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Downregulation of *SlIAA15* in tomato altered stem xylem development and production of volatile compounds in leaf exudates

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The *Aux/IAA* family genes encode short-lived nuclear proteins that function as transcriptional regulators in auxin signal transduction. *Aux/IAA* genes have been reported to control many processes of plant development. Our recent study showed that downregulation of *SlIAA15* in tomato reduced apical dominance, altered pattern of axillary shoot development, increased lateral root formation and leaves thickness. The *SlIAA15* suppressed lines display strong reduction of trichome density, suggesting that *SlIAA15* is involved in trichome formation. Here, we reported that *SlIAA15*-suppressed transgenic lines display increased number of xylem cells compared with wild-type plants. Moreover, the monoterpene content in trichome exudates are significantly reduced in *SlIAA15* downregulated leaves. The results provide the roles of *SlIAA15* in production of volatile compounds in leaf exudates and xylem development, clearly indicating that members of the *Aux/IAA* gene family can play distinct and specific functions.

Auxin plays important roles in many aspects of plant growth and development, including cell division, vascular differentiation, apical dominance, lateral/adventitious root formation, fruit set and development, and embryogenesis.[1] *Aux/IAA* family genes are early auxin-induced genes that encode short-lived nuclear proteins. Aux/IAA proteins can act as transcriptional repressors through interaction with auxin response factor (ARF) proteins. Ubiquitin-dependent degradation of the Aux/IAAs activates ARF, which can be either transcriptional activators or repressors of primary/early auxin-responsive genes.[2]

It has been reported that tomato *AUX/IAA* genes control many processes of plant development. In potato, downregulation of *StIAA2* resulted in altered phenotypes including petiole hyponasty, curvature of developing leaf primordia and increased plant height.[3] In tomato, genome-wide analysis identifies 26 putative *AUX/IAA* genes.[4] Suppression of *SlIAA3* produced auxin and ethylene-related developmental defects, such as reduced apical dominance and exaggerated hook in etiolated seedlings.[5] Downregulation of *SlIAA9* altered apical dominance and leaf architecture, and resulted in parthenocarpic fruit.[6,7] *SlIAA15* was first cloned from tomato fruit using Aux/IAAs family-specific degenerate primers.[8] Expression studies revealed *SlIAA15* transcripts can be detected in all the tissues including roots, stems, leaves, seedlings, flowers and fruit. In a recent publication, downregulation of *SlIAA15* in tomato results in pleiotropic phenotypes including reduced apical dominance, altered pattern of axillary shoot development, increased lateral root formation and increased leaves thickness.[9] Our results indicate that *SlIAA15* has a common role in maintaining growth and developmental processes.

In this work, the effect of downregulation of *SlIAA15* on anatomical characterization of stem was investigated in embedded sections. The *SlIAA15*-suppressed transgenic lines display increased number of xylem cells compared with wild-type plants (WT) (**Fig. 1**). This result indicates that *SlIAA15* is involved in stem xylem development in tomato. It has been reported that auxin is a key signal for stem xylem development.[10] Moyle et al. found hybrid aspen five *Aux/IAA* genes (*PttIAA1*, *2*, *3*, *4* and *8*) are upregulated in xylem. In Arabidopsis, eight of the *Aux/IAA* genes (*IAA19*, *IAA28*, *IAA22*, *IAA2*, *IAA12*, *IAA8*, *IAA13* and *IAA26*) were upregulated in xylem.[12] The data describe the roles of *SlIAA15* gene in xylem development and provide new evidence supporting that xylem formation requires a functional auxin signaling pathway.

Tomato trichomes are a variety of multicellular glandular and non-glandular structures which act as both physical and chemical barriers against biotic and abiotic stresses.[13] Tomato trichomes are categorized as types I to VII, with types I, IV, VI and VII being glandular while types II, III and V are non-glandular.[14] In our recent study, the *SlIAA15*-suppressed transgenic plants display a remarkable reduction in numbers of type I, V and VI trichomes, suggesting that auxin-dependent transcriptional regulation is involved in trichome formation.[9] Since trichome glands are an important site of volatile compounds production,

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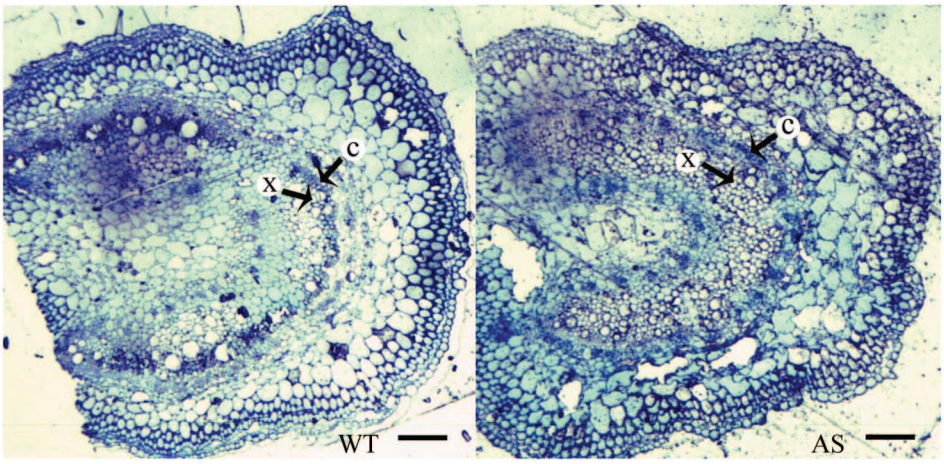

Figure 1. The increased number of xylem cells in *SlIAA15*-suppressed transgenic plants. Samples (fifth internode from top) from ten-weeks-old WT and transgenic plants were embedded in LR white resin. Semi-thin sections were stained with toluidine blue and viewed with the light microscope. WT, wild-type plants; AS, *SlIAA15* downregulated plants; C, Cambia; X, Xylem. Bars, 40 μm.

in this study the content of volatile terpenes in trichome exudates was analyzed by gas chromatography-mass spectrometry (GC-MS) following extraction with 3 mL of *tert*-butyl methyl ether (MTBE) for 5 min. Important differences were found between WT and *SlIAA15*-suppressed transgenic plants in the content of five compounds identified as monoterpenes α-Terpinene, β-Phellandrene, γ-Elemene, β-Caryophyllene and α-Humulene (**Fig. 2**). Notably, these compounds were shown previously to be major volatile components of tomato leaf aroma.[15,16] These data indicate that monoterpene content in trichome exudates are significantly reduced in *SlIAA15* downregulated leaves compared with wild-type leaves.

Li et al.[17] reported that a tomato homolog of CORONATINE-INSENSITIVE1 (COI1), an F-box protein required for JA-signaled processes, positively regulates both glandular trichome development and the production of monoterpenes stored in this structure. In this study, the reduction in monoterpene content of *SlIAA15*-suppressed transgenic plants is consistent with our finding that downregulation of *SlIAA15* expression significantly reduced the density of type I and VI glandular trichomes.[9] We speculate that the reduction of glandular trichomes' density may lead to the low exudation of monoterpene from tomato leaves. This data provide the functions of *SlIAA15* in production of volatile compounds in leaf exudates, clearly demonstrating that members of the *Aux/IAA* genes can play distinct and specific functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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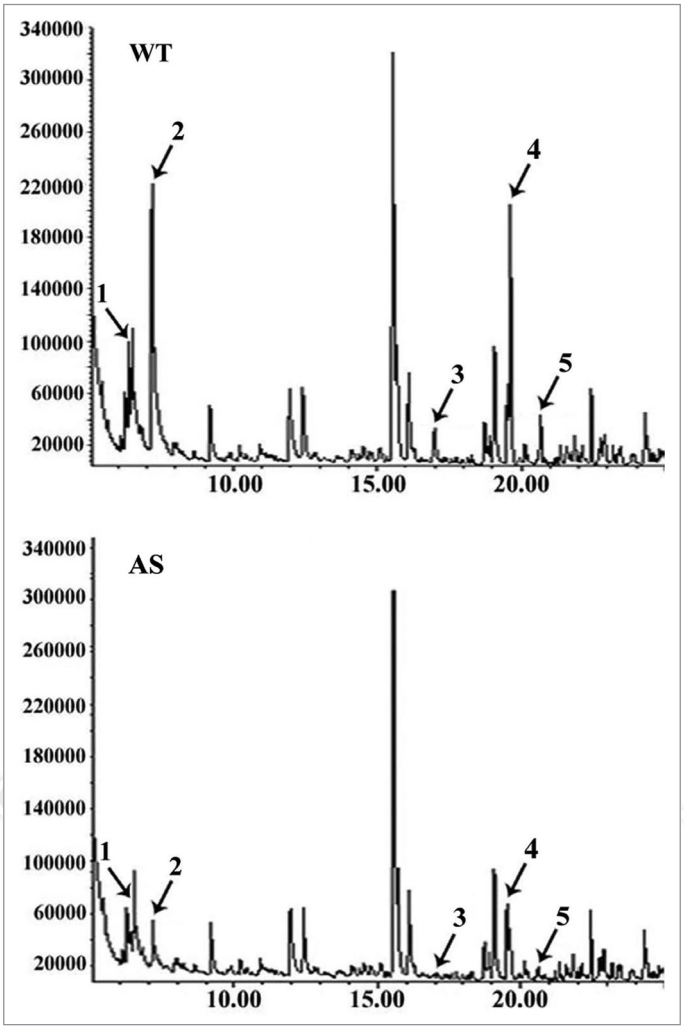

Figure 2. The reduced contents of volatile compounds in leaf exudates of transgenic plants. Gas chromatography-mass spectrometry (GC-MS) was used to measure the young-leaf exudates from ten-weeks-old WT and transgenic plants. The exudates were obtained by brief extraction with tert-methyl butyl ether buffer (MTBE). WT, wild-type plants. AS, *SlIAA15* downregulated plants. Numbers 1–5 represent the different monoterpenes respectively: 1, α-Terpinene, 2, β-Phellandrene, 3, γ-Elemene, 4, β-Caryophyllene, 5, α-Humulene.

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