New Phytologist

Research

Plant growth-promoting rhizobacterium *Pseudomonas* sp. CM11 specifically induces lateral roots

Qian Li[1,2,3]*, Huchen Li[1,2]*, Zhuang Yang[2], Xu Cheng[2], Yaceng Zhao[4], Ling Qin[1,4], Ton Bisseling[1,2], Qingqin Cao[1,4] and Viola Willemsen[2]

[1]Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing University of Agriculture, Beijing 102206, China; [2]Cluster of Plant Developmental Biology, Laboratory of Molecular Biology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; [3]Beijing Bei Nong Enterprise Management Co. Ltd, Beijing 102206, China; [4]College of Plant Science and Technology, Beijing Key Laboratory for Agricultural Application and New Technique, Beijing University of Agriculture, Beijing 102206, China

Authors for correspondence:
Viola Willemsen
Email: Viola.Willemsen@WUR.NL

Qingqin Cao
Email: caoqingqin@bua.edu.cn

Received: *13 January 2022*
Accepted: *28 April 2022*

New Phytologist (2022)
doi: 10.1111/nph.18199

Key words: lateral roots, plant growth-promoting, PLETHORA, *Pseudomonas*, Rhizobacteria, root architecture, secondary roots.

Summary

- Plant growth-promoting rhizobacteria are involved in altering secondary root (SR) formation, but hitherto there has been no distinction between the different types of SRs upon induction of soil biota, and the genetic pathways involved.
- By using plate and soil systems, we studied the effects of the *Pseudomonas* strains CM11 and WCS417 on plant performance with a focus on root development. Through a combination of cellular, molecular and genetic analyses, we investigated the type of SRs induced upon CM11 and WCS417 root inoculation using genetic pathways associated with specific SR types.
- CM11 was shown to affect the root architecture differently from WCS417. CM11 inoculation leads to primary root arrest, whereas WCS417 reveals a longer primary root. Both CM11 and WCS417 activate the *PLETHORA 3,5,7*-controlled lateral root pathway, rather than the *WUSCHEL-RELATED HOMEOBOX 11,12*-controlled adventitious (lateral) root pathway. In addition, CM11 promotes plant growth in model and various crop species. It improves plant fitness traits, such as bigger shoots, faster bolting and higher yield in terms of seeds.
- Our results indicate that the root system architecture can be promoted by activation of *PLETHORA 3,5,7* dependent primed lateral pre-branch sites upon inoculation with CM11, which creates great potential to gain a better understanding of root plasticity.

Introduction

Plants roots are colonized by an enormous number of microorganisms that can have profound effects on, for example, plant growth and development, nutrient uptake, conferring tolerance to abiotic stress and suppression of diseases (Philippot *et al.*, 2013). The microorganisms that are in contact with the plant are collectively referred to as the plant microbiome (Mendes *et al.*, 2013). The microbiome composition of the rhizosphere, which is a narrow zone surrounding plant roots, is different from that of bulk soil (Kuzyakov, 2002). It has been proposed that the rhizosphere microbiome can be modulated by plants to enrich beneficial microorganisms (Cook *et al.*, 1995; Mendes *et al.*, 2013; Huang *et al.*, 2019; Song *et al.*, 2021). This is supported by the characterization of several rhizosphere bacteria that have been well documented for their beneficial effects on plant growth and health (Berendsen *et al.*, 2012; Lakshmanan *et al.*, 2014; Tkacz & Poole, 2015). In the model plant Arabidopsis, it has been shown that plant growth-promoting rhizobacteria (PGPR), for example *Bacillus*, *Pseudomonas* and *Phyllobacteria* strains, can alter the root system by producing volatile and secondary metabolites, or by affecting phytohormone homeostasis and/or signaling, (Zhang *et al.*, 2007; Contesto *et al.*, 2010; Ortiz-Castro *et al.*, 2011; Zamioudis *et al.*, 2013; Cheng *et al.*, 2017; Kudoyarova *et al.*, 2019; Dahmani *et al.*, 2020; Jiménez-Vázquez *et al.*, 2020; Li *et al.*, 2021).

The root system of dicotyledons, for example in Arabidopsis, is composed of a primary root (PR) that is formed during embryogenesis and several types of side roots that are formed post-embryonically and can be classified further depending on the tissue which they branch from (Scheres *et al.*, 1994; Casimiro *et al.*, 2003; Verstraeten *et al.*, 2014). Side roots can be subdivided into secondary roots (SRs) that branch from the PR, higher order roots, or adventitious roots that formed from nonroot tissues. SRs can be further subdivided in lateral roots (LRs) and adventitious lateral roots (adLRs) (Bellini *et al.*, 2014; Sheng *et al.*, 2017; Ge *et al.*, 2019; Motte *et al.*, 2019). Side roots are morphologically undistinguishable from the PR. They are both maintained by the stem cell niche composed of a quiescent center (QC) and its surrounding stem cells that will form different tissue

*These authors contributed equally to this work.

layers (Dolan *et al.*, 1993; van den Berg *et al.*, 1997; Bennett & Scheres, 2010; Torres-Martinez *et al.*, 2019; Pardal & Heidstra, 2021). LRs originate from a subset of xylem pole associated pericycle cells, which are primed by temporal oscillations in auxin signaling that correlates with cell growth in the root meristem (Laskowski *et al.*, 1995; Dubrovsky *et al.*, 2000; Beeckman *et al.*, 2001; Dubrovsky *et al.*, 2008; Dubrovsky & Rost, 2012; Beeckman & De Smet, 2014; van den Berg *et al.*, 2021). In these primed cells, auxin can activate the plant-specific AP2 transcription factors PLETHORA (PLT) 3, PLT 5, and PLT 7. These PLTs are essential for subsequent LR initiation, emergence and spacing of LR primordia (Hofhuis *et al.*, 2013; Du & Scheres, 2017, 2018). In response to wounding or other environmental signals, Arabidopsis activates the adventitious rooting pathway to produce another class of side roots, adLRs or ARs (Geiss *et al.*, 2009; Sheng *et al.*, 2017; Karlova *et al.*, 2021). adLRs are not primed in the root meristem, but are initiated *de novo* between LRs in a non-acropetal sequence (Sheng *et al.*, 2017). In adLR and AR, auxin promotes the fate transition of the *de novo* root founder pericycle cells by upregulating the expression of *WUSCHEL-RELATED HOMEOBOX 11* (*WOX11*) and the partially redundant *WOX12* to initiate adventitious root primordia (Liu *et al.*, 2014; Sheng *et al.*, 2017; Xu, 2018).

The well-known PGPR *Pseudomonas simiae* WCS417 and *Bacillus amyloliquefaciens* SQR9, have been shown to be able to induce SR formation (Sukumar *et al.*, 2013; Zamioudis *et al.*, 2013; Verbon & Liberman, 2016; Li *et al.*, 2021). Although it has been demonstrated that these PGPR can induce the formation of SRs, it has not yet been reported what kind of SRs are induced and which genetic pathways are involved. Furthermore, certain rhizobacteria are capable of modulating the root system architecture and significantly impact aboveground plant growth (Zamioudis *et al.*, 2013; Jiménez-Vázquez *et al.*, 2020; Li *et al.*, 2021), which is a highly desirable trait for obtaining more efficient and sustainable plant growth.

Recent studies on the rhizosphere of various crops confirm the frequent occurrence of species belonging to the *Pseudomonas* genus, and which can represent a source of PGPR (Ofek *et al.*, 2014; Walters *et al.*, 2018; Chiniquy *et al.*, 2021). Previously, we have shown that *Pseudomonas* spp. formed the most abundant OTU (operational taxonomic unit) in the rhizosphere of a long-living tree, *Castanea mollissima* (CM). Eleven *Pseudomonas* strains (CM1 to 11) belonging to this OTU were isolated and the CM11 strain was > 50-fold more abundant than the other individual strains. CM11 induces plant growth promotion of Arabidopsis seedlings (Cheng *et al.*, 2020).

Here, we studied the CM1–CM11 strains and selected the CM11 strain based on its enhancement of plant performance. We compared the effects of the CM11 strain to those of the WCS417 strain with a focus on root development by using different growing conditions. Through a combination of cellular, molecular, and genetic analysis, we show what type of SRs are induced by CM11 and WCS417. These new insights into an apparent diversity in induction mechanisms provide a stepping stone for the application potential of combinations of PGPR for crop improvement.

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana L. cv Columbia-0 (Col0) was used as wild-type (WT). For lettuce (*Lactuca sativa* L. cv 'beisansheng no. 4') and for tomato (*Solanum lycopersicum* L. cv 'Ailsa Craig'), seeds were used. The Arabidopsis mutants used were the following, *plt3plt5plt7* (Hofhuis *et al.*, 2013), *wox11wox12* (*wox11-2* (SALK_004777), *wox12-1* (*SALK_087882*) (Alonso *et al.*, 2003)). Reporter lines used were *pCYCB1;1::GFP* (Ubeda-Tomas *et al.*, 2009), *pSCR::SCR::GFP* (Heidstra *et al.*, 2004), *pWOX5::GFP* (Haecker *et al.*, 2004), *pPLT7::GUS* (Hofhuis *et al.*, 2013), *pWOX11::GUS* and *35S::WOX11-SRDX* (Liu *et al.*, 2014) and *DR5::vYFP* (Benková *et al.*, 2003).

Arabidopsis seeds were surface-sterilized with 5% (v/v) sodium hypochlorite. Sterile seeds were sown on plates containing 50 ml ½ Murashige & Skoog (MS) medium supplemented with 5 g l^{-1} sucrose or not. After 2 d of stratification, plates were positioned vertically in a growth chamber under a long-day photoperiod (16 h : 8 h, light : dark, relative humidity 60%) at 22°C (Willemsen *et al.*, 1998). Lettuce and tomato seedlings both were grown on ½MS medium with 5 g l^{-1} sucrose under the same conditions as Arabidopsis seedlings, the only difference being that tomato was growing at 25°C.

Plant–bacteria co-cultivation

Bacterial strains used for the experiments were *Pseudomonas simiae* WCS417 and *Pseudomonas Castanea mollissima* (CM) strain 1–11 (Zamioudis *et al.*, 2013; Cheng *et al.*, 2020). Individual *Pseudomonas* strains were cultured in liquid King's B medium (KB) for 12 h as described in Cheng *et al.* (2020). Bacterial cells were centrifuged, washed and re-suspended in 10 mM $MgSO_4$ and adjusted to a final density of 10^9 CFU ml^{-1} (OD_{600} = 1.0).

For the plate experiment, each Arabidopsis seedling was inoculated at the same position with 2-µl suspensions containing a single strain or a combination (1 : 1, v/v), or with 2 µl 10 mM $MgSO_4$ as mock or with heat-inactivated (5 min 100°C) CM11 bacteria (Supporting Information Fig. S1b,c), and co-cultivated for the indicated duration. Each lettuce and tomato seedlings was inoculated at the root tip with 5-µl CM11 suspensions, or with 5-µl 10 mM $MgSO_4$ as a mock.

For pot experiment, 7 d post-germination (dpg) Arabidopsis seedlings (one seedling per pot), or 10 dpg lettuce and tomato seedlings (10 seedlings per box) were transferred to containers carrying vermiculite : soil mixture (1 : 1, v/v) that was autoclaved twice for 120 min at 120°C with an interval of 24 h. Immediately after transplanting, the soil was inoculated with 50 ml bacterial suspension (10^8 CFU ml^{-1}) or with 50 ml 10 mM $MgSO_4$ as a mock. Plants were grown in the growth chamber and periodically watered to maintain a 50–60% soil humidity. For each treatment, three independent experiments were carried out and for each experiment ≥ 10 plants were used.

Growth phenotype analyses

The inoculation sites were marked at the positions of plant root tip when the plants were inoculated. After co-cultivation of seedlings with bacteria for an indicated duration on agar plates, digital images (600 dpi) of Arabidopsis, lettuce and tomato seedlings were captured using an Expression 12000XL Photo Scanner system (Epson, Nagano, Japan). The length of the PR below the inoculation point was quantified with the ImageJ analysis program v.1.52a (Hartig, 2013).

The number of emerged SR (> 0.5 mm) was counted from the high-resolution images scanned produced by the experiment described above. Because the CM11-treated plants harbor a much shorter PR, we could only count the SR numbers above the inoculation sites. The nonemerged SR called LR primordia initiation (LRI) or adLR primordia initiation (adLRI) were quantified using *pPLT7::GUS* or *pWOX11::GUS* lines at 2 dpi, respectively (GUS, β-glucuronidase).

The meristem length and cell number in the root meristem were determined by counting cortical cells from the QC cells to the first elongated cortical cell at 5 dpi. The size of fully differentiated cells was determined by measuring the length of cortical cells in the differentiation zone.

For Arabidopsis root hair observation, digital images were obtained from the PR segment located above the root tip with a microscope at magnification ×12.5 (Zeiss).

For FW measurements of Arabidopsis, lettuce and tomato, the shoot and the root of seedlings were cut at the junction between root and shoot. Directly after cutting, the FWs of shoot and root were measured.

Leaves of 10 Arabidopsis pot seedlings per treatment were imaged using an Expression 12000XL Photo Scanner system (Epson). Leaf areas per plant were measured using ImageJ v.1.52a (Hartig, 2013).

In order to evaluate the effect of CM11 on plant fitness in soil, the bolting time was determined by the date that the first-developed flower meristem was separated from the rest of the rosette by at least a few mm.

Plant height was measured with a ruler on the day that the first silique and its petiole was dry and yellow. Plants were harvested at least one month after their drying date, when all siliques were dry. The number of seeds was calculated by counting and weighing a sample of 100 seeds; weight of 100 seeds/100 = weight per seed and weighing the total amount of seed per plant/weight per seed = no. of seeds per plant.

The digital images displaying phenotypes of 100 dry seed per treatment were acquired via stereomicroscopy (Zeiss). Seed sizes were measured using ImageJ v.1.52a (Hartig, 2013).

Mature seeds of mock and CM11-inoculated plants were harvested on the same day. Surface-sterilized seeds were plated on the medium described above, stratified and then transferred to the growth chamber described above. The percentage of seeds with radicle emergence (germination) was calculated at 5 d after stratification. One hundred seeds per treatment were performed with three biological replicates.

Microscopy and histology

Part of the confocal images were acquired using a SP8 confocal laser scanning microscope (Leica). Fresh transgenic roots were mounted on slides with 10 μM propidium iodide for cell outline staining. Green and yellow fluorescent protein (GFP and YFP) were detected with an excitation wavelength of 488 nm and propidium iodide was detected with an excitation wavelength of 543 nm. To visualize amyloplast in columella cells, mPS-PI staining was performed according to Truernit *et al.* (2008).

The meristem length was visualized by SCRI staining using the LSM 710 (Zeiss) with 405 nm laser (Kerstens *et al.*, 2020).

β-glucuronidase activity was visualized after incubation of transgenic plants for 3 h at 37°C in a GUS reaction buffer (Willemsen *et al.*, 1998). Then roots were mounted on slides in chloral hydrate solution (8 chloralhydrate : 3 H_2O : 1 glycerol) and analyzed under an Axio Imager A1 microscope (Zeiss) with Nomarski optics (Willemsen *et al.*, 1998).

GFP labeling and CM11 colonization

The CM11 strain was labeled with the GFP that was driven by *lac* promoter (P_{lac}*::GFP*/pBBR1MCS-2) according to Li *et al.* (2017). Seven-day-old Arabidopsis seedlings grown on plates were inoculated at corresponding positions with 2-μl suspension containing CM11-GFP or 2-μl 10 mM $MgSO_4$ as a mock. The roots were imaged at 1 dpi by confocal laser scanning microscope as described above.

Results

Colonization by CM strains affects plant growth in Arabidopsis

In order to test whether the 1 different *Pseudomonas* CM strains, recently identified by Cheng *et al.* (2020), play a role in plant growth, Arabidopsis WT seedlings were grown on agar plates and at 7 d post-germination (dpg) inoculated with the individual strains at the root tip. At 10 dpi, a strong reduction of PR growth was observed in all 11 CM inoculated plants, compared with the mock (Figs 1a,b, S1a). Inoculation of heat-inactivated (dead) CM11 could not suppress PR growth (Fig. S1b,c). Additionally, all plants inoculated with the 11 CM strains developed more SRs, but it was striking that the SRs of the CM6–10 inoculated plants stayed very short compared with those emerged from CM11 and CM1–5 inoculated plants, as well as the mock (Figs 1a,c, S1a).

Besides the effect on root architecture, we observed an increased root and shoot size in CM11- and CM5-treated plants (Fig. 1a). To quantify the effect of the 11 CM strains on root and shoot biomass, we measured the FW of roots and shoots at 10 dpi. A significant difference in root and shoot FW was present between CM11 and CM5 inoculated and mock inoculated plants. Root FW of CM11 and CM5 inoculated plants was increased 2.5- and 1.9-fold, respectively, compared to the mock (Fig. 1d). Correspondingly, an increase of 1.9-fold for CM11 and 1.5-fold for CM5 was observed for shoot FW. CM1–4 only

New Phytologist

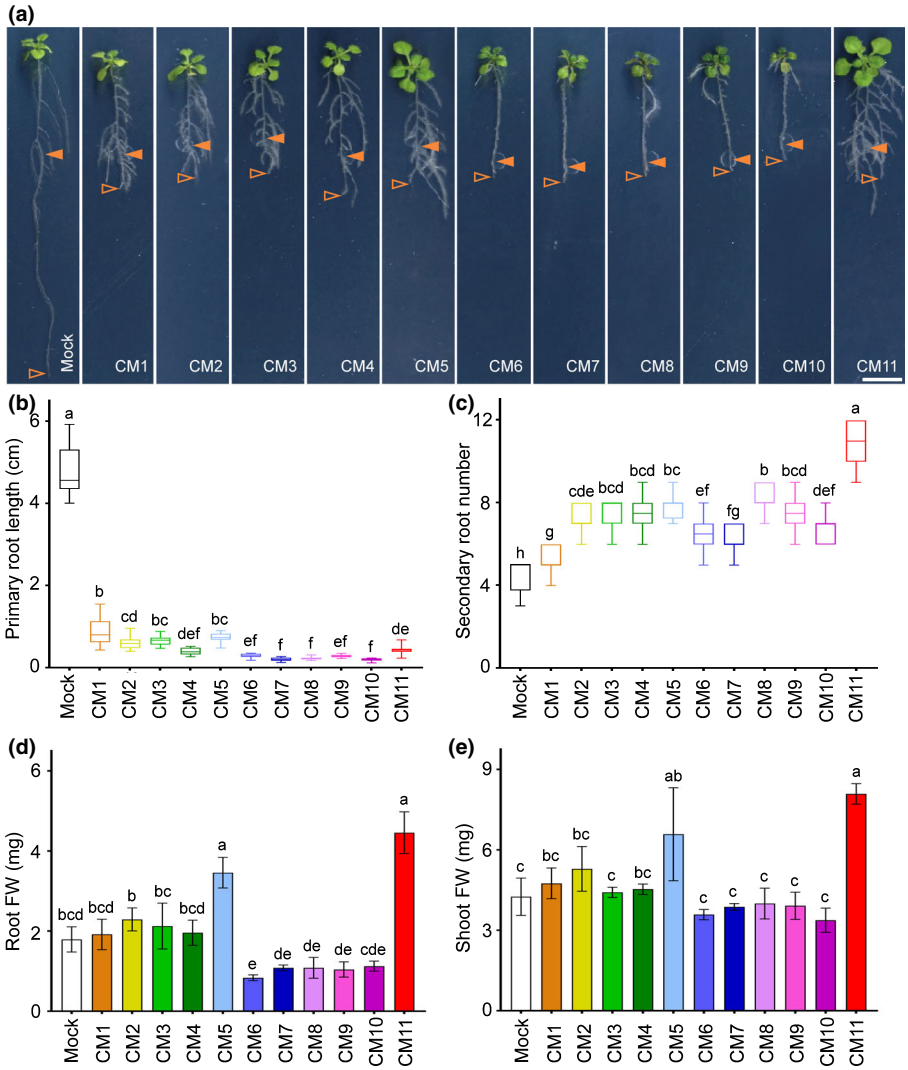

Fig. 1 Inoculation of *Pseudomonas* spp. strains CM1–CM11 affects plant growth. (a) Representative images of Arabidopsis wild-type (WT) seedlings growing on ½ Murashige & Skoog (MS) agar plates with CM1–CM11 strains or mock. Closed arrowheads, inoculation sites; open arrowheads, tips of primary roots (PRs). Bar, 1 cm. (b–c) Quantification of PR length (newly grown PR below the inoculation sites, b) and number of emerged secondary roots (SRs) (emerged SRs above the inoculation sites, c) in mock and CM1–CM11 inoculated plants. (d–e) Root (d) and shoot (e) FW production per Arabidopsis seedling with the indicated bacterial strains or mock. In all cases, 7-d-old seedlings were inoculated with mock or CM1-CM11 bacterial suspensions at the root tip and grown 10 d post-inoculation (dpi). Box plots in (b) and (c) indicate the lower and upper quartiles, bars represent the maximum and minimum values. The line in the box indicates the median. Data in (d) and (e) represent mean ± SD of three biological replicates, each consisting of eight seedlings. Different letters indicate statistically significant differences (LSD and Tukey's honestly significant difference (HSD) tests; $P \leq 0.05$).

slightly increased the root and shoot FW. By contrast, CM6–10 showed a decrease in both root and shoot FW (Fig. 1d,e). Of the different strains, CM11 showed the strongest increase in SR number, root and shoot biomass production. In the following experiments, we selected this strain for in depth analysis.

Beneficial *Pseudomonas* strains CM11 and WCS417 alter root architecture

The previously identified PGPR model strain *Pseudomonas simiae* WCS417 also inhibits PR elongation and promotes SR formation of Arabidopsis seedlings grown on agar plates (Zamioudis *et al.*, 2013). This resembles the phenotype upon inoculation with CM11. In addition, recently it has been shown that WCS417 mainly colonize root tips (Pieterse *et al.*, 2021), and by using CM11 strain expressing GFP (CM11-GFP) inoculated on root tips, hypocotyl and whole roots, we observed that CM11 showed the same root tip colonization pattern (Figs 2a, S2a). Then we asked whether CM11 alters root architecture in the same way as WCS417.

In order to study this we inoculated an equal amount of CM11 or WCS417 on 7 dpg seedling root tips growing on ½MS medium without sugar, and compared the growth response with that of the mock. PR growth was reduced upon CM11 inoculation, whereas WCS417 significantly increased PR growth (Figs 2b,c, S2b). Both strains induced more SRs than the mock, but CM11 resulted in the highest number of SRs compared to the mock treatment (Figs 2b,d, S2b). To create an 'ideal' root system that would root deeper – as has been found with WCS417 inoculation – and has a higher SR density – as has been seen in CM11 inoculated roots – we co-inoculated CM11 and WCS417 (Co-inoc). Inoculation of WT seedlings roots with the Co-inoc resulted in an intermediate root phenotype: a longer PR than CM11 and mock inoculated roots, but a shorter PR compared to WCS417 inoculated roots; and more SRs than both the mock and the WCS417 inoculated roots, but fewer SRs than in CM11 inoculated roots (Figs 2b–d, S2b). Together, these data demonstrate that CM11 and WCS417 differently affect PR growth and SR initiation.

It is noteworthy that WCS417 was reported to repress PR growth (Zamioudis *et al.*, 2013), which contradicts with our study. This might be caused by the differences in inoculating position (at root tips or at 5 cm below the root tips) and/or

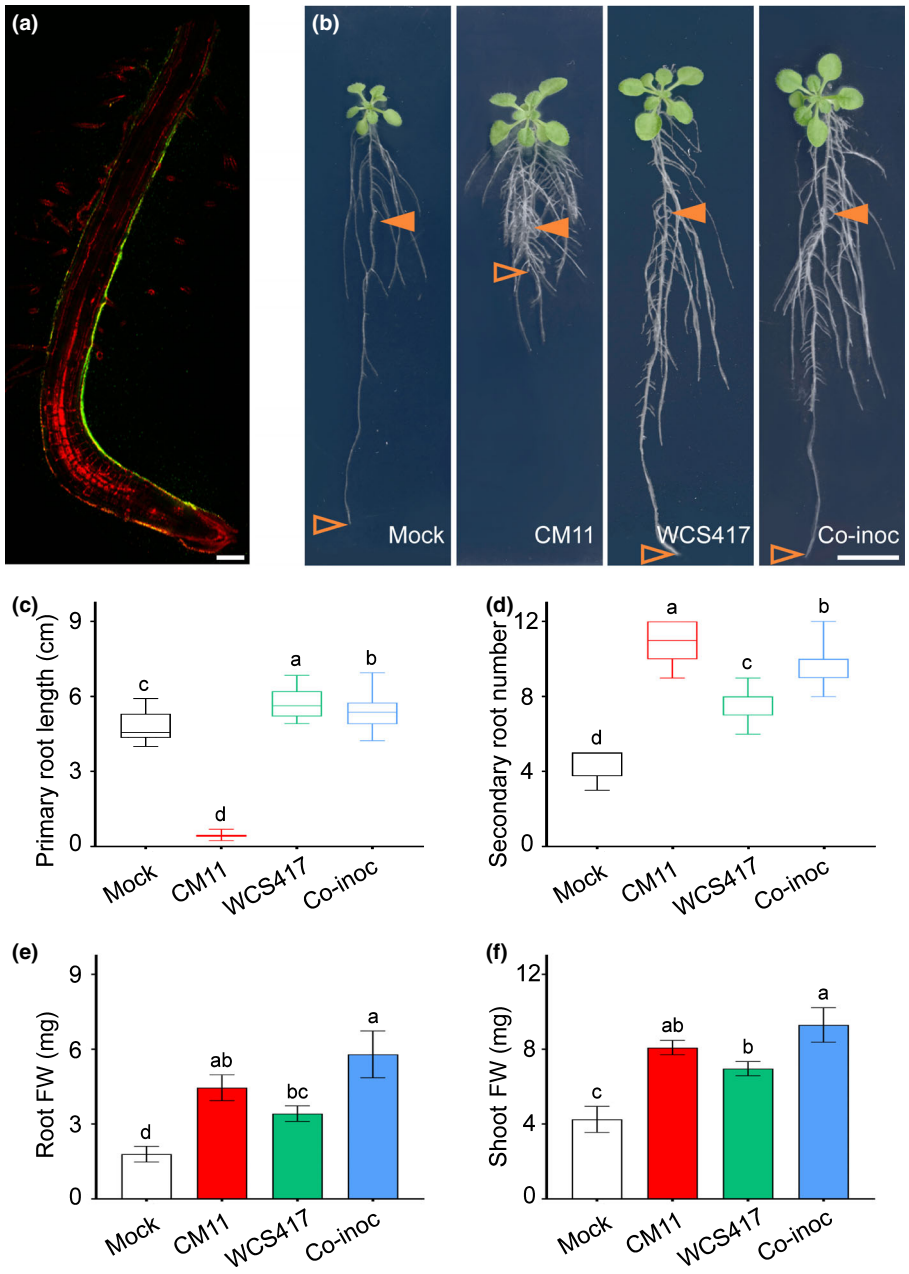

Fig. 2 CM11 and WCS417 play additive roles in plant growth promotion. (a) Colonization of CM11 in the rhizosphere of Arabidopsis seedlings grown on plates. 7-d-old seedlings were inoculated with CM11-GFP bacterial suspensions at the root tip and grown 1 d post-inoculation (dpi). Red, propidium iodide; green, green fluorescent protein (GFP). (b) Representative images of seedlings growing on ½ Murashige & Skoog (MS) agar plates with CM11, WCS417 and CM11+WCS417 (Co-inoc) or mock. Closed arrowheads, inoculation sites; open arrowheads, root tips of primary root (PR). (c–d) Quantification of PR length (newly grown PR below the inoculation sites, c) and number of emerged secondary roots (SRs) (emerged SRs above the inoculation sites, d) in mock and bacteria inoculated plants. (e–f) Root (e) and shoot (f) FW production per Arabidopsis seedling with the indicated bacterial strains or mock. In (b–f), 7-d-old seedlings were inoculated with mock or bacterial suspensions at the root tip and grown 10 dpi. Box plots in (c–d) indicate the lower and upper quartiles, bars represent the maximum and minimum values. The line in the box indicates the median. Data in (e–f) represent mean ± SD of three biological replicates, each consisting of eight seedlings. Different letters indicate statistically significant differences (LSD and Tukey's honestly significant difference (HSD) tests; $P \leq 0.05$). Bars: (a) 100 μm; (b) 1 cm.

growth medium (with sugar or without sugar) between the two studies. We compared the effect of CM11 and WCS417 inoculation – with equal amounts (10^9 CFU ml^{-1}) of bacteria – on 4-d-old Arabidopsis root architecture alteration under these different conditions. When the Arabidopsis seedlings were growing on ½MS medium containing sugar, we showed that inoculation of WCS417 either at root tips or at 5 cm below root tips both inhibited PR growth, the latter inoculation position as well as the growing condition are consistent with the previous study and showed the same result (Zamioudis *et al.*, 2013). CM11 only inhibited PR growth when it was inoculated at root tips, but not at 5 cm below root tips (Fig. S2c). Inoculation of WCS417 or CM11 at the two positions both induced SRs formation (Fig. S2d).

When the Arabidopsis seedlings were grown on ½MS medium without sugar, inoculation of CM11 at root tips inhibited the PR growth, whereas this did not occur when inoculation was at 5 cm below root tips. Inoculation of WCS417 did not show an obvious difference in PR growth between the two inoculation positions on ½MS (Fig. S2c). SR formation was induced when CM11 or WCS417 were inoculated at the root tips, this was not observed when the inoculation was done 5 cm below root tips (Fig. S2d). Similar results on PR growth and SR formation were obtained by Desrut *et al.* (2020), where WT seedlings grown on ½MS medium without sugar were inoculated at 1 cm below the shoot–root junction with WCS417. These data demonstrate that the effect of WCS417 and CM11 on root architecture is environment- or system-dependent.

Natural interactions occur in a sugar-limited environment and to minimize the amount of additions to the medium, but maintaining the same effect on root architecture for CM11, the sugar-free ½MS medium was chosen as growing medium for further experiments.

In addition to the alterations in root architecture, inoculation with CM11, WCS417 or the Co-inoc at the root tip increased the root FW (Fig. 2e) and shoot FW compared to the mock (Fig. 2f). The additive role of CM11 and WCS417 in plant biomass promotion, observed in the Co-inoc, together with the variation in the root architecture phenotypes, demonstrate that the two *Pseudomonas* strains CM11 and WCS417 affect plant growth promotion differently.

CM11 inoculation causes loss of the stem cell niche in primary roots

CM11 inoculation induced a reduction of the PR length different from WCS417 inoculation. To unravel the effect of CM11 inoculation on the kinetics of root growth, root growth was analyzed over time. Within the first 24 h post-inoculation, PR length was indistinguishable between CM11 inoculated seedlings and mock seedlings. However, a reduction in PR length after 24 h resulting in later growth arrest was observed in CM11 inoculated seedlings, whereas mock plants did not show a reduction in PR growth (Fig. S3a).

In order to test the possibility that the reduction of root length in CM11 inoculated seedlings was due to effects on reduced meristem activity, cell division or cell elongation, the meristem length, cell numbers and length of differentiated cells were assessed, respectively. The meristem of 5 dpi roots was analyzed by measuring the length and counting the number of cortical cells, starting from the QC up to the first expanding cortical cell. The meristem length and the number of cells were significantly lower (39% and 49% of the mock, respectively), correlating with a slightly smaller meristematic cell size (89% of the mock) in CM11 inoculated roots compared to the mock roots (Figs 3a,b, S3b). The same measurements were done for WCS417 and Co-inoc inoculated roots. Comparing these results to the meristems of CM11 and mock inoculated roots showed that WCS417, as well as Co-inoc have an increased meristem length correlating with an increase in the number of meristematic cells at 5 dpi (Figs 3a,b, S3b). Furthermore, loss of meristem activity in CM11 inoculated roots was confirmed by the lack of expression of the G2/M cell cycle reporter *pCYCB1,1::GFP* in 3 dpi CM11 inoculated roots, whereas this reporter showed a patchy expression pattern in mock roots (Fig. 3d,e) (Ubeda-Tomas *et al.*, 2009). To analyze whether the reduction of the PR length by CM11 inoculation was caused solely by a reduction of meristem activity, sizes of 10 cortical cells above the meristem of 10 individual seedlings were determined. A reduction of 0.35-fold in CM11 inoculated roots compared to the mock inoculated roots at 3 dpi was observed (Fig. 3c). However, WCS417 inoculation did not affect the mature cortical cell size (Fig. S3h). These results indicate that besides the effect on the meristem size caused by reduced cell divisions, CM11 also affects elongation of differentiating root cells, different from WCS417.

Root hairs normally are formed on differentiated epidermal cells above the meristem and this landmark can be used to confirm the root meristem length (Grierson & Schiefelbein, 2002). In CM11 inoculated roots, the root hairs are present in higher density and emerge closer to the root tip (Fig. S3c). This supports the observation of the loss of the elongation zone and the reduced activity of the meristematic zone.

The stem cell niche is a source for meristematic cells (van den Berg *et al.*, 1997). The loss of stem cell maintenance could account for a reduction in meristem size and thereafter affect the PR length. To investigate the maintenance of the stem cell niche, the reporter lines *pSCR::SCR::GFP* marking the endodermis and the QC, and *pWOX5::GFP* marking the QC were used. At 3 dpi a reduction in SCR expression and an extended expression of *pWOX5::GFP* in divided QC layers were observed (Fig. 3f–i). At 5 dpi, the root meristem had 'collapsed' and expression of *pSCR::SCR::GFP* and *pWOX5::GFP* was absent (Fig. S3d–g). Columella stem cells divide and form a new stem cell and a daughter cell that differentiates and can be shown by amyloplast staining (van den Berg *et al.*, 1995, 1997). To analyze columella stem cell maintenance, mPS-PI staining was used (Truernit *et al.*, 2008). The staining showed that the columella stem cells were still present at 3 dpi, but compared to the mock the amount of amyloplast in columella cells was markedly reduced upon CM11 inoculation (Fig. 3j,k).

In order to maintain the stem cell niche an auxin maximum is required. Alteration in the auxin maximum reflected by *DR5::vYFP* was studied after CM11 inoculation. The *DR5::vYFP* expression was reduced starting from 1 dpi and was hardly detected at 3 dpi (Fig. 3l–o), whereas a normal *DR5::vYFP* expression was seen upon WCS417 inoculation at 2 dpi (Fig. S3i–j). The loss of the auxin maximum before the differentiation of the columella stem cells and the absence of *pWOX5::GFP* expression in the stem cell niche, indicate that the loss of the auxin maximum is the primary cause of the disturbance of the stem cell niche and not caused by the loss of the stem cell niche that would disturb the auxin reflux loop. Taken together, these data indicate that the loss of the stem cell niche and the decline of cell divisions and elongation account for the observed CM11 mediated arrest of the PR growth.

CM11 induces the *PLT3PLT5PLT7*-mediated LR pathway

The root system architecture in Arabidopsis is formed by SRs that can be divided into LR, adLR and ARs (Bellini *et al.*, 2014; Sheng *et al.*, 2017; Ge *et al.*, 2019; Motte *et al.*, 2019). To determine the nature of the induced SRs by CM11 inoculation, we studied the root system architecture using high resolution scans of the *plt3plt5plt7* triple mutant, in which the LR formation is severely compromised, and the *wox11wox12* double and *35S::WOX11-SRDX* mutants, that are defective in adLR and AR formation, compared with the WT (Liu *et al.*, 2014; Du & Scheres, 2017; Sheng *et al.*, 2017). In the *plt3plt5plt7* mutant inoculated with CM11, no emerged SR were observed at 7 dpi (Figs 4a,b, S4a). By contrast, in *wox11wox12* and *35S::WOX11-SRDX* mutants a significant increase in the SR number was observed

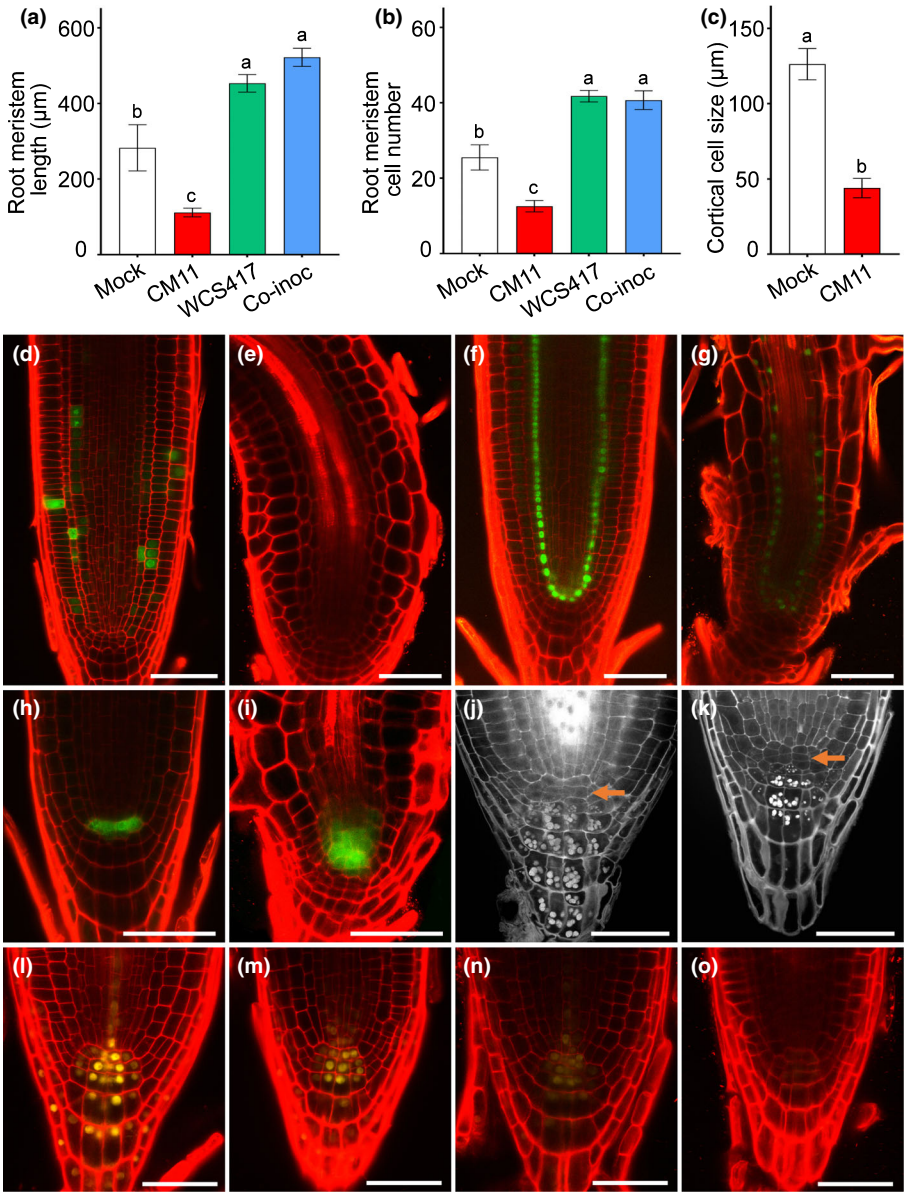

Fig. 3 Effects of CM11 on primary root development of Arabidopsis. (a, b) Root meristem length (a) and cell numbers (b) of Arabidopsis seedlings inoculated with CM11, WCS417 and CM11+WCS417 (Co-inoc) bacteria or mock. Data represent mean ± SD of four biological replicates, each consisting of five seedlings; (c) Cortical cell size in the differentiation zone of mock and CM11 inoculated roots. Data represent means ± SD of 10 individual seedlings. In (a–c), 4-d-old seedlings were inoculated at the root tip and grown 5 d post-inoculation (dpi) in (a) and (b), or 3 dpi in (c). Different letters indicate statistically significant differences (LSD and Tukey's honestly significant difference (HSD) tests; $P \leq 0.05$). (d–e) Representative confocal images showing the expression patterns of the cell cycling reporter *pCYCB1;1::GFP* in roots under mock (d) and CM11 inoculated conditions (e) (GFP, green fluorescent protein). (f–g) Representative confocal images showing the expression pattern of the endodermis/quiescent center (QC) localized reporter *pSCR::SCR::GFP* under mock (f) and CM11 inoculated conditions (g). (h–i) Representative confocal images showing the expression patterns of the QC-localized reporter *pWOX5::GFP* under mock (h) and CM11-inoculated conditions (i). (j–k) mPS-PI stained root tips show the absence of amyloplasts in the columella stem cell layer (orange arrows) in mock (j) and CM11 inoculated conditions (k); (l–o) Effects of CM11 on auxin distribution in the Arabidopsis root tips. Representative confocal images of *DR5::vYFP* expression in the Arabidopsis root tip of mock (l) and CM11 inoculated conditions at 1 (m), 2 (n) and 3 (o) dpi (YFP, yellow fluorescent protein). In (d–o), 4-d-old seedlings were inoculated with mock or CM11 bacterial suspensions at the root tip and confocal images were captured at 3 dpi (d–k) or at the indicated time (l–o). All imaging experiments were repeated twice and showed similar results. Bar: (d–o) 50 μm.

after 7 dpi, comparable to the increase observed after CM11 inoculation on WT roots (Figs 4a,b, S4a,d,e).

In order to include the initiated primordia in the analysis as well, the number of LRI and adLRI were quantified by using the reporter lines *pPLT7::GUS* and *pWOX11::GUS*, respectively (Figs 4c, S4b,c). Compared with the mock, CM11 significantly increased the *pPLT7::GUS* expressing LRI (3.6-fold) at 2 dpi. Additionally, the emerged LRs (LRE) increased 1.4-fold, leading to a total LR increase by 1.9-fold compared to mock (Fig. 4c). Notably, after inoculation of the *pWOX11::GUS* reporter line,

New Phytologist

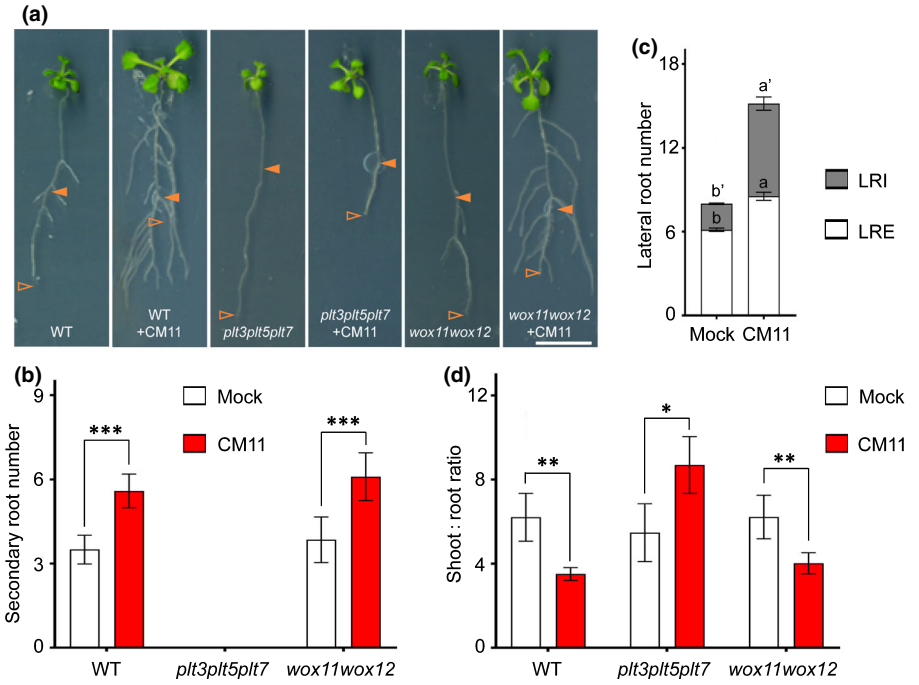

Fig. 4 CM11 induces the *PLT3PLT5PLT7*-mediated lateral root pathway. (a) Representative images of wild-type (WT), *plt3plt5plt7*, *wox11wox12* seedlings growing on ½ Murashige & Skoog (MS) agar plates with CM11 or mock. Closed arrowheads, inoculation sites; open arrowheads, tips of primary roots. (b) Quantification of secondary root (SR) number in WT, *plt3plt5plt7*, *wox11wox12* seedlings under mock and CM11 inoculated conditions. Only the SRs formed above the inoculating sites were counted. (c) Quantification of the numbers of emerged lateral roots (LRE) and initiated lateral root primordia (LRI) using the *pPLT7::GUS* reporter line under mock and CM11 inoculated conditions. Different letters indicate statistically significant differences (LSD and Tukey's honestly significant difference (HSD) tests; $P \leq 0.05$); (d) Shoot : root ratio of WT, *plt3plt5plt7* and *wox11wox12* Arabidopsis seedlings under mock and CM11 inoculated conditions. In all cases, 7-d-old seedlings were inoculated with mock or bacterial suspensions at the root tip. In (a), (b) and (d), images and data were analyzed 7 d post-inoculation (dpi), whereas in (c) they were analyzed at 2 dpi. Data represent mean ± SD of three biological replicates, each consisting of eight seedlings in (b) and (d), and 20 seedlings in (c). Asterisks in (b) and (d) indicate significant differences between inoculated and mock plants: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Student's *t*-test). Bar, 1 cm.

almost no GUS expressing adLRI was identified at 2 dpi (Fig. S4c). Together, these results show that the SRs induced by CM11 inoculation are LRs using the *PLT3PLT5PLT7* pathway.

We wondered whether the induction of additional SRs through the LR formation pathway is a common effect of *Pseudomonas* beneficial strains. Therefore, WCS417 and the Co-inoc were used to inoculate the *plt3plt5plt7* and *wox11wox12* mutants and *pPLT7::GUS* and *pWOX11::GUS* reporter lines. Comparable to the CM11 inoculations, the SR formation after WCS417 and the Co-inoc inoculation was completely abolished in the *plt3plt5plt7* mutant, whereas SRs were still formed in the *wox11-wox12* mutant (Fig. S4a). Accordingly, these inoculated roots formed exclusively *pPLT7::GUS* marked LRIs and LREs (Fig. S4b) and almost no *pWOX11::GUS* could be detected (Fig. S4c). In all inoculation experiments, adLRs were scarcely observed, which suggests that both CM11 and WCS417 strains induced the *PLT3PLT5PLT7*-mediated LR pathway.

CM11 promotes shoot growth regardless root size

Besides the inhibiting effect on primary root growth and the induction of LRs, CM11 promoted shoot growth in WT seedlings resulting in an increased biomass (Fig. 1e). To analyze whether the increase in shoot biomass is the effect of an enlarged root system, the shoot : root ratio was determined of *plt3plt5plt7* mutant seedlings – which have a reduced root system – that were inoculated with CM11 and compared with *plt3pl5plt7* untreated seedlings. Interestingly, upon CM11 inoculation, *plt3plt5plt7* mutants exhibited a slight increase in the shoot biomass (1.2-fold), whereas the root biomass was reduced (0.7-fold) compared to the *plt3plt5plt7* untreated seedlings, leading to a significantly higher shoot : root ratio (Fig. 4a,d). CM11-mediated root and shoot growth promotion in WT were both increased as presented before, but the shoot : root ratio became significantly lower (Figs 1e, 4a,d). A similar decrease of shoot : root ratio was observed in the *wox11wox12* mutant inoculated seedlings, compared with the untreated seedlings. These results suggest that CM11 treatment promoted the shoot growth, which is not solely dependent on the size of the total root system.

CM11 inoculum improves Arabidopsis fitness

The promoting ability of CM11 on plant growth was tested on plates, but to evaluate whether CM11 promotes total plant fitness cannot be studied in this way, it requires a more natural situation where plants can fulfill their lifecycle. Therefore, a soil experiment was conducted to evaluate the effect of CM11 on plant growth. Within the life cycle, different traits were studied such as

the amount of leaves and the leaf area, bolting time, the height of the plants, as well as the seed yield, which all are important indexes for plant fitness. For these experiments, Arabidopsis seedlings were transferred to soil which was inoculated with CM11. At 10 d after transfer, an increase of 5.0- and 3.2-fold in root and shoot FW, respectively, was observed when compared to the mock treated plants (Fig. 5). Comparison of the leaf area and amount of leaves, showed that the effect on shoot FW was caused by an increase of leaf area (3.3-fold) and not by the number of leaves (with an average of 10.9 and 11.3 in mock and CM11, respectively; Figs 5, S5a–c).

Furthermore, the CM11 inoculated plants started bolting at 18.6 d, which is 1.7 d earlier than the mock treated plants (Figs 5, S5d). At the moment the first yellow silique was present, the stem height was measured and showed a 1.6-fold increase of CM11 inoculated plants compared to the mock treated plants. The most important trait of plant fitness is the number of seeds and their weight. The total amount of seeds

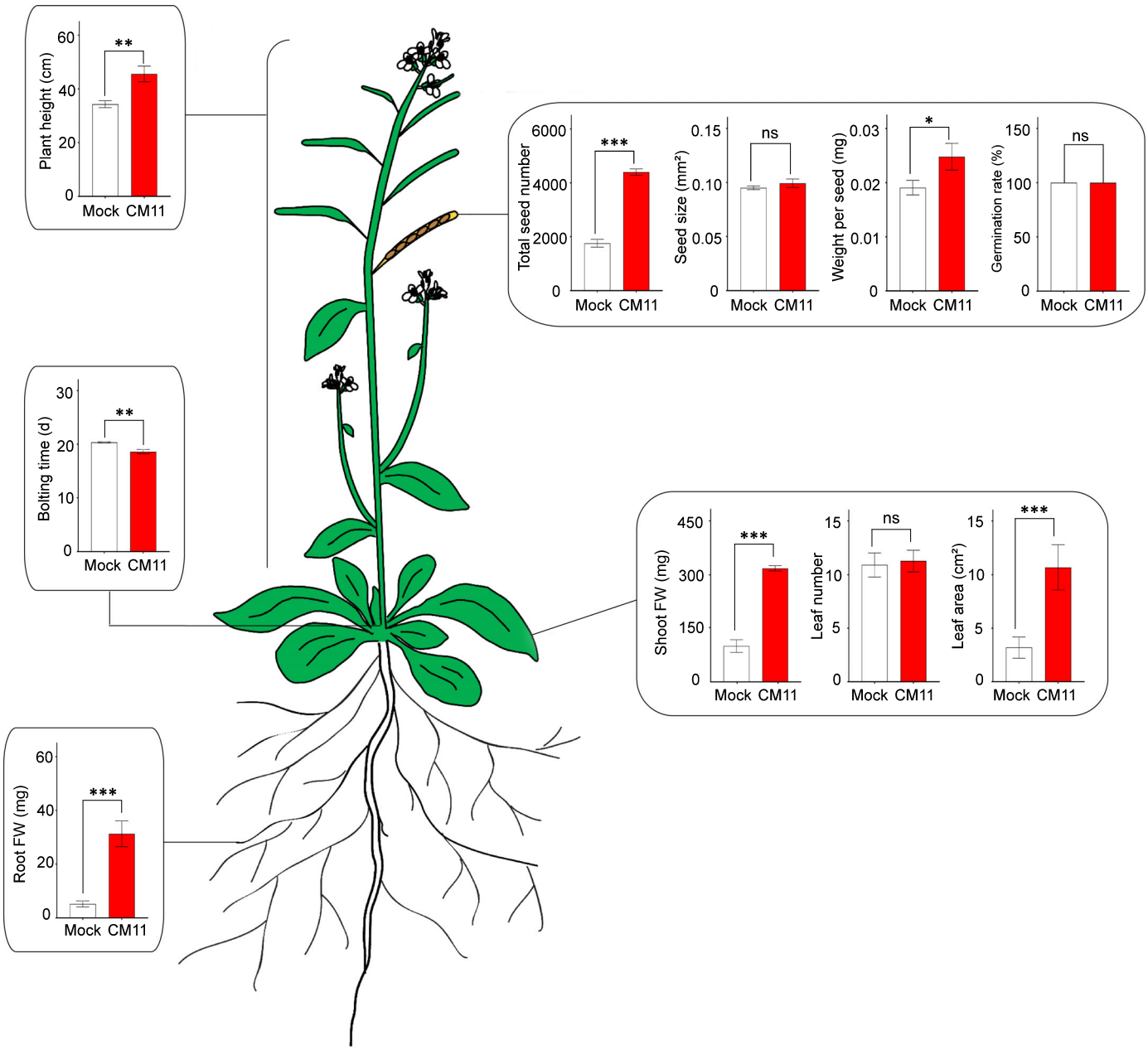

Fig. 5 CM11 inoculation improves Arabidopsis plant fitness. On the left of Arabidopsis plant mode downwards are comparisons in plant height, bolting time and root FW of plants grown in soil between CM11 inoculated and mock conditions. On the right are comparisons in total seed number, per seed size, per seed weight and seed germination rate (top), in shoot FW, leaf number and total leaf area (bottom) of plants grown in soil between mock and CM11 inoculated conditions. In all cases, 7-d-old Arabidopsis wild-type (WT) seedlings were transferred to pots with CM11 bacterial suspension or mock in soil. Each parameter was quantified as described in the Materials and Methods section. Data represent mean ± SD of three biological replicates, each consisting of 10–12 seedlings. Asterisks indicate SDs between inoculated and mock plants (ns, no significance): *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ (Student's *t*-test).

was weighed and divided by the weight of 100 counted seeds, assuming that the size of the seeds is equal (Fig. 5), and this revealed the amount of seeds formed per plant, which reflects plant productivity. The number of seeds was increased 2.5-fold and the weight per seed increased 1.3-fold in CM11 inoculated plants compared to the mock treated plants (Fig. 5). To test the viability of the seeds, 100 seeds per treatment were plated and in both cases showed 100% germination (Fig. 5). Taken together, these data show that inoculation of CM11 improves plant fitness for all analyzed traits.

CM11 promotes plant growth in crop species

In order to explore whether the plant growth promoting effect of CM11 does not only occur in the plant model system Arabidopsis, but also in other species such as commercially important horticulture crops, CM11 was used to inoculate plate-grown lettuce and tomato seedling at root tips. At 8 dpi and 3 dpi, respectively, CM11 inoculated roots showed altered root architecture with the inhibited PR growth and increased SRs formation above the inoculating site, which resembled the observed effects in Arabidopsis seedlings upon CM11 inoculation (Figs 6a–f, S6a–b). The number of SRs was significantly increased, 1.5-fold in lettuce and 1.4-fold in tomato compared to the mock, respectively (Fig. 6c,f).

We next conducted soil experiments to investigate the effect of CM11 inoculation on lettuce and tomato growth in soil. Seedlings were planted into soil mixed with the CM11 strain. At 10 d after transfer, a shoot growth promotion effect of CM11 inoculated plants was observed in lettuce and tomato seedlings. We quantified the root and shoot biomass and these showed a 8.2- and 2.4-fold increase for lettuce and a 1.6-and 2.2-fold increase for tomato FW, respectively (Figs 6g–l, S6c–d), compared with the corresponding mock treated plants. These results indicate that the plant promoting effects of CM11 are not species-specific which would make this strain a new candidate biological inoculum for agriculture.

Discussion

Rhizobacteria are known to alter side root (SR) formation, but hitherto there was no distinction between different types of SR upon induction of soil biota, nor have the genetic pathways involved been reported up to now. Here, we show that newly formed SRs upon CM11 inoculation are lateral roots (LRs), because SR formation after CM11 inoculation was completely abolished in the *plt3plt5plt7* triple mutant, whereas many SRs were still formed in the *wox11wox12* double mutant. Accordingly, the significant increase of LR primordia initiation (LRI) identified by *pPLT7::GUS* further confirmed a specific induction of meristem derived LRs instead of *WOX11*-mediated adventitious (ad)LRs by CM11. These data indicate that, to our knowledge for the first time, by using mutants involved in SR-specific pathways, *Pseudomonas* strains specifically induce the *PLT3*, *PLT5* and *PLT7*-mediated LR pathway to produce LRs.

In our studies we show that additional SRs upon WCS417 induction also require the *PLT3PLT5PLT7* pathway. These roots were previously referred to as LRs, based on their emergence from the primary root (PR) (Zamioudis *et al.*, 2013), and here we confirm this conjecture by genetic analysis. Our results suggest that LR induction upon plant growth-promoting rhizobacteria (PGPR) induction is a conserved pathway for PGPR to alter the root system architecture.

CM11 and WCS417 both induce the formation of LRs, but CM11 induction leads to arrest of the PR whereas WCS417 induces a longer and still active meristem. How can this be explained? LRs originate from primed pericycle cells, which originate from spatial–temporal variation in cell size, resulting in differences of auxin loading and leading to specific gene expression activation in the root transition zone. Through growth, the primed sites transform into repetitive pre-branch sites (De Smet *et al.*, 2007; Moreno-Risueno *et al.*, 2010; Xuan *et al.*, 2015, 2016). Nevertheless, although the pre-branch sites are constantly initiated, only about 25% of priming events will result in LR formation (Moreno-Risueno *et al.*, 2010; Kircher & Schopfer, 2016; Kircher & Schopfer, 2018; van den Berg *et al.*, 2021). Even though CM11 causes arrest of the PR meristem, extra LRs are formed between the already existing LRs, indicating that these are derived from the pre-branch sites that were programmed before inoculation. Our results therefore indicate that CM11 can activate part of these primed pre-branch sites.

Different from CM11, WCS417 treated plants showed a longer meristem (Zamioudis *et al.*, 2013). Based on the reflux-and-growth mechanism (van den Berg *et al.*, 2021), this would suggest that these roots have more cells that are able to load more auxin to form more primed sites. A recent study using *DR5::Luciferase* reporter line proved that *Bacillus* SQR9, which also promoted PR growth, induced higher frequencies of *DR5* oscillation and pre-branch sites formation (Li *et al.*, 2021). These data suggest the possibility that strains like WCS417 and SQR9 use the 'native' system to induce LRs by producing more primed sites, because these roots maintain an active meristem (this study; Zamioudis *et al.*, 2013; Li *et al.*, 2021).

CM11 induced roots had a positive impact not only on root biomass, but also on shoot biomass and plant fitness of Arabidopsis. This can be explained in different ways: (1) By increasing the root system the plant can take up more nutrients and water, which results an increase in shoot biomass. However, the observation that the shoot biomass is still increased in a *plt3plt5plt7* mutant, which has a reduced root system, indicates that the increase of shoot biomass upon CM11 inoculation is not fully dependent on the size of the root system. In addition, CM11 also could play a role in nutrient mobilization leading to enhanced nutrient uptake. (2) CM11 colonizes the whole plant and induces a growth-promoting effect in this way. However, when the CM11-green fluorescent protein (GFP) strain was inoculated on root tips, hypocotyl and whole roots, a bacterial biofilm formed mostly on the new part of the growing PR regardless of the inoculation sites (Figs 2, S2). (3) A long-distance signal travels from the root to the shoot to promote growth. Indeed, heat-inactivated CM11 does not show any effect on plant performance

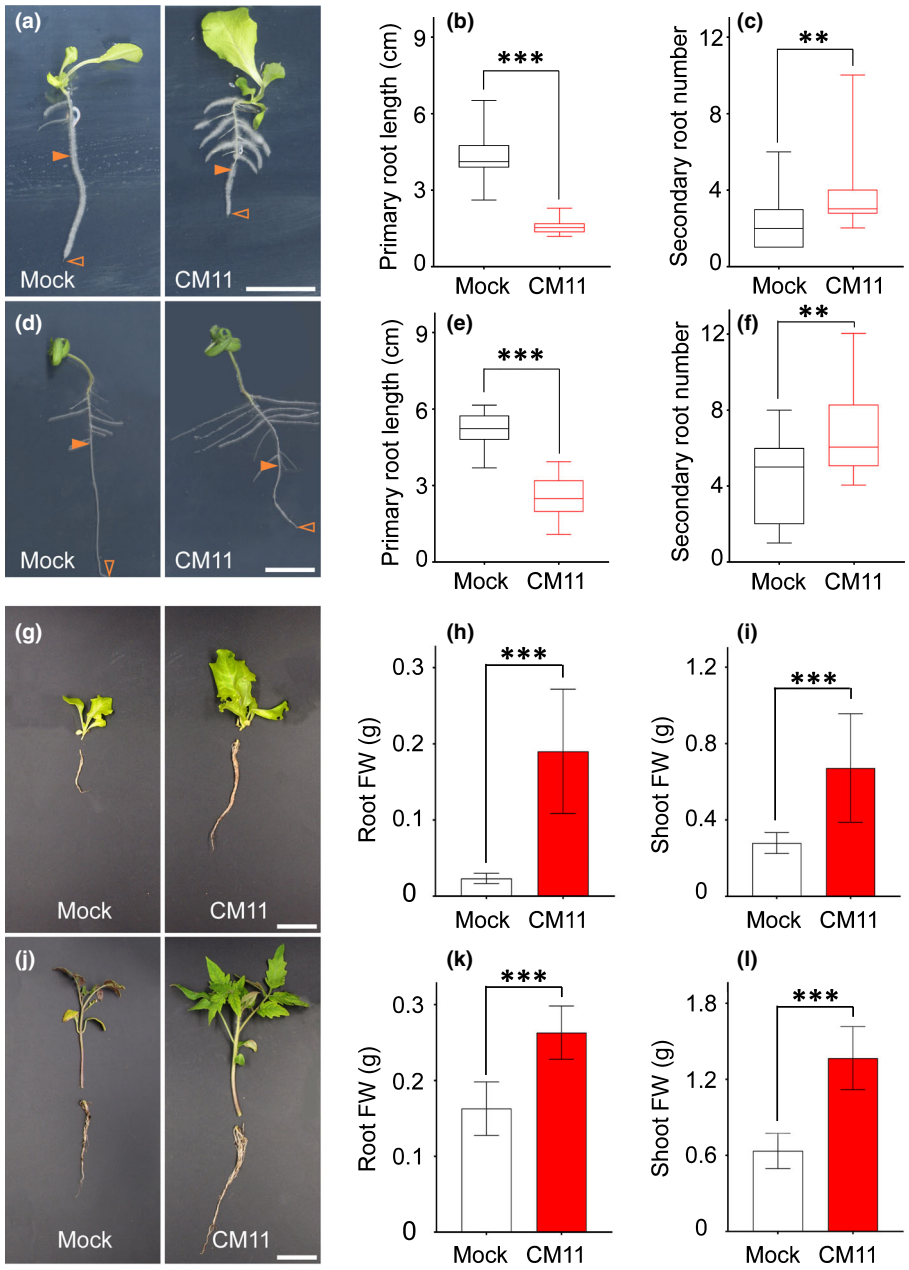

Fig. 6 CM11 alters root system architecture and promotes growth of lettuce and tomato seedlings. (a) Representative images of lettuce seedlings growing on ½ Murashige & Skoog agar plates with CM11 strains or mock at 10 d post-inoculation (dpi). (b–c) Quantification of primary root (PR) length (newly-grown PR below the inoculation sites, b) and number of emerged secondary roots (SRs) (emerged SRs above the inoculation sites, c) of lettuce seedlings under mock and CM11 inoculated conditions at 8 dpi. (d) Representative images of tomato seedlings growing on ½MS agar plates with CM11 strains or without (mock) at 10 dpi. (e–f) Quantification of PR length (newly grown primary root below the inoculation sites, e) and number of emerged SRs (emerged SRs above the inoculation sites, f) of tomato seedlings under mock and CM11 inoculated conditions at 3 dpi. Box plots in (b, c, e, f) indicate the lower and upper quartiles, bars represent the maximum and minimum values. The line in the box indicates the median; (g) Representative photographs of lettuce seedlings grown in soil inoculated with CM11 bacterial suspension or without (mock). (h–i) Root (h) and shoot (i) FW production per lettuce seedling with CM11 inoculation or mock. (j) Representative photographs of tomato seedlings grown in soil inoculated with CM11 bacterial suspension or mock. (k–l) Root (k) and shoot (l) FW production per tomato seedling with CM11 inoculation or mock. In (a–c) 3-d-old lettuce seedlings and in (d–f) 4-d-old tomato seedlings were inoculated with mock or CM11 bacterial suspensions at the root tip. In (g–l) 10-d-old lettuce or tomato seedlings were transferred to soil with CM11 bacterial suspension or mock, and measured at 10 dpi. Data in (h, i, k, l) represent mean ± SD of three biological replicates, each consisting of 8–10 seedlings. Asterisks indicate SDs between inoculated and Mock plants: **, $P \leq 0.01$; ***, $P \leq 0.001$ (Student's *t*-test). Bar, 2 cm.

and solely colonizes the root, and does not move up to the shoot tissue, suggesting that the induction or secretion of a long-distance signal by CM11 to promote shoot growth.

In addition, using different Arabidopsis genotypes, bacterial strains and growing conditions indicate that even though the plants are grown on sealed agar plates the plant growth promotion effects are very specific and not just a consequence of factors such as microbially produced elevated CO_2 concentrations in the plates.

Taken together, our results suggest that CM11 activates pre-branch sites to modulate the root system and generates or activates a long distance signal that affects the shoot performance. Regardless of the mechanism of CM11 action, to our knowledge this report shows for the first time that PGPR – that stimulate LR emergence – can specifically activate existing pre-branch sites and specifically induce *PLT3PLT5PLT7* dependent LR formation. Future experiments are required to identify the factors involved, which then can be used to improve our understanding on how plants respond are more flexible in different environmental circumstances and to improve plant performance.

Acknowledgements

The authors would like to thank Renze Heidstra and Ben Scheres for carefully reading the manuscript. We would like to thank the funding agencies for financial support: The National Key Research & Development Program of China (2018YFD1000605) (QL, HL, YZ, LQ, QC); The China Scholarship Council (CSC) grant no. 202007565029 (ZY); the Netherlands Organization for Scientific Research NWO-TTW (grant no. 14220, Microbial support of plant growth under

abiotic stress) (XC, TB). We thank Corné Pieterse for sharing the WCS417 strain, Lin Xu for sharing the *wox11wox12; pWOX11::GUS* lines and Liqun Zhang for sharing the plasmid pBBR1MCS-2 (P_{lac}*::GFP*).

Author contributions

VW, QL, HL conceived the project; VW, QL, HL, TB, QC designed the experiments; QL, HL, ZY, YZ performed the experiments; QL, HL, ZY performed statistical analysis; QL, HL, VW wrote the manuscript; and TB performed editing; XC, LQ, QC provided essential materials. QL and HL contributed equally to this work.

ORCID

Ton Bisseling https://orcid.org/0000-0001-5494-8786
Qingqin Cao https://orcid.org/0000-0002-1550-7440
Xu Cheng https://orcid.org/0000-0003-0693-7147
Huchen Li https://orcid.org/0000-0002-5388-4124
Qian Li https://orcid.org/0000-0002-0239-9153
Ling Qin https://orcid.org/0000-0002-9814-5771
Viola Willemsen https://orcid.org/0000-0002-6420-0605

Data availability

Data available on request from the authors.

References

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R *et al.* 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**: 653–657.

Beeckman T, Burssens S, Inzé D. 2001. The peri-cell-cycle in Arabidopsis. *Journal of Experimental Botany* **52**: 403–411.

Beeckman T, De Smet I. 2014. Pericycle. *Current Biology* **24**: R378–R379.

Bellini C, Pacurar DI, Perrone I. 2014. Adventitious roots and lateral roots: similarities and differences. *Annual Review of Plant Biology* **65**: 639–666.

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**: 591–602.

Bennett T, Scheres B. 2010. Root development-two meristems for the price of one? In: Timmermans MCP, ed. *Plant development: current topics in developmental biology*. San Diego, CA, USA: Academic Press, 67–102.

Berendsen RL, Pieterse CMJ, Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* **17**: 478–486.

van den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B. 1995. Cell fate in the Arabidopsis root meristem determined by directional signalling. *Nature* **378**: 62–65.

van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B. 1997. Short-range control of cell differentiation in the Arabidopsis root meristem. *Nature* **390**: 287–289.

van den Berg T, Yalamanchili K, de Gernier H, Santos Teixeira J, Beeckman T, Scheres B, Willemsen V, ten Tusscher K. 2021. A reflux-and-growth mechanism explains oscillatory patterning of lateral root branching sites. *Developmental Cell* **56**: 2176–2191.

Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett MJ. 2003. Dissecting Arabidopsis lateral root development. *Trends in Plant Science* **8**: 165–171.

Cheng X, Etalo DW, van de Mortel JE, Dekkers E, Nguyen L, Medema MH, Raaijmakers JM. 2017. Genome-wide analysis of bacterial determinants of plant growth promotion and induced systemic resistance by *Pseudomonas fluorescens*. *Environmental Microbiology* **19**: 4638–4656.

Cheng X, Yan Z, Schmitz L, Schneijderberg M, Yan J, Ge Y, Lan Y, Wang Y, Li G, Yang L *et al.* 2020. Longevity of Chinese chestnut correlates with stable root microbiomes. *Research Square*. doi: 10.21203/rs.3.rs-36590/v1.

Chiniquy D, Barnes EM, Zhou J, Hartman K, Li X, Sheflin A, Pella A, Marsh E, Prenni J, Deutschbauer AM *et al.* 2021. Microbial community field surveys reveal abundant *Pseudomonas* population in sorghum rhizosphere composed of many closely related phylotypes. *Frontiers in Microbiology* **12**: 598180.

Contesto C, Milesi S, Mantelin S, Zancarini A, Desbrosses G, Varoquaux F, Bellini C, Kowalczyk M, Touraine B. 2010. The auxin-signaling pathway is required for the lateral root response of Arabidopsis to the rhizobacterium *Phyllobacterium brassicacearum*. *Planta* **232**: 1455–1470.

Cook RJ, Thomashow LS, Weller DM, Fujimoto D, Mazzola M, Bangera G, Kim D-S. 1995. Molecular mechanisms of defense by rhizobacteria against root disease. *Proceedings of the National Academy of Sciences, USA* **92**: 4197–4201.

Dahmani MA, Desrut A, Moumen B, Verdon J, Mermouri L, Kacem M, Coutos-Thévenot P, Kaid-Harche M, Bergès T, Vriet C. 2020. Unearthing the plant growth-promoting traits of *Bacillus megaterium* RmBm31, an endophytic bacterium isolated from root nodules of *Retama monosperma*. *Frontiers in Plant Science* **11**: 124.

De Smet I, Tetsumura T, De Rybel B, Freidit Frey N, Laplaze L, Casimiro I, Swarup R, Naudts M, Vanneste S, Audenaert D *et al.* 2007. Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. *Development* **134**: 681–690.

Desrut A, Moumen B, Thibault F, Le Hir R, Coutos-Thevenot P, Vriet C. 2020. Beneficial rhizobacteria *Pseudomonas simiae* WCS417 induce major transcriptional changes in plant sugar transport. *Journal of Experimental Botany* **71**: 7301–7315.

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B. 1993. Cellular organisation of the *Arabidopsis thaliana* root. *Development* **119**: 71–84.

Du Y, Scheres B. 2017. PLETHORA transcription factors orchestrate de novo organ patterning during Arabidopsis lateral root outgrowth. *Proceedings of the National Academy of Sciences, USA* **114**: 11709–11714.

Du Y, Scheres B. 2018. Lateral root formation and the multiple roles of auxin. *Journal of Experimental Botany* **69**: 155–167.

Dubrovsky JG, Doerner PW, An C-C, Rost TL. 2000. Pericycle cell proliferation and lateral root initiation in Arabidopsis. *Plant Physiology* **124**: 1648–1657.

Dubrovsky JG, Rost TL. 2012. Pericycle. *eLS*. [WWW document] URL http://www.els.net/WileyCDA/ [accessed 16 July 2012].

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Friml J, Shishkova S, Celenza J, Benková E. 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proceedings of the National Academy of Sciences, USA* **105**: 8790–8794.

Ge Y, Fang X, Liu W, Sheng L, Xu L. 2019. Adventitious lateral rooting: the plasticity of root system architecture. *Physiologia Plantarum* **165**: 39–43.

Geiss G, Gutierrez L, Bellini C. 2009. Adventitious root formation: new insights and perspectives. In: Beeckman T, ed. *Annual plant reviews-root development*. Hoboken, NJ, USA: Wiley-Blackwell, 127–156.

Grierson C, Schiefelbein J. 2002. Root hairs. In: Somerville CR, Meyerowitz EM, eds. *The Arabidopsis book, vol. 1*. Rockville, MD, USA: American Society of Plant Biologists, e0060.

Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T. 2004. Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. *Development* **131**: 657–668.

Hartig SM. 2013. Basic image analysis and manipulation in ImageJ. *Current Protocols in Molecular Biology* **102**: 14.15.11–14.15.12.

Heidstra R, Welch D, Scheres B. 2004. Mosaic analyses using marked activation and deletion clones dissect Arabidopsis SCARECROW action in asymmetric cell division. *Genes & Development* **18**: 1964–1969.

Hofhuis H, Laskowski M, Du Y, Prasad K, Grigg S, Pinon V, Scheres B. 2013. Phyllotaxis and rhizotaxis in Arabidopsis are modified by three PLETHORA transcription factors. *Current Biology* **23**: 956–962.

Huang AC, Jiang T, Liu YX, Bai YC, Reed J, Qu B, Goossens A, Nützmann HW, Bai Y, Osbourn A. 2019. A specialized metabolic network selectively modulates Arabidopsis root microbiota. *Science* **364**: eaau6389.

Jiménez-Vázquez KR, García-Cárdenas E, Barrera-Ortiz S, Ortiz-Castro R, Ruiz-Herrera LF, Ramos-Acosta BP, Coria-Arellano JL, Sáenz-Mata J, López-Bucio J. 2020. The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. *The Plant Journal* **103**: 1639–1654.

Karlova R, Boer D, Hayes S, Testerink C. 2021. Root plasticity under abiotic stress. *Plant Physiology* **187**: 1057–1070.

Kerstens M, Strauss S, Smith R, Willemsen V. 2020. From stained plant tissues to quantitative cell segmentation analysis with MorphoGraphX. In: Bayer M, ed. *Plant embryogenesis. Methods in molecular biology, vol. 2122*. New York, NY, USA: Humana, 63–88.

Kircher S, Schopfer P. 2016. Priming and positioning of lateral roots in Arabidopsis. An approach for an integrating concept. *Journal of Experimental Botany* **67**: 1411–1420.

Kircher S, Schopfer P. 2018. The plant hormone auxin beats the time for oscillating light-regulated lateral root induction. *Development* **145**: dev169839.

Kudoyarova G, Arkhipova T, Korshunova T, Bakaeva M, Loginov O, Dodd IC. 2019. Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in Plant Science* **10**: 1368.

Kuzyakov Y. 2002. Review: factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science* **165**: 382–396.

Lakshmanan V, Selvaraj G, Bais HP. 2014. Functional soil microbiome: belowground solutions to an aboveground problem. *Plant Physiology* **166**: 689–700.

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM. 1995. Formation of lateral root meristems is a two-stage process. *Development* **121**: 3303–3310.

Li YJ, Li Q, Zhang ZX, Li SF. 2017. Screening and identification of peach endophytic bacteria with antagonism against *Agrobacterium tumefaciens*. *Scientia Agricultura Sinica* **50**: 3918–3929.

Li Y, Shao J, Xie Y, Jia L, Fu Y, Xu Z, Zhang N, Feng H, Xun W, Liu Y *et al.* 2021. Volatile compounds from beneficial rhizobacteria *Bacillus* spp. promote periodic lateral root development in Arabidopsis. *Plant, Cell & Environment* **44**: 1663–1678.

Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, Xu L. 2014. *WOX11* and *12* are involved in the first-step cell fate transition during de novo root organogenesis in Arabidopsis. *Plant Cell* **26**: 1081–1093.

Mendes R, Garbeva P, Raaijmakers JM. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews* **37**: 634–663.

Moreno-Risueno MA, Norman J, Moreno A, Zhang J, Ahnert SE, Benfey PN. 2010. Oscillating gene expression determines competence for periodic Arabidopsis root branching. *Science* **329**: 1306–1311.

Motte H, Vanneste S, Beeckman T. 2019. Molecular and environmental regulation of root development. *Annual Review of Plant Biology* **70**: 465–488.

Ofek M, Voronov-Goldman M, Hadar Y, Minz D. 2014. Host signature effect on plant root-associated microbiomes revealed through analyses of resident vs. active communities. *Environmental Microbiology* **16**: 2157–2167.

Ortiz-Castro R, Díaz-Pérez C, Martínez-Trujillo M, del Río RE, Campos-García J, López-Bucio J. 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proceedings of the National Academy of Sciences, USA* **108**: 7253–7258.

Pardal R, Heidstra R. 2021. Root stem cell niche networks: it's complexed! Insights from Arabidopsis. *Journal of Experimental Botany* **72**: 6727–6738.

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews: Microbiology* **11**: 789–799.

Pieterse CMJ, Berendsen RL, de Jonge R, Stringlis IA, Van Dijken AJH, Van Pelt JA, Van Wees SCM, Yu K, Zamioudis C, Bakker PAHM. 2021. *Pseudomonas simiae* WCS417: star track of a model beneficial rhizobacterium. *Plant and Soil* **461**: 245–263.

Scheres B, Wolkenfelt H, Willemsen V, Terlouw M, Lawson E, Dean C, Weisbeek P. 1994. Embryonic origin of the Arabidopsis primary root and root meristem initials. *Development* **120**: 2475–2487.

Sheng L, Hu X, Du Y, Zhang G, Huang H, Scheres B, Xu L. 2017. Non-canonical *WOX11*-mediated root branching contributes to plasticity in Arabidopsis root system architecture. *Development* **144**: 3126–3133.

Song YI, Wilson AJ, Zhang X-C, Thoms D, Sohrabi R, Song S, Geissmann Q, Liu Y, Walgren L, He SY *et al.* 2021. *FERONIA* restricts *Pseudomonas* in the rhizosphere microbiome via regulation of reactive oxygen species. *Nature Plants* **7**: 644–654.

Sukumar P, Legue V, Vayssieres A, Martin F, Tuskan GA, Kalluri UC. 2013. Involvement of auxin pathways in modulating root architecture during beneficial plant-microorganism interactions. *Plant, Cell & Environment* **36**: 909–919.

Tkacz A, Poole P. 2015. Role of root microbiota in plant productivity. *Journal of Experimental Botany* **66**: 2167–2175.

Torres-Martinez HH, Rodriguez-Alonso G, Shishkova S, Dubrovsky JG. 2019. Lateral root primordium morphogenesis in angiosperms. *Frontiers in Plant Science* **10**: 206.

Truernit E, Bauby H, Dubreucq B, Grandjean O, Runions J, Barthélémy J, Palauqui JC. 2008. High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in Arabidopsis. *Plant Cell* **20**: 1494–1503.

Ubeda-Tomas S, Federici F, Casimiro I, Beemster GT, Bhalerao R, Swarup R, Doerner P, Haseloff J, Bennett MJ. 2009. Gibberellin signaling in the endodermis controls Arabidopsis root meristem size. *Current Biology* **19**: 1194–1199.

Verbon EH, Liberman LM. 2016. Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in Plant Science* **21**: 218–229.

Verstraeten I, Schotte S, Geelen D. 2014. Hypocotyl adventitious root organogenesis differs from lateral root development. *Frontiers in Plant Science* **5**: 495.

Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, González-Peña A, Peiffer J, Koren O, Shi Q *et al.* 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of Sciences, USA* **115**: 7368–7373.

Willemsen V, Wolkenfelt H, de Vrieze G, Weisbeek P, Scheres B. 1998. The *HOBBIT* gene is required for formation of the root meristem in the Arabidopsis embryo. *Development* **125**: 521–531.

Xu L. 2018. De novo root regeneration from leaf explants: wounding, auxin, and cell fate transition. *Current Opinion in Plant Biology* **41**: 39–45.

Xuan W, Audenaert D, Parizot B, Möller BK, Njo M, De Rybel B, De Rop G, Van Isterdael G, Mähönen A, Vanneste S *et al.* 2015. Root cap-derived auxin pre-patterns the longitudinal axis of the Arabidopsis root. *Current Biology* **25**: 1381–1388.

Xuan W, Band LR, Kumpf RP, Van Damme D, Parizot B, De Rop G, Opdenacker D, Moller BK, Skorzinski N, Njo MF *et al.* 2016. Cyclic programmed cell death stimulates hormone signaling and root development in Arabidopsis. *Science* **351**: 384–387.

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CM. 2013. Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiology* **162**: 304–318.

Zhang H, Kim M-S, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu C-M, Allen R, Melo IS *et al.* 2007. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in Arabidopsis. *Planta* **226**: 839–851.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Overview of plant growth regulation by *Pseudomonas* CM1-11 strains.

Fig. S2 Beneficial *Pseudomonas* strains CM11 and WCS417 alter root architecture.

Fig. S3 Effect of CM11 and WCS47 on Arabidopsis primary root development.

Fig. S4 CM11 induces the *PLT3PLT5PLT7*-mediated lateral root pathway instead of the *WOX11WOX12*-mediated adventitious lateral root pathway.

Fig. S5 CM11 inoculation alters aboveground phenotypes in Arabidopsis.

Fig. S6 Effects of CM11 on root system architecture and aboveground growth of lettuce and tomato seedlings.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.