Spatiotemporal signatures of plant immune responses to *Pseudomonas syringae* pv. tomato DC3000 (*Pto*)

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In vivo monitoring and quantification of plant immune responses using Genetically Encoded Biosensors:

Treatment with flg22 induces acidification of the cytosol, alkalinization of the apoplast and oxidation in all the organs

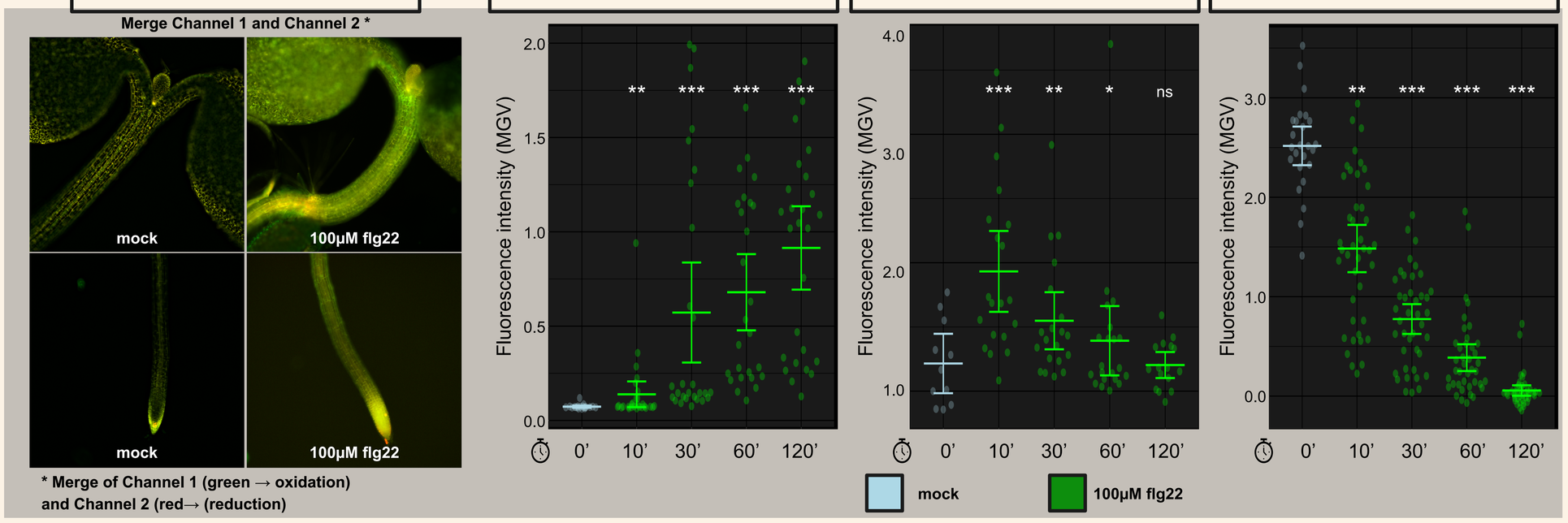
PARAMETERS

Time points *[in mins]*:
10 - 30 - 60 - 120

Treatments:
- 100µM flg22
- *Pseudomonas syringae* pv. tomato DC3000 (*Pto*)
- *fliC Pto* mutant

Organs:
- Petioles
- Hypocotyl
- Root

Genetically encoded biosensors (GEBs):
- *pro35S::roGFP2-Orp1* (for REDOX)
- *proUB10::pHGFP* (for cyt-pH changes)
- *proUB10::syp122-pHusion* (for apo-pH changes)



pro35S::roGFP2-Orp1 5-day old seedlings treated with 100µM flg22 compared to mock.

Samples are alternatively excited at 390 and 475nm and detected at 535nm. Merged pictures (of the two channels) show different fluorescent intensity.

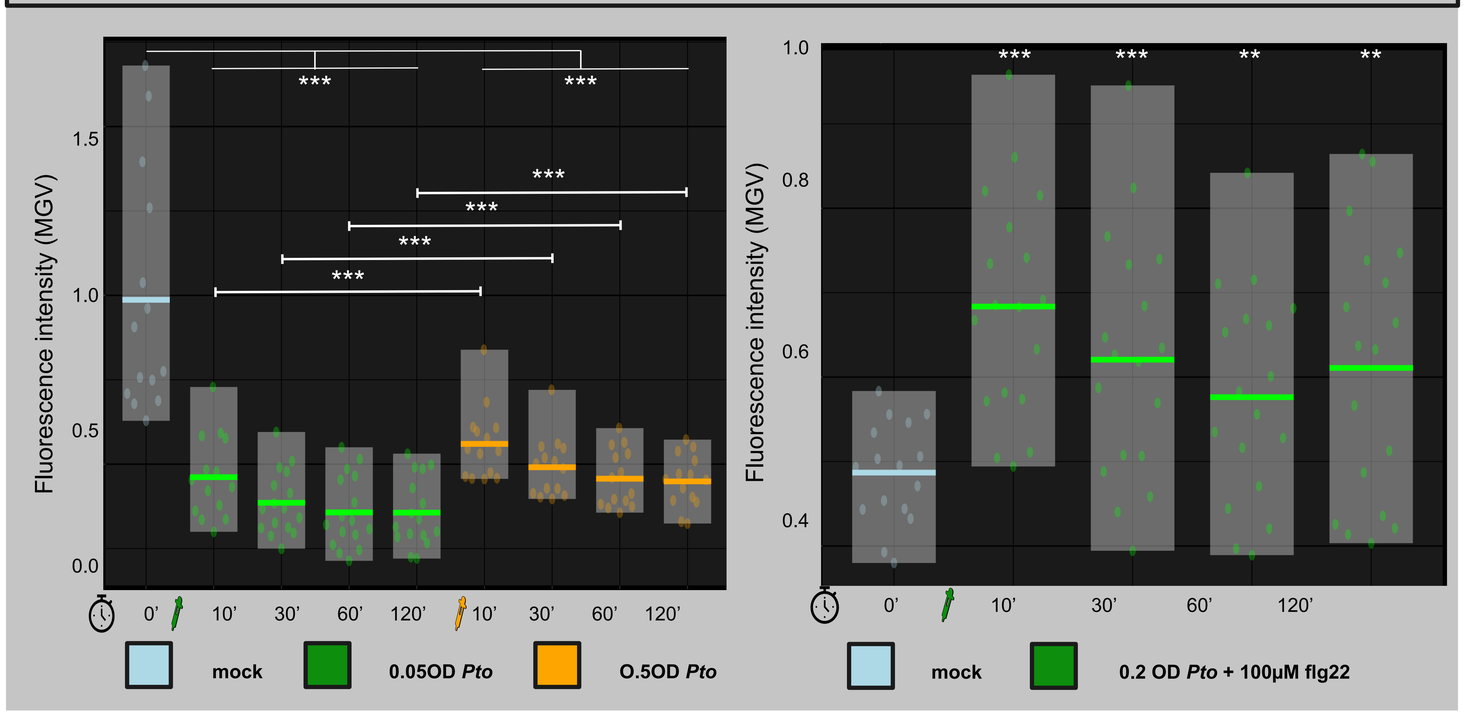
Quantification of fluorescence intensity of the root of three different biosensors over time (0-2h).

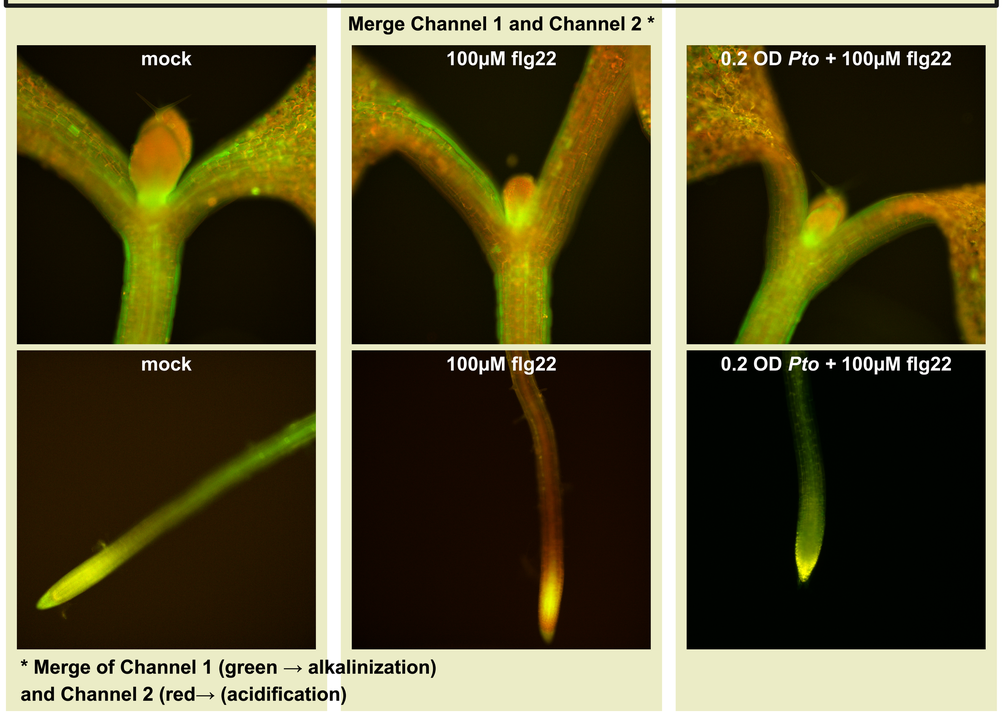
Fluorescence intensity is measured as the ratio between the fluorescence detected at EX1/EX2. Plots represent acquired measurements for root samples over time and with three different biosensors. In particularly, roGPF2-Orp1 is a cytosolic redox sensor (Nietzel *et al.*, New Phytol. 2019)**, increased value indicates oxidation; syp122-pHusion and pHGFP are respectively an apoplastic or cytosolic pH sensor** (Kesten *et al.*, EMBO J. 2019)**, measurements with syp122-pHusion show alkalinization of the apoplast (increased value), while pHGFP-measurements show acidification of the cytosol. Wilcoxon Rank Sum Exact Test was don for statistical significance (* p-value<0.05, ** p-value<0.01, *** p-value<0.001)**

Pseudomonas evades PTI responses:

Higher concentration of *Pto* results in decreased of acidification in the cytosol

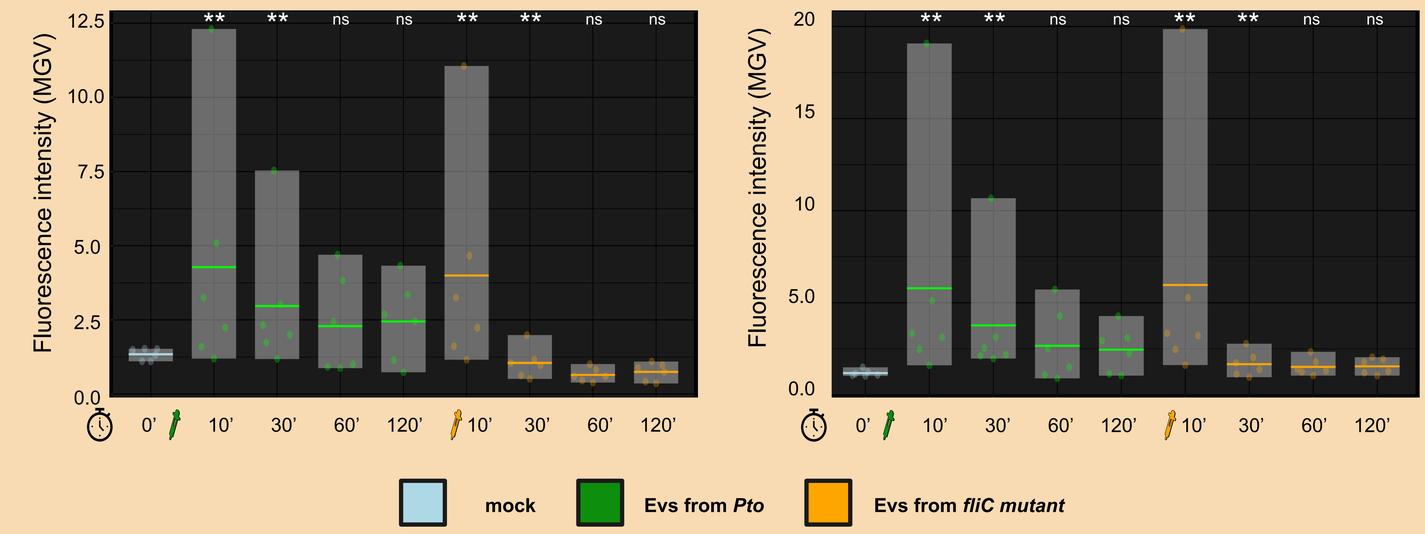
Co-treatment of Pto and flg22 attenuates or suppressed the responses, resulting in alkalinization of the cytosol





Extracellular Vesicles - EVs- are contributing to Pto immunogenic responses (not only flg22)

proUB10::syp122-pHusion - (left) Petiole, (right) Hypocotyl)



(Top left) **pHGFP-seedlings were treated with different concentration of *Pto* (0.05-0.5) and the fluorescence intensity was measured, showing significant acidification of the cytosol. Interestingly, higher concentration (orange) induces less responses compared to lower concentration (green). This suggests that *Pto* is able to evade PTI responses. When treated simultaneously with 100µM flg22 and O.2 OD of *Pto*, the observed responses triggered by flg22 were significantly suppressed.**

(Top right) **Merged pictures of the two channels, show different fluorescence intensity between seedlings treated with flg22, and *Pto* and flg22 and mock.**

(Bottom Right) **The potential role of extracellular vesicles in *Pto* immunogenicity was tested on syp122-pHusion seedlings using EVs from wild type or *fliC* mutant (mutant lacking of the flagellum). This analysis shows that both treatments induce alkalinization of the apoplast, suggesting that not only flg22 but also EVs are contributing to trigger PTI responses.**

Wilcoxon Rank Sum Exact Test was don for statistical significance (* p-value<0.05, ** p-value<0.01, *** p-value<0.001, ns=not significant).

In conclusion, we established a pipeline to use GEBs to study *in vivo* plant immunity responses triggered by *Pseudomonas syringae* pv. tomato DC3000. We aim to characterize such responses in a spatiotemporal manner, extending the analysis to other pathogens, in particularly to the xylem-limited bacteria *Xylella fastidiosa*. Further, a detailed tissue specific analysis will be conducted using the same biosensors driven by cell file specific promoters.

BIO MENTORING

erc Advanced Grant MultiX