# SUPPLEMENTARY MATERIAL

## Supporting experimental procedure: S1

Three spinach leaves from each treatment were used to estimate leaf area (LSA) by computer analysis of scanned images. Use of a scanner eliminates leaf overlap and minimizes distortions. The scanner lid was protected with a plain white sheet of paper. LA was measured in ImageJ v. 1.47 software (Aboukarima *et al.* 2017; Chaudhary *et al.* 2012).

Root hair surface area (RHSA, cm2) was also estimated by computer image analysis (Aboukarima *et al.* 2017; Chaudhary *et al.* 2012). One plant from each pot was randomly selected and the shoot was cut off. A stainless steel soil corer (diameter 4.8 cm, height 5 cm) was inserted fully into the soil surface, centered on the plant stem. Soil cores were soaked in water for a day, and the roots were carefully separated from the soil. The clean roots were suspended in a beaker filled with water and photographed with a digital camera (Cyber-shot DSC-T77 MKM, 10.1 megapixels; Sony Corp., Japan) mounted on a fixed platform. The lens faced the beaker horizontally, 1 m above the floor. A 10-cm × 10-cm area was defined in the centre of the lens to ensure a consistent geometry of all images. Four images were taken from a 4 different sites of the beaker and used for analysis by ImageJ and they average were used.

The images were pre-processed in ImageJ before analysis. First, the geometry of each image was corrected by polynomial methods using the “Analysis” tool and the “Set Scale” command (Figure 2 (a), Figure 3 (a)), and then cropped using the “Image” tool and the “Crop” command (Figure 2 (b), Figure 3 (b)). Second, each colour image was transformed into a grayscale image by using the “Image” tool, the “Type” command, and the “8-bit” subcommand (Figure 2 (c), Figure 3 (c)). Third, the grayscale image was binarized to a black-and-white image by using the “Image” tool, the “Adjust” command, and the “threshold” subcommand, which made the leaf and root areas black (Figure 2 (d), Figure 3 (d)). Finally, LA and RHA were measured by using the “Analysis” tool and the “Measure” command. The data were recorded in a Microsoft Excel table. LA and RHA were expressed in cm2.



**Figure 1** The RGB image of spinach leaf with scale (a), cropped image (b), grayscale image (c) and inverted binary image (black and white image; d).

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**Figure 2** The RGB image of spinach hair root with scale (a), cropped image (b), grayscale image (c) and inverted binary image (black and white image) (d).

# REFERENCES

Aboukarima AM, Zayed MF, Minyawi M, Elsoury HA, Tarabye HHH 2017: Image analysis-based system for estimating cotton leaf area. *Asian Res. J. Agric.*, **5**(1), 1-8

Chaudhary P, Godara S, Cheeran AN, Chaudhari AK 2012: Fast and accurate method for leaf area Measurement. Int. J. Comput. Appl., **49**(9), 22-25.