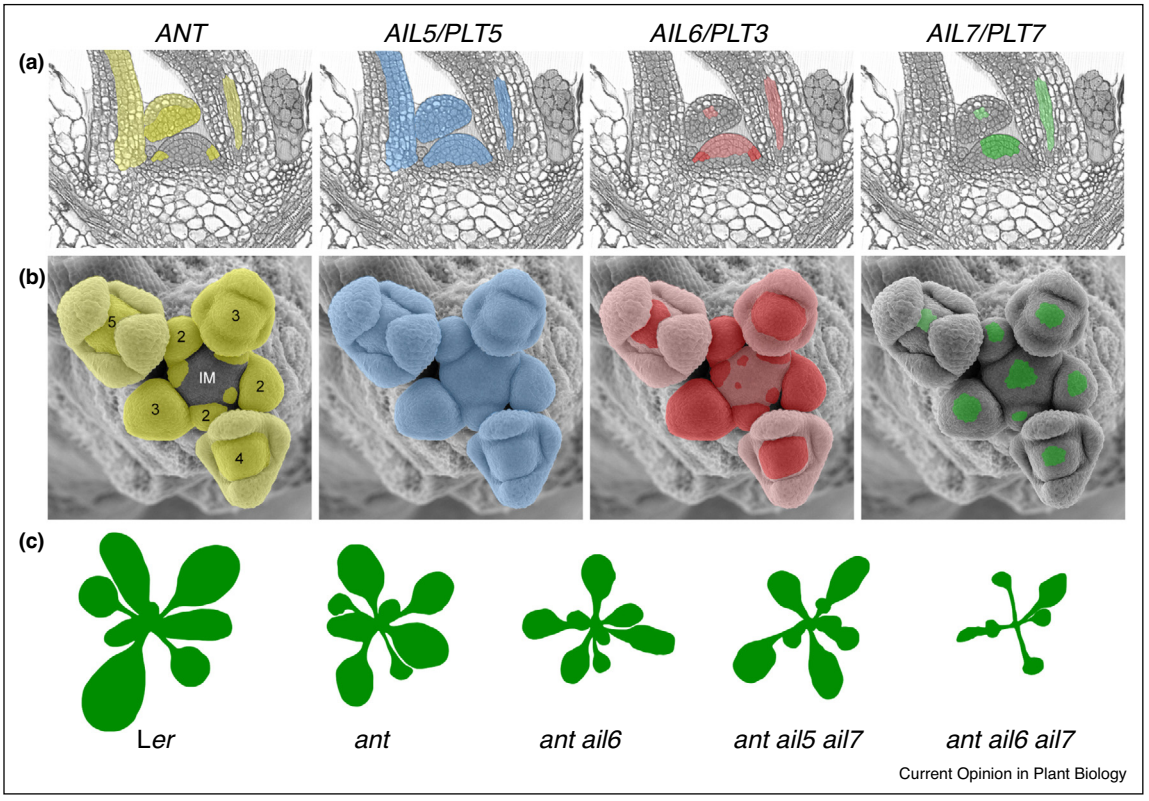
Peak levels of *PLT1* and *PLT2* transcripts correlate well with the position of the root auxin response maximum and transcription of *AIL/PLT* genes in the embryonic root pole depends on two Auxin Responsive Transcription Factors (ARFs), ARF5 (MONOPTEROS) and ARF7 (NON-PHOTOTROPIC HYPOCOTYL4) [9]. Induction of *AIL/PLT* gene transcription in lateral root primordia occurs after auxin response and depends on ARF7 and ARF19 [16]. These observations indicate that auxin accumulation and response defines the spatial domain of *AIL/PLT* transcription in primary and lateral roots. It is noteworthy that auxin-mediated *AIL/PLT* transcription is a slow response, indicating the existence of intermediate ARF-dependent transcription factors which delay AIL/PLT induction [11]. In turn, *ARF5* is a direct target of AIL/PLT transcription factors [12••], suggesting feedback control, and the ARF5 target TMO7, like AIL/PLTs, regulates vascular cell division in roots [17], showing how global and local patterning events are intertwined.

The shape of an auxin gradient in root tips, produced by auxin efflux and influx carriers and local auxin metabolism, has been inferred from reporter gene expression [18]. How can a dynamic and variable auxin gradient steer a progressive and unidirectional process such as the transition from cell division to cell expansion and differentiation? A key observation to understand this is the slow induction time of *AIL/PLT* transcription, which decorrelates auxin and AIL/PLT gradients [11]. The AIL/PLT protein gradient forms by the distribution of AIL/PLT protein to daughter cells during cell division and limited movement of AIL/PLT protein across cells (Figure 1a). In addition, peptide growth factors and their receptors which themselves are PLT targets regulate the span of the AIL/PLT gradient [12••,19••,20]. Gradient formation therefore only has an indirect and delayed dependency on auxin distribution. In its simplest interpretation, this mechanism translates a persistently present auxin maximum into an independent AIL/PLT gradient that establishes developmental boundaries. Fast auxin effects have independent contributions to cell division, expansion and differentiation rates, which have important roles in immediate growth responses [21]. Computational modelling reveals that such fast auxin effects in turn influence the speed and range of AIL/PLT distribution and thereby modify the span of the AIL/PLT protein gradient. Even with this cross-talk, the persistent detection of an auxin maximum by its decoding through local *AIL/PLT* gene transcription and the resulting AIL/PLT gradient is capable of translating a noisy upstream auxin signal, for example during gravitropic response cycles, into a reliable unidirectional differentiation process [11].

AIL/PLT proteins and regulation of shoot development

Differences in *AIL/PLT* gene expression patterns are more dramatic in shoot tissues as compared with the root tip. *ANT* is expressed at much higher levels than *AIL5/PLT5*, *AIL6/PLT3* and *AIL7/PLT7* and each gene shows unique spatial and temporal mRNA expression patterns

Figure 2



AIL/PLT expression and function in shoots. **(a)** *ANT*, *AIL5/PLT5*, *AIL6/PLT3* and *AIL7/PLT7* mRNA expression in the shoot apex of seven day seedlings. Differences in expression levels are indicated with yellow, red and green scales. **(b)** *ANT*, *AIL5/PLT5*, *AIL6/PLT3* and *AIL7/PLT7* mRNA expression in the inflorescence meristem (IM) and developing flowers. Numbers indicate the stage of flower development. Yellow, red and green scales as in (a). **(c)** Size comparison of 20 day old rosettes of wild type (L*er*), *ant*, *ant ail6*, *ant ail5 ail7*, and *ant ail6 ail7*.

(Figure 2a,b) [22–25]. AIL/PLT protein distributions match those shown for the corresponding mRNAs in both vegetative and inflorescence shoot apical meristems. Thus, there is no overall AIL/PLT protein gradient within the shoot apex.

AIL/PLTs regulate both cell proliferation and differentiation in shoot tissues

ANT, a key regulator of organ size in the shoot, is both necessary and sufficient for lateral organ growth and is thought to control the length of the cytoplasmic growth/cell division phase of primordium growth [22,26–28]. Genetic analysis of double and triple *ant ail* mutants have revealed varying contributions of other AIL/PLTs to organ growth. Mutations in *AIL6/PLT3* combined with mutations in *ANT* produce shorter plants with smaller leaves and flowers than *ant* single mutants (Figure 2c) [29]. The contribution of *AIL5/PLT5* or *AIL7/PLT7* on lateral organ growth and plant height are only apparent when combined together with *ant* or in the *ant ail6* background (Figure 2c) [30]. In addition, transgenic plants overexpressing *ANT*, *AIL5/PLT5* or *AIL6/PLT3* result in larger flowers [24,27,31,32].

The molecular mechanisms by which AIL/PLTs promote lateral organ growth remain a mystery. ANT was proposed to regulate cell division via direct regulation of *CYCD3;1* based on its persistent expression in *35S:ANT* plants [28]. However, *CYCD3;1* expression is normal in *ant* mutants, ANT was not able to bind the *CYCD3;1* promoter in a yeast one-hybrid assay, and a steroid activated ANT-GR was not able to induce *CYCD3;1* expression in flowers suggesting that *CYCD3;1* is not a direct ANT target [33•,34]. Another possibility suggested by RNA-seq on the *ant ail6* double mutant is that AILs promote cellular growth via regulation of the mechanical properties of the cell wall. Many genes encoding cell wall modifying enzymes are differentially expressed in the double mutant, and *ant ail6* inflorescences show reduced levels of demethylesterified homogalacturonan (HG), a type of pectin [33•]. HG demethylesterification is required for cell wall loosening associated with flower primordium outgrowth from the inflorescence meristem

[35,36], although the effects of this chemical change on cellular and organ growth are complex and dependent on the local environment [37]. Thus, further work is needed to discern any relevance of the cell wall changes in *ant ail6* to organ growth defects. The activation of cell wall remodeling genes by AIL/PLTs in the shoot contrasts with repression of cell wall biogenesis/organization genes by AIL/PLTs in the root, suggesting a potential rewiring of the mechanisms used by AIL/PLT proteins to promote growth in root and shoot contexts [12••,33•].

Phenotypic analyses of *ant ail* mutants indicate that AIL/PLTs also inhibit cellular differentiation in the shoot. While *ant ail6* flowers produce fewer floral organs and cells within the floral meristem differentiate prematurely, transgenic plants accumulating high levels of *AIL6/PLT3* mRNA produce flowers in which epidermal cells do not terminally differentiate [29,32]. *ant ail6 ail7* triple mutants exhibit termination of the vegetative shoot apical meristem after the production of several leaves [23]. Meristem termination is associated with reduced cell proliferation, loss of the stem cell marker *CLAVATA3* (*CLV3*), and premature differentiation. The molecular mechanisms by which AIL/PLTs promote cell division and inhibit cellular differentiation within the shoot apical meristem have not been elucidated, but the recent identification of AIL/PLT target genes involved in DNA replication and cell cycle regulation suggests candidate genes for testing [12••].

Rather than acting in an additive manner, *ANT*, *AIL6/PLT3* and *AIL7/PLT7* likely have different functions within the shoot apical meristem that together are needed for proper meristem activity. These three genes are expressed in distinct regions within the shoot apex (Figure 2a) and show differential genetic interactions with meristem regulators such as the stem cell promoting factor *WUSCHEL* (*WUS*) [23]. *AIL7/PLT7* is expressed in stem cells in the meristem center, while *ANT* and *AIL6/PLT3* are expressed in the meristem periphery suggesting that meristem termination in the triple mutant may result from the combined disruption of pathways operating in distinct cell populations with the shoot tip.

AIL/PLTs control positioning of lateral organ primordia within the shoot

In addition to maintaining stem cell identity in the meristem center, AIL/PLTs regulate the positioning of lateral organ initiation in the meristem periphery. *plt5 plt3 plt7* triple mutants are delayed in the transition of the vegetative shoot apical meristem from an initial decussate phyllotaxis to spiral phyllotaxis and show a conversion from spiral phyllotaxis within the inflorescence meristem to a metastable distichous phyllotaxis [25,38]. AIL/PLTs promote auxin biosynthesis in the center of the inflorescence meristem, perhaps ensuring a sufficient level of auxin for the regular initiation of flower primordia in a spiral pattern [38]. In flowers, floral organ primordia arise with whorled phyllotaxis, and each primordium is situated at a characteristic position within a whorl. In *ant ail6* flowers, sepal primordia in the outer whorl do not arise in a cross pattern and later-arising primordia show no regular phyllotaxis [29]. Less severe positioning defects are observed in *ant ail5* and *ant ail7* flowers [30]. The absence of phyllotaxis patterns in *ant ail6* flowers differs from the nonrandom changes in phyllotaxis of *plt5 plt3 plt7* and seem unlikely to be a consequence of too little auxin as *ant ail6* flowers show increased expression of an auxin-responsive reporter [33•].

ARF5 activates ANT and AIL6/PLT3 expression at sites of flower initiation

Auxin utilizes different mechanisms for regulation of *AIL/PLT* gene expression in roots and shoots. In inflorescence meristems, ARF5 directly regulates *ANT* and *AIL6/PLT3* to mediate flower primordia initiation [39]. Evidence for this regulation is the spatial overlap of *ARF5*, *ANT* and *AIL6/PLT3* expression within the inflorescence meristem, the induction of *ANT* and *AIL6/PLT3* expression by steroid activation of ARF5-GR, and direct binding of ARF5 to *ANT* and *AIL6/PLT3* regulatory regions. ARF5 also directly binds and activates expression of the floral meristem identity gene *LEAFY* (*LFY*) and together *ANT*, *AIL6/PLT3* and *LFY* promote the initiation of flower primordia from the inflorescence meristem.

Conclusions

It is intriguing that *AIL/PLT* genes regulate cell division and the timing of cell differentiation in both roots and shoots despite utilizing distinct genetic mechanisms to do so. While *AIL/PLT* genes in the root exhibit partial genetic redundancy and largely function in an additive and dose-dependent manner, *AIL/PLT* genes in the shoot largely exhibit unequal genetic redundancy, except in shoot phyllotaxis [25]. In all other aspects of shoot development, *ANT* is the key regulator and *AIL5/PLT5*, *AIL6/PLT3* and *AIL7/PLT7* functions are only apparent when *ANT* function is lost [29,30]. *AIL/PLT* functions within shoot tissues are not always additive, with *AIL5/PLT5*, *AIL6/PLT3* and *AIL7/PLT7* each possessing some unique functions [23,30]. The role of auxin as an upstream regulator of AIL/PLTs is shared in roots and shoots but the mechanisms by which auxin response factors activate their expression appear different. Finally, the molecular mechanisms by which AIL/PLTs carry out their roles in regulating the cell division and differentiation phases of organ development may involve distinct target genes in the root and shoot, perhaps due to the different structures and growth strategies of these organ systems. A more detailed analysis of the AIL/PLT gene regulatory networks in roots and shoots of Arabidopsis and other species will be required to elucidate how evolution positioned these growth regulators in diverse developmental contexts.

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