Expanded View Figures

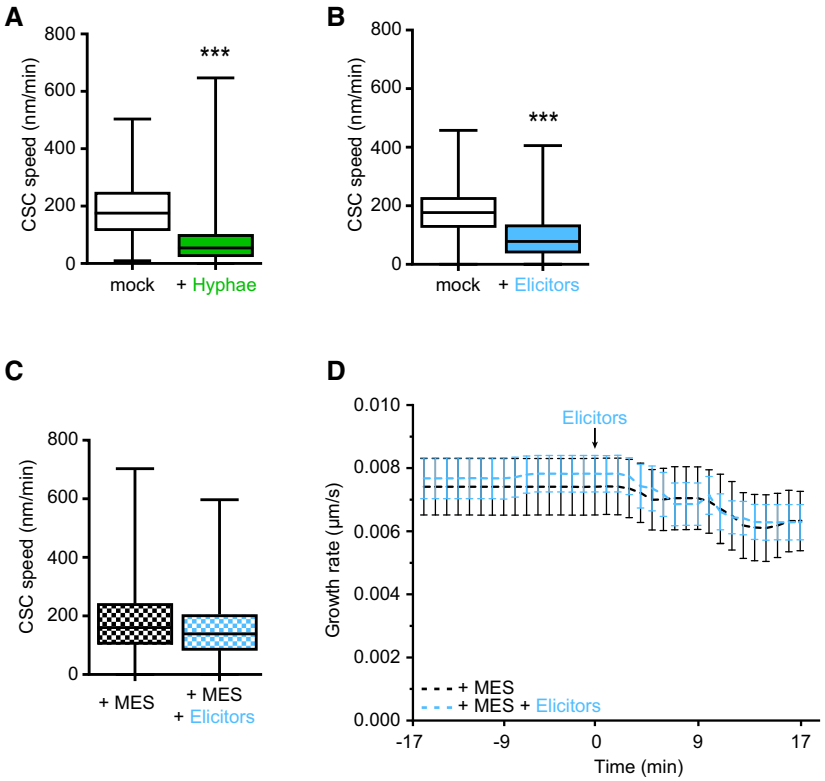


Figure EV1. Fo5176 hyphae and elicitors cause an immediate speed decrease of plasma membrane localized cellulose synthase complexes.

A GFP-CesA3 speed at the plasma membrane of WT (Col-0) root cells 5 min after half MS treatment or Fo5176 contact (as depicted in Fig 1A). Box plots: centerlines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum. $N \geq 369$ particles from 6 cells and 6 roots and three independent experiments; Welch's unpaired *t*-test; ***P-value ≤ 0.001.

B GFP-CesA3 speed at the plasma membrane of WT (Col-0) root cells 5 min after half MS or half MS + elicitor treatment (as depicted in Fig 1F). Box plots as described in (A). $N \geq 647$ particles from 9 cells and 6 roots and three independent experiments; Welch's unpaired *t*-test; ***P-value ≤ 0.001.

C GFP-CesA3 speed at the plasma membrane of WT (Col-0) root cells 5 min after being exposed to half MS + 5 mM MES and half MS + 5 mM MES + elicitor treatment (as depicted in Fig 2A). Box plots as described in (A). $N \geq 786$ particles from 13 cells and 11 roots and three independent experiments.

D Growth rate of WT (Col-0) roots exposed to fungal elicitors and 5 mM MES. After 17 min of growth in half MS + 5 mM MES, H_2O or elicitors were added and the growth rate was measured for an additional 17 min. Average growth rate before treatment (−17 to 0 min): 5 mM MES: 0.0074 ± 0.0009 µm/s; elicitors + 5 mM MES: 0.0068 ± 0.0010 µm/s. Average growth rate after treatment (0–17 min): 5 mM MES: 0.0077 ± 0.0024 µm/s; elicitors + 5 mM MES: 0.0069 ± 0.0020 µm/s. Values are mean ± SEM, $N \geq 11$ seedlings from three independent experiments. Welch's unpaired *t*-test for roots before and after elicitor treatment; P-value = 0.33.

Source data are available online for this figure.

Figure EV2. Fo5176-induced depletion of the cellulose synthase machinery and acidification of the plasma membrane interface, measured with newly designed sensors, can be buffered.

A Representative images of 6-day-old WT (Col-0) root epidermal and cortex cells expressing apo-pHusion or SYP122-phusion. Much less intracellular signal of pHusion is observed for SYP122-pHusion than for apo-pHusion. Scale bar = 20 μm.

B *In vivo* calibration of SYP122-pHusion in 6-day-old roots. Dots represent individual samples with $N \geq 5$ seedlings per standard buffer. Data points were fitted using sigmoidal regression.

C Representative images of 6-day-old WT (Col-0) root epidermal and cortex cells expressing pHGFP-Lti6b. Cell walls of seedlings were counterstained with 3 μg/ml propidium iodide to illustrate plasma membrane localization of pHGFP-Lti6b of two adjacent cells (white arrow). Scale bars = 10 μm.

D *In vivo* calibration of pHGFP-Lti6b in 6-day-old roots. Dots represent individual samples with $N \geq 5$ seedlings per standard buffer. Data points were fitted using sigmoidal regression.

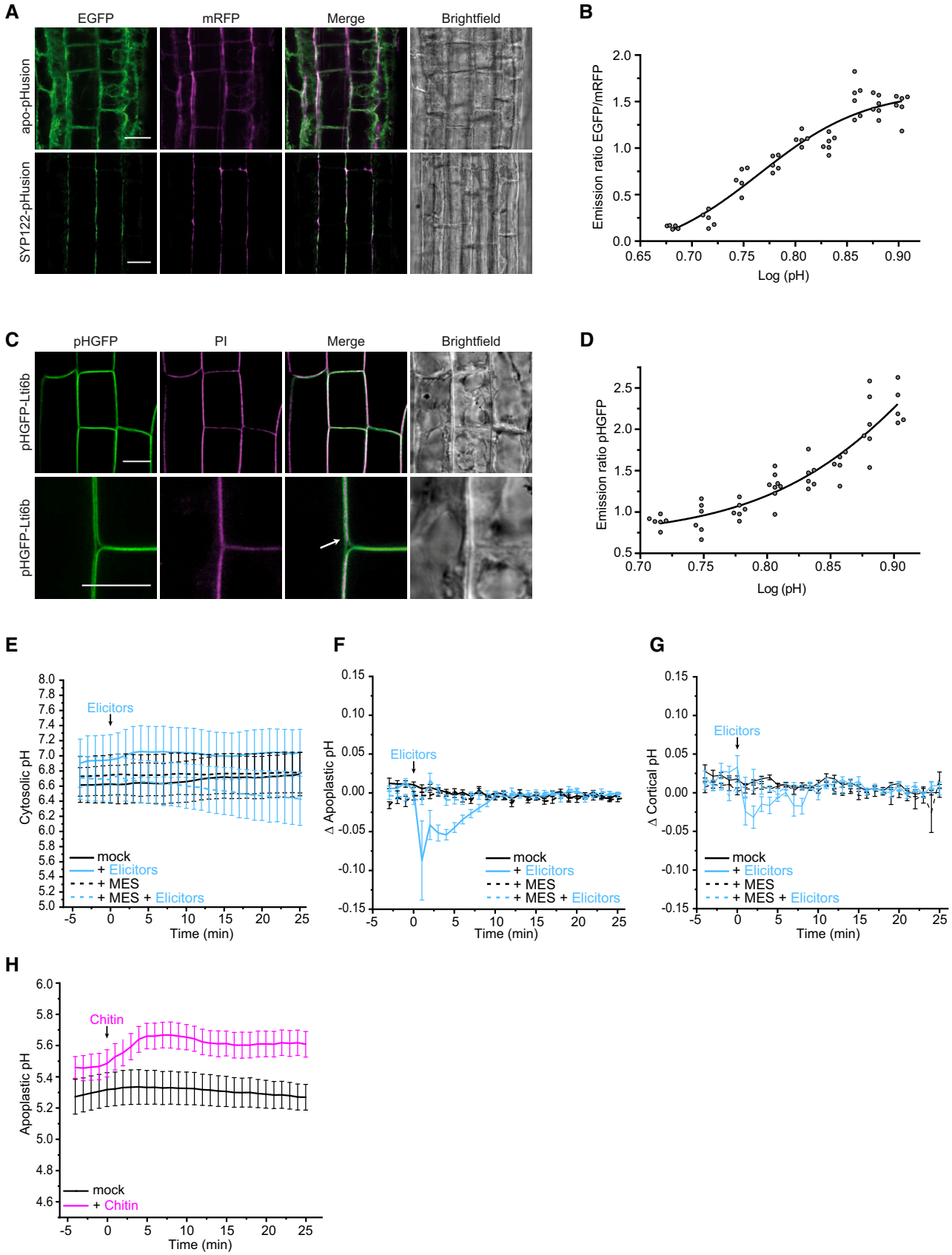
E Cytoplasmic pH variation of WT roots expressing the pH_{cyto} free pHGFP sensor over time, either in half MS or in half MS + 5 mM MES. Imaging started 5 min before either H_2O or a fungal elicitor mix was added (0 min). Values are mean ± SEM, $N \geq 12$ seedlings from three independent experiments. RM two-way ANOVA on half MS + H_2O versus half MS + elicitors: $P = 0.42$ (treatment), $P = 0.06$ (time), $P = 0.08$ (treatment × time).

F Apoplastic ΔpH of WT roots expressing the pH_{apo} SYP122-pHusion sensor over time, either in half MS or in half MS + 5 mM MES. Imaging started 5 min before either H_2O or a fungal elicitors were added (0 min). Values are mean ± SEM, $N \geq 16$ seedlings from three independent experiments. RM two-way ANOVA on half MS + H_2O versus half MS + elicitors: $P \leq 0.001$ (treatment), $P \leq 0.05$ (time), $P \leq 0.001$ (treatment × time).

G Cortical ΔpH of WT roots expressing the $pH_{cortical}$ pHGFP-Lti6b sensor over time, either in half MS or in half MS + 5 mM MES. Imaging started 5 min before either H_2O or a fungal elicitor mix was added (0 min). Mixed-effects model on half MS + H_2O versus half MS + elicitors: $P \leq 0.01$ (treatment), $P \leq 0.001$ (time), $P \leq 0.001$ (treatment × time).

H Apoplastic pH of WT roots expressing the pH_{apo} SYP122-pHusion sensor over time in half MS. Imaging started 5 min before either H_2O (mock) or 1 mM chitin were added (0 min). Values are mean ± SEM, $N = 15$ seedlings from three independent experiments. RM two-way ANOVA on half MS + H_2O versus half MS + chitin: $P \leq 0.05$ (treatment), $P \leq 0.001$ (time), $P \leq 0.001$ (treatment × time). This chitin assay was done simultaneously to the elicitor treatments (Fig 2E); therefore, they share the same mock (H_2O) curve.

Source data are available online for this figure.


A
EGFP
mRFP
Merge
Brightfield
apo-pHusion
SYP122-pHusion
B
Emission ratio EGFP/mRFP
Log (pH)
C
pHGFP
PI
Merge
Brightfield
pHGFP-Lti6b
pHGFP-Lti6b
D
Emission ratio pHGFP
Log (pH)
E
Cytosolic pH
Elicitors
mock
+ Elicitors
+ MES
+ MES + Elicitors
Time (min)
F
Δ Apoplastic pH
Elicitors
mock
+ Elicitors
+ MES
+ MES + Elicitors
Time (min)
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Elicitors
mock
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Time (min)

Figure EV2.

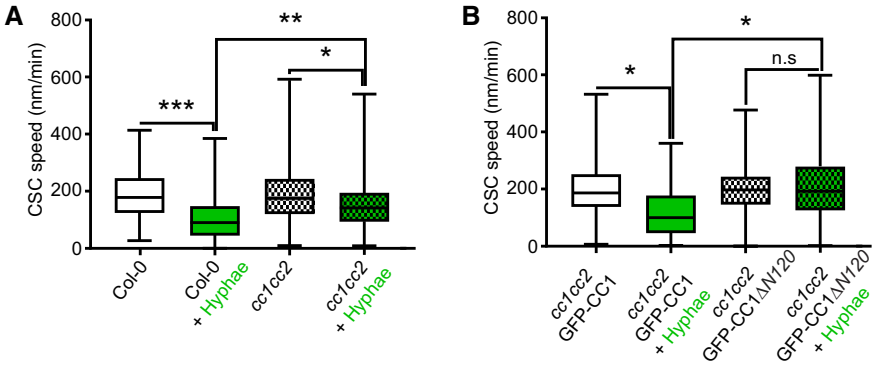

Figure EV3. The speed of plasma membrane localized cellulose synthase complexes is differentially affected by Fo5176 hyphae contact in *cc1cc2* mutants compared to wild-type.

A CSC speed at the plasma membrane of WT (Col-0) and *cc1cc2* root cells, as depicted in Fig 3A and B, upon Fo5176 hyphae contact. Box plots: centerlines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum. $N \geq 270$ particles from 8 cells and 7 roots and three independent experiments; Welch's unpaired *t*-test; **P*-value $\leq$ 0.05, ***P*-value $\leq$ 0.01, ****P*-value $\leq$ 0.001.

B GFP-CC1 and GFP-CC1ΔN120 speed (as proxy for the CSC) at the plasma membrane of *cc1cc2* root cells upon Fo5176 hyphae contact, as depicted in Fig 3F and G. Box plots as described in (A). $N \geq 288$ particles from 12 cells and 7 roots and three independent experiments; Welch's unpaired *t*-test; **P*-value $\leq$ 0.05, n.s. = not significant.

Source data are available online for this figure.

Figure EV4. *cc1cc2* resistance to Fo5176 root colonization can be mimicked in wild-type plants by buffering the growth media.

A Root elongation at various days post-transfer to Fo5176 spore containing plates. Values are mean ± SEM, $N \geq 33$ plants from three independent experiments. RM two-way ANOVA: $P \leq 0.01$ (genotype), $P \leq 0.001$ (time), $P \leq 0.05$ (genotype × time).

B Left panel: Monosaccharide elution profile of a 1 μg/ml standard (black) compared to 1:10 dilutions of hydrolyzed samples (Saeman hydrolysis, as indicated in methods section) derived from roots after 7 days of either mock (pink) or Fo5176 treatment (blue). The N-acetylglucosamine (GlcNAc) peak (4) is only identifiable in hydrolyzed infected samples (blue). Right panel: Strong and weak hydrolyses of fungal-derived cell wall material indicate negligible presence of glucose non-extractable with the matrix hydrolysis method, as the glucose peak is similar in either hydrolysis method. In plants, there is a clear difference between the glucose peaks in either hydrolysis method.

C Quantification of the N-acetylglucosamine peak of Fo5176 pSix1::GFP-infected roots derived from the fungal cell wall as shown in (B). Box plots: centerlines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum. $N \geq 5$ biological replicates; Welch's unpaired *t*-test; $P = 0.34$.

D Quantification of Fo5176 colonies developing after surface sterilization of infected roots. Box plots as described in (C). $N = 5$ biological replicates; Welch's unpaired *t*-test; $P = 0.75$.

E Vascular penetration of WT (Col-0) and *cc1cc2* plants at various days post-inoculation with Fo5176 pSIX1::GFP spores with or without 5 mM MES. Values are mean ± SEM, $N \geq 33$ plants from three independent experiments. RM two-way ANOVA (Col-0 half MS versus Col-0 half ME + MES): $P \leq 0.001$ (genotype), $P \leq 0.001$ (time), $P \leq 0.001$ (genotype × time).

F Root elongation of WT (Col-0) and *cc1cc2* plants at various days post-inoculation with Fo5176 pSIX1::GFP spores with or without 5 mM MES. Values are mean ± SEM, $N \geq 33$ plants from three independent experiments. RM two-way ANOVA (Col-0 half MS versus Col-0 half MS + MES): $P \leq 0.001$ (genotype), $P \leq 0.001$ (time), $P \leq 0.001$ (genotype × time).

G Cellulose content of roots 7 days post-transfer to Fo5176 pSIX1::GFP spore containing half MS + 5 mM MES plates or post-transfer to control (half MS +5 mM MES) plates, represented as μg of D-glucose derived from crystalline cellulose per mg of dried alcohol-insoluble residue (AIR). Box plots as described in (C). $N \geq 5$ biological replicates; 2 technical replicates per biological replicate. Welch's unpaired *t*-test; ***P*-value $\leq$ 0.01.

H CSC ($N \geq 27$ cells from 11 roots) and microtubule ($N \geq 17$ cells from 7 roots) density and CSC speed ($N \geq 366$ particles from 17 cells and 9 roots) at the plasma membrane of WT (Col-0) or *cc1cc2* root cells expressing YFP-CesA6 and mCh-TUA5 grown on half MS + 5 mM MES. Data are from three independent experiments. Box plots as described in (C). Welch's unpaired *t*-test indicates no significant difference for all measurements.

I Quantification of the N-acetylglucosamine peak of Fo5176 infected plants grown on half MS + 5 mM MES derived from the fungal cell wall as shown in (B). Box plots as described in (C). $N = 3$ biological replicates; Welch's unpaired *t*-test; $P = 0.41$.

Source data are available online for this figure.

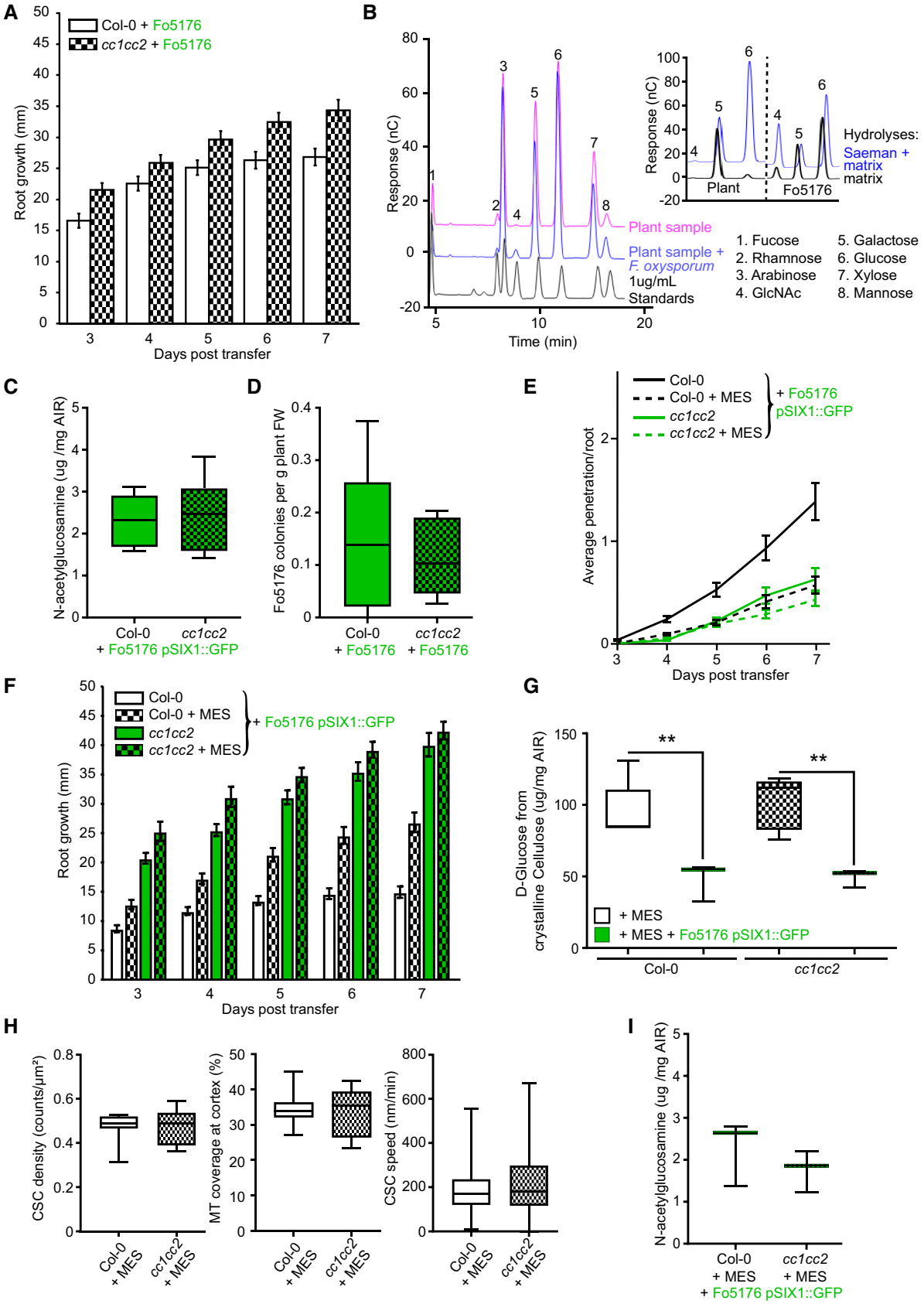
A
Col-0 + Fo5176
cc1cc2 + Fo5176
Root growth (mm)
Days post transfer
B
Response (nC)
Time (min)
Plant sample
Plant sample + F. oxysporum 1ug/mL
Standards
Hydrolyses:
Saeman + matrix
matrix
Plant
Fo5176
1. Fucose
2. Rhamnose
3. Arabinose
4. GlcNAc
5. Galactose
6. Glucose
7. Xylose
8. Mannose
C
N-acetylglucosamine (ug /mg AIR)
Col-0 + Fo5176 pSIX1::GFP
cc1cc2 + Fo5176 pSIX1::GFP
D
Fo5176 colonies per g plant FW
Col-0 + Fo5176
cc1cc2 + Fo5176
E
Col-0
Col-0 + MES
cc1cc2
cc1cc2 + MES
+ Fo5176 pSIX1::GFP
Average penetration/root
Days post transfer
F
Col-0
Col-0 + MES
cc1cc2
cc1cc2 + MES
+ Fo5176 pSIX1::GFP
Root growth (mm)
Days post transfer
G
D-Glucose from crystalline Cellulose (ug/mg AIR)
**
**
+ MES
+ MES + Fo5176 pSIX1::GFP
Col-0
cc1cc2
H
CSC density (counts/µm²)
MT coverage at cortex (%)
CSC speed (nm/min)
Col-0 + MES
cc1cc2 + MES
I
N-acetylglucosamine (ug /mg AIR)
Col-0 + MES + Fo5176 pSIX1::GFP
cc1cc2 + MES + Fo5176 pSIX1::GFP

Figure EV4.

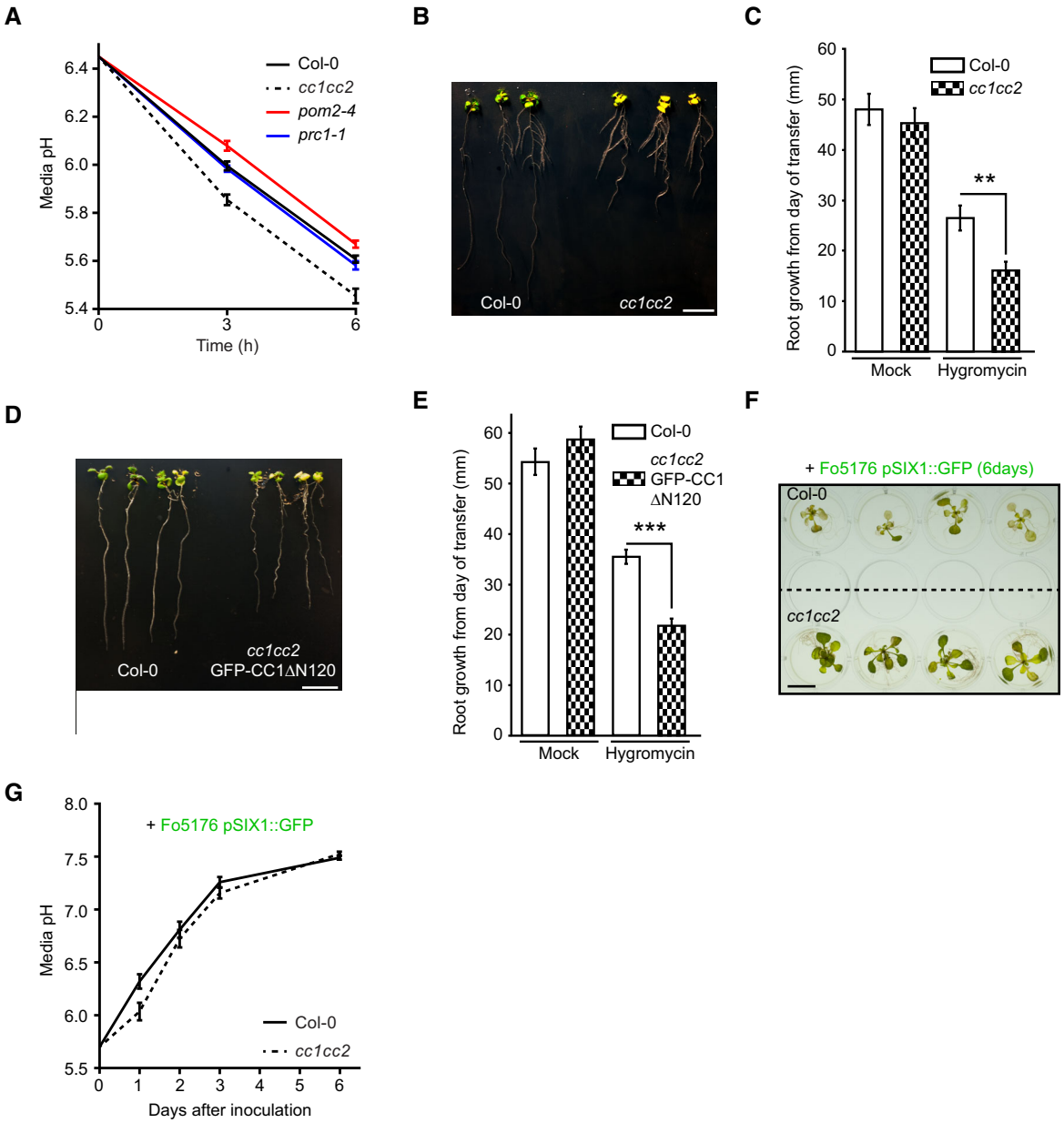

Figure EV5. *cc1cc2* mutants rapidly acidify the media, are hypersensitive to hygromycin B, and show a delay of Fo5176-induced media alkalinization.

A Media pH development over time when 10-day-old WT (Col-0), *cc1cc2*, *pom2-4*, and *prc1-1* plants were transferred to a liquid, alkaline media (pH 6.45). Values are mean ± SEM, $N \geq 17$ plants from three independent experiments. Welch's unpaired *t*-test versus Col-0 at 6 h: *cc1cc2* *P*-value ≤ 0.001; *pom2-4* *P*-value ≤ 0.01; *prc1-1* *P*-value = 0.29.

B Representative image of WT (Col-0) and *cc1cc2* plants 7 days post-transfer to half MS + 5 μg/ml hygromycin B plates. Scale bar = 10 mm.

C Root growth of plants grown as in (B). Values are mean ± SEM, $N \geq 15$ plants from three independent experiments. Welch's unpaired *t*-test; **P-value ≤ 0.01.

D Representative image of WT (Col-0) and *cc1cc2* GFP-CC1ΔN120 plants 7 days post-transfer to half MS + 5 μg/ml hygromycin B plates. Scale bar = 10 mm.

E Root growth of plants grown as in (D). Values are mean ± SEM, $N \geq 16$ plants from three independent experiments. Welch's unpaired *t*-test; ***P-value ≤ 0.001.

F Representative image of WT (Col-0) and *cc1cc2* plants 7 days post-inoculation with Fo5176 pSIX1::GFP spores in liquid half MS media. Scale bar = 10 mm.

G Media pH development of an experiment as depicted in (F). Values are mean ± SEM, $N = 36$ plants from three independent experiments. RM two-way ANOVA: $P = 0.069$ (genotype), $P \leq 0.001$ (time), $P \leq 0.05$ (genotype × time).

Source data are available online for this figure.