The auxin-signaling pathway is required for the lateral root response of Arabidopsis to the rhizobacterium Phyllobacterium brassicacearum

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Abstract
Plant root development is highly responsive both to changes in nitrate availability and beneficial microorganisms in the rhizosphere. We previously showed that *Phyllobacterium brassicacearum* STM196, a plant growth-promoting rhizobacteria strain isolated from rapeseed roots, alleviates the inhibition exerted by high nitrate supply on lateral root growth. Since soil-borne bacteria can produce IAA and since this plant hormone may be implicated in the high nitrate-dependent control of lateral root development, we investigated its role in the root development response of *Arabidopsis thaliana* to STM196. Inoculation with STM196 resulted in a 50% increase of lateral root growth in Arabidopsis wild-type seedlings. This effect was completely abolished in *aux1* and *axr1* mutants, altered in IAA transport and signaling, respectively, indicating that these pathways are required. The STM196 strain, however, appeared to be a very low IAA producer when compared with the high-IAA-producing *Azospirillum brasilense* sp245 strain and its low-IAA-producing *ipdc* mutant. Consistent with the hypothesis that STM196 does not release significant amounts of IAA to the host roots, inoculation with this strain failed to increase root IAA content. Inoculation with STM196 led to increased expression levels of several IAA biosynthesis genes in shoots, increased Trp concentration in shoots, and increased auxin-dependent GUS staining in the root apices of *DR5::GUS* transgenic plants. All together, our results suggest that STM196 inoculation triggers changes in IAA distribution and homeostasis independently from IAA release by the bacteria.

Keywords
Arabidopsis, Indole-3-acetic acid, Lateral root, *Phyllobacterium*, Plant growth-promoting rhizobacteria

Abbreviations
AAO1 Arabidopsis aldehyde oxidase 1
AIR Auxin-induced in root cultures
ASA1 Anthranilate synthase alpha subunit 1
ASB1 Anthranilate synthase beta subunit 1
AXR Auxin resistant
AUX1 Auxin resistant 1
AUX/IAA Auxin/indole acetic acid
CYP Cytochrome P450
DFL1 Dwarf in light 1

GUS β-Glucuronidase
IAA Indole-3-acetic acid
IAAsp Indole-3-acetylaspartate
IAGlu Indole-3-acetylglutamate
PAS1 Pasticcino 1
PGPR Plant growth-promoting rhizobacteria
PIN1 Pin-formed 1
PXA1 Peroxisomal ABC transporter 1
SCF Skp1/cullin/F-box ubiquitin ligase
SUR Superroot
TIR1 Transport inhibitor response 1
YUCCA Flavin monooxygenase gene family

Introduction

Plant roots live in close association with a large set of bacteria that thrive in the rhizosphere. Some of these microorganisms have a significant impact on root morphogenesis and, consequently, the architecture of the root system (Kapulnik et al. 1985; Larcher et al. 2003). Considering the pleiotropic effects of rhizospheric bacteria in general and the possible relative specificity of plant responses to different bacterial classes, extending such studies to other bacterial genera interacting with the model plant *Arabidopsis thaliana* is particularly relevant to us. In this viewpoint, we used the *Phyllobacterium brassicacearum* STM196 strain which was selected for its ability to naturally colonize the rhizosphere of *Brassica napus*, a close relative of Arabidopsis with high agronomical interest (Bertrand et al. 2001). The *Phyllobacterium* genus lies in the Phyllobacteriaceae family in the Rhizobiales order of *α-Proteobacteria*, in close phylogenetic relationship with *Mesorhizobium* (Mantelin et al. 2006b). In both *B. napus* and *A. thaliana* seedlings grown in gnotobiotic conditions, the inoculation with the STM196 strain resulted in increased lateral root length (Larcher et al. 2003; Mantelin et al. 2006a). This response differs from that triggered by the most studied plant growth-promoting rhizobacteria (PGPR), *Azospirillum brasilense*, which consists in a lateral root proliferation rather than a stimulation of lateral root growth (Kapulnik et al. 1985). This shows that PGPR have very diverse effects on plants.

Remarkably, STM196 antagonized the repressive effect of high nitrate concentrations on lateral root development (Mantelin et al. 2006a). It has been shown that the negative control of lateral root development by high nitrate involves a systemic regulatory mechanism that acts at a very early stage around lateral root emergence (Zhang et al. 1999; Tranbarger et al. 2003). Under non-inoculated conditions, a negative correlation has been found between the leaf nitrate content and lateral root growth in tobacco and Arabidopsis, suggesting that a leaf nitrate pool could act as a sensor of the N status to control root development (Zhang et al. 1999; Touraine et al. 2001). The phloem-translocated signal(s) and the signaling pathway(s) responsible for this systemic NO_3^--dependent inhibitory effect are not definitely identified yet. Transferring Arabidopsis plants from a high nitrate supply (50 mM) to a low nitrate supply (1 mM) resulted in concomitant decrease of shoot IAA content and increase of root IAA content, while the growth of short lateral roots was restored (Walch-Liu et al. 2006). Reasoning that shoot-derived auxin is important for stimulating lateral root growth (rather than initiation) in Arabidopsis (Bhalerao et al. 2002), the authors proposed that NO_3^- accumulation in the shoot could inhibit the transport of IAA to the root, leading to an arrest of lateral root development at a stage close to emergence. If this hypothesis is correct, IAA would be the shoot-derived signal involved in the NO_3^- systemic lateral root development inhibition. Consistent with this hypothesis, the inhibition of maize root growth by high nitrate correlates with reduced IAA levels in roots (Tian

et al. 2008). Considering the similarity of the effects of STM196 and nitrate supply lowering on lateral root development, shoot-to-root transport of IAA would be a good candidate to explain the lateral root response to STM196.

Plant growth factors, including IAA, cytokinins, and gibberellins, have been found in the supernatant of soil microbes cultures (for review, see Persello-Cartieaux et al. 2003). It has been proposed that these microbial originating phytohormones, especially IAA, would be responsible for the root development changes induced by rhizospheric bacteria (Xie et al. 1996; Dobbelaere et al. 1999; Spaepen et al. 2008). Plant-exuded tryptophan would enter the IAA biosynthesis pathways of bacteria living in the rhizosphere, and in return, a significant part of the bacterial IAA would be delivered to the plant root (Spaepen et al. 2007). This hypothesis is supported by studies that used IAA-deficient bacterial mutants. In the first study published, a low-IAAproducer mutant of *Azospirillum brasilense* was less efficient than the wild-type strain in increasing lateral root number and length, and the root hair density and length of *Triticum durum* (Barbieri and Galli 1993). Similar results were obtained with a *Pseudomonas putida* indolepyruvate decarboxylase mutant (Patten and Glick 2002). Furthermore, the auxin-related effect of *A. brasilense* on *Triticum aestivum* seedlings was enhanced by adding Trp, a precursor of IAA biosynthesis in *A. brasilense*, to the culture medium. Although the data obtained with *A. brasilense* and *P. putida* suggest that bacteria thriving in the rhizosphere may alter root development due to their production of IAA, plant studies are required to assess the role of bacterialoriginating auxin in this plant response. Only a few reports provide genetic evidence about the mechanisms elicited by soil-borne bacteria in plants (Persello-Cartieaux et al. 2001; Lopez-Bucio et al. 2007; Zhang et al. 2007). In the earliest of these two studies (Persello-Cartieaux et al. 2001), a screen for Arabidopsis mutants insensitive to the inoculation with a *Pseudomonas thivervalensis* strain was developed based on the reduction of primary root length upon inoculation. This screen resulted in the isolation of two mutants allelic to *aux1-100*, which is impaired in the influx component of the auxin polar transport. However, it is significant that inoculation with *P. thivervalensis* actually decreased the primary root growth rate, but does not affect lateral root development in Arabidopsis seedlings. Recently, Lopez-Bucio et al. (2007) found that *Bacillus megaterium* is able to increase lateral root number and root hair length in auxin- and ethylene-signaling mutants as well as in wild-type Arabidopsis plants, leading the authors to conclude that *B. megaterium* alters root architecture through an auxin- and ethylene-independent mechanism. Zhang et al. (2007) showed that the PGPR strain *B. subtilis* GB03 can trigger growth promotion in Arabidopsis plants independent of the microbial auxin production. Although they gave no quantitative figure on the root architecture changes triggered by exposure to GB03, they show that this rhizobacterium favors lateral root proliferation and growth rate (Figure 2 in Zhang et al. 2009). Their combined transcriptional and pharmacological study showed that volatile organic compounds emitted by the GB03 strain promotes the growth of Arabidopsis seedlings by regulating auxin homeostasis in plants (Zhang et al. 2007). Specifically, the expression level of some auxin synthesis genes was up-regulated in the shoot of GB03-exposed seedlings while auxin accumulation decreased in leaves and increased in roots as revealed by the DR5::GUS auxinresponsive Arabidopsis line. These results suggest that PGPR (in that case, via VOCs) can modify the basipetal transport of endogenous auxin in host plant. Finally, a survey of recent literature thus shows that the classical paradigm according to which rhizospheric bacteria alter root development due to their production of auxin does not apply to all cases. Depending on the PGPR strain, the root architecture responses may rely on auxin-independent mechanisms or involve the auxin-signaling pathway as a consequence of changes in auxin homeostasis within plant tissues without the provision of microbial auxin.

To investigate the involvement of auxin in the lateral root response of Arabidopsis to STM196, we performed a functional study using mutants altered in auxin biosynthesis, transport and

signaling. We chose to use the *aux1-100* mutant (Bennett et al. 1996) where the major IAA influx carrier is knocked-out and the primary root inhibition by *P. thivervalensis* is blocked (Persello-Cartieaux et al. 2001), and the *axr1-3* mutant (Estelle and Somerville 1987) which lacks the RUB1-activating enzyme required for the activity of the ubiquitination complex that plays a central role in IAA transduction (del Pozo et al. 2002). Finally, we also used the *sur1-3* IAA-overproducing mutant (Boerjan et al. 1995) in which phenotype is expected to be mimicked by the action of STM196 if the effect of this bacterium actually depends on IAA. In addition, we assayed free IAA accumulation in roots and shoots, and we used a transgenic line expressing the auxinreporter gene *DR5::GUS* to characterize the impact of STM196 on auxin homeostasis. To get independent indications on the role of auxin in the plant response, we analyzed the expression response patterns of genes involved in auxin biosynthesis, transport, and signaling. All together, our results show that the involvement of auxin signaling independent of auxin production is a PGPR feature that is shared by *P. phyllocbacterium* STM196 and *B. subtilis* GB03.

Materials and methods

Plant material and growth conditions

All *Arabidopsis thaliana* (L.) Heynh lines used in this study are in the Columbia-0 (Col-0) ecotype background. The wild-type Col-0 and the *axr1-3* mutant (Estelle and Somerville 1987) were obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, Columbus, OH, USA). Seeds of the *sur1-3* mutant (Boerjan et al. 1995) were kindly supplied by Dr. P. Nacry (Biochimie et Physiologie Moléculaire des Plantes, CNRSINRA- SupAgro-Université Montpellier 2, Montpellier, France), and seeds of the *aux1-100* mutant (Bennett et al. 1996) by Dr. L. Nussaume (Laboratoire de Biologie du Développement des Plantes, CEA-CNRS-Université Marseille 2, St Paul Lez Durance). All seeds were surfacesterilized by immersion in a mixture of 0.57% sodium hypochlorite (v/v) and 0.095% Tween 20 (v/v) for 15 min. Seeds were washed five times in sterile distilled water and sown in square Petri dishes on a 1% (w/v) agar (Sigma- Aldrich, St Louis, MO, USA) mineral medium containing 0.5 mM $CaSO_4$, 5 mM KNO_3, 0.5 mM $MgCl_2$, 1mM KH_2PO_4, 0.05 mM $Na_2FeEDTA$, 0.03 lM $(NH_4)_6Mo_7O_{24}$, 1 lM CuSO4, 1 lM $ZnSO_4$, 15 lM $MnCl_2$, 50 lM H_3BO_3, 2.5 mM Mes, with pH adjusted to 5.7 with 10 mM KOH. The plates were sealed with Micropore^{TM} tape and stored at 4 C for 5 days. Seeds were vertically germinated in a controlled environmental chamber (22 C/20 C for the 16 h/8 h light/dark photoperiod). Six days after germination, uniform seedlings were transplanted into new Petri dishes filled with the standard agar mineral medium supplemented or not with *Phyllobacterium brassicacearum* STM196 (10^8 cfu ml^{-1}). Plants were grown vertically another 6 days in the controlled environmental chamber before being harvested.

Bacteria growth conditions

The *Phyllobacterium brassicacearum* STM196 strain was at first cultivated for 3 days on a rich and sterile 1.5% agar (w/v; Sigma-Aldrich) medium (E0) containing 2.86 mM K_2HPO_4, 0.81 mM $MgSO_4$, 1.71 mM NaCl, 7.91 mM KNO_3, 0.34 mM $CaCl_2$, 30 lM $FeCl_3$, 1% mannitol (w/v), and 0.3% yeast extract (w/v; Sigma-Aldrich), and pH was adjusted to 6.8 with 1 mM HCl. For inoculum preparation, the bacteria were grown aerobically in 500 ml E0 medium (initial A580nm = 0.1) on a rotary shaker (145 rpm) at 25 C for 24 h to obtain bacteria in exponential phase. Culture of bacterial cells was pelleted by centrifugation (3,200g, 15 min, 20 C), washed twice and resuspended in sterile liquid plant medium.

Root architecture analysis

For root system architecture analysis, transplanted seedlings were grown on mineral agar medium inoculated or not with 108 cfu ml-1 of the *P. brassicacearum* STM196 strain as described above. Numerical images of plates were recorded 6 days after transplantation using a flatbed scanner (Epson Perfection 1250; Epson, Owa, Suwa, Nagano, Japan) at the resolution of 300 dpi. Images of root system of healthy seedlings were later analyzed using the ImageJ software (available at http://rsb.info.nih.gov/ij/; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) with the plugging NeuronJ (available at http://www.imagescience.org/meijering/; developed by Erik Meijering, University Medical Center Rotterdam, NL). The lengths of the primary root (PR) and lateral roots (LR) and the number of visible growing lateral roots were measured. The proportion of LR\1 or [1 cm was determined for each genotype in inoculated or non-inoculated condition. For all experiments, the overall data were statistically analyzed with the Statistica 6.0 software (StatSoft, Tulsa, OK, USA). The analysis of variance using the two-factor ANOVA with a LSD post hoc test was used for assessing differences at $P = 0.05$ in PR length, total LR length, LR density, and proportion of LR\1 or[1 cm.

GUS assay

The GUS histochemical staining (adapted from Lagarde et al. 1996) was performed on whole transgenic *DR5::GUS Arabidopsis thaliana* seedlings inoculated or not with STM196. Twelve-day-old seedlings were incubated in 1.5% formaldehyde, 0.05% Triton X100, and 50 mM phosphate buffer pH 7.2 at room temperature for 45 min. After three washes of 5 min in 0.05% Triton X100 and 50 mM phosphate buffer pH 7.2, tissues were vacuum infiltrated in 1 mM X-Gluc (Sigma-Aldrich), 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 0.058% Triton X100, and 50 mM phosphate buffer pH 7.2. The enzymatic reaction was performed for 15 h at 37 C in the dark. Samples were fixed in 1:1 glacial acetic acid–methanol for 15 min three times. Tissues were cleared and dehydrated by two washes in 95% ethanol (w/v) for 5 min, and stored in 95% ethanol at 4 C. The observation of the sample was made with a binocular (Olympus SZH10, Olympus Corporation, Tokyo, Japan) at 7–70 magnification.

Analysis of transcript levels by real-time PCR

For quantitative RT-PCR amplification, total RNA was isolated from 12-day-old plants, inoculated or not with 108 cfu ml-1 of the *P. brassicacearum* STM196 strain for the last 6 days. Roots and shoots were harvested separately, frozen in liquid nitrogen, and stored at -80 C. For each treatment, 30–100 mg of frozen tissues was ground in liquid nitrogen using the mixer mill MM200 (Retsch, Haan, Germany). RNA isolation was performed with the SV Total RNA Isolation System (Promega, Madison, WI, USA), according to the manufacturer recommendations. The quality and quantity of the recovered total RNA were assayed by UV spectrophotometry. The absence of genomic DNA contamination was checked by PCR using an intron containing partial *AtAKT1* sequence (At2G26650). The PCR was performed according to the GoTaq™ DNA polymerase manufacturer's protocol (Promega) from 1 ll of the RNA preparation, with gene-specific primers (AKT1for 50-G ACTTGGTGGATGTAGATACTGG-30 and AKT1rev 50-G CAATTGGGGTCTGCGTGGTAC-30) under the following conditions: 95 C for 5 min, 40 cycles (94 C for 30 s, 60 C for 30 s, and 72 C for 1 min), and 72 C for 7 min. After the reaction, the complete PCR reaction mix (20 µl) was loaded on 1% agarose gel (Sigma-Aldrich), and the PCR products were separated by electrophoresis.

First strand cDNA were synthesized from 3 lg of total RNA using the Super-Script II reverse transcriptase (Invitrogen, Carlsbad, CA, USA), according to manufacturer recommendations.

The real-time PCR was performed on the MX3000P™ Real-Time PCR System (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA) with the qPCR™ Mastermix Plus for Sybr™ Green I kit (Eurogentec, Liège, Belgium), according to manufacturer recommendations. Each reaction was performed on 5 ll of 1:200 (v/v) dilution of the first cDNA strands, in final reaction volume of 20 ll. The reactions were incubated at 50 C for 2 min and 95 C for 10 min, followed by 40 cycles of 95 C for 15 s and 60 C for 1 min. The specificity of the real-time PCR amplification products was checked with the following dissociation protocol: heating at 95 C for 15 s, cooling at 60 C for 20 s, slowly heating up to 95 C within 20 min, and a heating plateau at 95 C for 15 s. Specific primer sets were designed with Beacon Designer 4 (PREMIER Biosoft international, Palo Alto, CA, USA) for genes involved in Trp and auxin biosynthesis, in auxin transport and signaling, and in auxin response and lateral root development (Table 1). To assess further the specificity, we separated the various PCR products on a gel to measure their sizes. We then checked that the relative differences of the observed sizes were comparable to in silico predicted ones (Czechowski et al. 2004). The results obtained on the different treatments were standardized to the constitutive Actin (At3g18780) and Ubiquitin (At4g05320) genes expression level.

Tryptophan analysis

Amino acids were extracted from 12-day-old Arabidopsis Columbia wild-type seedlings inoculated or not with *P. brassicacearum* STM196 strain (108 cfu ml-1) for the last 6 days of culture. Shoots and roots were harvested separately. Free amino acids were extracted from fresh tissues at 4 C, first in 80% ethanol over-night, then in 60% ethanol for 1 h, and finally in H_2O for 24 h. The supernatants of each sample were pooled, aliquoted, and kept at -20 C. Free amino acids were essayed by HPLC (gradient pump SP8800, Spectra Physics, Santa Clara, CA, USA; fluorimeter 821-FP, Jasco, Easton, MD, USA; SP4270 integrator piloted by SP-LABNET software, Spectra Physics) as described (Muller and Touraine 1992).

Analysis of plant endogenous IAA content

Shoots and roots of 12-day-old Arabidopsis Columbia wild-type seedlings inoculated or not with *P. brassicacearum* STM196 strain (10^8 cfu ml^{-1}) for the last 6 days of culture were harvested separately. Each sample consisted of shoots or roots from 20 independent seedlings to get 15–20 mg FW per sample. Ten independent samples were obtained from a single culture in order to perform the analyses of free IAA, conjugates, and catabolites from five replicates. The experiment was repeated three times and provided similar results. Samples were extracted, purified, and analyzed by gas chromatography–selected reaction monitoring–mass spectrometry (GC–SRM–MS) as previously described (Edlund et al. 1995). Calculation of the isotopic dilution factors was based on the addition of 50 pg [13C6]IAA/mg tissue. *t* test was performed according to http://graphpad.com/quick calcs/ttest1.cfm.

Samples were extracted, purified, and analyzed for IAA conjugates and catabolites by LC–MRM–MS as previously described (Kowalczyk and Sandberg 2001). t test was performed according to http://graphpad.com/quickcalcs/ttest1.cfm.

Analysis of IAA production by bacterial strains

Auxin production by the *P. brassicacearum* STM196 strain was determined in the absence or presence of L-Trp at concentrations ranging from 10 to 1,000 mg l-1 by colorimetry, in comparison with auxin production by the high auxin-producing *Azospirillum brasilense* Sp 245 strain and its *ipdc* knockout mutant which is classified as very low auxin producer (see Costacurta et al. 1994). Both strains have been kindly supplied by Pr Yvan Moe¨nne-Loccoz (Ecologie Microbienne, CNRS-INRA-Université Claude Bernard Lyon 1, Lyon, France). For this purpose, 9 ml of King's B medium (King et al. 1954) supplemented with L-Trp was added

in 50 ml Erlenmeyer flasks, autoclaved, and cooled. The flask contents were inoculated by adding 1 ml of bacterial suspension in exponential growth. The flasks were plugged and incubated at 28 C at 150 rpmshaking for 24 h in a dark room. After incubation, an aliquot of bacterial suspension was taken tomeasure the bacteria concentration by spectrophotometry at 595 nm. In the remaining bacterial suspension, bacteria were pelleted by centrifugation (10,000g, 5 min, 20 C). 500 µl aliquotswere taken fromsupernatants, and indole compounds were colorimetrically assayed using the Salkowski reagent (Sarwar et al. 1992). Indole concentrations have been normalized for a bacterial suspension concentration of OD = 1. Three replicates were performed at each L-Trp concentration, and the experiment was repeated twice.

Results

The sur1, aux1, and axr1 mutants are impaired in the lateral root response to Phyllobacterium brassicacearum STM196

Six-day-old *Arabidopsis thaliana* wild-type and *aux1-100*, *axr1-3*, or *sur1-3* mutant plants were transferred to fresh agar medium inoculated or not with STM196 rhizobacteria for additional 6 days. Root architecture was analyzed using three parameters: the primary root length, lateral root number, and lateral root length.

Under non-inoculated conditions, the three auxin mutants exhibited significantly greater primary root length than the wild type, *axr1-3* being the longest (Fig. 1). The *axr1-3* mutant and wild-type plants had identical lateral root length, while the lateral root length of *sur1-3* mutant was significantly higher, and the *aux1-100* mutant had the highest lateral root length (Fig. 1). Earlier reports mention that lateral roots are less numerous in the axr1 mutant than in the *aux1* mutant, and less numerous in this latter than in the wild type (Hobbie and Estelle 1995; Marchant et al. 2002). In our study, the *axr1-3* mutant seedlings did bare a reduced number of lateral roots (6.9 ± 1.3 lateral roots per plant compared to 11.5 ± 1.0 in the wild-type seedlings), but the *aux1-100* seedlings had the same number of lateral roots than the wild-type ones (11.8 ± 1.3). We thus observed quantitatively smaller reductions in lateral root numbers due to axr1 and aux1 mutations than reported earlier. This difference may be due to the absence of a carbon source in our nutrient media while 1% sucrose was included in those used in earlier reports. Indeed, the presence of sugar has been shown to be directly or indirectly involved in root architecture and to interfere with both hormonal signaling pathways (Leon and Sheen 2003; Moore et al. 2003) and nitrogen control of root development (Malamy and Ryan 2001; Touraine et al. 2001). When calculating the lateral density (number of lateral roots per cm of primary root), the mutant root architecture phenotypes observed in the present study do resemble those described previously, with lateral root densities of *axr1-3* smaller than that of *aux1-100*, in turn smaller than that of the wild type (Fig. 1). The sur1-3 seedlings bore a slightly higher number of lateral roots per plant (13.3 ± 1.9), which is consistent with the higher number of lateral root primordia found in this mutant by Boerjan et al. (1995). Because of a small difference in the primary root length between *sur1-3* and wild-type seedlings, however, there was no significant difference in the lateral root density of either seedling (Fig. 1). Again, the differences between our figures and those previously published on the *sur1-3* mutant may reflect differences in culture conditions, especially the presence/absence of sucrose.

The inoculation with STM196 resulted in no significant change in the primary root length, in either the wild type or mutant seedlings (Fig. 1). The inoculation with STM196 did not alter the number of lateral roots per plant, hence did not change the lateral root density, in either the mutant or wild-type plants (Fig. 1). Consistent with our earlier study (Mantelin et al. 2006a), the total lateral root length was increased by ca. 50% upon inoculation with STM196 in the wild-type plants (Fig. 1). Since STM196 had no effect on lateral root number, this figure is due to an increase in the length of individual lateral roots. In contrast, the lateral root length

remained unchanged upon inoculation with STM196 in all of the three mutants (Fig. 1), indicating that mutations in the auxin pathway block the lateral root response to STM196.

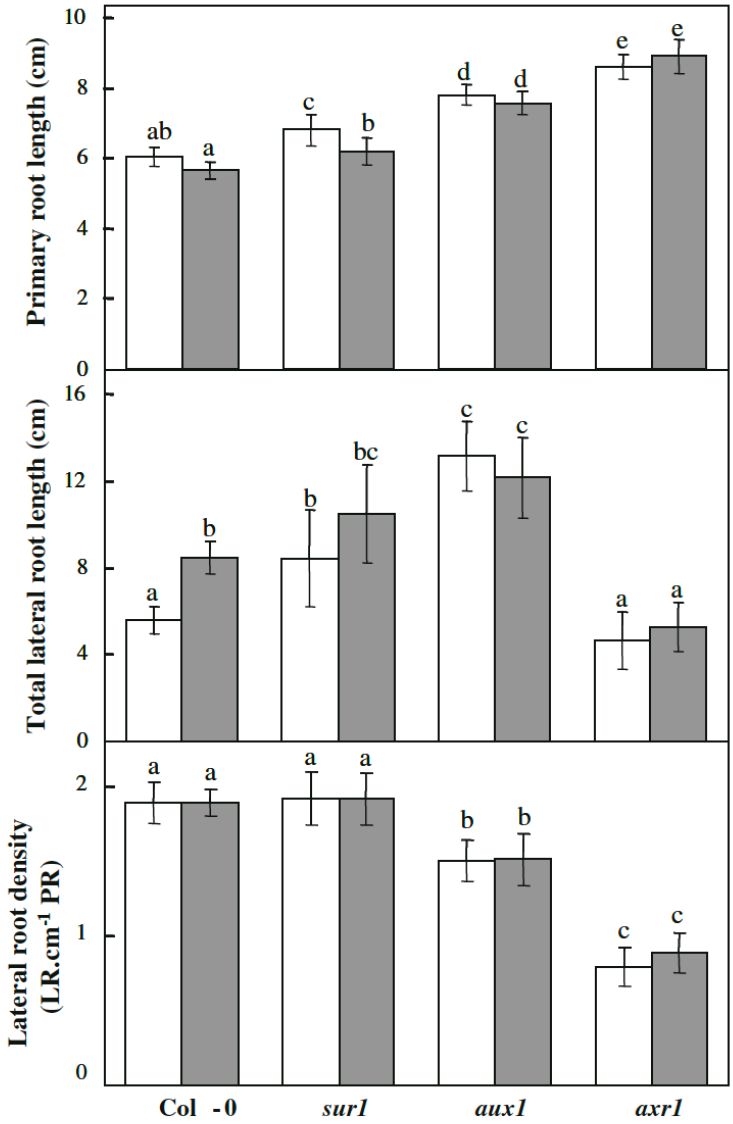

Fig. 1 Effect of *Phyllobacterium brassicacearum* STM196 on the root system architecture of Arabidopsis wild-type and *sur1*, *aux1*, *axr1* mutant plants. Seedlings were grown on a mineral medium in vertically oriented Petri dishes for 6 days and transferred to Petri dishes containing the same medium inoculated or not with the STM196 strain. Primary root length, total lateral root length, and lateral root density were measured 6 days later (n = 16–20). Open bars represent control plants and gray bars inoculated ones. The experiment was repeated threefold, and representative results of one experiment are shown (average values ± interval of confidence calculated for a P value of 0.05). Letters represent a significant difference of the means at P = 0.05, according to Fisher's LSD test.

To get a more precise indication on the effect of STM196 on lateral root growth rate in the mutant and wildtype wildtype plants, we compared the relative frequencies of shorter and longer lateral roots (\1 and [1 cm, respectively) in inoculated and non-inoculated plants (Fig. 2). In the wildtype plants, the frequency of longer lateral roots increased upon inoculation, confirming that STM196 had a positive effect on individual lateral root growth rate. Non-inoculated *aux1-100* and *axr1-3* mutant plants had a higher proportion of longer roots than wild-

type plants, but this frequency was not changed upon inoculation. This indicates that the effect of STM196 on the lateral root growth rate is blocked in these two mutants. In the *sur1-3* mutant, the frequency of longer lateral roots slightly increased in response to STM196, but to a lesser extent than in wildtype plants.

Overall, the root architecture analysis shows that, in our culture conditions, STM196 stimulated the lateral root growth rate in Arabidopsis wild-type plants, but that this response is blocked in the *aux1* and *axr1* mutants, and nearly abolished in the sur1 mutant. In contrast, the bacteria had no significant effect on the primary root growth, in either the wild type or mutant plants.

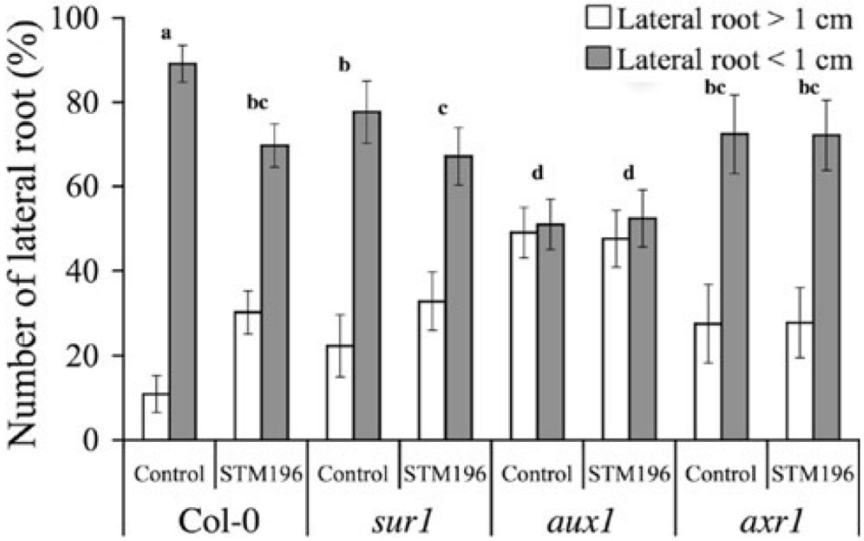

Fig. 2 Effect of *Phyllobacterium brassicacearum* STM196 on the percentage of lateral root smaller or longer than 1 cm in Arabidopsis thaliana wild-type plants and *sur1*, *aux1* and *axr1* mutants. Seedlings were grown as mentioned in Fig. 1 (‘‘Control’’ and ‘‘STM196’’ for non-inoculated and STM196-inoculated seedlings, respectively). Values are the means of the percentages of each class of roots measured for 16–20 individual plants ± interval of confidence (calculated for a P value of 0.05). Letters represent a significant difference of the means at $P = 0.05$, according to Fisher’s LSD test

Inoculation with Phyllobacterium brassicacearum STM196 affects the homeostasis of endogenous IAA in Arabidopsis seedlings

To get additional evidence on auxin involvement in plant responses toSTM196, we looked at the effects of inoculation on expression pattern of *DR5::GUS* auxin-responsive gene (Ulmasov et al. 1997). Microscopic observations showed that the GUS staining was stronger and expanded in larger zones, in both shoots and roots of STM196-inoculated transgenic plants than in the non-inoculated plants (Fig. 3). This GUS activity pattern, indicating a stronger peak of accumulation in the meristematic regions of both primary and lateral roots, is consistent with the involvement of IAA in root responses to STM196.

The auxin accumulating in root apices could originate from the bacterial cells. In order to assess the ability of the *P. brassicacearum* STM196 strain to secrete auxin, the amount of auxin released by a STM196 bacterial culture was compared to the amount of auxin released by a culture of *Azospirillum brasilense* Sp245 as an auxin-producing positive control since this strain is producing significant level of IAA (Steenhoudt and Vanderleyden 2000) and its *ipdc* mutant as a very low auxin-producing control (Barbieri and Galli 1993). For this purpose, the three strains were grown in parallel in Erlenmeyer flasks on King’s B medium containing 0–1,000

mg l^{-1} L-Trp for 24 h, and indoles released in the medium were assayed using the Salkowski assay. The knockout mutation of the *ipdc* gene led to a dramatic decrease in IAA secretion by *A. brasilense* pure culture, in the presence or absence of Trp (Fig. 4), consistent with earlier report (Barbieri and Galli 1993; Costacurta et al. 1994). The amount of IAA produced by P. *brassicacearum* STM196 was not significantly higher, or hardly higher, than that produced by the *ipdc* mutant strain of *A. brasilense*, at any external L-Trp concentration tested (Fig. 4). Compared to the higher auxin producing *A. brasilense* Sp245 wild-type strain, both the *ipdc* mutant and the *P. brassicacearum* STM196 strain released 7–20 times less indole compounds in the bacterial growth medium. The STM196 strain is thus unlikely to release significant amounts of auxin in the rhizosphere. Consistent with this assumption, neither the *A. brasilense ipdC* mutant nor the STM196 strain showed the root phenotype typically induced by exogenous auxin application, while the wild-type *A. brasilense* strain did (Supplemental Fig. S1).

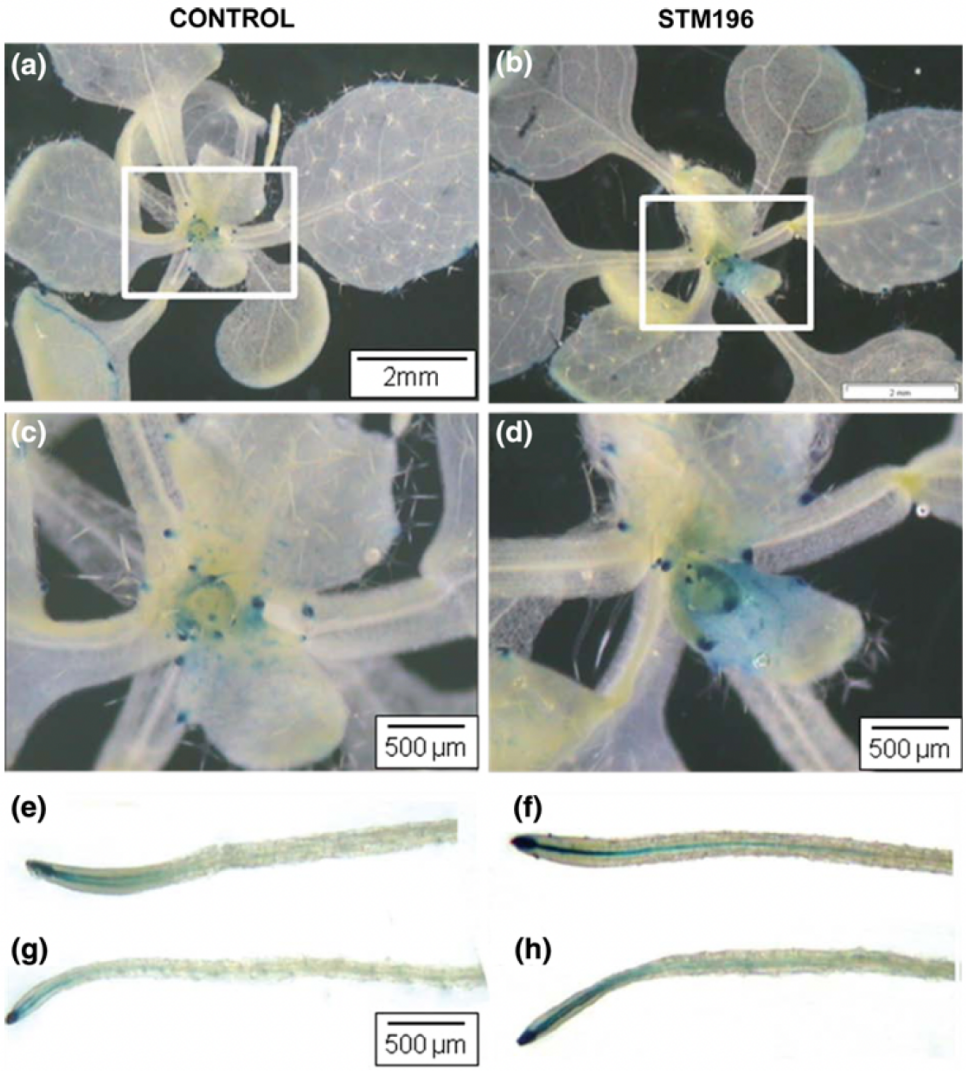

Fig. 3 Effect of *Phyllobacterium brassicacearum* STM196 on GUS staining in the roots of *DR5::GUS* transgenic line of *Arabidopsis thaliana*. a, b Shoots of non-inoculated and STM196-inoculated plants, respectively. c, d Magnification of zones shown by white rectangles in a and b, respectively. e, f Primary root tips of non-inoculated and STM196-inoculated plants, respectively. g, h Lateral root tips of non-inoculated and STM196-inoculated plants, respectively. Seedlings were grown as mentioned in Fig. 1

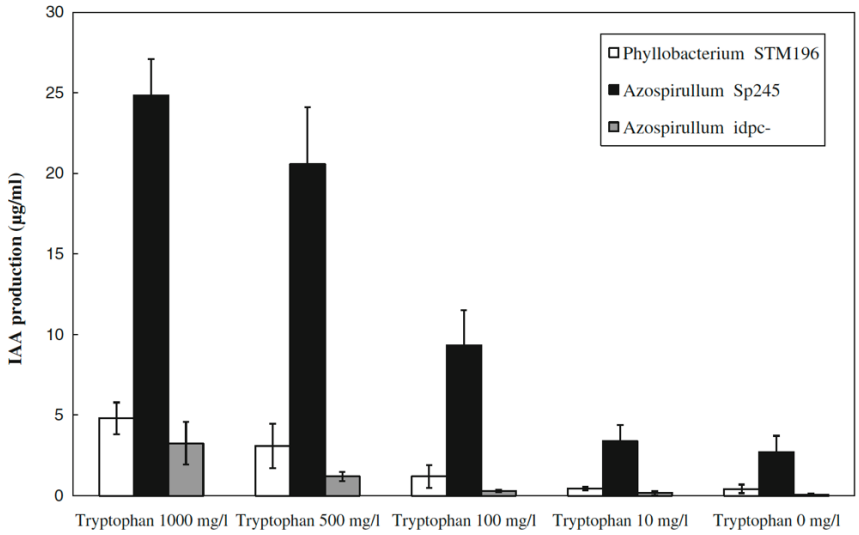

Fig. 4 Production of indole-3- acetic acid by *Azospirillum brasilense* Sp245 (black bars), its *ipdc* mutant (gray bars), and *Phyllobacterium brassicacearum* STM196 (white bars). Bacteria were grown in King's B medium supplemented with tryptophan at various concentrations as indicated. Supernatants were taken 24 h after the addition of tryptophan, and indoles were analyzed using the Salkowski assay. Values are the mean of three replicates ± SD

In order to investigate whether the inoculation with STM196 affects the IAA levels in Arabidopsis, we assayed IAA in the shoots and roots of inoculated and non-inoculated plants. Surprisingly, our results revealed that the amount of free IAA was not significantly affected by the inoculation with STM196 in shoots, while it was markedly reduced in roots (ca. twofold, Fig. 5). The level of oxoindole- 3-acetic acid was not statistically different between the roots of STM196-inoculated and non-inoculated plants (data not shown). The levels of the amide conjugates IAGlu and IAAsp remained more than one order of magnitude lower than the free IAA levels, in both shoots and roots (data not shown) and, therefore, would not explain the differences measured in free IAA levels. In conclusion, STM196 did not trigger a significant change in the average IAA shoot content while it led to a decrease in the average IAA root content. The GUS activity pattern, therefore, may reflect changes in IAA distribution within each plant part without any increase in total auxin content.

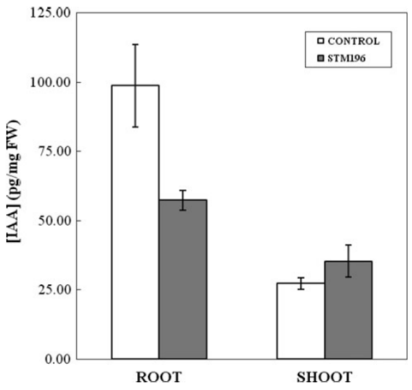

Fig. 5 Free IAA content in 12-day-old *Arabidopsis thaliana* wildtype seedlings inoculated or not with *Phyllobacterium brassicacearum* STM196. Seedlings were grown as mentioned in Fig. 1, harvested, and washed with care to remove any medium or bacterial contamination, and roots and shoots were separated prior to endogenous free IAA level measurement. White bars represent control plants and gray bars inoculated ones. Five independent biological replicates were used for each data point. Error bars indicate SD

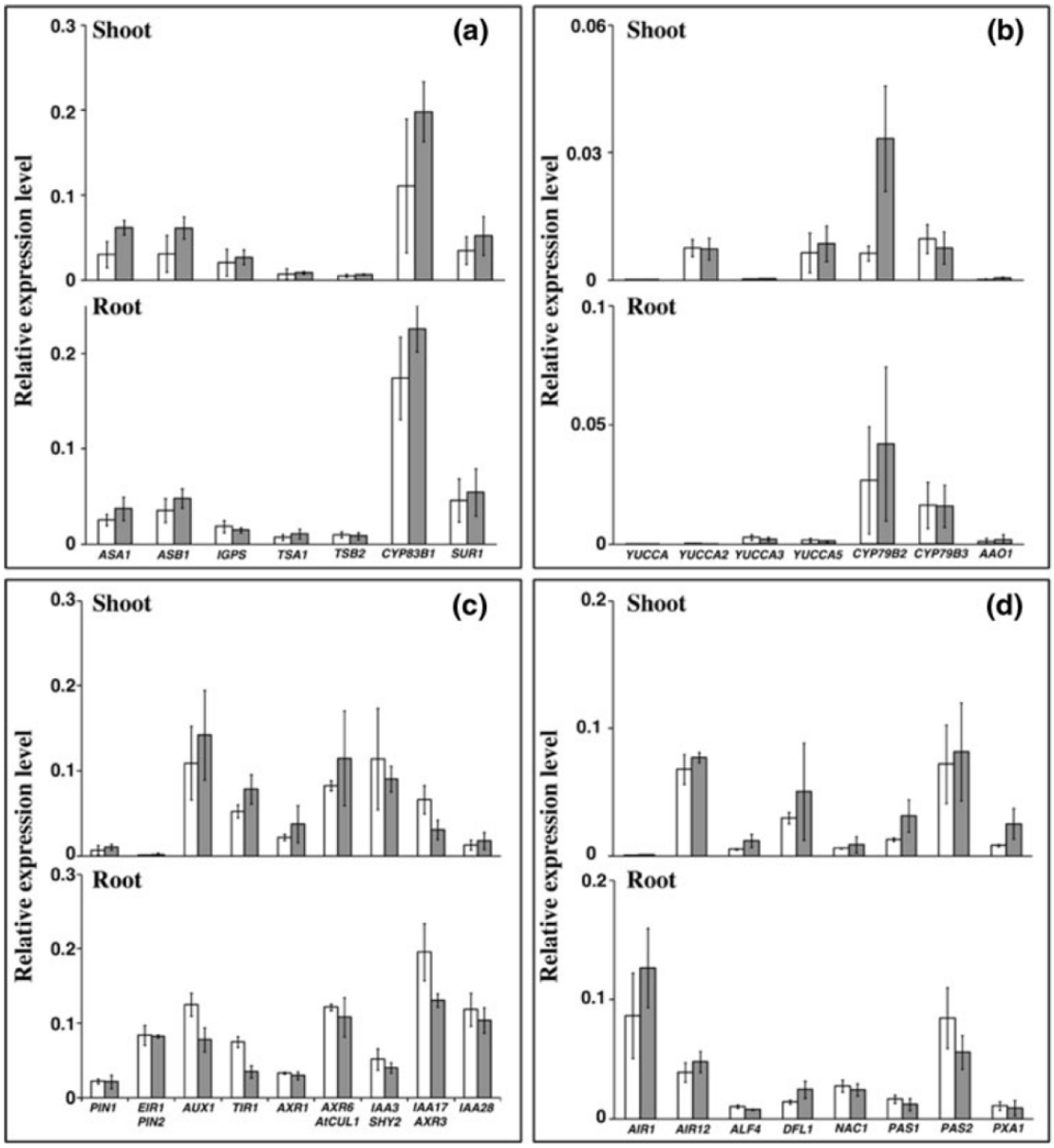

Fig. 6 Effect of inoculation with *Phyllobacterium brassicacearum* STM196 on the expression levels of genes involved in tryptophan and auxin biosynthesis (a, b), auxin transport and transduction (c), and auxin-dependent lateral root development regulation (d). RT-PCR analyses of gene expression have independently been performed on Arabidopsis shoot and root tissues, as indicated. The values presented are the mean expression levels ± SD relative to the Actin (At3g18780) and Ubiquitin (At4g05320) genes, for three biological independent experiments. White bars non-inoculated plants, gray bars STM196-inoculated plants

Since STM196 is very unlikely to provide IAA to the roots but qualitatively alters endogenous IAA levels, we investigated its effects on the expression levels of genes coding for enzymes involved in IAA biosynthesis (Table 1). STM196 repeatedly stimulated the expression of *ASA1* and *ASB1* in the shoots (Fig. 6a), suggesting an enhancement of Trp biosynthesis in these organs. In agreement with this prediction, the content of Trp, which can serve as a precursor for IAA biosynthesis, was 80% higher in the shoots of STM196-inoculated plants than in those of non-inoculated plants (Fig. 7). The expression levels of the *YUCCA*, *YUCCA2*, *YUCCA3*, and *YUCCA5* genes were essentially unchanged upon inoculation with STM196 (Fig. 6b). On the other hand, the expression levels of the *CYP79B2* and *AAO1* genes that code for enzymes

involved in IAA biosynthesis through the Trp-dependent pathway were stimulated by 5- and 3-fold, respectively, in shoots upon STM196 inoculation. Finally, the *CYP83B1* and *SUR1* genes that code for enzymes involved in glucosinolate synthesis showed a very weak up-regulation in response to STM196 inoculation (Fig. 6a). Thus, the inoculation with STM196 triggered slight but simultaneous up-regulation of the expression of genes involved in IAA and glucosinolate biosynthesis, in the shoots, the main site of auxin production (Ljung et al. 2001). In contrast to what has been observed in shoots, the expression levels of genes coding for enzymes involved in IAA metabolism were found globally unresponsive to STM196 in the roots (Fig. 6a, b). The overall picture, therefore, is that STM196 is unlikely to stimulate the root IAA production.

Effects of Phyllobacterium brassicacearum STM196 on the expression of genes involved in auxin signaling and auxin responses

To investigate the effects of STM196 on the auxin-signaling pathway, we analyzed the accumulation of transcripts of genes coding for components of the IAA transport and transduction pathways. Inoculation with STM196 induced a weak increase in the transcript amounts of the *PIN1* and *PIN2/EIR1* auxin efflux facilitators in the shoots (1.6- and 2.0-fold, respectively), but not in the roots (Fig. 6c). The expression of *AUX1*, coding for an auxin influx transporter, was not significantly affected in the shoots while it was down-regulated in the roots (Fig. 6c). Similarly the *TIR1* gene expression level was down-regulated in the roots (2-fold) while slightly up-regulated in the shoots (1.5-fold) (Fig. 6c). The transcript levels for two other components of the SCF ubiquitin ligase complex analyzed, *AXR1* and *AXR6*, showed no significant changes. Five of the six *AUX/IAA* genes tested, namely *IAA7*, *IAA14* and *IAA19* (data not shown), *IAA3/SHY2* and *IAA28* (Fig. 6c), remained essentially unchanged upon inoculation, whereas *IAA17/AXR3* was down-regulated in both shoots and roots (by 2- and 1.5-fold, respectively). Most of the auxin-responsive genes remained essentially unchanged in shoots and roots, except for *AIR1* and *DFL1* which were slightly up-regulated in both roots and shoots (1.7–3-fold), PXA1 that was up-stimulated by 3-fold in shoots and *PAS2* that was down-regulated by 1.5-fold in roots (Fig. 6d). All together, STM196 induced only very weak changes in the expression of genes related to auxin transport, transduction, and response, but the slight changes in transcript accumulation observed draw a consistent pattern (genes involved in similar functions are affected in the same way in a given organ).

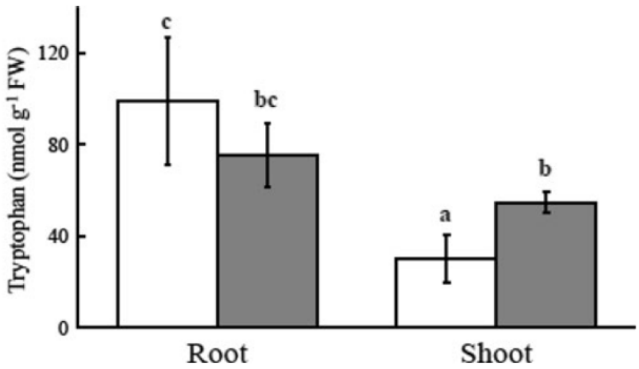

Fig. 7 Tryptophan tissue concentration in 12-day-old *Arabidopsis thaliana* wild-type seedlings inoculated or not with *Phyllobacterium brassicacearum* STM196. Seedlings were grown as mentioned in Fig. 1. White bars represent control plants and gray bars inoculated ones. Data are means (n = 8) ± interval of confidence calculated for a P value of 0.05 (drawn from Mantelin et al. 2006a). Letters represent a significant difference of the means at P = 0.05, according to Fisher's LSD test

Discussion

Inoculation with the *P. brassicacearum* STM196 strain led to increased lateral root length in wild-type Arabidopsis seedlings (Fig. 1) consistent with our earlier observations (Mantelin et al. 2006a). This effect was abolished in the *aux1-100* mutant (Fig. 1). The root system of the *aux1* mutant has been found to be insensitive to two different rhizobacteria, *P. brassicacearum* (this study) and *Pseudomonas thivarvalensis* (Persello-Cartieaux et al. 2001). This is all the more interesting since the morphological modifications induced in Arabidopsis by these two bacteria are quite different: the former promoting plant growth while the latter had a negative effect on plant growth. The fact that the disruption of the *AUX1* gene blocks root morphology changes induced by these two rhizobacteria strongly suggests that IAA is involved in the plant response to PGPR. To investigate further the involvement of the auxin signal transduction pathway in the root developmental response of Arabidopsis to STM196, we used an *axr1* mutant. The *auxin resistant 1* (*AXR1*) gene of Arabidopsis codes for a subunit of a RUB-activating enzyme that is required for the activity of the TIR1-SCF ubiquitination complex (del Pozo et al. 2002). Because of the crucial role of this complex and the unique role of *AXR1* in its activity, *axr1* mutations block the IAA signaling pathway, leading to primary defects in auxin responses (Leyser 2006). The complete abolishment of lateral root growth stimulation by STM196 in the *axr1-3* mutant (Fig. 1) thus reveals that the auxin-signaling pathway is required for this response. To our knowledge, this is the first genetic evidence that the IAA transduction pathway is required for a plant response to a soil-borne beneficial bacterium.

Another piece of evidence in favor of the involvement of auxin in the root architecture response to *Phyllobacterium* can be drawn from the comparison with nitrate effects on lateral root development. We previously showed that the inoculation of wild-type Arabidopsis plants with the STM196 strain alleviates the nitrate-dependent inhibition of lateral root development (Mantelin et al. 2006a) observed in non-inoculated Arabidopsis plants grown with nitrate supplies above 5–10 mM (Zhang et al. 1999; Tranbarger et al. 2003). These antagonistic effects of high nitrate and STM196 on lateral root development suggest the existence of common element(s) in the two signaling pathways involved. Interestingly, Walch-Liu et al. (2006) recently reported an increase in the root IAA concentration 24 h after transferring Arabidopsis seedlings from 50 to 1 mM nitrate, followed by the restoration of lateral root development in the subsequent 24 h. All together, these data strongly suggest that a partially overlapping signaling pathway that involves IAA polar transport operates to control lateral root development by either nitrate nutrition or PGPR.

Remarkably, equal lateral root length was measured in STM196-inoculated wild-type plants and the non-inoculated IAA biosynthesis mutant sur1-3 plants (Fig. 1). This suggests that the effect of the PGPR strain relies on increased IAA accumulation in the roots as it occurs in non-inoculated sur1-3 mutant compared to the wild-type plants (Boerjan et al. 1995; Ljung et al. 2005). Given the widespread ability of PGPR to produce auxin (Thuler et al. 2003), one possibility would have been that the STM196 released IAA in the rhizosphere, thereby increasing endogenous levels of this hormone in the roots of inoculated plants. Alternatively, as reported for the *B. subtilis* GB03 PGPR strain, STM196 could activate plant auxin basipetal transport from its biosynthesis sites in shoot independent of bacterial auxin production (Zhang et al. 2007). Different pathways to synthesize IAA exist in bacteria (Spaepen et al. 2007). The predominant pathway in rhizobacteria that have been well described for their effects on plant root development, like *Azospirillum brasilense*, is the indole-3-pyruvate pathway. The rate-limiting step is catalyzed by the indole-3-pyruvate decarboxylase (IPDC), and mutation of the ipdC gene results in a strong reduction in IAA production (Barbieri and Galli 1993; Costacurta et al. 1994). We, therefore, used an *ipdC* mutant of *A. brasilense* and its wild-type counterpart

Sp245 to assess the IAA production capacity of the P. *brassicacearum* STM196 strain. The results obtained showed that the IAA production rate of this latter was not significantly higher than that of the *ipdC* knockout mutant of *A. brasilense* (Fig. 4). Given that an average concentration of 100 lM Trp was measured in our Arabidopsis root tissues (Fig. 7), the rhizospheric Trp concentration is unlikely to reach the millimolar range. In this condition, STM196 should not release auxin to the plant root in substantial amount, and STM196 behaved as an IAA-nonproducing bacterium. Finally, we showed that the free IAA level was significantly lower in the roots of STM196-inoculated wild-type plants than in the roots of non-inoculated ones (Fig. 5). The hypothesis of a strong provision of IAA originating from STM196 to roots, therefore, must be ruled out. Consistently, the root phenotypic response was much more drastic in the presence of the IAA-producing *A. brasilense* Sp245 than in the presence of its *ipdC* mutant or of STM196 (Supplemental Fig. S1). Bacteria-produced auxin released by Sp245 in the rhizosphere is likely to enter the root and be taken into the polar auxin transport; the activity of primary root meristem and the initiation and activity of lateral root meristems will then be affected as a consequence of the large increase in IAA flow. On another hand, here, with the STM196 strain, we provide evidence, after Zhang et al. (2007), that a PGPR strain can trigger changes in endogenous plant auxin homeostasis without providing microbial-originating auxin. This feature is thus not unique for *B. subtilis* GB03, and considering the phylogenetic distance between *Bacillus* and *Phyllobacterium*, it is likely to be widespread for various soil bacteria. In addition, our root system analysis and the use of the *axr1-3* mutant line more specifically show that a bacterium thriving in the rhizosphere can induce root morphogenetic changes that rely on the auxin-signaling pathway without supplying exogenous IAA to roots.

Inoculation with STM196 led to coordinated up-regulation of several genes involved in Trp and IAA biosynthesis in the shoots of Arabidopsis plants inoculated for 6 days (Fig. 6a, b) as summarized in Fig. 8. Consistent with the increased expression levels of genes involved in Trp biosynthesis, the shoots of STM196-inoculated plants accumulated Trp at higher amounts than the shoots of non-inoculated plants (Fig. 7). The IAA level in shoots, however, remained essentially unchanged upon inoculation with STM196 (Fig. 5). The apparent discrepancy between the IAA biosynthesis gene expression profiles and the actual IAA levels can reflect the fact that rather modest changes in gene expression levels do not necessarily lead to changes in biosynthesis rates. Interestingly Zhang et al. (2007) reported lower auxin accumulation in B. subtilis GB03-exposed leaves despite increased expression levels of auxin biosynthesis genes including ASA1 (Zhang et al. 2007). Although free IAA amounts in shoots did not change upon inoculation, STM196-inoculated *DR5::GUS* transgenic plants displayed stronger GUS staining in the shoot regions, where IAA accumulates (Fig. 3), indicating that the bacterium is likely to affect the tissue distribution of auxin, hence its polar transport. Similar observations with *B. subtilis* GB03-exposed DR5::GUS plants led Zhang et al. (2007) to propose that not only the biosynthesis of auxin but also its transport and tissue homeostasis must be affected by this PGPR strain.

In roots, the transcript levels of IAA biosynthesis genes remained mostly unchanged (Fig. 6), and the free IAA level decreased (Fig. 5) upon inoculation. At first sight, when considering the root system as a whole, the STM196 bacterium thus seems to act as a sink rather than as a source of auxin. However, *DR5::GUS* transgenic plants revealed higher auxin levels in the regions, where it normally accumulates in roots (primary and lateral root meristems), and an extension in size of these regions toward older tissues along the central cylinder (Fig. 3). Therefore, it is likely that STM196 induced changes in IAA content at the tissue level that are due neither to the supply of supra-auxin nor to the increase in total IAA production in the plant. The only explanation would rely on the effects on IAA transport. Consistently, inoculation with STM196 slightly stimulated the expression of *PIN1* and *PIN2/EIR1* efflux facilitators in shoots which would be consistent with an increased delivery of shoot-synthesized auxin to roots (Fig.

6). The lack of response of the *aux1-100* mutant is consistent with this hypothesis as well. Since shoot-derived IAA has been shown to be essential for lateral root initiation and development in Arabidopsis seedlings (Xie et al. 2000; Bhalerao et al. 2002), changes in auxin polar transport that would lead to an accumulation of IAA in the stele up the root tip may account for the positive effect of STM196 on lateral root development.

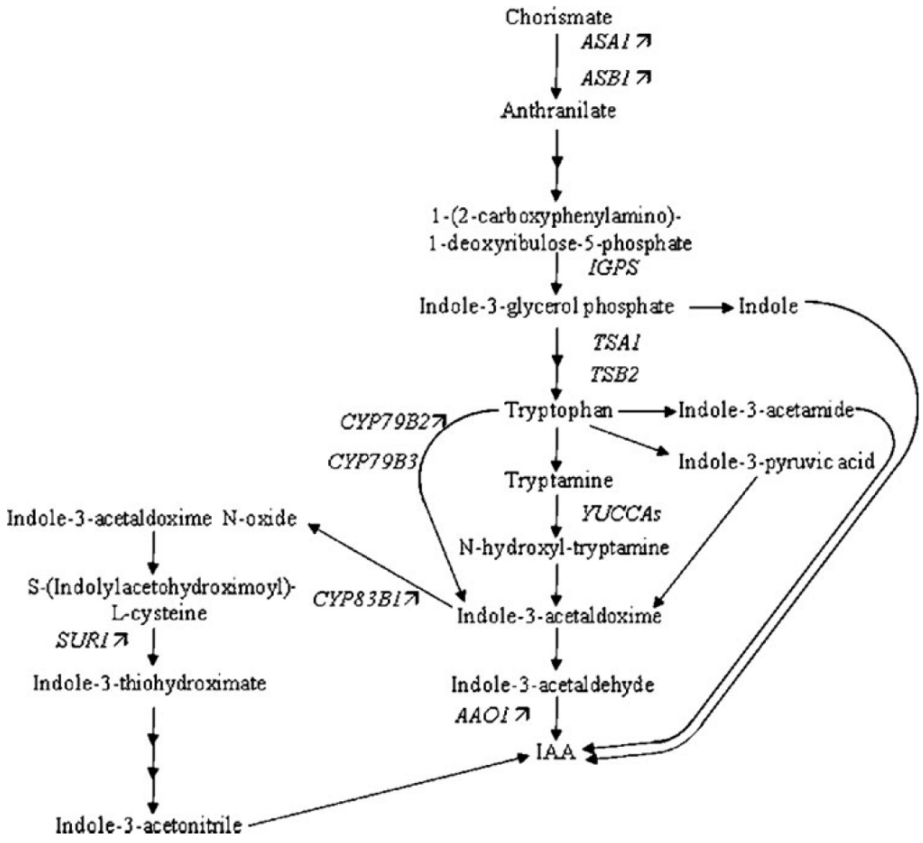

Fig. 8 Schematic representation of the impact of *Phyllobacterium brassicacearum* STM196 on the expression of genes involved in IAA biosynthesis in Arabidopsis shoots. The IAA biosynthesis pathway is adapted from Woodward and Bartel (2005). Analyzed genes are indicated with an arrow when upregulated in the shoots of inoculated plants.

Despite the effects of STM196 on IAA biosynthesis and/ or transport, we found no increase in the transcript levels of the *Aux/IAA* genes tested (Fig. 6c). However, this does not conflict with an involvement of the IAA transduction pathway since these genes that were first characterized as early auxin-responsive genes (Abel and Theologis 1996) appeared marginally affected by long-term exposure to exogenous auxin (Zhao et al. 2002; Goda et al. 2004). The expression of some genes described as involved in auxindependent root morphogenesis, including *TIR1*, *AIR1*, *DFL1*, and *PXA1* (Neuteboom et al. 1999; Casimiro et al. 2003), was found to be stimulated in shoots upon inoculation (Fig. 6c, d). Surprisingly, these genes were either upregulated at a lower extent or unchanged, or even slightly down-regulated (*TIR1*) in roots. Due to the effects of the rhizobacteria on IAA distribution within the root system, however, it is hard to conclude whether these transcript accumulation patterns measured at the whole root system scale reliably reveals the expression response patterns within the cells, where they act to control the lateral root development.

In conclusion, the results presented here demonstrate that functional IAA signaling pathway is required for the root architecture response to *P. brassicacearum* STM196. Although the action of PGPR on plant roots has long been said to involve auxin hypothetically originating from the

bacteria, this is the first demonstration that a key step of auxin transduction pathway (AXR1-dependent) is required in the plant response. We show that a PGPR strain may affect endogenous IAA homeostasis in the host plant in a complex manner, without directly providing IAA. Since an extension of the stained zone has independently been observed in the stele of *DR5::GUS* transgenic plants inoculated with *Pseudomonas fluorescens* strain C7R12 (Gérard Vansuyt and Philippe Lemanceau, personal communication), similar to what we found with STM196 (Fig. 3), the alteration of endogenous auxin homeostasis could be a more generalized response to PGPR. This conclusion concurs with that by Zhang et al. (2007) on volatile organic compounds (VOCs) from the PGPR *Bacillus subtilis* strain GB03 devoid of auxin regulating auxin homeostasis in plants. However, the *P. brassicacearum* strain STM196 is unlikely to emit substantial amounts of VOCs and is not able to alter root architecture at distance as *B. subtilis* strain GB03 does (data not shown). Our study thus provides new evidence, with another PGPR, that soil-borne bacterium can trigger developmental changes in plants via modifying auxin distribution in plant tissues; these plant responses thus involve the auxin transduction pathway. Nevertheless, all the developmental effects induced by rhizobacteria in Arabidopsis cannot be attributed to auxin as shown by the root hair response to STM196 (Desbrosses et al. 2009). The ''Additive Hypothesis'', according to which multiple mechanisms are employed by PGPR to stimulate plant growth, is most likely to be valid (Bashan et al. 2004). Not only IAA but other phytohormones, like the gibberellins and ethylene, potentially play important roles in the various and complex plant responses triggered by PGPR (Persello-Cartieaux et al. 2003). Due to extensive cross-talk between various plant hormones signaling pathways, the STM196-induced changes in root architecture may involve auxin signaling interplay with one or several other hormones. Especially, it has been reported that both ethylene and IAA are involved in the effects of *P. putida* GR12-2 on adventitious root length and number in mung bean cuttings (Mayak et al. 1999). In Arabidopsis, it has been shown that ethylene regulates lateral root formation by altering AUX1-mediated uptake and hence polar IAA transport (Negi et al. 2008). However, although the involvement of ethylene signaling in STM196-induced root architecture changes cannot be ruled out, this possibility seems to be unlikely because inoculation with STM196 triggered no change in ethylene production in Arabidopsis seedlings (Desbrosses, unpublished data), and an *acdS* mutant of STM196, that lacks ACC deaminase activity, induced similar changes in lateral root development as the wild-type strain (Contesto et al. 2008).

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Table 1 Accession number, name, description, and gene-specific primer pairs used for quantitative RT-PCR

AGI code	Name	Description	Forward and reverse primers
At1g04240	*IAA3/SHY2*	Regulates multiple auxin responses in roots, induced by IAA	50-GCCAAAGGTTTAGGCTGTGGTG-30 (F) 50-TAGATCTTGCCCGAGAAACCCG-30 (R)
At1g04250	*1AA17/AXR3*	Auxin-inducible AUX/IAA gene, transcription regulator acting as repressor of auxin-inducible gene expression	50-TGGATTTGGTGAATAGCTGGG-30 (F) 50-TGTATCGACGAACATTGGCC-30 (R)
At1g04610	*YUCCA3*	Flavin monooxygenase	50-TCACAACCAGACAAGCAAACTC-30 (F) 50-GCTGCCTTCGGGGATGAG-30 (R)
At1g05180	*AXR1*	Ubiquitin-activating enzyme E1, involved in auxin action	50-CTTATGGCCTTGCTGGGTTTG-30 (F) 50-TGGATTATTCAGGCGGAGGTC-30 (R)
At1g25220	*ASB1*	Anthranilate synthase beta subunit 1	50-ACCACTCGCCGCCTAAACC-30 (F) 50-ATTGGACCATGCTGCTTAGAGG-30 (R)
At1g56010	*NAC1*	Transcription factor involved in shoot apical meristem formation and auxin-mediated lateral root formation	50-GCCTCCGGGATTCAGATTTCA-30 (F) 50-TCAGGACAAGAGGTGGTCGATG-30 (R)
At1g73590	*PIN1*	Auxin efflux carrier involved in shoot and root development	50-CGCTTCAGAGTTCAAGAAACCC-30 (F) 50-CACAGCTTCTCCAGGACCAAAG-30 (R)
At2g04400	*IGPS*	Indole-3-glycerol-phosphate synthase	50-GGACCATTTGAGTTGAGATTGC-30 (F) 50-CCTAAGAGCCCCAACAAAATCC-30 (R)
At2g20610	*SUR1*	C-S lyase of the glucosinolate biosynthesis	50-ACCGAGATCTTCCGCACAAGT-30 (F) 50-GTTTGGTCGAGCCAACGATT-30 (R)
At2g22330	*CYP79B3*	Cytochrome P450 that is involved in tryptophan metabolism	50-TCTCGGAATGTCGTCGTTTACC-30 (F) 50-TGGGTCCTGGCGGGAGAG-30 (R)
At2g38120	*AUX1*	Auxin permease	50-AAGGGCTTTGGCTAGATTGCC-30 (F) 50-CAAGAAGAGCACCGACAGCG-30 (R)
At3g07390	*AIR12*	Auxin-responsive protein	50-TGAATCAGGTATGGCAGATCGG-30 (F) 50-TGAAACTCAACACACGGTGGG-30 (R)
At3g54010	*PAS1*	Immunophilin-like protein	50-CGGAGCAGAATTACTTGCCGA-30 (F) 50-TCGCACCACAGCTTTCAACAA-30 (R)
At3g54640	*TSA1*	Tryptophan synthase alpha chain	50-CGTTGGAGAGGGGAACAAACC-30 (F) 50-TTGGTGTGGTTGGTGTAGTGAG-30 (R)
At3g62980	*TIR1*	E3 ubiquitin ligase SCF complex F-box subunit	50-GCAGAAGTGCGACCAGATGTTT-30 (F) 50-TAGGCAGGAACAGCATCCCAA-30 (R)
At4g02570	*ATCUL1/AXR6*	Cullin, a component of SCF ubiquitin ligase complexes	50-CTAAGATGGAGGGCATGGTGA-30 (F) 50-AATCCCTGGGTTTGCAGCA-30 (R)
At4g12550	*AIR1*	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein/auxin-responsive gene	50-CCTAACATTGGGAAACCCACCT-30 (F) 50-TAGCTTTGAGCGCAGTGCAGA-30 (R)
At4g13260	*YUCCA2*	Flavin monooxygenase	50-AGGCGAAAGCGGATTGTATGC-30 (F) 50-AATGTTGAGGACGAGCCAATGG-30 (R)
At4g27070	*TSB2*	Tryptophan synthase beta chain	50-TTGAAAGACTACGTTGGTCGGG-30 (F) 50-TTGAGGTATATGAGCGGCCC-30 (R)
At4g31500	*CYP83B1*	Cytochrome P450 monooxygenase 83B1	50-ACGAGACGCAAGCACTTTTGG-30 (F) 50-GGGCGGTTAGGGTCAAGAGTC-30 (R)
At4g32540	*YUCCA*	Flavin monooxygenase	50-AGCCGTGGAGTCCCTTCTTTG-30 (F) 50-TTGTTGAACCTTGGAGCGATGC-30 (R)
At4g39850	*PXA1*	Peroxisomal ABC transporter	50-GAATTGCCAGCGATGTACCAAG-30 (F) 50-CAGGCGCCATGCATACAAA-30 (R)
At4g39960	*CYP79B2*	Cytochrome P450 that is involved in tryptophan metabolism	50-CCATGCAGAGACAACAGAAACC-30 (F) 50-AGGTGCTAAAGGACGATGTTTC-30 (R)
At5g05730	*ASA1*	Anthranilate synthase alpha subunit 1	50-GCGTTGGTCGTTATAGCGTTG-30 (F) 50-TGGGATCTCCATTGGATCTTCG-30 (R)

At5g10480	*PAS2*	Protein tyrosine phosphatase-like involved in cell division and differentiation	50-TGGATCGTCTTTGCTGGATGG-30 (F) 50-AGAGGCTTCTCAATGGCGTCA-30 (R)
At5g11030	*ALF4*	alf4-1 prevents initiation of lateral roots,cannot be rescued by IAA	*50-AAAGCGGCCTCTTGTATTCCT-30 (F)* *50-TTTTCAGTGTCATTCCCCGTG-30 (R)*
At5g20960	*AAO1*	Arabidopsis aldehyde oxidase 1 with the specificity for indole-3-acetaldehyde	*50-TGTGGCGAAGGTGGTTGTGG-30 (F)* *50-GCTGCGAGGAGGTGGATGAG-30 (R)*
At5g25890	*IAA28*	Auxin-responsive protein, negative regulator of lateral root formation in response to auxin	*50-AAGGAGATAAAGTTCTGGTCGGG-30(F)* *50-TGAGAGTGAGAAGGCGTGGG-30 (R)*
At5g43890	*YUCCA5*	Flavin monooxygenase	*50-TGTGTCCAGTCTGCTCGATACG-30 (F)* *50-CCCGTCGCCACCACTAACC-30 (R)*
At5g54510	*DFL1*	IAA-amido synthase that conjugates Ala, Asp, Phe, and Trp to auxin	*50-CACCAAAGATCGCAGCTTTG-30 (F)* *50-ATCATCTGCGTTCGTGGTCAC-30 (R)*
At5g57090	*PIN2/EIR1*	Auxin efflux carrier	*50-CCAATGTTCACGGGGTCAACG-30 (F)* *50-GAAGCACTCGAACTCCACACG-30 (R)*

The set of genes studied included: genes involved in IAA biosynthesis (anthralinate synthases *ASA1* and *ASB1*, the indole-3-glycerol-phosphate synthase *IGPS*, Trp synthases *TSA1* and *TSB2*, disulfite oxidoreductases *YUCCA*, *YUCCA2*, *YUCCA3*, and *YUCCA5*, cytocrome P450s *CYP79B2* and *CYP79B3* and the aldehyde oxidase AAO1) (Zhao et al. 2001, 2002; Woodward and Bartel 2005; Cheng et al. 2006), two genes coding for enzymes of the glucosinolate biosynthesis pathway that have been shown to be involved in IAA homeostasis (*SUR1* and *CYP83B1/SUR2*) (Boerjan et al. 1995; Bak and Feyereisen 2001), genes involved in IAA transport (*AUX1* auxin influx transporter, and *PIN1* and *PIN2/EIR1* auxin efflux facilitators), and genes involved in auxin signaling and root responses