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REGULAR ARTICLE

A soil-free root observation system for the study of root-microorganism interactions in maize

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Abstract
Background and aims The root surface of a plant usually exceeds the leaf area and is constantly exposed to a variety of soil-borne microorganisms. Root pathogens and pests, as well as belowground interactions with beneficial microbes, can significantly influence a plants' performance. Unfortunately, the analysis of these interactions is often limited because of the arduous task of accessing roots growing in soil. Here, we present a soil-free root observation system (SF-ROBS) designed to grow maize (*Zea mays*) plants and to study root interactions with either beneficial or pathogenic microbes.

Methods The SF-ROBS consists of pouches lined with wet filter paper supplying nutrient solution.
Results The aspect of maize grown in the SF-ROBS was similar to soil-grown maize; the plant growth was similar for the shoot but different for the roots (biomass and length increased in the SF-ROBS). SF-ROBS-grown roots were successfully inoculated with the hemi-biotrophic maize fungal pathogen *Colletotrichum graminicola* and the beneficial rhizobacteria *Pseudomonas putida* KT2440. Thus, the SF-ROBS is a system suitable to study two major belowground phenomena, namely root fungal defense reactions and interactions of roots with beneficial soil-borne bacteria.
Conclusions This system contributes to a better understanding of belowground plant microbe interactions in maize and most likely also in other crops.

Keywords Corn · *Zea mays* · Root infection · Pathogen · Rhizobacteria · *Colletotrichum graminicola* · *Pseudomonas putida*

Abbreviations

SF-ROBS	soil-free root observation system
PE	polyethylene
MNS	maize nutrient solution
GFP	green fluorescent protein
dpi	day(s) post infection
LB	Luria-Bertani
CFU	colony-forming unit(s)

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Introduction

Soil-borne pathogens are estimated to cause an annual monetary loss of US$4 billion in the US (Okubara and Paulitz 2005). Root physiology under biotic and abiotic stress conditions is a field of increasing importance, specifically in view of improving crop yield and diminishing the possible negative environmental impact of agricultural practices (Gewin 2010). However, most studies on plant defense have been essentially focusing on aboveground plant parts. The root system plays a key role for the whole plant: roots are not only essential for nutrient and water uptake, they also contribute to adequately anchor the plant and have an important impact on its capacity to react to stress (Rasmann and Agrawal 2008; Erb et al. 2009). In tobacco for example, nicotine is produced in the roots and translocated to the leaves in response to aboveground herbivore attack (Kaplan et al. 2008). *Colletotrichum graminicola* (Ces.) Wilson, the causal agent of corn anthracnose, infects both aboveground and belowground maize parts (Sukno et al. 2008). This pathogen is responsible for significant yield losses worldwide. Soil-borne pathogens, such as some species of *Fusarium*, *Phytophtora* or *Pythium,* have also an important economic impact. This stresses the need for studies focused on belowground plant interactions.

In addition to pathogenic interactions, beneficial interactions between microorganisms such as rhizobacteria or endophytic fungi and roots can have an impact on belowground stress reactions. The growing demand for sustainable alternatives to the massive input of pesticides in agriculture has led to an increase of interest concerning beneficial interactions between plants and soil-borne microbes. Such beneficial microbes are able to stimulate plant growth and to induce aboveground systemic resistance against different types of stresses (Pineda et al. 2010). For example, filamentous fungi such as *Trichoderma virens* or *Piriformospora indica* induce resistance against biotrophic and necrotrophic pathogens in some cereals (Deshmukh et al. 2006; Djonovic et al. 2007). Various rhizobacteria such as some *Pseudomonas spp.* or *Bacillus spp.* also protect plants against above- or belowground stresses (reviewed in De Vleesschauwer and Höfte 2009). Selected rhizobacteria have been tested for their capacity to enhance defense reactions against biotic and abiotic stress and to promote growth of maize plants (Huang et al. 2010; Nadeem et al. 2009). *Pseudomonas putida* KT2440 has been recently tested for its close interaction with maize seeds and roots (Neal et al. 2012) and for its capacity to induce resistance in Arabidopsis against the pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Matilla et al. 2010).

Research focusing on belowground plant defense is limited by the difficult accessibility of roots growing in soil. For some plants such as Arabidopsis (Gibeaut et al. 1997; Hétu et al. 2005; Ishiga et al. 2011), tomato (Ahn et al. 2011), or rice (Kim et al. 2005) well-established growing systems circumventing the presence of soil are available as aeroponic, hydroponic, solid or semi-liquid cultures. A recently described aeroponic culture system allows even the study of root herbivory on Arabidopsis (Vaughan et al. 2011). For maize plants, most of the soil-free systems are based on a solid substrate like quartz sand (Hund et al. 2009a, Schulze and Pöschel 2004) or glass beads (Boeuf-Tremblay et al. 1995). However, none of these maize systems allows an easy access to the root during all steps of development. Moreover, these substrates tend to stick to the roots and can interfere with some measurements and manipulations.

The aim of our study was to establish a growth system well adapted to maize. Moreover, this system should allow microorganism-root interactions and an easy access to the root system for in vivo observations, root inoculations with microbes and harvesting of material with the smallest possible damage to the roots. The soil-free root observation system (SF-ROBS) we finally established is adapted from the model of Hund et al. (2009b) created for maize root morphology analysis. The system has been used to study the response of maize roots to abiotic stresses, such as extreme temperature (Hund et al. 2012), low water potential induced by polyethylene glycol (Ruta et al. 2009) or aluminum toxicity (Trachsel et al. 2010) but was not tested for its suitability to study plant-microbe interactions.

In the following we report on the effect of the SF-ROBS on maize plants. Plants from standard soil-pot conditions were compared to plants grown in the SF-ROBS. Different parameters including the general aspect (habitus) of plants and measurements of different plant parts were assessed. Since one of the main reasons for adapting the soil-free system was to obtain an easier access to the roots for specific studies in plant-microorganism interactions, we tested the suitability

of the SF-ROBS for a pathogenic interaction with the hemibiotrophic fungus *C. graminicola* and a beneficial interaction with the rhizobacteria *P. putida* KT2440.

Materials and methods

Growth of maize in the soil-free system

For sterilization, maize seeds (var. Golden Jubilee, West Coast Seeds, Canada) were rinsed in 70 % ethanol, incubated for 5 min in 10 % bleach and washed three times with sterile distilled water. The sterilized seeds were pre-germinated for 2–4 days between humid filter paper sheets (Filterkrepp Papier braun, 100 $g m^{-2}$, E. Weber & Cie AG, 8157 Dielsdorf, Switzerland) in a plant growth chamber (Percival AR-95 L, CLF Plant Climatics GmbH, Wertingen, Germany) with the following conditions: 16 h day at 26 °C, 8 h night at 22 °C, 60 % relative humidity and an irradiance of 400 $\mu mol m^{-2} s^{-1}$. They were then transferred to a soil-free root observation system (SF-ROBS). The SF-ROBS is based on the model of Hund et al. (2009b) with the following modifications: a larger size of pouches, the quality and quantity of the nutrient solution, the type of filter paper and an increased number of plants per pouch. In details, the SF-ROBS consists of a 34x64 cm polyethylene foil (PE-Teichfolie WA-1200, 0.5 mm, Walser AG, 8575 Bürglen, Switzerland), which was folded in half to form a 34x32 cm pouch (Fig. 1). Each pouch had three 2x1 cm slits, allowing the growth of the shoots; 3 cm below that aperture, a bulge made with a polyurethane bumper (3 M Bumpon protective products, 12.7 mm diameter, 3.5 mm depth, 3 M Europe, 1831 Diegem, Belgium) was pasted to one side of the pouch to keep the seedling in place. The seedling was fixed between two 33x33 cm filter papers (Filterkrepp) in the closed pouch with two standard paper clips (43 mm long) on each side of the bulge. The pouch was attached to an aluminum rod with two foldback clips (Büroline, 51 mm, 69198 Schriesheim, Germany), one on each side of the upper edge of the pouch. The filter paper was humidified with maize nutrient solution (MNS). The MNS (Ruakura solution adapted from Smith et al. 1983) consists of the following solutions: macronutrient stock solution A (2.31 g $MgSO_4.7H_2O$, 16.78 g $Ca(NO_3)_2.4H_2O$, 8.48 g NH_4NO_3, 2.28 g KNO_3, 2.31 g $(NH_4)_2SO_4$ per liter); macronutrient stock solution B (2.67 g KH_2PO_4, 1.64 g K_2HPO_4, 6.62 g K_2SO_4, 0.60 g Na_2SO_4, 0.33 g NaCl per liter); micronutrient supplement (128.80 mg H_3BO_3, 4.48 mg $CuSO_4$, 81.10 mg $MnCl_2.4H_2O$, 0.68 mg MoO_3, 23.45 mg $ZnCl_2$, 809.84 mg $C_6H_5FeO_7$ per liter); each solution was sterilized by autoclaving; 200 mL of each of the macronutrient stock solutions was mixed with 100 mL of the micronutrient supplement and finally diluted with deionized water to a volume of 4.5 L. The pouches were put into polypropylene containers (60x40x32.5 cm, Rako, Migros, Switzerland), containing 4.5 L MNS, so that the bottom of the filter paper protruding from the pouches was constantly submerged in the nutrient solution. The filter paper was replaced every 3–4 days by new, moistened paper. The plastic containers with the growing maize plants were placed in the climate chamber at the same conditions as for seed germination. The SF-ROBS is also explained in the video available in the online supplementary material. To test an alternative nutrient solution, plants were grown as described above in Hoagland's No. 2 (Sigma-Aldrich, 3050 Spruce St., St. Louis, Missouri 63103, H2395) and compared with plants grown in MNS. Shoot and root fresh weights were measured for each plant (10 replicates per treatment) and possible symptoms of nutrient deficiencies were recorded.

Plant growth in soil

For experiments in soil, maize plants were potted in polypropylene pots, 11 cm high, 4 cm in diameter (Semadeni, 3072 Ostermundingen, Switzerland). Seeds were sterilized and pre-germinated as for the SF-ROBS. Germinated seeds were then transferred into a 50:50 (vol:vol) soil (25 % compost, 12 % sand and 63 % peat; Ricoter Erdaufbereitung AG, 3270 Aarberg, Switzerland): sand (washed sand 0–4 mm, Jumbo, Switzerland) mixture. The soil:sand mixture was autoclaved 1 day before use. The plants were grown under the same conditions as for plants in the SF-ROBS.

Root infections with *Colletotrichum graminicola-gGFP*

To facilitate fungal detection and quantification a GFP-expressing strain of *C. graminicola* (gGFP; Erb

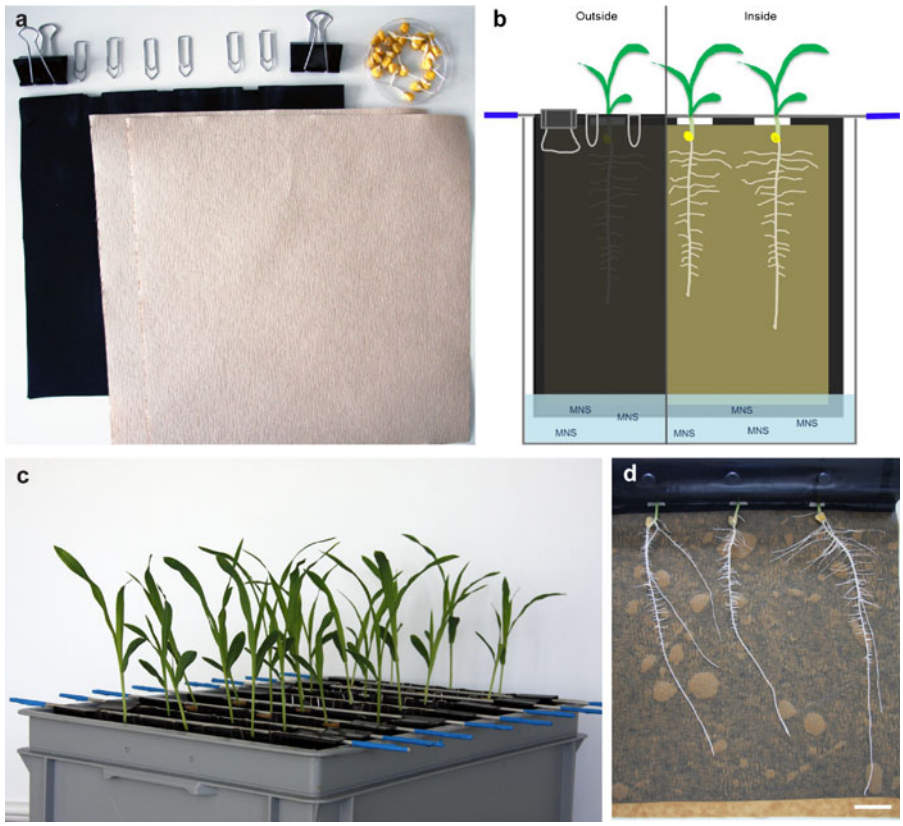

Fig. 1 Details of the SF-ROBS. a Elements of the pouch system with germinated maize seeds in the upper right corner. The SF-ROBS consists in pouches formed from black PE foil folded on itself and two humid filter papers. The layers are held together by paper clips. These pouches are attached to an aluminium rod with two foldback clips and placed in polypropylene containers that contain the maize nutrient solution (MNS). **b** View of the outside and the inside of the black PE foil pouch in which plants are growing. **c** Nine-day old maize plants. **d** Root systems of 5-day old plants in the SF-ROBS. Bar=2 cm

et al., 2011) was used. *C. graminicola-gGFP* was maintained on potato dextrose agar under continuous light (70 $\mu mol\,m^{-2}s^{-1}$) and 25 °C. Maize plants were removed from the SF-ROBS just before the inoculation process and the roots were inoculated by submerging the entire root system in a spore suspension (10^6 spores mL^{-1}, harvested from a 3 weeks old fungal culture) for 30 min in the dark at room temperature. Immediately afterwards, plants were put back in the SF-ROBS. Inoculations were performed at the end of a day period. Fungal root colonization was observed over a time period of 1–6 days post infection (dpi).

Plant inoculation with *Pseudomonas putida* KT2440

The rifampicin-resistant strain *Pseudomonas putida* KT2440 was grown on Luria-Bertani (LB; Difco LB, Becton, Dickinson and Company, 38800 Le Pont de Claix, France) agar supplemented with 100 $\mu g mL^{-1}$ rifampicin (Fluka, Sigma-Aldrich, 9471 Buchs, Switzerland) at 25 °C in the dark for 2–10 days. A single colony was picked and transferred to 100 mL of LB liquid medium with 100 $\mu g mL^{-1}$ rifampicin for an overnight culture at 28 °C, under continuous shaking at 150 rpm. The bacterial culture was divided in two, centrifuged at 3700 rpm, washed twice with sterile $MgSO_4$ 10 mM and the pellet was re-suspended in 25 mL of sterile M9 minimal medium (Sambrook and Russell 2001). Maize seeds were first sterilized and pre-germinated for 2 days between Filterkrepp paper sheets as described above. The germinated seeds were then either mock-inoculated with sterile M9 minimal medium as control or with a fresh overnight bacterial suspension ($1–3\times10^{12}$ colony-forming units (CFU) mL^{-1}) by shaking for 30 min at 35–40 rpm at room temperature. Bacterial root colonization of such treated plants grown in the standard soil-pot system and in the SF-ROBS was then compared. Roots from 11-day old plants were harvested and cleaned from remaining soil under running tap water. Roots were then quickly dried and 100 mg of fresh weight per sample were ground in 600 µL sterile $MgSO_4$ 10 mM. For each plant (12 replicates), two root samples were collected: one near the seed, for the upper part of roots, and the other one in the primary root tip area, for the lower root parts. Serial dilutions of each sample were plated

on solid King's medium B (Pseudomonas agar F, Merck KGaA, 64271 Darmstadt, Germany) supplemented with 100 $\mu g\,mL^{-1}$ rifampicin to quantify the rifampicin-resistant *P. putida* KT2440 strain. After 18–20 h of incubation at 25 °C in the dark, the number of colony-forming units per gram of fresh root was determined.

Assessment of growth parameters

In order to compare plants from the SF-ROBS with plants grown in the standard soil system we measured shoot and root length as well as fresh weight of 12-day old plants. The part considered as shoot reached from the seed to the tip of the longest leaf, whereas the root part was from the seed to the tip of the primary root. After these measurements, roots and shoots were dried separately in an oven at 70 °C (Hybridisation oven/shaker SI20H, Stuart Scientific, UK) in coffee filter papers until sample weight remained constant. The dry weight of shoots and roots was then assessed.

Imaging

Microscopy of *C. graminicola-gGFP*-infected roots was performed using a (Eclipse E800, Nikon Corporation, Tokyo, Japan) microscope and a dissecting microscope (C-BD230, Nikon Corporation, Tokyo, Japan). Images were captured using a digital sight device (DS-L1, Nikon Corporation, Tokyo, Japan). GFP fluorescence of the fungal structures was excited with blue light (430–470 nm).

Statistical analysis

For the comparison of plants from the soil-free system with plants from the standard system, measurements were analyzed using a Student *t*-test, after passing a Shapiro-Wilk test as a normality test. All analyses were performed using the R software v2.12.1 (R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/). For comparison of plants grown in different nutrient solutions and for bacterial root colonization, the data were processed in SigmaPlot 11.0 (Systat Software, Inc., San Jose California USA, URL http://www.sigmaplot.com). Depending on the distribution of data, a Student *t*-test or a Mann–Whitney Rank Sum Test were performed.

Results

The SF-ROBS favors maize root development

To evaluate the effect of the SF-ROBS on maize growth in comparison to soil, we assessed the habitus of the plants (Fig. 2a and b) and measured parameters linked to plant fitness: the plant length (Fig. 3a), fresh and dry weight (Fig. 3b and c). Both planting methods yielded healthy plants with a similar habitus (Fig. 2a). This visual observation is supported by no significant differences for either shoot length or weight measurements between both systems (shoot length $p=0.607$; shoot fresh weight $p=0.415$; shoot dry weight $p=0.106$). Interestingly, plants from the SF-ROBS had a better developed root system with enhanced branching compared to plants from the soil system (Fig. 3). This was reflected in root length (44.82 cm), root fresh weight (1.68 g) and root dry weight (90.26 mg) of SF-ROBS-grown plants, which were significantly higher compared to soil-grown plants (root length 34.68 cm, $p=0.002$; root fresh weight 0.77 g, $p=3.325\times10^{-6}$; root dry weight 50.38 mg, $p=7.576\times10^{-5}$). Two different nutrient solutions were tested in the SF-ROBS: the MNS and the standard Hoagland's No. 2 solutions. Whereas plants with MNS were healthy, plants with Hoagland's No. 2 exhibited leaf chlorosis (Fig. 4). Moreover, plants grown in MNS had a significantly higher fresh weight of leaves (0.315 g; Mann–Whitney Rank Sum Test, $p=<0.001$) compared to plants grown in the standard solution (0.160 g). The root fresh weight of plants in MNS (0.496 g) was similar to plants in Hoagland's No. 2 (0.432 g; Student *t*-test, $p=0.849$).

The SF-ROBS facilitates colonization of maize root by *Colletotrichum graminicola*

To investigate whether the SF-ROBS can be used for fungal infection assays, roots were inoculated with the GFP-expressing pathogenic fungus *C. graminicola-gGFP* (Fig. 5). By monitoring the colonization over time, we found a characteristic infection pattern: four days after inoculation, the appearance of acervuli was observed (Fig. 5a, c and d). In later infection stages,

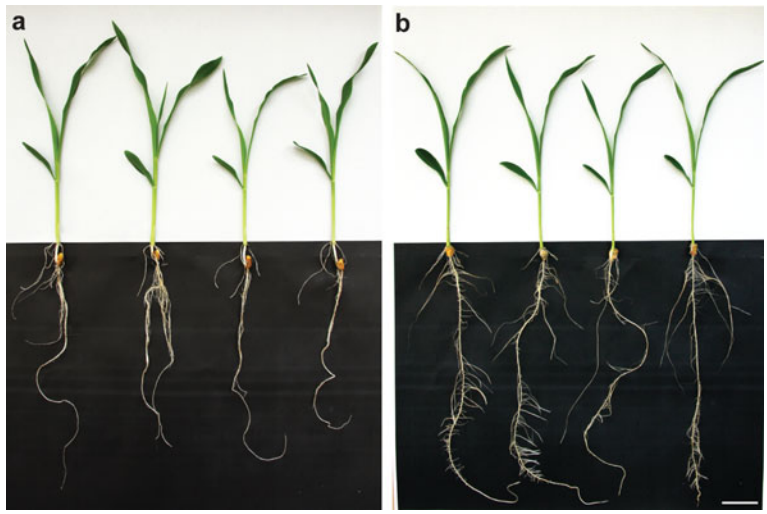

Fig. 2 Comparison between plants grown in standard soil conditions or in the SF-ROBS. a Nine-day old plants up-rooted from standard soil conditions. **b** Plants grown in the SF-ROBS. Bar=4 cm

epidermal cells packed with falcate conidia were detected (Fig. 5e). Mature roots, root caps and the root elongation zones were rapidly and consistently colonized by *C. graminicola-gGFP* (data not shown), suggesting that there was no fungal penetration preference for the different zones of the roots. During advanced infection stages (>4 dpi), colonized roots showed a brown discoloration (not shown).

The SF-ROBS provides accurate conditions for root colonization by rhizobacteria

In order to test whether the SF-ROBS can also be used in research with beneficial root colonizing bacteria, the plants were inoculated with *P. putida* KT2440. The number of colony-forming units of *P. putida* KT2440 extracted from such inoculated roots of 11-day old maize plants was assessed to evaluate the capacity of the rhizobacteria to efficiently colonize roots in the SF-ROBS compared to the soil-pot standard system. There were no significant differences in root colonization of plants in the SF-ROBS ($3{,}53\times10^5$ $CFU\,g^{-1}$ of fresh roots) and in soil ($2.5\times10^5\,CFU\,g^{-1}$ of fresh roots; Mann–Whitney Rank Sum Test, $p=0.29$). The amount of bacteria differed between the upper and lower root parts. This difference was observed in both growing systems. There were more bacteria present in the upper parts ($3.47\times10^6\,CFU\,g^{-1}$ of fresh roots in the SF-ROBS and $5.85\times10^5\,CFU\,g^{-1}$ of fresh roots in soil) than in the lower parts ($3.60\times10^4\,CFU\,g^{-1}$ of fresh roots in the SF-ROBS and $8.7\times10^4\,CFU\,g^{-1}$ of fresh roots in soil; Mann–Whitney Rank Sum Test, $p=<0.001$ for SF-ROBS and $p=0.002$ for soil).

Discussion

The SF-ROBS facilitates the growth of young maize plants

Several culture methods have been described to allow growth of plants without soil. However, soil-free

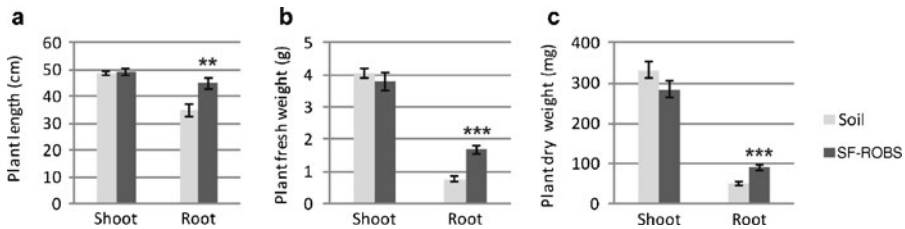

Fig. 3 Comparison of root and shoot length and weight. Shoot and root dry and fresh weight of 12-day old maize plants grown in standard soil conditions or in SF-ROBS were assessed: **a** shoot and root length (*cm*), **b** shoot and root fresh weight fresh weight (*g*), and **c** shoot and root dry weight (*mg*). Error bars indicate the standard errors for the average values of 18 replicates. Asterisks indicate a significant difference in a Student *t*-test (**=p<0.01, ***=p<0.001)

Fig. 4 Leaves of 12 day-old maize plants grown in the SF-ROBS containing Hoagland's No. 2 (H) or MNS. Leaves grown in Hoagland's No. 2 exhibit a chlorotic phenotype. Bar=1 cm.

systems to study plants with higher biomass than Arabidopsis and demanding greenhouse care such as the monocotyledon model plant maize are less established. Gunning and Cahill (2009) described a method by which *Lupinus angustifolius* was cultivated in a system using blotting paper that was embedded between two plastic plates with a given spacing. They reported to have successfully tested maize in this system. Nevertheless, we found that this system was less viable in our hands for growing maize compared to our system. A major advance in this field was achieved with the development of a soil-free phenotyping platform for maize (Hund et al. 2009b). This system facilitates non-destructive digital assessments of the root morphology. Modification and adaptation of this method led to the development of the SF-ROBS presented here. Three critical elements of the SF-ROBS were identified: the pouches, in which the roots are growing, the filter paper providing the nutrients for the roots, and the nutrient solution itself. The pouches should shield the roots from light, and their surface should remain as aseptic as possible and not be toxic to plants. PE foil, which is commonly used to make garden ponds, was chosen since it satisfied these criteria. Choice of the right filter paper was found to be even more crucial. Standard white filter or blotting paper inhibited growth (data not shown), therefore we used filter paper that had not been treated with bleaching chemicals. Similarly, the nutrient solution had to be optimized for maize. Modified Ruakura solution (adapted from Smith et al. 1983) was identified as the most appropriate solution. Commonly used standard media such as Hoagland's No. 2 were found to be insufficient leading to visual chlorosis of the leaves. The leaf color and growth rate were similar in both SF-ROBS and soil conditions. Roots grown in the SF-ROBS were longer and exhibited enhanced branching. Consistently, the fresh weight and dry weight of roots was found to be higher for SF-ROBS-grown plants, indicating that the SF-ROBS favors root development. The reason for this may lie in the reduced contact between the roots and the substrate in the SF-ROBS compared to soil. To counteract this situation, the plant will enhance the root surface contact for nutrients uptake by inducing a higher production of roots. Taken together, we demonstrate that the SF-ROBS is a convenient system to cultivate young maize plants. The main advantage of the SF-ROBS is the easy root handling. Roots can be accessed during any early developmental stage, and they can easily be removed from the system to perform treatments such as inoculations or microscopic observations. Moreover, harvesting roots for further experiments such as gene expression analysis or metabolomic fingerprinting is simplified using the SF-ROBS. Removing soil residues from roots is time-consuming and often leads to tissue damage, which might interfere with downstream experiments. However, the SF-ROBS requires regular changes of filter papers and a growth environment to limit the risk of contamination favored by the presence of a constant humid filter paper. Another limiting factor of the SF-ROBS is the root growth and age of the plants. Limited by the size of the pouch in our system, we were able to keep the plants no longer than about 21 days in the SF-ROBS (corresponding to a maize plant with four developed leaves). The short growth period does not allow studies on plant-microbe interactions which need a longer time to establish. An extended culture time would require a size modification of the pouches to accommodate a larger root system. Efforts to increase the size of such paper-based rhizotron systems are in progress.

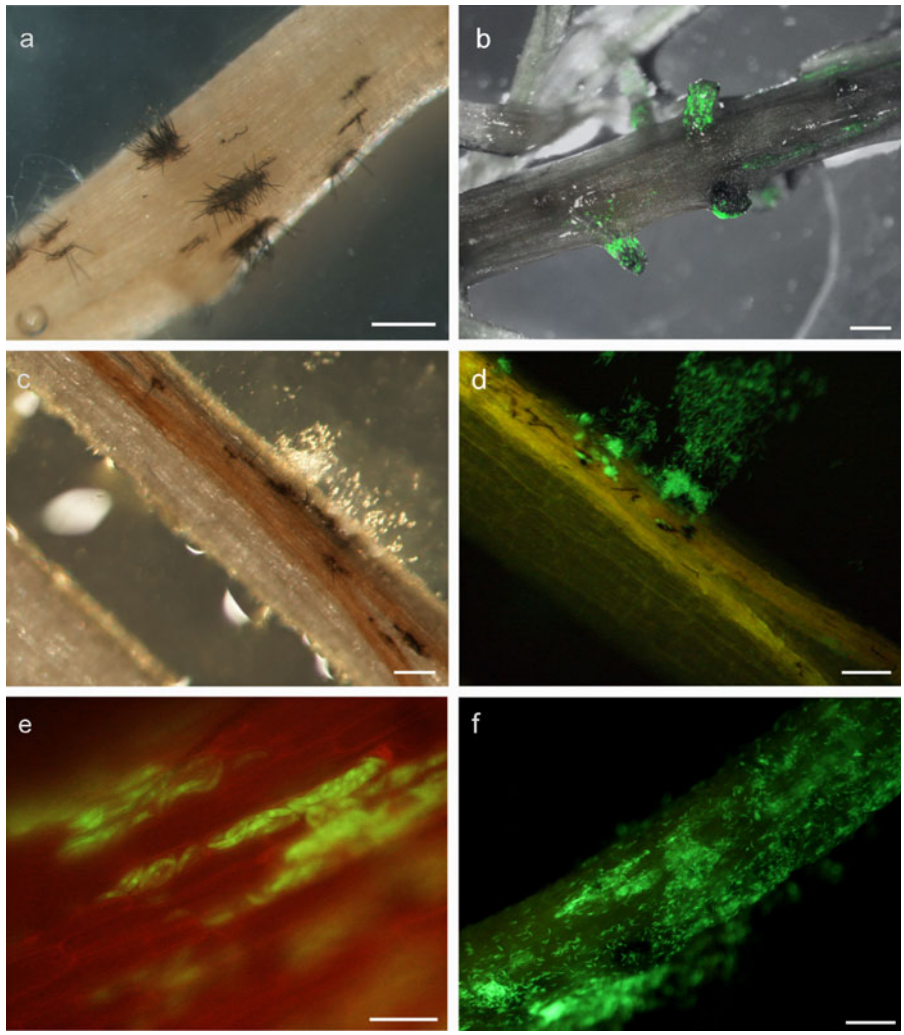

Fig. 5 ***C. graminicola-gGFP*-infection of maize roots grown in the SF-ROBS**. Roots of 10-day old plants were inoculated by dipping in a spore suspension and kept in the SF-ROBS for further development. Colonization was observed under epifluorescence **b**, **d**, **e** and **f** and bright field illumination **a**, **c**. **a** Acervuli with characteristic setae on infected roots, 4 dpi. **b** Early infection stage on an inoculated root (1 dpi); the fluorescence-image is superimposed over the bright field picture. **c**, **d** The same infected root viewed under UV and visible light at 5 dpi, showing conidia flowing out of acervuli. **e** Root epidermal cell packed with falcate conidia, 5dpi. **f** Heavily infected root at late stage, 6dpi. Bars=100 μm **a**, **b**, **c**, **d**, **f** and 30 μm **e**

The SF-ROBS is convenient to study fungal root infections

Thus far, described culture methods of soil-free systems in combination with pathogens are scarce and often limited to in vitro analysis. Here, we present a soil-free growth system allowing in vivo observations of fungal root infections of maize. The *C. graminicola* infection assays performed in the SF-ROBS resulted in a colonization behavior and pattern which are similar to observations made for soil-grown plants (Sukno et al. 2008), indicating that the SF-ROBS does not alter the natural infection process. We observed an earlier development of acervuli on infected roots in the SF-ROBS compared to published data from a soil system (Sukno et al. 2008). This suggests that the infection process might be favored in the SF-ROBS, possibly through the constant humidity of the system but also by the enhanced infection efficiency or a decreased antiphytopathogenic potential in the paper compared to natural soil. Normally, maize roots are inoculated by either soaking seedlings (2 days after germination) in a spore suspension or by growing older seedlings in vermiculite mix containing agar plugs from *C. graminicola* cultures (Sukno et al. 2008). Especially the agar plug method makes it difficult to control the colonization of a specific root part, which results in a less efficient infection rate. The SF-ROBS allows the infection of specific root parts and enables easy sampling for downstream plant-pathogen interaction analysis such as gene expression profiling or hormone quantification.

The SF-ROBS allows the study of root-bacteria interactions

Despite the fact that plant-beneficial microbe interactions are an emerging research field, the molecular and chemical mechanisms underlying these interactions remain largely unknown. As for root-pathogen interactions, the arduous accessibility of roots is also an issue when studying beneficial root microbes.

The ease of root harvesting makes the SF-ROBS an advantageous system to study the interactions of roots and beneficial root-colonizing microbes. Instead of a bacterial inoculation of soil, it is possible to inoculate the roots directly without damaging them. Here, we show that the SF-ROBS is suitable for cultivating maize roots inoculated with the root beneficial bacterium *P. putida* KT2440. *P. putida* KT2440 was successfully recovered from 11-day old roots grown either in the SF-ROBS or in the soil, showing that the SF-ROBS does not inhibit bacterial development in plant roots. A similar amount of bacteria was found in roots of SF-ROBS-grown plants as well as in roots of soil-grown plants, indicating that growth conditions do not affect the potential of bacterial root colonization. Bacterial colonization along the length of the root was similar in maize plants grown in the SF-ROBS as for plants grown in soil and as previously described in other systems (Simons et al. 1996) with a decreased gradient of bacteria from the root base towards the root tip. Furthermore, the density of *P. putida* KT2440 on roots is critical for bacterial contribution to plant defense. Raaijmakers et al. (1995) showed that a bacterial density of approximately 10^5 CFU per gram of root is required for direct disease suppression and induction of plant resistance. Hence, the SF-ROBS could be used for studies on maize resistance mechanisms induced by rhizobacteria.

Since the availability of nutrients for the plant is controlled through the supply by the MNS growth medium in the SF-ROBS, it would be also easily possible to study the importance of selected nutrients or combinations thereof on root colonization, on direct bacterial inhibitory effects or on plant induced resistance mechanisms.

To sum it up, we have presented here a soil-free growth system that allows the non-destructive study of interactions of roots with pathogenic and beneficial microorganisms. Moreover, our system is suitable for crop plants such as maize and could therefore contribute to a better understanding, and finally management, of belowground stress situations of plants.

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References

Ahn IP, Lee SW, Kim MG, Park SR, Hwang DJ, Bae SC (2011) Priming by rhizobacterium protects tomato plants from biotrophic and necrotrophic pathogen infections through multiple defense mechanisms. Mol Cells 32:7–14. doi:10.1007/s10059-011-2209-6

Boeuf-Tremblay V, Plantureux S, Guckert A (1995) Influence of mechanical impedance on root exudation of maize seedlings at two development stages. Plant Soil 172:279–287. doi:10.1007/BF00011330

Deshmukh S, Hückelhoven R, Schäfer P, Imani J, Sharma M, Weiss M, Waller F, Kogel KH (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc Natl Acad Sci USA 103:18450–18457. doi:10.1073/pnas.0605697103

De Vleesschauwer D, Höfte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281. doi:10.1016/S0065-2296(09)51006-3

Djonovic S, Vargas W, Kolomiets M, Horndeski M, Wiest A, Kenerley C (2007) A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol 145:875–889. doi:10.1104/pp. 107.103689

Erb M, Flors V, Karlen D, de Lange E, Planchamp C, d'Alessandro M, Turlings TCJ, Ton J (2009) Signal signature of aboveground-induced resistance upon belowground herbivory in maize. Plant J 59:292–302. doi:10.1111/j.1365-313X.2009.03868.x

Erb M, Balmer D, De Lange ES, Von Merey G, Planchamp C, Robert CAM, Röder G, Sobhy I, Zwahlen C, Mauch-Mani B, Turlings TCJ (2011) Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. Plant Cell Environ 34:1088–1103. doi:10.1111/j.1365-3040.2011.02307.x

Gewin V (2010) Food: an underground revolution. Nature 466:552–553

Gibeaut DM, Hulett J, Cramer GR, Seemann JR (1997) Maximal biomass of *Arabidopsis thaliana* using a simple, low-maintenance hydroponic method and favorable environmental conditions. Plant Physiol 115:317–319

Gunning T, Cahill DM (2009) A soil-free plant growth system to faciliate analysis of plant pathogen interactions in roots. J Phytopathol 157:497–501. doi:10.1111/j.1439-0434.2008.01503.x

Hétu MF, Tremblay LJ, Lefebvre DD (2005) High root biomass production in anchored Arabidopsis plants grown in axenic

sucrose supplemented liquid culture. Biotechniques 39:345–349

Huang CJ, Yang KH, Liu YH, Lin YJ, Chen CY (2010) Suppression of southern corn leaf blight by a plant growth-promoting rhizobacterium *Bacillus cereus* C1L. Ann Appl Biol 157:45–53. doi:10.1111/j.1744-7348.2012.00408.x

Hund A, Reimer R, Stamp P, Walter A (2012) Can we improve heterosis for root growth of maize by selecting parental inbred lines with different temperature behavior? Philos Trans R Soc B-Biol Sci 367:1580–1588. doi:10.1098/rstb.2011.0242

Hund A, Ruta N, Liedgens M (2009a) Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. Plant Soil 318:311–325. doi:10.1007/s11104-008-9843-6

Hund A, Trachsel S, Stamp P (2009b) Growth of axile and lateral roots of maize: I development of a phenotyping platform. Plant Soil 325:335–349. doi:10.1007/s111104-009-9984-2

Ishiga Y, Ishiga T, Uppalapati SR, Mysore KS (2011) Arabidopsis seedling flood-inoculation technique: a rapid and reliable assay for studying plant-bacterial interactions. Plant Methods 7:32. doi:10.1186/1746-4811-7-32

Kaplan I, Halitschke R, Kessler A, Rehill BJ, Sardanelli S, Denno RF (2008) Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory. Ecol Lett 11:841–851. doi:10.1111/j.1461-0248.2008.01200.x

Kim DW, Rakwal R, Agrawal GK, Jung YH, Shibato J, Jwa NS, Iwahashi Y, Iwahashi H, Kim DH, Shim IS, Usui K (2005) A hydroponic rice seedling culture model system for investigating proteome of salt stress in rice leaf. Electrophoresis 26:4521–4539. doi:10.1002/elps.200500334

Matilla MA, Ramos JL, Bakker PAHM, Doornbos R, Badri DV, Vivanco JM, Ramos-González MI (2010) *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in Arabidopsis root exudation. Environ Microbiol Rep 2:381–388. doi:10.1111/j.1758-2229.2009.00091.x

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Can J Microbiol 55:1302–1309. doi:10.1139/w11-044

Neal AL, Ahmad S, Gordon-Weeks R, Ton J (2012) Benzoxazinoids in root exudates of maize attract *Pseudomonas putida* to the rhizosphere. PLoS ONE 7(4):e35498. doi:10.1371/journal.pone.0035498

Okubara PA, Paulitz TC (2005) Root defense responses to fungal pathogens: a molecular perspective. Plant Soil 274:215–226. doi:10.007/s11104-004-7328-9

Pineda A, Zheng SJ, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci 15:507–514. doi:10.1016/j.tplants.2010.05.007

Raaijmakers JM, Leeman M, van Oorschot MMP, van der Sluis I, Schippers B, Bakker PAHM (1995) Dose–response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85:1075–1081. doi:10.1094/Phyto-85-1075

Rasmann S, Agrawal AA (2008) In defense of roots: a research agenda for studying plant resistance to belowground herbivory. Plant Physiol 146:875–880. doi:10.1104/pp.107.112045

Ruta N, Liedgens M, Fracheboud Y, Stamp P, Hund A (2009) QTLs for the elongation of axile and lateral roots of maize in response to low water potential. Theor Appl Genet 120:621–631. doi:10.1007/s00122-009-1180-5

Sambrook J, Russell DW (2001) Molecular cloning. Cold Spring Harbor, NY

Schulze J, Pöschel G (2004) Bacterial inoculation of maize affects carbon allocation to roots and carbon turnover in the rhizosphere. Plant Soil 267:235–241. doi:10.1007/s11104-005-4980-7

Simons M, van der Bij AJ, de Weger LA, Wijffelman CA, Lugtenberg BJJ (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol Plant Microbe In 9:600–607

Smith GS, Johnston CM, Cornforth IS (1983) Comparison of nutrient solutions for growth of plants in sand culture. New Phytol 94:537–548. doi:10.1111/j.1469-8137.1983.tb04863.x

Sukno SA, Garcia VM, Shaw BD, Thon MR (2008) Root infection and systemic colonization of maize by *Colletotrichum graminicola*. Appl Environ Microbiol 74:823–832. doi:10.1128/AEM.01165-07

Trachsel S, Stamp P, Hund A (2010) Effect of high temperatures, drought and aluminum toxicity on root growth of tropical maize (*Zea mays* l.) Seedlings. Maydica 55:249–260

Vaughan MM, Tholl D, Tokuhisa JG (2011) An aeroponic culture system for the study of root herbivory on *Arabidopsis thaliana*. Plant Methods 7:5. doi:10.1186/1746-4811-7-5