

Sl-IAA27 gene expression is induced during arbuscular mycorrhizal symbiosis in tomato and in *Medicago truncatula*

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Aux/IAA genes play a pivotal role in auxin transcriptional regulation. Their functions were mainly studied in *Arabidopsis* through analysis of gain-of-function mutants. In the tomato, the Solanaceae reference species, different studies on *Sl-IAA* down-regulated lines showed specific role for *Sl-IAA* genes. Our recent work revealed that the *Sl-IAA27* gene displays a distinct behavior compared with most *Aux/IAA* genes, being down-regulated by auxin. Interestingly, the silencing of *Sl-IAA27* leads to altered chlorophyll accumulation in leaves, reduced fertilization, altered fruit development and altered root formation. Here we report that *IAA27* could be a key auxin signaling gene involved in AM in tomato and also in *Medicago* model plant. Indeed both *Sl-IAA27* and its closest homolog in *Medicago truncatula*, *Mt-IAA27*, are overexpressed in mycorrhized roots. These data are in line with the putative role of auxin in arbuscular mycorrhization.

The plant hormone auxin has an essential role in plant growth and development processes. Auxin is involved in the regulation of cell growth affecting both cell division and cell elongation and also in specific differentiation events such as embryogenesis, root development, vascular differentiation, apical dominance, gravitropic and phototropic response, fruit set, and fruit development.^{1,2} The auxin signaling pathway is modulated by the interaction of 3 main multigenes family coding for the TIR1/AFB receptors, the Aux/IAA repressors and the auxin response factor (ARF) transcription factors. The binding of auxin to the TIR1/AFB nuclear receptors leads to the degradation of the Aux/IAA proteins via the ubiquitin-proteasome pathway and allows the release of the ARF activators that could activate or repress their target auxin responsive genes.³⁻⁶

In the tomato, a reference species for Solanaceae plants, 25 Aux/IAA genes were identified and the understanding of their function in planta was achieved for 4 of them through the characterization of down-regulated plants.⁷⁻¹⁴ Our recent publication aims with the functional characterization of a specific tomato Aux/IAA gene, the *Sl-IAA27* gene.⁸ Indeed, the *Sl-IAA27* protein harbors the 4 typical Aux/IAA domains but we also identified another motif in the protein that is conserved among

putative orthologous *IAA27* proteins in monocot and dicot species. Moreover, the *Sl-IAA27* auxin expression is atypical being downregulated by auxin treatment whereas the expression of Aux/IAA genes is generally overexpressed by auxin. Interestingly, our functional analysis of *Sl-IAA27* by reverse genetic revealed an implication of this gene in various plant developmental processes. First, the level of chlorophyll content was reduced in leaves of *Sl-IAA27* downregulated lines and was correlated with downregulation of many genes involved in chlorophyll synthesis. Second, the reproductive development of the *Sl-IAA27* RNAi lines was altered at different levels. Indeed, the size of the *Sl-IAA27* RNAi fruits was smaller with an enlarged placenta and the fertility of both ovule and pollen were dramatically reduced compared with wild-type plants. Moreover, the transgenic lines displayed an alteration of root development with an increased primary root length and more lateral roots. Interestingly, *Sl-IAA27* overexpressing plants displayed a reduced primary root growth and no lateral root formation at the opposite of *Sl-IAA27* RNAi lines.⁸

Previous studies have shown that AM symbiosis positively affects tomato plant productivity and tomato phenology. Indeed, mycorrhization accelerates flowering and fruit development, increases fruit yield and enhances the nutritional and

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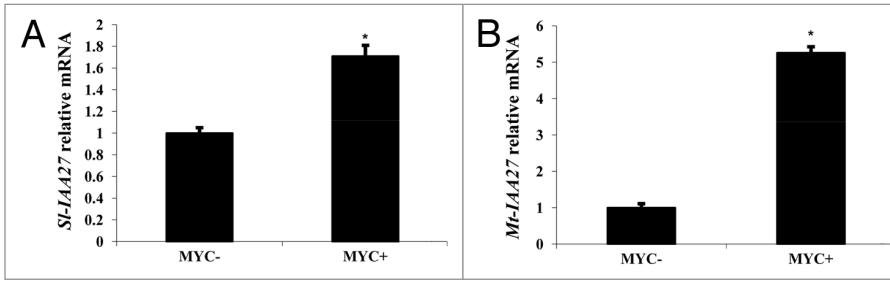


Figure 1. Accumulation of *SI-IAA27* (A) and *Mt-IAA27* (B) transcripts in uninoculated roots (MYC-) and in mycorrhizal roots (MYC+) of 12-week-old tomato plants and 8-week-old *Medicago truncatula* plants, respectively. qRT-PCR analyses were performed according to Laressergues et al.,²³ with n = 6, error bars represent SEM. Stars indicate significant differences when compared with corresponding control, according to the Kruskal–Wallis test ($P < 0.05$).

nutraceutical value of the fruit.^{15–17} Following inoculation with AM fungi, a significant increase in the level of free auxin was observed in different species.¹⁸ However, even though such an increase in auxin levels has not been reported yet in tomato, analysis of mycorrhization in 2 auxin tomato mutants revealed that auxin signaling is required for normal fungal infection.¹⁹ Partial transcriptomic analysis using the TOM2 microarray showed that several genes involved in auxin metabolism, such as GH3-like protein, are differentially expressed in mycorrhized tomato plants compared with the controls.²⁰ All these data suggest a role for auxin during the process of tomato mycorrhization. Since our previous work indicated that modulation of *SI-IAA27* expression impacts root development, and considering that AM symbiosis greatly impacts root development, we investigated the potential effect of mycorrhization on *SI-IAA27* expression. Transcript accumulation of *SI-IAA27* assessed by qRT-PCR revealed a slight but significant upregulation of *SI-IAA27* in tomato mycorrhized roots with a level of expression 1.7 fold higher than in uninoculated roots (Fig. 1A). To uncover whether this induction of *SI-IAA27* expression is a

common feature during AM symbiosis, we also assessed the expression of the putative ortholog of *SI-IAA27* in *Medicago truncatula*, a plant species widely used as a model for the study of the mycorrhization process. We performed TBLASTN analysis on the *M. truncatula* genome using *SI-IAA27* protein sequence (www.plantgdb.org/MtGDB/). This in silico search identified Medtr2g122570.1, here named *Mt-IAA27*, as the closest homolog *SI-IAA27* in *M. truncatula*. The *Mt-IAA27* protein displays 67% of amino acid identity with *SI-IAA27* and presents the specific domain (YxGLS) of tomato Aux/IAA clade B comprising *SI-IAA27* (Fig. 2).⁸ Interestingly, *Mt-IAA27* transcript accumulation is significantly induced in mycorrhized roots with a level more than 5-fold higher than in uninoculated roots (Fig. 1B). The upregulation of *IAA27* expression in mycorrhized roots uncovered in this study in 2 different plant species, strongly suggests a role of this gene during AM symbiosis. These data add putative new functions to Aux/IAA members and open novel opportunities to study auxin signaling during AM symbiosis. Future work will focus on the response of the *IAA27* gene to the Myc-LCOs and COs and its role during AM symbiosis.^{21,22}

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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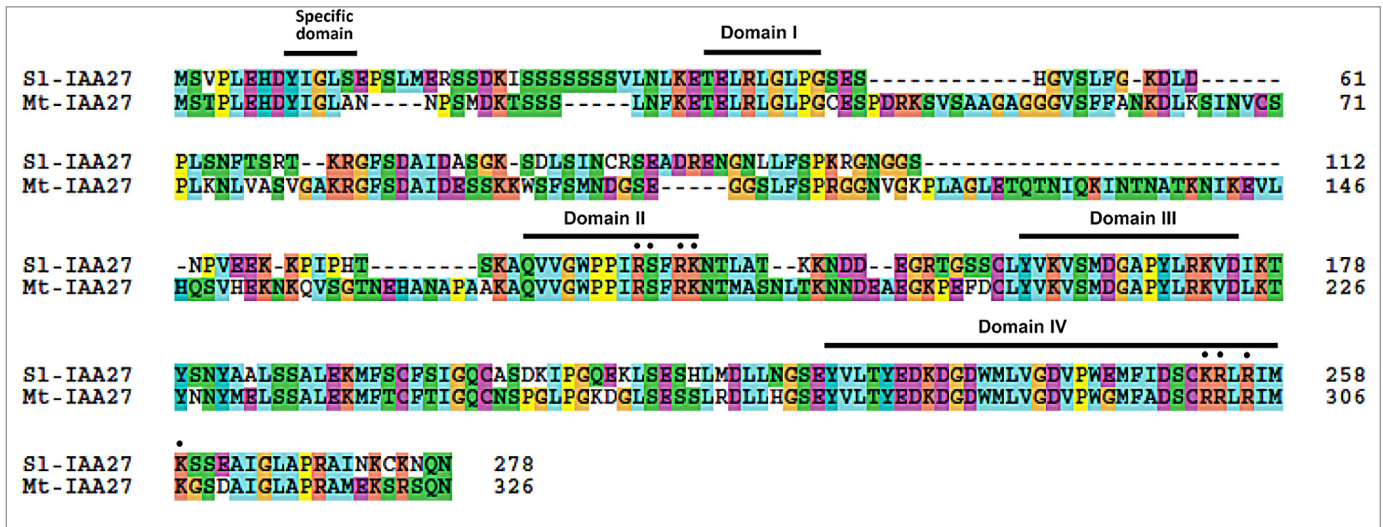


Figure 2. Sequence alignment of *SI-IAA27* protein and its closest homolog in *Medicago truncatula*, *Mt-IAA27* obtained with ClustalX and manual correction. Amino acid residues, part of nuclear localization signal, are indicated by stars. The amino acid position is given on the right of each sequence.

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