**1. Callose staining**

Callose staining was performed using 0.1% aniline blue (415049, Sigma-Aldrich) in

1xPBS, pH 7.4. For staining of adult leaves, aniline blue was syringe-infiltrated in the

leaf just before imaging. For staining of cotyledons, cotyledons were detached from

soil grown 8-10 day old seedlings and placed on a microscope slide on a droplet of

aniline blue solution. A coverslip was then gently pressed on the cotyledons so that

the staining solution penetrated in the apoplastic space. The cotyledons were then rinsed in sterile water prior to be mounted on a microscope slide.

**2. Acquisition and quantification of aniline blue staining data**

To quantify the levels of callose deposited at plasmodesmata, leaves were stained

with aniline blue as described above (1) and then mounted on microscopy slides

immobilized by sticking on double-sided tape and covered by a coverslip. Images

were acquired on a LSM Zeiss 800 confocal microscope using a water immersion 63x

objective (C-Apocromat 63x/1.20WKorr UV VIS IR-water). Aniline blue was excited

with the 405 nm laser at 2% power and fluorescence was collected in the range 410-470

nm. Z-stacks were acquired in two regions of each cotyledon, with 8-10 slices for each

stack and an interval between slices of 0.8-0.2 μm kept constant within one experiment.

To ensure acquisition of high quality images, the following settings were used: image

size 1024x1024 pixels, 16 bit depth and 4x line averaging.

Aniline blue signal at plasmodesmata was automatically quantified in Fiji (Schindelin

et al., 2012), using a macro developed for the purpose by Matthew Johnston.

In this macro, first, the image is made binary using a user-defined threshold and the

’convert to mask’ command. The threshold was kept consistent within an experiment

and it was set so that aniline blue stained plasmodesmata at the cell periphery were

visible and the noise or background signal was cut off. Then the ’analyze particles’

command was used to analyse the number of particles still visible in the binary image

that matched the criteria of maximum and minimum size. These criteria were set to

make sure that spots of signal that were not plasmodesmata but may have escaped

the threshold filter were not detected. The maximum and minimum size criteria

were user-defined and kept consistent within an experiment. The ’analyze particles’

command extracted number of particles, size and integrated density (the sum of all the

pixel intensities) of the particles. The data obtained in this way were analysed in R (R

Core Team, 2020) following a script developed by Matthew Johnston, where summary

was obtained for the mean number, size and integrated density of the detected particles

in each z-stack. Data displayed in this thesis are the mean integrated density of a

particle in a z-stack, in other words the mean intensity of aniline blue staining signal

per plasmodesma in each z-stack, which correlates with the levels of callose.