Rapid Auxin-mediated Growth Inhibition in *Arabidopsis* root

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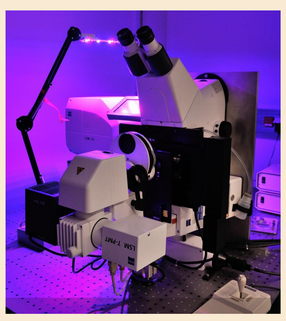
BACKGROUND

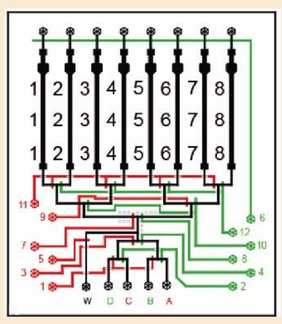
- Phytohormone auxin participates in diverse developmental processes and physiological response. Particularly, auxin regulates growth significantly and faster than other hormones. For decades, it is known that auxin triggers opposite growth response- promotes shoot cell but inhibits root cell growth. How auxin promotes shoot growth has been studied thoroughly and in line with the Acid Growth Theory[1]. However, how auxin inhibits root growth is largely unrevealed.
- By using microfluidics and vertical confocal microscope, auxin was recently discovered to inhibit root growth in 30 s through TIR1 receptor[2], the time frame of which suggests a fast mechanism. Here, we study which cellular processes and further molecular player mediates the rapid response.

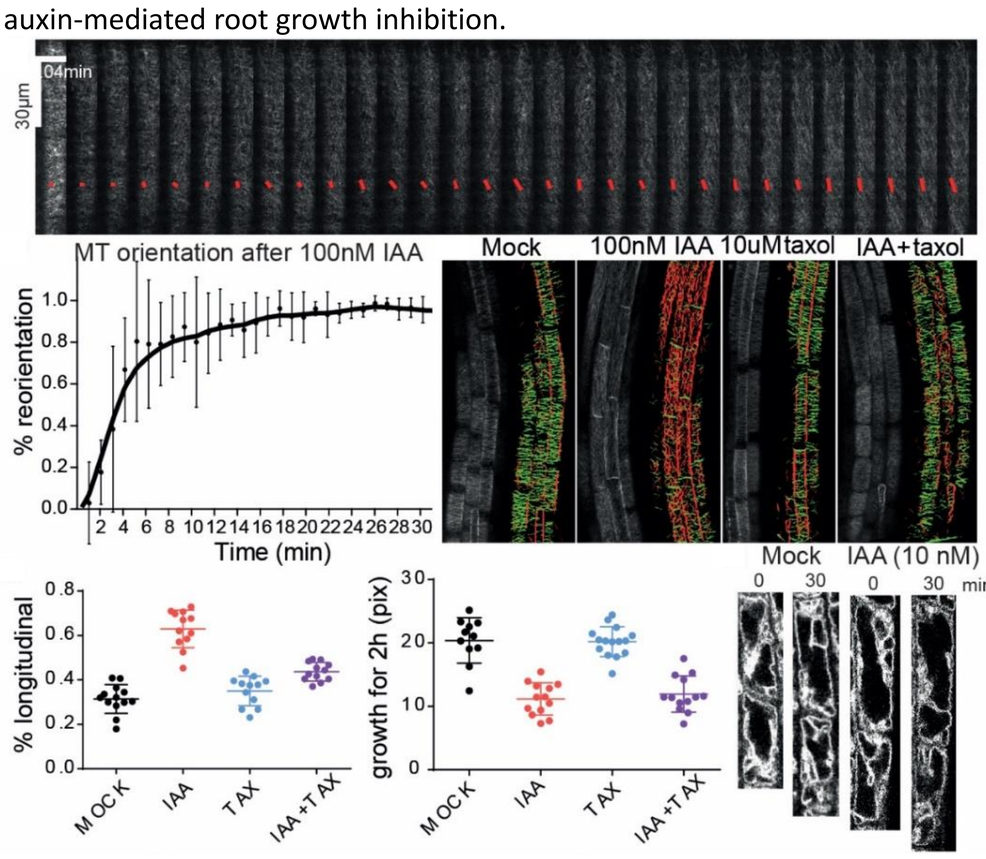
Microtubule and vacuole don't trigger auxin-mediated growth inhibition

- After auxin, cortical microtubule(CMT) reorients from horizontal to longitudinal, and vacuole morphology changes as well. Both cellular processes have been proposed to be the mechanism of auxin-mediated root growth inhibition[3,4].
- We analysed the CMT dynamics after auxin with Eb1b marker line in the microfluidics vRootchip in vertical microscope. The CMT reorientation in elongating cells took > 4 min, longer than growth inhibition takes place. Further, we inhibited auxin-mediated microtubule reorientation by microtubule stabilizer taxol, but auxin-mediated root growth inhibition was unaffected.
- We used the SYP22-YFP marker line and analysed the vacuole morphology in the elongating cells not significantly changed after 30 min auxin.
- Conclusion: CMT and vacuole are not the trigger for auxin-mediated root growth inhibition.

Vertical confocal & Microfluidics

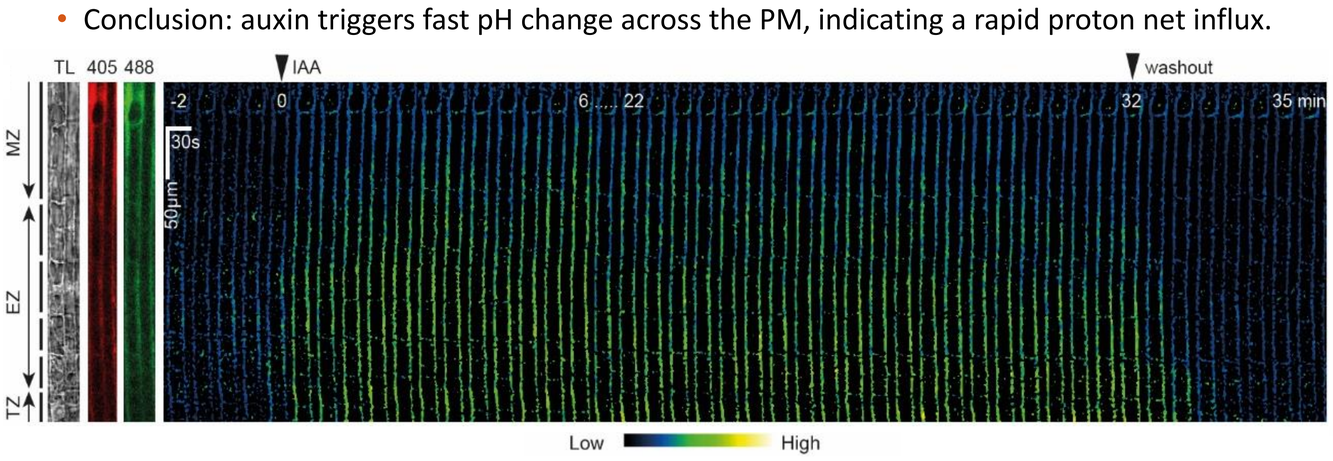


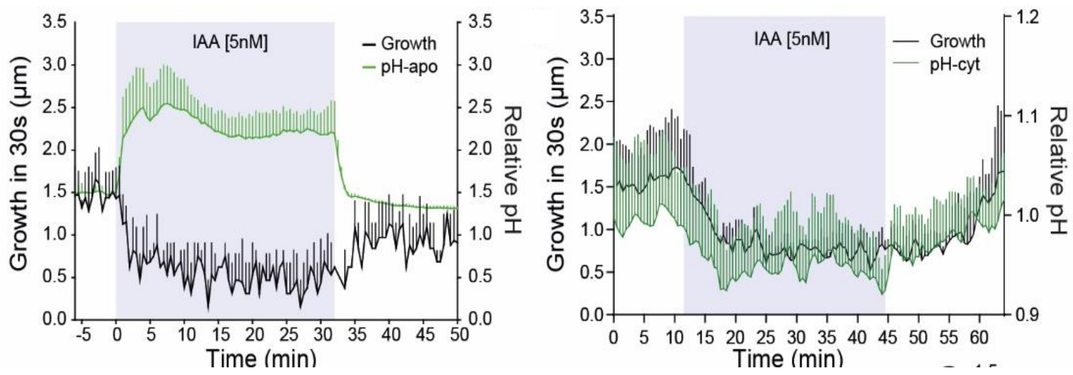




Apoplast alkalization correlates with auxin-mediated growth inhibition

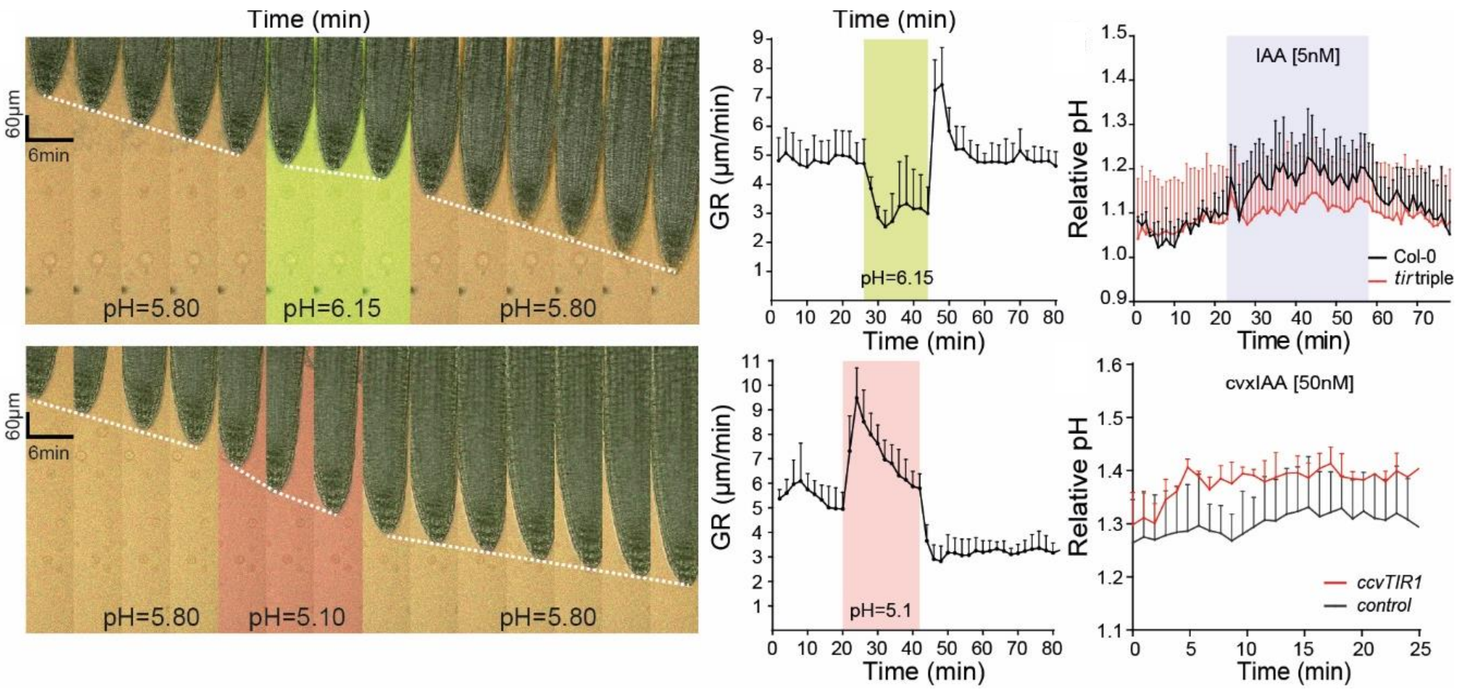
- We analysed the apoplastic pH by HPTS(8-Hydroxypyrene-1,3,6-Trisulfonic Acid)[5] dye in the vRootchip.
- Apoplast in the elongating cells was alkalized in 30 s after auxin, correlated with root growth inhibition.
- The cytosol near the plasma membrane was acidified after auxin.
- Conclusion: auxin triggers fast pH change across the PM, indicating a rapid proton net influx.





Auxin through TIR1 alkalizes cell apoplast to rapidly inhibit root growth

- Manipulation of medium pH regulated root growth in 30 s. The alkaline medium inhibited root growth rapidly and reversely. In contrast, the acidic medium promoted root growth rapidly as well. These indicates that apoplastic pH regulates root growth, and auxin regulates apoplastic pH to modulate root growth.
- TIR1 receptor mediates auxin-induced apoplastic alkalization. The tir triple mutant was resistant to auxin in both growth and apoplastic pH. The cvxIAA triggered apopalstic alkalization in the ccvTIR1 but not in the control line[6].



CONCLUSION

- We rejected the possibility of CMT orientation and vacuole constriction which have been proposed before as the trigger of the auxin-mediated root growth inhibition.
- We confirmed the apoplastic pH response closely correlates with and causes rapid auxin-mediated root growth inhibition.
- We discovered that TIR1 receptor mediates auxin-induced apoplastic alkalization.
- In the future, we aim to dissect how TIR1 receptor conducts rapid apoplastic alkalisation.

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