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A sister of PIN1 gene in tomato (*Solanum lycopersicum*) defines leaf and flower organ initiation patterns by maintaining epidermal auxin flux

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ABSTRACT

The spatiotemporal localization of the plant hormone auxin acts as a positional cue during early leaf and flower organogenesis. One of the main contributors to auxin localization is the auxin efflux carrier PIN-FORMED1 (PIN1). Phylogenetic analysis has revealed that PIN1 genes are split into two sister clades; *PIN1* and the relatively uncharacterized *Sister-Of-PIN1* (*SoPIN1*). In this paper we identify *entire-2* as a loss-of-function *SlSoPIN1a* (Solyc10g078370) mutant in *Solanum lycopersicum*. The *entire-2* plants are unable to specify proper leaf initiation leading to a frequent switch from the wild type spiral phyllotactic pattern to distichous and decussate patterns. Leaves in *entire-2* are large and less complex and the leaflets display spatial deformities in lamina expansion, vascular development, and margin specification. During sympodial growth in *entire-2* the specification of organ position and identity is greatly affected resulting in variable branching patterns on the main sympodial and inflorescence axes. To understand how *SlSoPIN1a* functions in establishing proper auxin maxima we used the auxin signaling reporter DR5: Venus to visualize differences in auxin localization between *entire-2* and wild type. DR5: Venus visualization shows a widening of auxin localization which spreads to subepidermal tissue layers during early leaf and flower organogenesis, showing that *SoPIN1* functions to focus auxin signaling to the epidermal layer. The striking spatial deformities observed in *entire-2* help provide a mechanistic framework for explaining the function of the *SoPIN1* clade in *S.lycopersicum*.

1. Introduction

In plants, cell fate and subsequent tissue formation are mainly determined by positional information rather than cell lineage. The plant hormone auxin acts as a positional cue for proper patterning in many developmental processes, including embryogenesis (Friml et al., 2003; Cheng et al., 2007), leaf and leaflet initiation (Cheng et al., 2007; Reinhardt et al., 2000, 2003a, 2006; Koenig et al., 2009), vascular patterning (Cheng et al., 2006; Mattsson et al., 1999; Mattsson et al., 2003; Scarpella et al., 2006), root organogenesis (Overvoorde et al., 2010) and flower initiation (Reinhardt et al., 2000; Benková et al., 2003; Heisler et al., 2005). Auxin presence guides the organization of these processes by inducing changes in transcriptional responses and by affecting cell wall physical properties. The multifaceted role of auxin necessitates a coordinated regulation of auxin influx and efflux carriers that guide auxin transport in a polar fashion that together make up the Polar Auxin Transport (PAT) network.

PAT facilitates auxin action to be precisely coordinated in both a localized and concentration dependent manner. Unlike other known plant hormones, auxin is actively transported in a directional fashion, allowing the creation of spatio-temporally regulated auxin concentrations. The largest contributors of directional transport in the PAT system are the PIN-FORMED (PIN) auxin transporters (Gälweiler et al., 1998; Müller et al., 1998; Friml, 2003; Paponov et al., 2005). Most PINs (PIN1, PIN2, PIN3, PIN4 and PIN7) accomplish directional transport by localizing asymmetrically on the plasma membrane of a cell (Vieten et al., 2007), transporting auxin out of the cell in the direction of PIN localization. Auxin, as a weak acid, is freely taken up into a cell; therefore transport of auxin out of the cell by PIN proteins is the determining factor for directional auxin movement (Vieten et al., 2007; Leyser, 2005; Friml, 2010). The cumulative effect of PIN localization at the tissue level is spatial variation in auxin concentration across the developing organ and the generation of small regions of high auxin concentration called auxin maxima (Okada et al., 1991; Guenot et al., 2012; Scarpella et al., 2010).

The understanding of *PIN-FORMED* (*PIN1*) contribution to plant patterning began with the characterization of the *Arabidopsis*

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thaliana (*A. thaliana*) *pin1* (*atpin1*) loss of function mutant (Gälweiler et al., 1998; Okada et al., 1991; Guenot et al., 2012). The defining phenotype of *atpin1* is the presence of radialized "pin-like" structures that are unable to make lateral organs. The *atpin1* phenotype is a consequence of the mutant plants being unable to form auxin maxima required to specify and initiate lateral organs on the flanks of the inflorescence meristem (Gälweiler et al., 1998; Okada et al., 1991). The influence of *AtPIN1* on *A. thalian* a shoot organogenesis varies with developmental age, as the mutant only loses the ability to initiate organs after the floral transition. Prior to reproduction, leaves form on mutant plants, although there are spatial organization problems including aberrant phyllotactic patterning (Gälweiler et al., 1998; Okada et al., 1991; Guenot et al., 2012) and leaf and vein developmental abnormalities. These abnormalities, which increase in severity with each developmental stage (Guenot et al., 2012), clearly illustrate that *AtPIN1* contributes to organ establishment during development.

Reiteration of plant modules is a unifying theme during plant development and understanding the formation of these iterative patterns, especially phyllotaxy, has sparked multidisciplinary interest throughout history. The first molecular marker of leaf organ formation, and thus phyllotactic patterning, is PIN1 localization on the periphery of apical meristems which creates an auxin maximum, marking the site of leaf initiation (Reinhardt et al., 2000; Benková et al., 2003; Heisler et al., 2005). PIN1 predominantly localizes on the L1 (epidermal) layer directing auxin to convergence points, where an auxin maxima is formed and then auxin subsequently becomes directed subepidermally at the site of leaf initiation (Reinhardt et al., 2003a; Heisler et al., 2005). The transport of auxin through the center of a newly developing leaf continues as the tip of the leaf begins synthesizing auxin, further directing vascular tissue differentiation in its wake (Cheng et al., 2006; Mattsson et al., 1999; Mattsson et al., 2003; Scarpella et al., 2006). Vascular patterning begins as broad domains of auxin pathways become increasingly focused through a largely self-organizing process called canalization. This pattern of epidermal PIN1 convergence creating subepidermal auxin localization and further canalization repeats throughout development, establishing many plant reiterative processes including generation of the midvein of leaves (Reinhardt et al., 2003a; Heisler et al., 2005) and leaflets (Koenig et al., 2009), higher-order veins (Scarpella et al., 2010), margin development to form leaf serrations (Scarpella et al., 2006; Kawamura et al., 2010; Hay and Tsiantis, 2006) and floral organ specification (Reinhardt et al., 2000; Benková et al., 2003; Heisler et al., 2005). The importance of auxin in directing leaf development is exemplified by work using leaf developmental mutants illustrating that many genes vital for leaf development interact directly with auxin transport and signaling (Scarpella et al., 2010).

The importance of auxin transport by the PIN transporters in plant development is evidenced by the prevalence of *PIN* genes across the plant kingdom. *PIN* genes have been found in every plant species sampled and in the algal lineage from which terrestrial plants emerged, Charophyta (Křeček et al., 2009; De Smet et al., 2011; Hori et al., 2014). In light of their importance in most developmental processes, *PIN* genes have been described as one of the most important gene families guiding plant developmental evolution and plant colonization on land (De Smet et al., 2011; Cooke et al., 2004; Rensing et al., 2008; Ross and Reid, 2010; Pires and Dolan, 2012; Viaene et al., 2013). Recent phylogenetic analysis of *PIN* genes has revealed that most angiosperm species have multiple orthologs of *AtPIN1*, and recent work is in agreement that *A. thaliana* is rare amongst Angiosperm species, in that the Brassicaceae family has likely recently lost a representative in the "*Sister of PIN1 clade*" (*SoPIN1*) (Bennett et al., 2014; O'Connor et al., 2014; Abraham Juárez et al., 2015). Unfortunately there are only a handful of studies that characterize *PIN* gene function outside the model species *A. thaliana* and only one functional study, in *Medicago truncatula*, describing the *sopin1* mutant *smooth leaf margin 1* (*slm1*) (Zhou et al., 2011a). Conservation of PIN-regulated developmental modules is likely species-specific and the extent of divergence in these modules needs to be addressed by analysis of PIN gene function in other species.

Solanum lycopersicum is a model system for studying shoot organogenesis, owing to the large and easily accessible apical meristem and sympodial mode of shoot growth after floral transition. *S. lycopersicum* has also been used specifically to understand auxin directed developmental mechanisms such as *SlSoPIN1a* protein localization in developing organs (Koenig et al., 2009; Bayer et al., 2009; Shani et al., 2010) and effects of auxin application on organogenesis (Reinhardt et al., 2000, 2003a; Koenig et al., 2009; Naz et al., 2013). Although *S. lycopersicum* is used extensively as a model system for understanding auxin directed development there is little functional work on *PIN* genes within this species. RNAi knock-down experiments are difficult owing to sequence similarity of the target sequences and have yielded limited insight into *PIN1* function in *S. lycopersicum* (Pattison and Catalá, 2012). There are 10 *PIN* genes in *S. lycopersicum*, three of which reside in a highly supported phylogenetic clade with *AtPIN1* (Bennett et al., 2014; O'Connor et al., 2014; Pattison and Catalá, 2012; Nishio et al., 2010).

To determine *PIN1* function in a broader evolutionary context, we analyzed the function of *SoPIN1* outside the limited context of the Brassicaceae member *A. thaliana*. This study identifies *entire-2*, a previously uncharacterized *SlSoPIN1a* loss of function mutant in *S. lycopersicum*. Phenotypic characterization revealed the role of *SlSoPIN1a* in spatial organization during organogenesis and in leaf, flower, and fruit development. Auxin maxima and auxin-induced gene activity were visualized using an auxin inducible promoter-reporter system, DR5: Venus, and showed that *SlSoPIN1a* loss of function causes a broadening of auxin localization in the apical, inflorescence, and floral meristems and at sites of formation of vasculature causing aberrant developmental responses. We conclude that *SlSoPIN1a* regulates auxin patterning by allowing auxin movement in tissue specific cell layers to create a correct spatio-temporal pattern of auxin concentrations needed to guide organ initiation and subsequent morphogenetic processes.

2. Results

2.1. There was a SoPIN1 gene duplication event prior to the diversification of the Solanaceae

In *S. lycopersicum*, there is one true *AtPIN1* ortholog, *SlPIN1* (Solyc03g118740), and two *SoPIN1* genes, *SlSoPIN1a* (Solyc10g078370) and *SlSoPIN1b* (Solyc10g080880) (Fig. 1)(Pattison and Catalá, 2012; Nishio et al., 2010). Previous phylogenetic analysis places both *SlSoPIN1a* and *SlSoPIN1b* genes together on a single branch tip, suggesting a recent *SoPIN1* gene duplication event in the branch leading to *S. lycopersicum* (Bennett et al., 2014; O'Connor et al., 2014). These reports suggested that a duplication event in the *SoPIN1* clade in *S. lycopersicum* occurred roughly sometime after the divergence between *S. lycopersicum* and *Mimulas guttatus* (Bennett et al., 2014; O'Connor et al., 2014). To gain a more precise understanding of the history of the *SoPIN1* clade, we performed phylogenetic analysis on *PIN1* and *SoPIN1* genes sampling Solanaceae more extensively by including *Capsicum annuum, Nicotiana benthamiana, Solanum habrochaites, Solanum lycopersicum, Solanum pennellii, Solanum pimpinellifolium*, and *Solanum tuberosum*. In addition, we included seven other representative Eudicot species (*Arabidopsis thaliana, Citrus*