

Auxin and LANCEOLATE affect leaf shape in tomato via different developmental processes

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Elaboration of complex leaves depends on the morphogenetic activity of a specific tissue at the leaf margin termed marginal blastozone. In tomato (*Solanum lycopersicum*), prolonged activity of the marginal blastozone leads to the development of compound leaves. The activity of the marginal blastozone is restricted by the TCP transcription factor LANCEOLATE (LA). Plants harboring the dominant LA mutant allele *La-2* have simple leaves with a uniform blade. Conversely, leaves of *pFIL >> miR319* are more compound than wild type and grow indeterminately in their margins due to leaf overexpression of *miR319*, a negative regulator of LA and additional *miR319*-sensitive (LA-like) genes. We have recently shown that the auxin-response sensor *DR5::VENUS* marks and precedes leaflet initiation events in the marginal blastozone. Mutations in *ENTIRE* (*E*), an auxin signal inhibitor from the Aux/IAA family, lead to the expansion of the *DR5::VENUS* signal to throughout the leaf-primordia margin, and to a simplified leaf phenotype. Here, we examined the interaction between auxin, *E*, and LA in tomato leaf development. In *La-2* leaf primordia, the auxin signal is very weak and is diffused to throughout the leaf margin, suggesting that auxin acts within the developmental-context of marginal blastozone activity, which is controlled by LA. *e La-2* double mutants showed an enhanced simplified leaf phenotype and *e pFIL >> miR319* leaves initiated less leaflets than wild-type, but their margins showed continuous growth. These results suggest that *E* and auxin affect leaflet initiation within the context of the extended marginal blastozone activity, but their influence on the extent of indeterminate growth of the leaf is minor.

The diversity of leaf forms arises from flexible tuning of a common developmental program. Leaf development has been described by three successive and overlapping stages.^{1,2} At the initiation stage, the leaves arise at the flanks of the shoot apical meristem (SAM). During primary morphogenesis the leaf expands laterally and acquires its principal shape. In the final stage of secondary morphogenesis, the leaf grows substantially, mainly through cell expansion, and the functional tissues differentiate. The elaboration of compound leaves often depends on prolonged morphogenetic activity of a specific region at the leaf margin, termed marginal blastozone. During primary morphogenesis, the marginal blastozone is responsible for the organogenesis of structures such as leaflets and lobes.³ Studies of leaf development have identified several genes and hormones that promote the morphogenetic activity of the marginal blastozone. For example, class I knotted like homeobox (*KNOXI*) genes are important for the maintenance of morphogenetic activity during early stages of compound-leaf development in many species.⁴⁻⁸ Dominant mutations or transgenic overexpression of *KNOXI* genes lead to increased leaf complexity. In tomato, the *KNOXI* gene *TKN2* (also called *LeT6*) was proposed to promote the morphogenetic activity by inhibiting the transition between primary morphogenesis and secondary

morphogenesis. The plant hormone cytokinin was shown to act downstream of *KNOXI* proteins in prolonging the morphogenetic activity of the tomato leaf margin.⁹

Conversely, the tomato CIN-TCP transcription factor LANCEOLATE (LA), and additional *miR319*-regulated CIN-TCPs, were shown to restrict the duration of the marginal blastozone activity by promoting differentiation.¹⁰⁻¹⁴ Precocious elevation of LA expression in the gain-of-function mutant *La-2*, in which the *miR319*-recognition site is mutated, results in early differentiation and simplified leaf form (Fig. 2C).¹⁴ Downregulation of LA-like genes by leaf-specific expression of *miR319* leads to prolonged morphogenetic activity of the leaf margin and to partially indeterminate leaf growth.¹²⁻¹⁴ (Fig. 2D). The plant hormone gibberellin was shown to negatively regulate leaf complexity.¹⁵⁻¹⁹ Recently LA was shown to act in part by modulating gibberellin levels.²⁰

Auxin mediates the positioning of initiating lateral appendage such as leaflets and lobes via the establishment of auxin maxima in the initiation sites.²¹⁻²⁶ In tomato, the Aux/IAA protein *SIAA9/ENTIRE* (*E*) is a specific auxin-response inhibitor that affects fruit and leaf development.^{22,25,27-30} We and others have recently shown that in the leaf, *E* inhibits auxin response between initiating leaflets, which enables the establishment of

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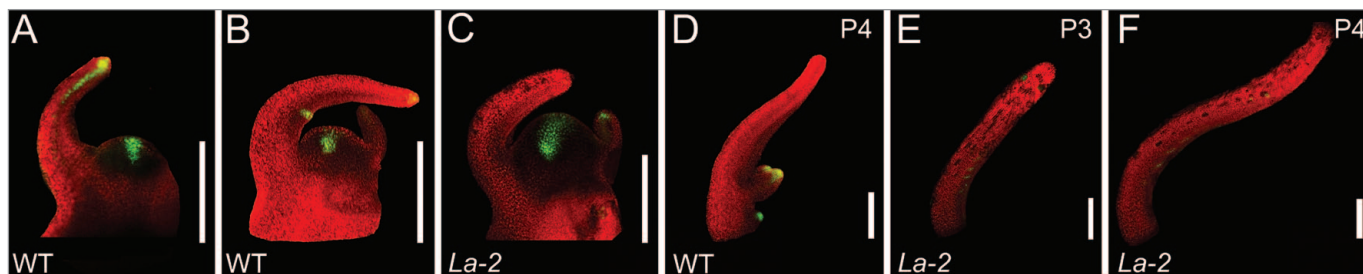


Figure 1. Leaf primordia of *La-2* exhibit weak and diffused auxin signal throughout their margins. Confocal micrographs showing the spatial expression of the auxin signal reporter *DR5::VENUS*. Genotypes are indicated at the bottom left corner of each panel. (A-C) Longitudinal view of the SAM and two young leaf primordia. (D-F) Longitudinal view of leaf primordia. The developmental stage is indicated at the top right corner. Scale bars: 200 μ m.

distinct auxin maxima and leaflet separation.^{22,23,25} In loss-of-function *e* mutants, leaflets initiate but the mature leaf is simplified as a result of expanded auxin response and ectopic blade outgrowth between leaflets (Fig. 2B).^{22,25,31}

We have recently shown that *E* mRNA expression is restricted to the intercalary area between initiating leaflets and that *E* restricts auxin maxima to leaflet initiation sites.²² While these studies show that *E* and auxin are important in leaf patterning, it is still unclear whether they also affect the extent of morphogenetic potential of the leaf margin, or act within the developmental window of morphogenetic potential to determine leaf initiation sites. To address this question, we tested the effect of the *La-2* mutant on the distribution of the *DR5::VENUS* signal and examined the genetic interaction between *e* and genotypes with altered LA activity.

We have recently shown that the auxin response sensor *DR5::VENUS* marks and precedes the initiation of marginal appendages.²² In *La-2* mutants, the *DR5::VENUS* signal appeared at the site of the incipient leaf primordia and in the prevascular tissue of young leaf primordia, similar to wild type (Fig. 1A-C). However, the auxin maxima at the leaflet-initiation sites that are typical to older wild-type leaf primordia (Fig. 1B, D) were lost, and a very weak signal appeared throughout the leaf margin (Fig. 1D-F). This is in accordance with the lack of leaflet initiation in this genotype. The loss of auxin maxima implies that a proper auxin gradient cannot be established in differentiated leaf-margins, and that the auxin maxima form within the developmental context defined by LA.

e La-2/+ double mutants show an enhanced phenotype of a single entire lamina (Fig. 2E), indicating that *E* and LA act through at least partially independent pathways. In *e pFIL >> miR319* leaves, *e* is epistatic to *pFIL >> miR319* with respect to the simple-leaf phenotype and the reduction in leaflet number, but the growth of the leaf margin was not affected by *e* and was indeterminate as in *pFIL >> miR319* (Fig. 2F). This implies

that *LA-like* and *E* affect different aspects of the compound-leaf development: Timing of *LA-like* activity defines the potential to elaborate marginal structures, but *E* is required for specification, localization and separation of these marginal structures. The initiation of leaflets in *e* may indicate that in young leaf primordia the marginal blastozone is active despite the expanded auxin signal. Thus, it seems that the maintenance of a morphogenetic potential in the leaf margin might be partially mediated by the antagonistic activity of gibberellin and cytokinin, and that auxin maxima are involved in the positioning and separation of leaflets within the morphogenetic active tissue.

The previously reported genetic interaction between *e* and *35S::kn1* plants supports these observations. Transgenic tomato plants overexpressing the maize *KNOX1* gene *Kn1* display a range of phenotypic abnormalities, including the formation of super-compound leaves featuring several orders of leaflet reiteration.⁵ In *e 35S::kn1* leaves leaflet number was reduced relative to *35S::kn1*, but the leaflet margin showed prolonged lamina growth.³² These results suggest that distinct programs affect the indeterminacy of the marginal blastozone, the first allows the initiation of leaf marginal appendages at early stages of leaf development and the other enables the growth of the lamina at the leaf margin and the formation of late arising leaflets after leaf expansion. Thus, auxin and *E* appear to affect the specification of marginal outgrowths within a developmental window of morphogenetic potential.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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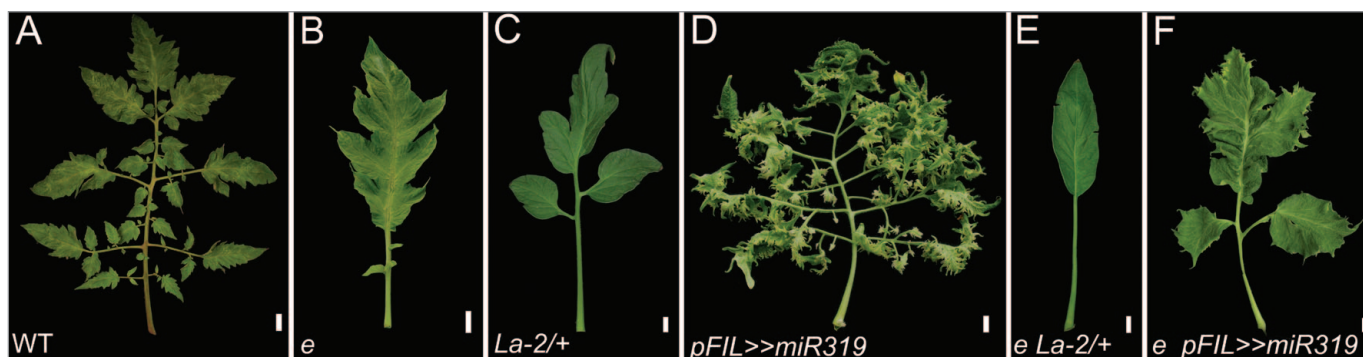


Figure 2. Interaction between *e* and genotypes with altered *LA* activity. Shown are mature fifth leaves of the indicated genotypes. Scale bars: 1cm.

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