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Auxin and salicylic acid signalings counteract during the adaptive response to stress

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In a previous publication, we performed a phenotypic characterization of Arabidopsis auxin receptor mutants grown under oxidative and salt stresses. In particular, the double mutant for TIR1 and AFB2 receptors, *tir1 afb2* displayed increased tolerance against salinity measured as germination rate, root elongation and chlorophyll content. Here, it is reported that salicylic acid (SA)-treated *tir1 afb2* mutant shows enhanced transcript level of a pathogenesis related gene, *PR1*. In addition, SA-mediated repression of auxin signaling was also demonstrated. All these findings allow us to suggest that down-regulation of auxin signaling may be a common mechanism within the plant adaptative response against both biotic and abiotic stresses.

Key words: *Arabidopsis thaliana*, auxin signaling, salicylic acid, stress

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The survival of plants to adverse conditions involves the accurate perception and transduction of environmental signals to switch on defense and adaptative responses. In general, the spread of information about environmental changes is through plant hormones, which integrate these stimuli into developmental programmes. Interestingly, auxin widely recognized as a key growth regulator is nowadays emerging as a new player in the defense response against biotic and abiotic stresses.[1,2] Auxin perception is due to members of a small family of F-box proteins, TIR1 and its paralogs AFB1-3.[3-5] Auxin binding to $SCF^{TIR1-AFBs}$ results in the targeted degradation of Aux/IAA transcriptional repressors via SCF E3-ubiquitin ligase proteasome pathway.[6] Thereafter, Aux/IAA degradation promotes activation of Auxin Response Factors (ARFs) and the consequent expression of auxin-responsive genes.[7]

Previously, we demonstrated the participation of TIR1/AFB auxin receptors in the plant defense response against oxidative and salt stresses by a genetic approach. We found that the double mutant, *tir1 afb2,* shows tolerance to salt stresses judged by increased germination rate, root elongation and chlorophyll content. Furthermore, *GST1*, *APX1* and *Zat12* were upregulated in *tir1 afb2* under salinity, suggesting an auxin-mediated negative regulation of the plant defense response against abiotic stress. Hence, in this addendum we explored the expression of the pathogenesis related (PR) marker, *PR1*, as a salicylic acid (SA)-induced gene in *tir1 afb2* mutant plants to get insights on the general role that auxin signaling may play in stressed plants. SA is a key mediator of basal resistance responses against biotrophic pathogens. Thus, ten-day-old *Arabidopsis thaliana* plants grown on ATS agar medium were transferred to liquid ATS medium containing 1 mM SA for 17 h and incubated in the growth chamber.

Figure 1 shows that *PR-1* was induced 3.5 folds in SA-treated *tir1 afb2* compared with SA-treated wild-type plants, indicating that auxin signaling might interfere with SA-regulated *PR-1* induction. Coincidently, *PR-1* is also significantly induced in Arabidopsis mutant plants with reduced IAA levels.[1] Thus, auxin and SA signaling pathways seem to be mutually antagonistic because a negative effect of SA on auxin signaling has been previously reported in reference 8. Multiple auxin-related genes, including

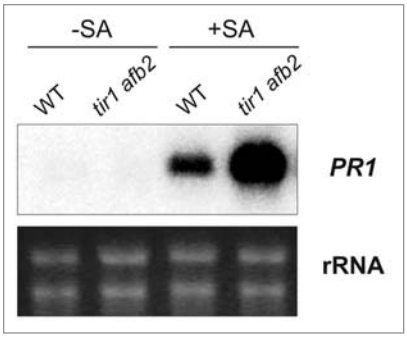

Figure 1. *PR1* expression is upregulated in SA-treated *tir1 afb2* mutant. Ten-day-old *tir1 afb2* and WT seedlings grown in ATS medium were transferred to 1 mM SA treatment for 17 h under 120 μmol photons m^{-2} s^{-1} with 16 h photoperiod at 23°C. *PR1* levels were revealed by Northern blot analysis. rRNA corresponds to ribosomal RNAs.

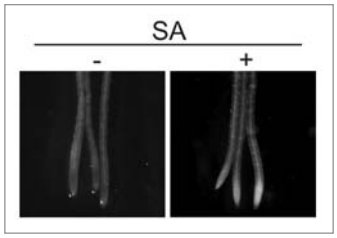

Figure 2. SA triggers repression of TIR1 receptor. Six-d-old *pTIR1:cTIR1-GUS* transgenic seedlings grown in ATS agar medium were transferred into liquid ATS medium supplemented with 1 mM SA and incubated with mild shaking for 6 h in the growth chamber. After treatment, seedlings were stained for GUS activity. Representative roots are shown.

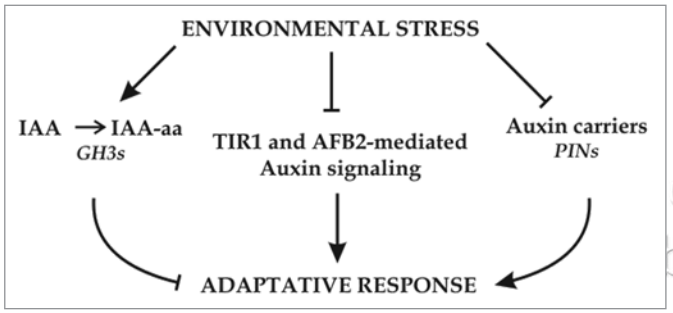

Figure 3. A hypothetical model shows the participation of TIR1/AFB2-mediated auxin signaling during environmental stress. Endogenous auxin pool may be regulated by *GH3* genes which encode auxin-conjugating enzymes. Auxin availability may be also linked to auxin transport by modulating the activity of auxin efflux carriers, PIN2 and PIN3. Repression of *TIR1* and *AFB2* expression may also occur in acclimated plants. Additionally, auxin may negatively regulate stress-responsive genes, such as *PR1*. In conclusion, modulation of growth programme as part of the adaptative plant strategy against both, biotic and abiotic stresses involves downregulation of TIR1/AFB2-mediated auxin signaling, as here described.

TIR1 are downregulated in response to SA. Therefore, we explored TIR1 level in TIR1 translational GUS fusion seedlings, *pTIR1:cTIR1-GUS* after SA treatment. In this line, GUS enzyme is fused to the C-terminal region of TIR1 receptor protein under the control of its natural promoter.[9] Six-day-old *pTIR1:cTIR1-GUS* transgenic seedlings were transferred into liquid ATS medium supplemented or not with 1 mM SA for 5 h and incubated under light and mild shaking. GUS activity was evidenced by the incubation with the substrate X-Gluc at 37°C in the dark.

Figure 2 shows a strong reduction of GUS activity in SA-treated seedlings, indicating a SA-mediated downregulation of TIR1. These data suggest that SA might inhibit auxin signaling by a translational repression of TIR1/AFB auxin receptors during stress. In the same direction, Wang et al.[10] demonstrated that the inhibitory effect on auxin response constitutes part of the SA-mediated disease resistance mechanism in Arabidopsis.

Thus, despite the differences in defense pathways between biotic and abiotic stresses, downregulation of auxin signaling may be considered a common strategy that plants adopt to deal with a broad spectrum of adverse conditions. It should be noted that SA-mediated repression of auxin response may be just one of the diverse molecular mechanisms that may operate throughout auxin in Arabidopsis plants. For instance, the microRNA miR393 which has emerged as a key regulator of *TIR1, AFB2* and *AFB3* is induced in Arabidopsis plants in response to flagellin and *Pseudomonas syringae* as well as under different abiotic stresses, such as cold, dehydration and salinity.[11] Furthermore, Navarro et al.[12] reported that miR393-mediated repression of auxin receptors constitutes a plant strategy to attenuate infection. In addition, different members of the auxin-responsive *GH3* gene family that encode IAA-conjugating enzymes and therefore modulate endogenous auxin pool have been associated to plant defense responses. For example, Arabidopsis mutant, *wes 1-D,* exhibites enhanced tolerance to drought, freezing temperature, salinity and pathogen infection.[1] *GH3.5* is reported as a regulator of both, auxin and SA signalings during plant-pathogen interactions.[13] The activation of different *GH3s* resulted in increased tolerance against pathogens and drought in rice.[14-17] Additionally, auxin availability could be perturbed through an effect on auxin carriers under stress.[18] Not surprisingly, auxin transport is inhibited by flavonoids, one of the plant metabolites synthesized in response to environmental signals.[19] Grunewald et al.[20] revealed that nematodes manipulate expression and subcelullar localization of PINs proteins to facilitate infection in Arabidopsis leaves.

In conclusion, all these findings allowed us to propose a hypothetical model where the adaptative response to biotic and abiotic stress seems to be mediated in part, through the negative regulation of auxin which may include auxin transport, homeostasis and signaling (**Fig. 3**). Reorganization of plant growth and developmental programs are critical to maximize plant survival under stress. Future research will reveal the intrincated mechanisms and switches that plants adopt to develop such highly dynamic responses.

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