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Supplementary Material

Calcium improves apoplastic–cytosolic ion homeostasis in salt-stressed *Vicia faba* leaves

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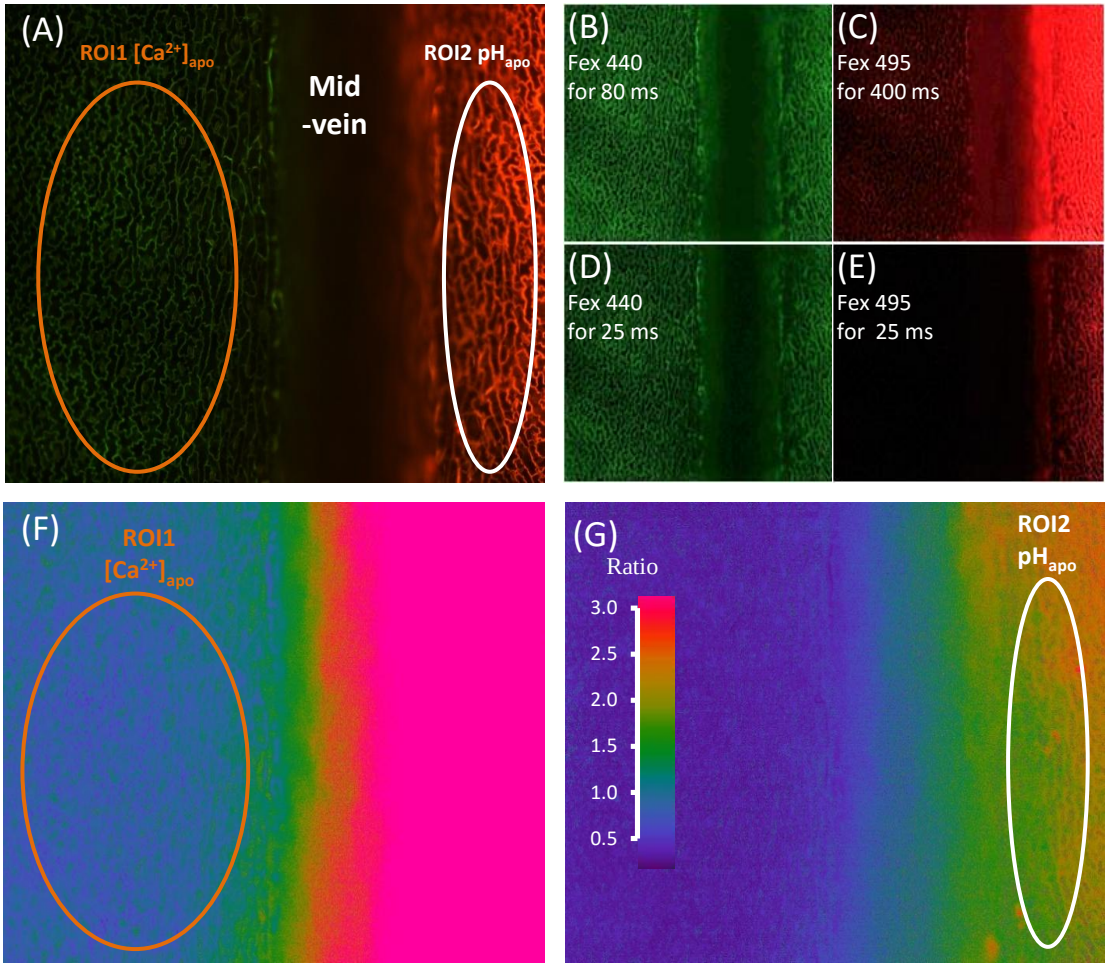
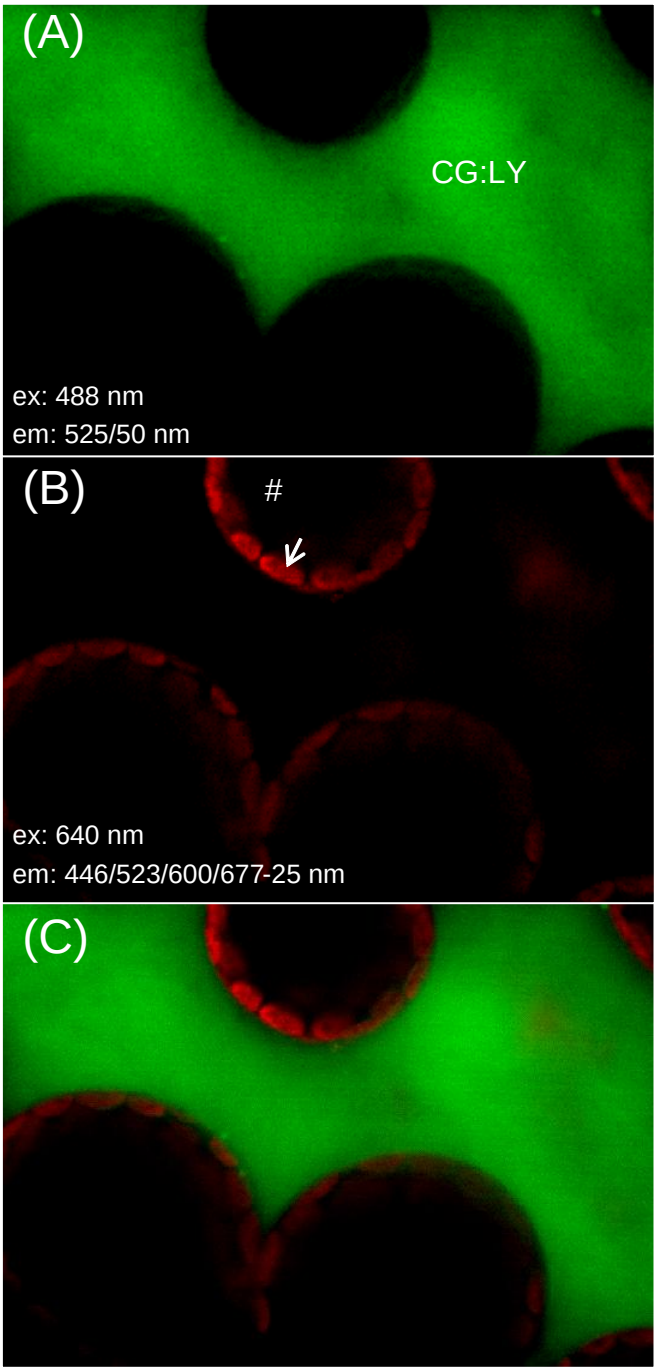
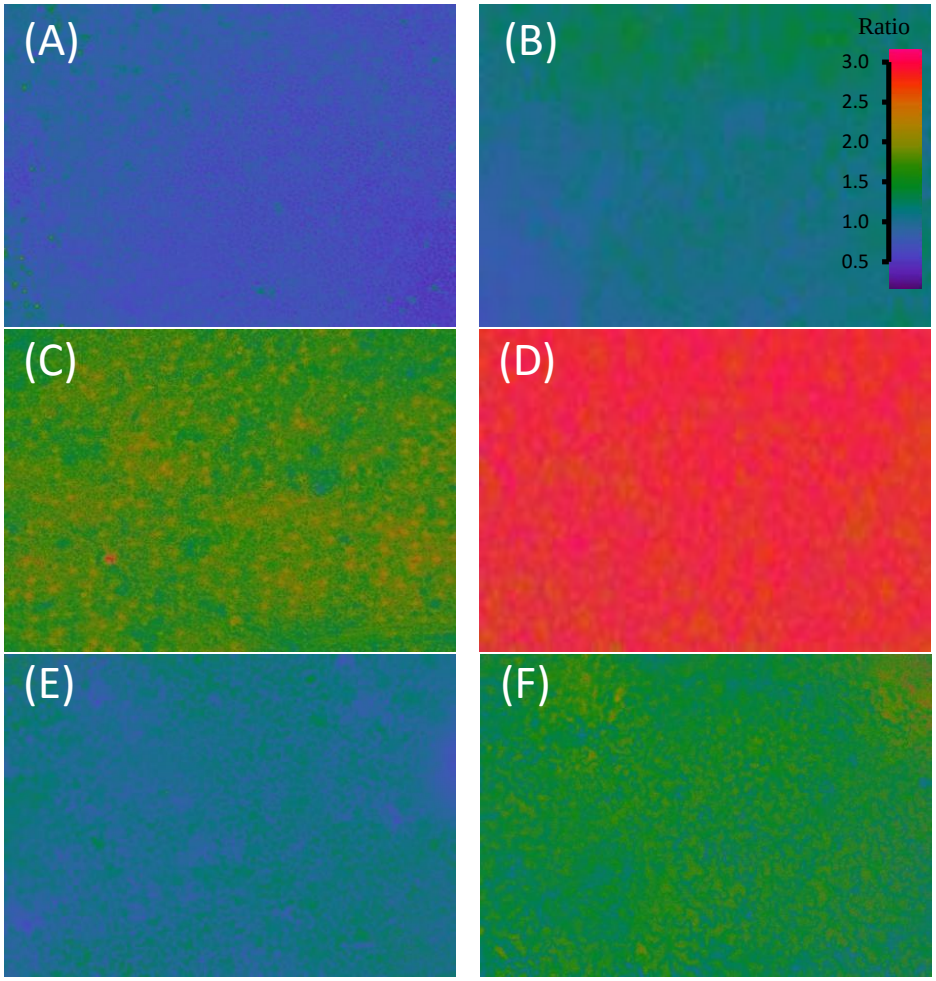


Sup. Fig. 1. Ratiometric calculation of apoplastic Ca^{2+} ($[Ca^{2+}]_{apo}$) and pH (pH_{apo}). Representative fluorescence- and ratio-images were captured from control plants. (A) The apoplast of intact second uppermost leaf was loaded with the Ca^{2+}-probe CG:LY (left of leaf vein) and with the pH-probe OG (right of leaf vein). Separation by leaf vein prevents mixture of Ca^{2+} and pH probe. The fluorescence images shown abaxial view of faba bean leaf as excited at (B) $F_{440\ for\ 80\ ms}$, (C) $F_{495\ for\ 400\ ms}$ (D) $F_{440\ for\ 25\ ms}$ and (E) $F_{495\ for\ 25\ ms}$. The fluorescence ratios (F) $F_{495\ for\ 400\ ms}/F_{440\ for\ 80\ ms}$ (CG:LY) and (G) $F_{495\ for\ 25\ ms}/F_{440\ for\ 25\ ms}$ (OG) were obtained as a measurement of $[Ca^{2+}]_{apo}$ and pH_{apo}, respectively. Thus, ratios were taken from different regions of interest (ROIs) in images (F) and (G). ROI1 from the ratiometric images (F) where CG:LY exists and ROI2 from the ratiometric images (F) where OG exists and used for quantitative $[Ca^{2+}]_{apo}$ and pH_{apo} calculation, respectively. The ratios were coded from purple (no signal) to blue (lowest signal) to pink (highest signal) as shown in (G).



Sup. Fig. 2. Calcium Green : Lucifer yellow (CG:LY) that is conjugated to 3 and 10 kDa dextran, respectively, does not enter the symplast. (A) Confocal image shows leaf apoplast of faba bean as labelled with CG:LY as excited at 488 nm by an diode laser (pseudo-green; CG:LY). Autofluorescence of the chloroplast as excited at 640 by a diode laser (pseudo-red; white arrow). #, palisade cells appear black as no CG:LY dextran has entered the cells. (C) Overlay of (A) and (B).



Sup. Fig. 3: Ratiometric images from *in situ* calibration of the fluorescence ratios (R=495/440 nm) versus free Ca^{2+} concentration in the apoplast obtained in the intact leaves of faba bean plants by using the pseudo-ratiometric probe Calcium Green: Lucifer Yellow (CG:LY). (A) and (B), R_{min} measured in leaves which were infiltrated with CG:LY that was dissolved in 10 mM EGTA adjusted by Tris to pH 4.8 (A) (R_{min} pH 4.8 = 0.64) or pH 5.3 (R_{min} pH 5.3 = 0.92) (B). (C) and (D), R_{max} measured in leaves which were infiltrated with CG:LY that was dissolved in 50 mM citric acid, 10 mM calcium gluconate and adjusted by Tris to pH 4.8 (C) (R_{max} pH 4.8 = 1.70) or pH 5.3 (D) (R_{max} pH 5.3 = 2.67). (E), $R_{control}$ measured in CG:LY infiltrated intact second uppermost leave of plants cultivated at 1 mM NaCl for 7d ($R_{control}$ = 0.98). (F), $R_{100\ mM\ NaCl}$ "Saline condition" measured in CG:LY infiltrated intact second uppermost leave of plants cultivated at 100 mM NaCl for 7d ($R_{100\ mM\ NaCl}$ = 1.53). Ratios (R=495/440 nm) were color-coded on a spectral color scale as show in image (B).