Supplemental Material

Groups of multi-cellular passage cells in the root exodermis of *Echinochloa crus-galli* varieties lack not only suberin lamellae but also lignin deposits

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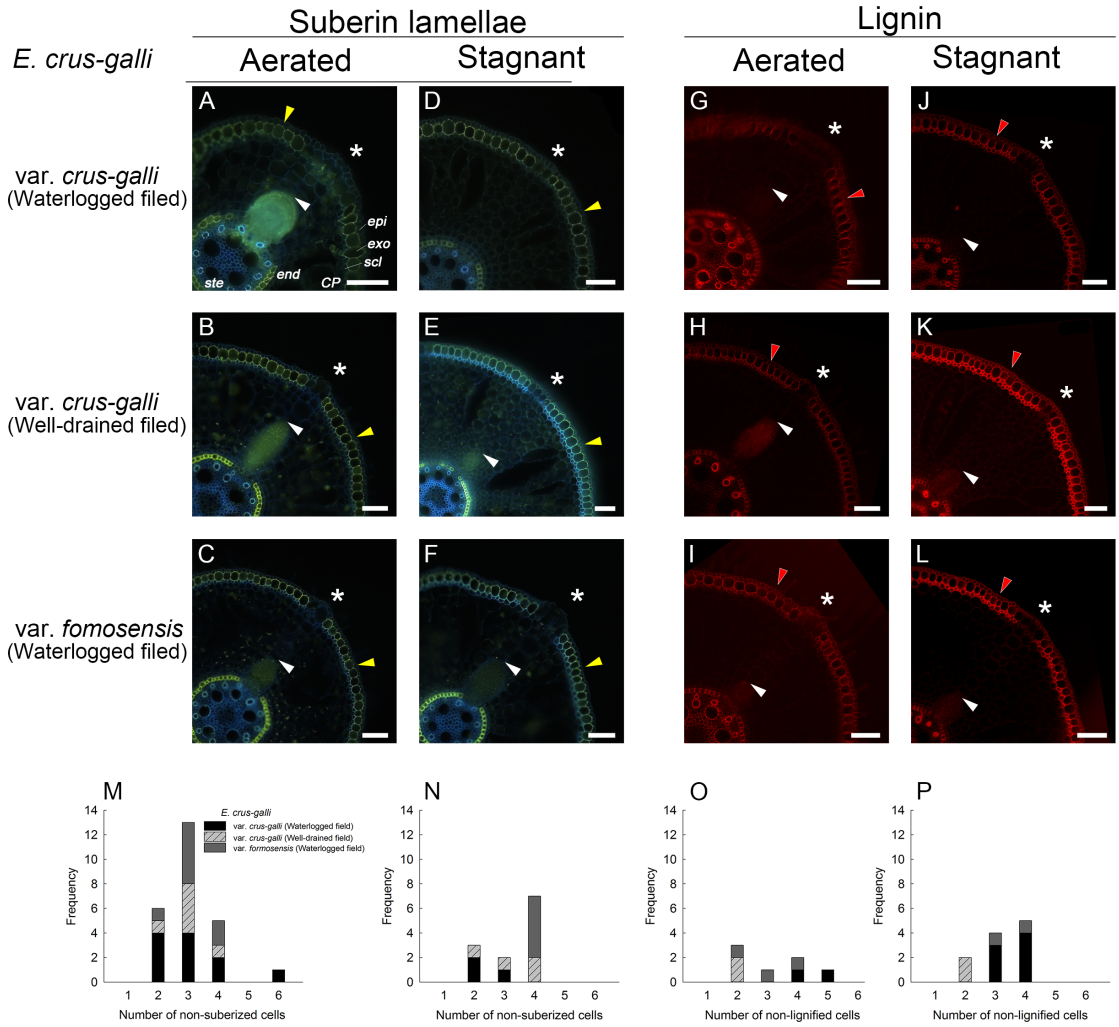
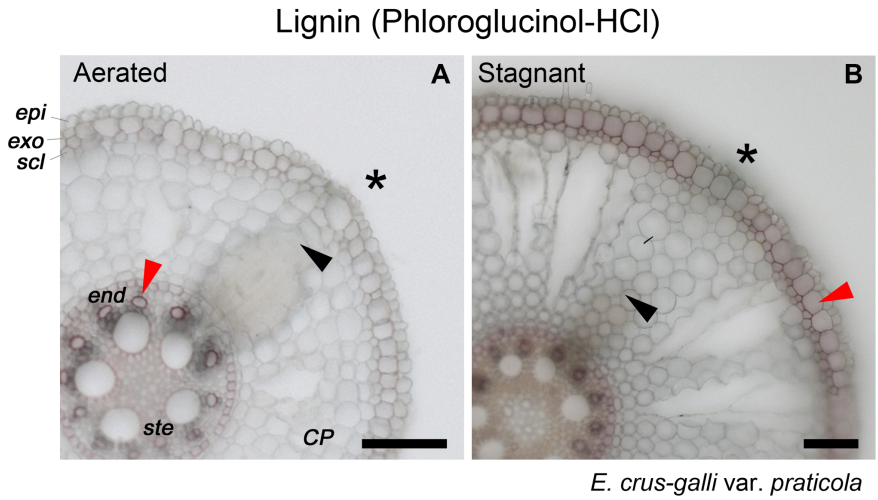


Supplemental Figure 1. Windows in three other *Echinochloa crus-galli* accessions lack suberin lamellae and lignin deposits. Plants were grown in aerated nutrient solution for 10 days, and then transferred to deoxygenated stagnant 0.1% agar solution or left in aerated nutrient solution for another 14 days. Basal parts (15-25 mm below root-shoot junction) of 100- to 120-mm-long adventitious roots were stained as described in Supplemental methods. (A-F) Suberin lamellae at the exodermis and endodermis. Suberin lamellae are indicated by yellow-green fluorescence with Fluorol Yellow 088 (Yellow arrowhead). (G-L) Lignin at the sclerenchyma, exodermis and endodermis. Basic Fuchsin fluorescence is shown by red color (Red arrowhead). (M, N) Number of non-suberized exodermal cells at window sites under aerated (M) and stagnant (N) conditions. (O, P) Number of non-lignified exodermal cells at window sites under aerated (O) and stagnant (P) conditions. Asterisks indicate areas of passage cells (windows) that lack both suberin lamellae and lignin deposits. White arrowheads indicate the apex of lateral root primordia. Abbreviations: *CP*, cortical parenchyma; *end*, endodermis; *epi*, epidermis; *exo*,

exodermis; *scl*, sclerenchyma; *ste*, stele. Scale bars, 100 μm.



Supplemental Figure 2. Lignin at the window site in *E. crus-galli* var. *praticola* detected by Phloroglucinol-HCl staining. Plants were grown in aerated nutrient solution for 10 days, and then transferred to deoxygenated stagnant 0.1% agar solution (B) or left in aerated nutrient solution for another 14 days (A). Lignin at the basal parts (15-25 mm below root-shoot junction) of 100- to 120-mm-long adventitious roots were stained by Phloroglucinol-HCl as described in Ejiri and Shiono (2019) [14]. Lignin are indicated by red color with Phloroglucinol-HCl (Red arrowheads).

Supplemental Methods

Histochemical Staining

Adventitious roots (100–120 mm long) without lateral roots were cut at the root–shoot junction. Their basal parts (15–25 mm below root–shoot junction) were embedded in 5% (w/v) agar. Root cross-sections of ca. 100 μm thickness were made using a vibrating microtome (Leica VT1200S, Leica Biosystems, Wetzlar, Germany). Before suberin staining, the cross-sections were made transparent by incubating them in lactic acid saturated with chloral hydrate at 70ºC for 60 min (Lux et al., 2005). To detect suberin lamellae in the basal parts, we used 0.01% (w/v) Fluorol Yellow 088 in polyethylene glycol 400 (Brundrett et al. 2005). Suberin lamellae were visualized as a yellowish-green fluorescence excited by UV light. For quantification, all cross-sections were photographed with a fluorescence microscope with the following settings [Exposure time: 1.41 sec; an 02 UV filter set, an Axio Imager.A2 and an AxioCam MRc CCD camera (all Carl Zeiss, Oberkochen, Germany)]. Before lignin staining, cross-sections were made transparent by incubating them overnight in ClearSee solution at room temperature (Kurihara et al., 2015). For Basic Fuchsin staining, the cross-sections were incubated overnight in 0.2% (w/v) Basic Fuchsin (Sigma-Aldrich) dissolved in ClearSee solution at room temperature (Ursache et al., 2018). Before the observation, the cross-sections were gently washed at least five times with ClearSee solution. Lignin was imaged on a confocal microscope (LSM510 META, Carl Zeiss; Excitation: 543 nm, Detection: 565-651 nm).

Quantification of fluorescence intensity

All images were obtained with the same section thickness and same exposure time. Fluorescence intensities were quantified using ImageJ (Version 2.0.0-rc-69/1.52n; https://imagej.nih.gov/ij/). To quantify Basic Fuchsin fluorescence quantification, intensities were converted to 8-bit values (0-255). Background noise was removed by subtracting the mean intensity of blank images (i.e., the solvent without root-cross section). To quantify Fluorol Yellow 088 fluorescence, the color root images and blank images were split into red, green and blue images. To quantify yellow fluorescence, the sum of the red and green intensities at each pixel was subtracted from the blue intensity. Background noise was removed by subtracting the mean intensity of blank images. The root images were then converted to 8-bit values. Fluorescence intensities of Basic Fuchsin or Fluorol Yellow 088 along the exodermal cells (i.e., from † to †† in Figure 1A, 1B, 1G and 1H) were measured by ImageJ using the ImageJ command “line plot”.

References

Brundrett MC, Kendrick B, Peterson CA. Efficient lipid staining in plant material with sudan red 7B or fluoral yellow 088 in polyethylene glycol-glycerol. Biotech Histochem 1991; 66:111-6.

Kurihara D, Mizuta Y, Sato Y, Higashiyama T. ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging. Development 2015; 142:4168-79.

Lux A, Morita S, Abe J, Ito K. An improved method for clearing and staining free-hand sections and whole-mount samples. Ann Bot 2005; 96:989-96.

Ursache R, Andersen TG, Marhavy P, Geldner N. A protocol for combining fluorescent proteins with histological stains for diverse cell wall components. Plant J 2018; 93:399-412.