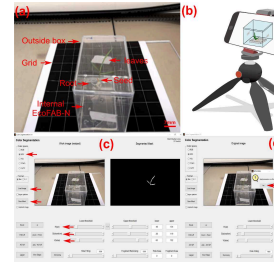




Non-invasive imaging of leaf area using a mobile phone in the EcoFAB-N

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Kuang Weiqi¹, Sanow Stefan², Kelm Jana M.², Müller-Linow Mark², Peter Andeer³, Kohlheyer Dietrich⁴, Trent Northen³, jpvogel⁵, Watt Michelle⁶, b.arsova²

¹College of Life and Environmental Sciences, Hunan University of Arts and Science, 415000 Changde, China;

²IBG-2 Plant Sciences, Institut für Bio- & Geowissenschaften, Forschungszentrum Jülich, Wilhelm-Johnen-Straße, 52428 Jülich, Germany;

³Environmental Genomics and Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, CA, USA;

⁴IBG-1 Biotechnology, Institut für Bio- & Geowissenschaften, Forschungszentrum Jülich, Wilhelm-Johnen-Straße, 52428 Jülich, Germany;

⁵The Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA;

⁶Faculty of Science, The University of Melbourne, Melbourne



Borjana Arsova

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Abstract

This protocol describes the non-invasive phenotyping steps in the EcoFAB-N chamber published in the study Kuang et al., "N-dependent dynamics of root growth and nitrate and ammonium uptake are altered by bacteria *Herbaspirillum seropedicae* in cereal model *Brachypodium distachyon*"

Figure legends (attached images):

Fig. SM2. EcoFAB-N imaged from three sides using the Plant screen mobile application on a mobile phone and analyzed with Color segmentation software. (a) EcoFAB-N on a grid attached to plastic plate on clean bench. Grid of known size (here, 20 mm x 20 mm squares, both black and white grid background work, black allows better visualization of roots, white is sufficient for leaves) (b) Shoot images (leaves) taken with a mobile phone positioned at three ways (1, 2, 3) with Plant screen mobile (PSM) software (Müller-Linow et al., 2019). Image analysis process with Color segmentation software guide. Steps for single (c) and multiple images analysis (d).

Fig. SM3 Plant screen mobile software (PSM) guide. (a) PSM App icon. (b-f) PSM settings.

Relevant references to the original EcoFAB chamber and the imaging software:

Gao J, Sasse J, Lewald KM, Zhalnina K, Cornmesser LT, Duncombe TA, Yoshikuni Y, Vogel JP, Firestone MK, Northern TR. 2018. Ecosystem Fabrication (EcoFAB) Protocols for The Construction of Laboratory Ecosystems Designed to Study Plant-microbe Interactions. *J Vis Exp*(134).

Müller-Linow M, Pinto-Espinosa F, Scharr H, Rascher U. 2015. The leaf angle distribution of natural plant populations: assessing the canopy with a novel software tool. *Plant Methods***11**(1): 11.

Müller-Linow M, Wilhelm J, Briese C, Wojciechowski T, Schurr U, Fiorani F. 2019. Plant Screen Mobile: an open-source mobile device app for plant trait analysis. *Plant Methods***15**(1): 2.

Sasse J, Kant J, Cole BJ, Klein AP, Arsova B, Schlaepfer P, Gao J, Lewald K, Zhalnina K, Kosina S, et al.,. 2019. Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. *The New phytologist* **222**(2): 1149-1160.

Attachments



[Fig. SM2.png](#)

1.1MB



[Fig. SM3.png](#)

400KB

Materials

- Mobile phone (e.g. Huawei p20)
- Mobile phone holder (Manfrotto, Germany), website: <https://www.manfrotto.com/us-en/mini-tripod-black-with-universal-smartphone-clamp-mkpixiclamp-bk/>.
- Plant screen mobile (PSM) applicaiton (Müller-Linow *et al.*, 2019)
- Color segmentation software (Müller-Linow *et al.*, 2015)
 - Install MCR_R2012b_win64_installer and
 - Start color Segmentation_Tool.exe
- Grid of known dimensions on A4 paper for photo background. Mark a position in the center of the grid for the seed reservoir hole.
- Plastic plate to attach the grid paper on the suitable plastic plate for stability (Fig. SM2a).

- 1 Sterilize a clean bench with UV light 15 min before use.
- 2 Place the EcoFAB-N on the clean bench under white light (Fig. SM2a), on the grid attached to the plastic plate, make sure the Seed reservoir hole of the EcoFAB-N overlaps with the black point depicted in the grid (Fig. SM2a).

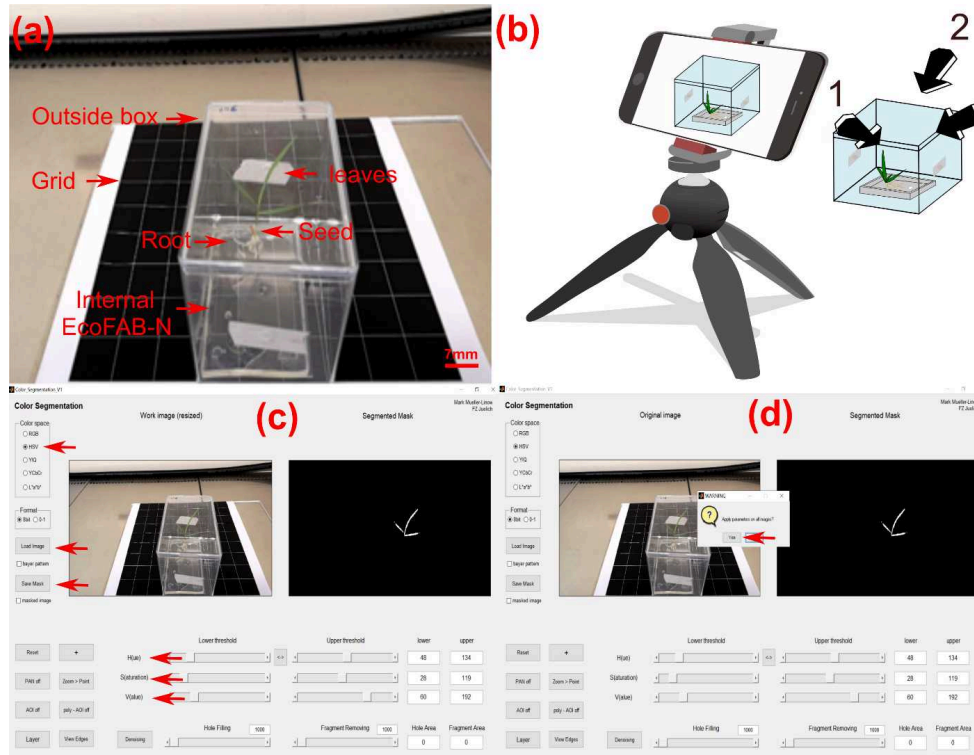


Fig. SM2. EcoFAB-N imaged from three sides using the Plant screen mobile application on a mobile phone and analyzed with Color segmentation software. (a) EcoFAB-N on a grid attached to plastic plate on clean bench. Grid of known size (here, 20 mm x 20 mm squares, both black and white grid background work, black allows better visualization of roots, white is sufficient for leaves) (b) Shoot images (leaves) taken with a mobile phone positioned at three ways (1, 2, 3) with Plant screen mobile (PSM) software (Müller-Linow et al., 2019). Image analysis process with Color segmentation software guide. Steps for single (c) and multiple images analysis (d).

- 3 Put the phone holder with mobile phone next to the EcoFAB-N, adjust distance and imaging angle to 45°, label the mobile phone tripod location (Fig. SM2b).
- 4 Rotate the EcoFAB-N, keeping the seed reservoir hole over the marked position on grid, and image the plant in the EcoFAB-N from 3 directions with PSM software (Fig. SM 2c).
- 5 Operation of Plant screen mobile software (PSM). Use the following steps: Open PSM application, Fig. SM3a; Select "settings" and press APP CONFIG, Fig. SM3b, c; Select

“project configuration”, input the Project Name and Project Folder. Select Confirm, Fig. SM3d, e; Press the camera button to imaging photo, Fig. SM3f.

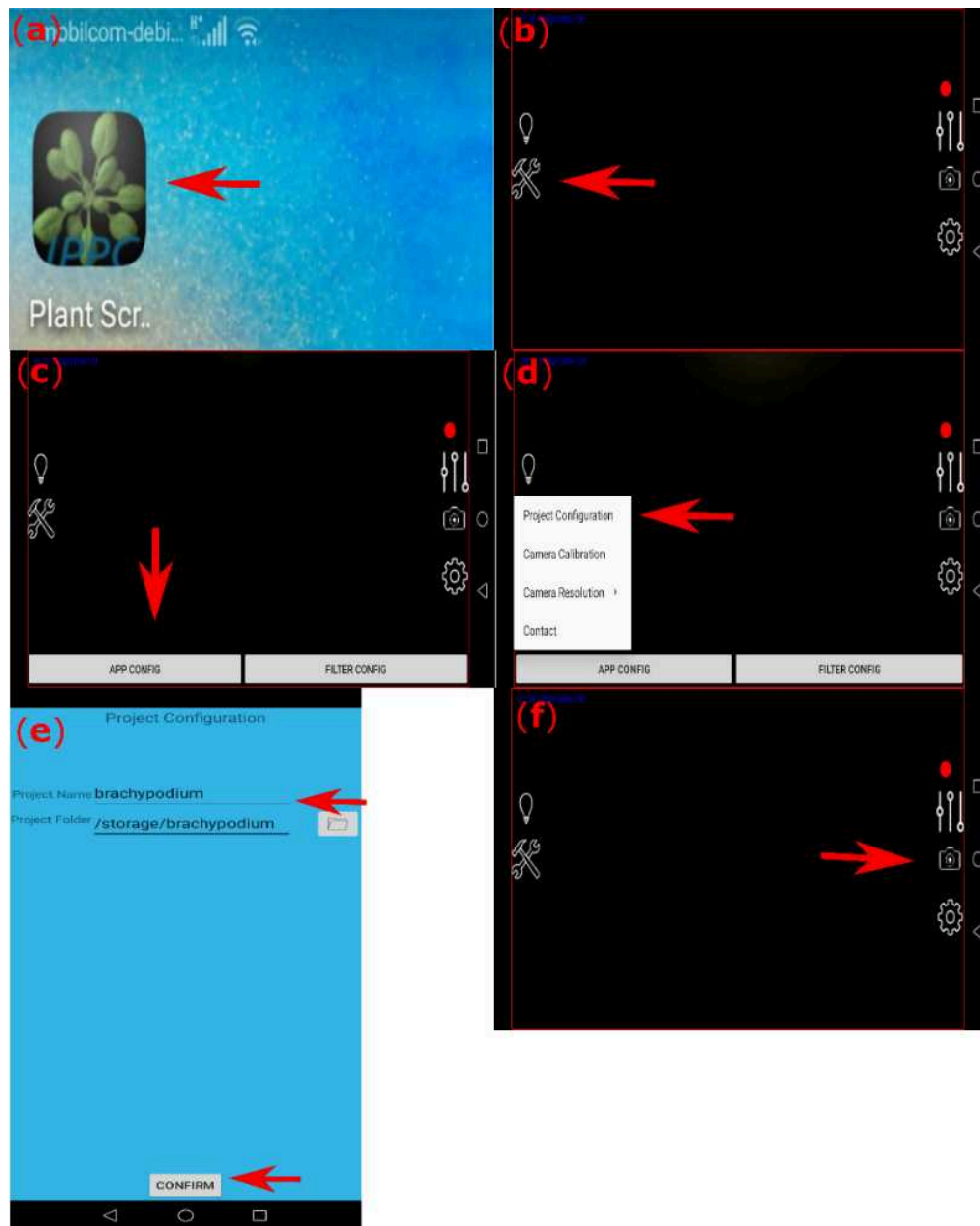


Fig. SM3 Plant screen mobile software (PSM) guide. (a) PSM App icon. (b-f) PSM settings.

- 6 Transfer photos from mobile phone to computer and analyze the images with Color Segmentation software (Müller-Linow et al., 2015) using the following steps: Open the color segmentation software; Press load image, Fig. SM2c; Select "HSV" color space, and adjust the best setting (Hue; Saturation; Intensity), For example: the used setting in Kuang et al., are Hue: 48-134; Saturation: 28-119; Intensity: 60-192; Select save mask, then the software will ask whether to imply the setting to all folder images, select yes, Fig. SM 3d; All the images will be analyzed and binarised from the masked image and data are deposited in excel files as projected leaf area in pixels (px).
- 7 Calibration and unit transformation: Project leaf area results in pixels (px) was converted to cm² using Equ 1; Project leaf area results were calibrated using Equ 2, to eliminate difference due to phone angle or distance. Equ. 1: plant leaf area (cm²) = (picture width in cm / picture width in px)² × plant leaf area (px) Equ. 2: Calibrated leaf area = plant leaf area (cm²) / [Width of EcoFAB (px) / Width of Ecofab mean (px)]²