The Genetic Relationship between Leaf Margin Regulation and Vascular Patterning in Arabidopsis thaliana

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Abstract: Little is understood regarding the development and evolution of leaf shape and vein pattern. In particular, many mechanisms of plant growth and adaptation remain unknown. By reviewing the effects of *ICK1*, an *Inhibitor of Cyclin-Dependent Kinase1*, on cell cycling in *Arabidopsis thaliana* leaf mutants, this study seeks to determine the relationship between leaf marginal serrations and secondary veins in *A. thaliana.* Analyzing the overexpression of *ICK1* in transgenic plants and the genetic pathways that regulate cell division will provide insight into the relationship between cell proliferation and margin development in *A. thaliana* leaves. Research has found that the overexpression of *ICK1* increased the depth of marginal serrations while decreasing the number of serrations, overall leaf areas, total cell numbers, and vein densities. These results suggest that the loss of serrations induced by the inhibition of cell division is directly related to the loss of secondary veins in *A. thaliana* leaves.

Introduction

During the process of embryogenesis, the primary shoot apical meristem (SAM), a region of tissue at the tip of the plant shoot consisting of pluripotent cells, is initiated. The SAM is comprised of the central zone (CZ), in which cells proliferate slowly and tend to remain undifferentiated, and the peripheral zone (PZ), in which cells proliferate rapidly and differentiate into various organs such as the leaves, stem, and flowers (Kalve et al. 2014). *WUS* (*Wuschel*), a homeodomain transcription factor that designates the stem cell niche, is expressed by some cells in the CZ. The slow rate of cell proliferation in the stem cell niche dislocates cells, enabling them to initiate active division. The PZ, on the other hand, gives rise to leaf primordia cells, whose locations are determined by local auxin maxima. The plant hormone auxin and its efflux carrier, *PIN1* (*Pin-Formed1*), modulate the differentiation of the progenitor cells into either lateral appendices or principal axis cells. *PIN1* directs the polarized transport of auxin through the epidermis to neighboring cells of high auxin concentrations (Rodriguez et al. 2014). In *Arabidopsis*, the developing leaves are positioned in a spiral phyllotaxy at approximately 137˚ apart from each other (Rodriguez et al. 2014). Expressed throughout the meristem, the *STM* (*Shoot Meristemless*) gene and other *KNOX1* (*Knotted-like homeobox*) transcription factors also regulate the formation of the leaf primordia by suppressing premature cell differentiation (Rodriguez et al. 2014). Cytokinin (CK), a plant hormone whose biosynthesis is stimulated by *KNOX1* genes, may also play an important role in facilitating the growth and preservation of the SAM, as high levels of CK inhibit cell differentiation to protect cell identity (Kalve et al. 2014).

Separated from the meristem by a boundary zone, differentiating cells within leaf primordia form along the edges of the SAM following germination. The presence of the boundary zone, distinguished by gene expression patterns and few cell divisions, facilitates leaf maturation and preserves the meristem's functions (Wang et al. 2015). Following the establishment of a polarity gradient, primordium outgrowth is activated by intensified cell division. The final shape of the leaf is in part determined by varying rates of cell growth along the developmental axes (Kalve et al. 2014). Leaf primordia may mature into compound leaves consisting of leaflets or simple leaves comprising undivided blades, as in *Arabidopsis*, depending on genetic expressions and environmental conditions (Wang et al. 2015). The establishment of secondary shoot apical meristems in leaf axils, the regions between the leaf stalks and the leaf stems, promote the initiation of new leaf

primordia after embryonic development; this new leaf primordia may then develop into a side-shoot or enter a resting phase. Through the regulation of both the secondary meristem and the side-shoot formations, a plant adapts to its environmental conditions (Wang et al. 2015).

Inhibitors of Cyclin-Dependent Kinases

At its core, the plant cell cycle, along with many other eukaryotic systems, is regulated by *cyclin-dependent kinases* (*CDK*), *cyclins*, and *inhibitors of cyclin-dependent kinases* (*ICK*). The activities of *cyclin-dependent kinases* are vital to the initiation of DNA replication during the G1/S phase transition of mitosis (Kalve et al. 2014). The *CDK* complex plays an integral role in plant development, and research has shown that its mutation results in altered organ sizes and shapes (Mizukami and Fischer 2000). In nature, abiotic stress conditions, such as treatment with abscisic acid or low temperatures, induce *ICK* expression (Wang et al. 2000). At low concentrations, *ICK*s inhibit the *CDK* complex to perpetuate the *CDK* oscillations necessary for DNA duplication, but at high concentrations, cell cycle arrest occurs (Kalve et al. 2014). Along with its specificity for plant kinases and its distinct sequences, this trait distinguishes *ICK1* from animal *CDK inhibitors*, suggesting that *ICK1* represents a divergence of plant cell cycle regulation from other eukaryotes. However, *ICK1* does share some characteristics with *CDK inhibitors* found in other species, such as its inhibitory effects, conserved region, and interactions with both a *CDK* and cyclin (Wang et al. 2000). Seven *ICK* genes have been found to be expressed in *Arabidopsis* tissues. The structure of the plant *ICK* protein sequence consists of a C-terminal domain that interacts with *CDK*s and a region that interacts with D-type cyclins, indicating that *ICK* seek to predominantly interact with complexes containing *CDKA* or *CYCD*. The *ICK* proteins are restricted to the nucleus in *Arabidopsis*, some of which transfer *CDKA* or *CYCD* proteins to the nucleus. It has been postulated that *ICK* regulates both the plant mitotic cycle and the endocycle (Cheng et al. 2013).

Studies in which researchers overexpressed *ICK1* in transgenic plants found that cell proliferation was inhibited throughout the developing plant, leading to transformed morphologies. The decreased cell numbers seen in *35S::ICK1* plants, transgenic plants expressing *ICK1* driven by the *35S* promoter, may be due to fewer cells engaging in mitosis in the meristems, prolonged cell division in the meristems, or a combination of these events (Wang et al. 2000). In addition, *ICK1* expression was found to repress lobe outgrowth when targeted to leaf margins and generate organs with extreme lobing when targeted to sinuses (Malinowski et al. 2011).

The decreased cell numbers in *35S::ICK1* plants affected not only the morphology of the leaves, but also all of the vein orders. The overexpression of *ICK1* led to significantly reduced secondary and minor vein densities, the absence of intersecondary and many higher order veins, and the cessation of most third order veinlets as freely ending. Mesophyll cells expanded to compensate for the reduced cell proliferation, resulting in the early termination of minor vein formation and an abnormal vascular pattern (Kang et al. 2007). These results have significant implications regarding the relationship between marginal leaf serrations, vascular patterning, and the genes that regulate them.

Marginal Serration Formation

The gene *CUC2* (*Cup-shaped cotyledon 2*) patterns serrations and promotes the separation of adjacent organs by suppressing growth in the boundary domain (Kawamura et al. 2010). Specifically, *CUC2* is expressed in a small group of cells at the boundary between the meristem and the emergent leaf primordia, and its expression is augmented by *STM* gene expression (Rodriguez et al. 2014). Double mutants exhibited an abnormal SAM and fused cotyledons. Acting early in plant development, *CUC2* regulates tooth size through cell proliferation at the sinuses primarily as a result of transcriptional regulation, while levels of *CUC2* expression are modulated by *MIR164A*. *CUC2* and *MIR164* promoter activities were acutely inhibited in *cuc2* mutants, suggesting that *CUC2* plays a vital role in the regulation of tooth outgrowth (Hasson et al. 2011).

The plant hormone auxin also defines the location of progenitor cells (cells capable of differentiation and limited division), regulates endoreduplication, and mediates blade expansion at the adaxial-abaxial boundary (Kalve et al. 2014). Auxin maxima, high

concentrations of auxin convergence, are markers of future tooth initiation sites that repress *CUC2* to regulate lamina growth and vein patterning (Kawamura et al. 2010). The convergence point formation of *PIN1* (*Pin-Formed1*) is promoted to produce auxin maxima. *PIN1* is a major auxin transporter, and its *pin1* mutant removes leaf serrations and reduces auxin accumulation at the base of leaves, exhibiting decreased polar transport of auxin (Bilsborough et al. 2010). *PIN1* expression is regulated by *CUC2*, suggesting that a mechanism involving *CUC2*, auxin, and *PIN1* is necessary for serration formation (Kawamura et al. 2010). Auxin has been found to down-regulate *CUC2* to position both *PIN1* convergence points and auxin maxima. It has been posited that *PIN1* convergence points are also regulated by *CUC2* during organogenesis (Bilsborough et al. 2010).

Polarizing Genes

Among the genes that coordinate the formation of auxin maxima is *AS2* (*Asymmetric 2*), a regulator of marginal outgrowth and polarity. Low auxin concentrations are found at the adaxial boundary of leaf primordia as *PIN1* polarity reverses and points towards the SAM, expending auxin and establishing the boundary (Wang et al. 2015). *AS2* functions in the AS1-AS2 complex to temporally repress *ARF3* (*Auxin response factor 3*), an abaxial (lower leaf domain) determinant, and non-repressively regulate *TAS3A*, an adaxial (upper leaf domain) determinant. *ARF3* is also repressed by a ta-siRNA (*trans-acting small interference RNA*) pathway, which influences several nodes of development and enables AS1-AS2 to maintain the flat surfaces and simple margins of the *A. thaliana* leaf. As a result, AS1-AS2 preserves the separation of the adaxial and abaxial domains by binding the promoters of its polarity targets as the patterning of polarity shifts from an external to an internal process (Husbands et al. 2015). The AS1-AS2 complex also suppresses *STM* and other *KNOX1* transcription factors in the leaf primordia through several pathways to separate the meristem from the leaf primordia (Rodriguez et al. 2014).

Regulators of the abaxial domain also contribute to the maintenance of bilateral symmetry through the establishment of leaf development processes. The polarizing gene *FIL* (*Filamentous flower*) determines the identities of cells on the abaxial leaf side. *FIL* is one of six *YABBY* genes found in the *Arabidopsis* genome. *YABBY* genes promote laminar growth, establish communication between growing leaves and the shoot apical meristem, regulate embryo patterning, and convert organ polarity to lamina-specific programs. Furthermore, *YABBY* establishes linear marginal auxin flow to shape modified shoot systems into flat leaves (Sorojam et al. 2010).

Vascular Patterning

In addition to regulating cell cycling and differentiation, auxin coordinates the formation of procambial strand (the precursor to mature xylem and phloem, cells that conduct water and carbohydrates, respectively). The expression of *AtHB8* (*Arabidopsis thaliana homeobox gene 8*), an early marker for vascularization that precedes vein differentiation, is modulated by auxin (Kang and Dengler 2002). During vascular formation, differential cell divisions promote procambial cell growth and arrange strands in a hierarchy of vein orders (Kang et al. 2007). Research has suggested that *AtHB8* may convert auxin signals into procambial strands and heighten tissue responsiveness to auxin (Kang and Dengler 2002). Furthermore, auxin may direct secondary vein formation and regulate serration formation by initiating localized growth (Kang and Dengler 2004).

Conclusion

Research has found that the overexpression of *ICK1* is directly related to the loss of secondary leaf veins in *A. thaliana*. The complex interactions between diverse genetic pathways of other regulators of cell proliferation have also been shown to influence the vascular patterning and morphological development of *Arabidopsis*. *CUC2* governs the patterning of marginal serrations, while polarizing genes such as *AS2* and *FIL* coordinate the establishment of the adaxial-abaxial domain. Furthermore, plant hormones such as auxin play significant roles in early plant development and the formation of leaf shape and vein pattern.

Implications

The implications of further studying these genetic pathways are both extensive and multifaceted. At the cellular level, targeted expression of *CDK* inhibitor

genes such as *ICK1* may be applied in the fields of agriculture, horticulture, and arboriculture to alter individual organs. It is known that mechanisms of cell division are maintained among crop species such as broccoli, cabbage, cauliflower, and kale, which belong to the *Brassicaceae* family along with *Arabidopsis* (Wang et al. 2000). Thus, researching the regulation of plant growth and organ development is essential to achieving a better understanding of crop yield and plant adaptation to both environmental and experimental settings (Kalve et al. 2014). In addition, the results of studying leaf morphology and vasculature may be employed to modify photosynthetic capacities and nutrient transport within plants, respectively. Finally, gaining insight into pathways involving auxin may lead to an enhanced comprehension of leaf development, specifically plant size, root systemization, and apical dominance (Kawamura et al. 2010).

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