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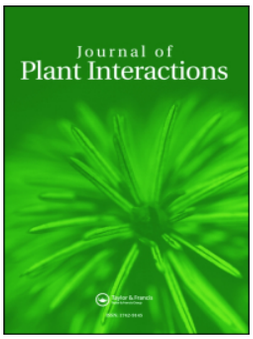

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Transcriptional regulation of plant sugar transporter genes by beneficial rhizobacteria

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ABSTRACT

In their natural environment, plants live in close interaction with complex populations of microorganisms, including rhizobacteria species commonly referred to as 'Plant Growth Promoting Rhizobacteria' (PGPR). A growing body of evidence demonstrates the importance of sugar transport in plant pathogen resistance and in plant-microorganism mutualistic symbioses. Using an *in vitro* experimental system, including the model plant species *Arabidopsis thaliana*, two PGPR strains (*Pseudomonas simiae* PICF7 and *Burkholderia phytofirmans* PsJN) and a non-PGPR strain (*Escherichia coli*), we conducted a comprehensive set of phenotypic and gene expression analyses to explore the role and regulation of sugar transporter genes in plant-PGPR interactions. In physical contact with the seedling roots, or solely *via* the emission of bacterial volatile compounds, the two PGPR strains tested improved the growth and development of the Arabidopsis seedlings and altered the expression of several plant sugar transporter genes. Our results also revealed both conserved and strain-specific transcriptional regulation mechanisms.

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Plant Growth Promoting Rhizobacteria; *Arabidopsis thaliana*; sugar transport; volatile compounds

1. Introduction

A growing body of evidence in the literature demonstrates pathogens are able to manipulate the sugar transport machinery of the host plant to increase the efflux of sugar toward them (Bezrutczyk et al. 2018). Notably, several studies shed light on the capacity of some leaf pathogens to gain sugars from the plant cells by upregulating the expression of genes coding for sugar facilitator proteins of the SWEET family (Chen et al. 2010; Chen 2014). Plants have also evolved mechanisms of regulation of their plant sugar transporters to restrict pathogen proliferation. For instance, expression of the glucose transporter gene *STP13* is upregulated in response to the necrotrophic fungus *Botrytis cinerea*, thereby limiting the availability of apoplastic sugars to the pathogen and thus the disease progression (Lemonnier et al. 2014; Yamada et al. 2016). Similarly, expression of the vacuolar sugar transporter gene *SWEET2* is induced in roots of Arabidopsis upon exposure with the soil-borne pathogen *Phythium irregular*, which limit the amount of sugar exuded from the roots and hence the infection (Chen et al. 2015).

Evidence also exists regarding the importance of sugar transport in plant-microorganism mutualistic symbioses. In Arbuscular Mychorriza (AM) symbiosis, the symbiotic fungus provides nutrients (in particular phosphorus and nitrogen) to the plant, and in return receives carbon derived from the plant host photosynthetic activity (Vandenkoornhuyse et al. 2007). It has been shown that *Medicago* host plants supply more carbohydrates to more cooperative AM fungal species that transfer them greater phosphorus resources (Kiers et al. 2011). Furthermore, some recent studies provide evidence that plant sugar transporter activities are functionally involved in this interaction and in regulating AM root colonization. Notably, this includes the sucrose transporter genes *SUT1* in potato plants (Gabriel-Neumann et al. 2011) and *SUT2* in tomato plants (Bitterlich et al. 2014). Reduced expression of *SlSUT2* led to an increased mycorrhization and the positive effect of the interaction on plant growth was partially abolished. These results suggest a sucrose retrieval function for *SlSUT2*, from the peri-arbuscular space back into the plant cell cytoplasm, to limit mycorrhiza fungal development and secure beneficial effects of the symbiosis on the host plant. Furthermore, another study suggests several SWEET sugar transporters (including SWEET7a and SWEET12a) are also involved in AM symbiosis (Manck-Gotzenberger and Requena 2016). Similarly, in legume-rhizobia N2 fixing interaction, several *SWEET* genes (including *LjSWEET3*, *MtSWEET11*, and *MtN3/MtSWEET15c*) are induced during nodule development and may have a role in symbiotic rhizobia nutrition (Kryvoruchko et al. 2016; Sugiyama et al. 2017).

Plants also live in close association with rhizobacteria species (often referred to as plant growth-promoting rhizobacteria; PGPR) that stimulate plant growth and/or protect plants against abiotic stresses and pathogen attacks. PGPR are known to enhance plant growth and development via the production of phytohormones and volatile compounds and by enhancing the plant nutrient uptake, notably through their ability to solubilize phosphate, produce siderophores and perform biological nitrogen fixation (Vacheron et al. 2013; Hardoim et al. 2015). Bacterial volatile compounds are known to promote plant growth by acting on phytohormone signaling pathways, modulating photosynthesis and sugar accumulation, and by increasing the uptake of

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nutrients. However, little is known about the underlying molecular mechanisms and volatile compound receptors involved in these biological processes (Fincheira and Quiroz 2018; Sharifi and Ryu 2018). Many PGPR species also enhance the host plant tolerance to various abiotic stress and/or display a biocontrol activity against a wide range of soil pathogens via direct antagonism mechanisms involving the production of antimicrobial substances (some of them volatile) or enzymes and/or by competition for nutrients (Vacheron et al. 2013). Lastly, PGPR can also trigger induced systemic resistance (ISR) which stimulates plant defenses against leaf pathogens (Pieterse et al. 2014). A large and diverse set of rhizobacteria have been reported for these beneficial effects on plant productivity. Several strains of the genera *Pseudomonas* and *Bacillus,* in particular, have been widely investigated as promising PGPR for application in agriculture (Hashem et al. 2019; Hakim et al. 2021). In the light of recent evidence that demonstrates the importance of sugar transport in plant microorganism interactions, changes in the plant carbon fluxes and sugar transporter activities may also play a key role in plant-PGPR interaction and their beneficial effects on plant productivity. A better understanding of the molecular mechanisms involved in these biological processes may help to expand the application of PGPR in sustainable agriculture.

With the aim to identify genes playing an essential role in the efficiency of plant-PGPR interaction, and investigate the importance of plant sugar transport in this biological process, we earlier designed (Desrut et al. 2020) an in vitro experimental system enabling the analysis of PGPR modes of action and molecular mechanisms involved in their beneficial effects on plant growth and development. Using this system, the model plant *Arabidopsis thaliana* (thereafter named Arabidopsis) was co-cultivated with the well-characterized PGPR strain *Pseudomonas simiae* WCS417r (PsWCS417r), and a comprehensive set of phenotypic and gene expression analyses (by RNA-sequencing and qRT-PCR) was carried out. Results from this previous study revealed that this strain induces major transcriptional changes of several plant sugar transporter genes. Using a reverse genetic approach, we also demonstrated that two of them, *SWEET11* and *SWEET12*, were functionally involved in the PGPR-triggered plant growth-promoting effects (Desrut et al. 2020).

In order to select additional candidate genes for functional characterization among the sugar transporter genes transcriptionally regulated in Arabidopsis by PsWCS417r (Desrut et al. 2020), and to identify conserved and specific molecular mechanisms of plant growth promotion and sugar transport regulation among PGPR, we aim now to extend this work to two other PGPR strains (*Pseudomonas simiae* PICF7 and *Burkholderia phytofirmans* PsJN) and a non-PGPR strain (*Escherichia coli* DH5α). Overall, our results reveal that the PGPR strains PICF7 and PsJN are able, like PsWCS417r, to alter the expression of several plant sugar genes (essentially genes of the SWEET and ERD6-like families) in both experimental conditions tested: (i) when the seedling roots were inoculated with the PGPR, and (ii) via the emission of volatile compounds only. Noteworthy, the *E. coli* strain also produced volatile compounds with phytobeneficial effects but did not induce any plant growth-promoting effects in physical contact with the seedling roots. Moreover, our results reveal both conserved and bacterial strain-specific transcriptional regulation of plant sugar transport.

2. Results

2.1. Plant growth-promoting activities of PICF7, PsJN and E. coli DH5α in physical contact with seedling roots

To study the plant growth-promoting effects of the PGPR strains PICF7 and PsJN using our in vitro experimental system, Arabidopsis seedlings were grown axenically on MS medium (0.5X, 0.5% MES, no sucrose) prior their inoculation. Five day old seedlings were inoculated with the bacterial strains and seven days post inoculation their shoot and root fresh weight were measured, and their root architecture parameters were analyzed. Under this experimental condition (PGPR in physical contact with the seedling roots), which included the putative action of diffusible and volatile substances, both strains displayed plant growth-promoting properties, but to a different extent (Figure 1). PICF7 significantly ($P < .0001$) enhanced the shoot and root biomass of the seedlings (76% and 228% increase, respectively), their lateral root length and number (584% and 254% increase, respectively) and significantly increased ($P < .001$) their root hair length and density (300% and 373% increase, respectively) in comparison to the control condition (Mock treatment). In contrast, PsJN displayed milder positive, yet significant ($P < .01$) effects, on the seedling shoot biomass (26%) in comparison to the mock treatment. PsJN also significantly ($P < .0001$) enhanced the root biomass (147% increase), the primary root length (55% increase), the lateral root length (225%) and the root hair density (126% increase) of the inoculated seedlings, but had non-significant effect on the lateral root number and root hair length parameters (Figure 1 and Table S1). In the same experimental conditions that the one described above, we also evaluated the plant growth-promoting effects of a non-PGPR strain, *E. coli* DH5α. In physical contact with the seedling roots, *E. coli* DH5α did not trigger any major plant growth-promoting effects (Figure 1 and Table S1).

2.2. Plant growth-promoting activities of PICF7, PsJN and E. coli DH5α volatile compounds

Volatile compounds produced by PICF7 and PsJN may also be involved in the phytostimulatory effects observed. In order to investigate their effects alone, we set up a second in vitro experimental system in which the PGPR strains were physically separated from the Arabidopsis seedlings (Figure 2). In this condition in which the phenotypic effects observed could only be triggered by the bacterial volatile compounds, those emitted by the PGPR strains PICF7 and PsJN led to marked and significant ($P < .0001$) beneficial effects on the seedling shoot (322% and 209% increase, respectively) and root fresh weight (587% and 415% increase, respectively). In addition, both PICF7 and PsJN volatile compounds had a significant ($P < .0001$) influence on the lateral root length (5 and 12.6 fold increase, respectively) and number (486% and 340% increase, respectively), and significantly ($P < .01$) increased the root hair density of inoculated plants (121% and 64% increase, respectively) in comparison to the control condition (Mock) (Figure 2 and Table S2). Via the production of volatile compounds only, *E. coli* DH5α also significantly ($P < .0001$) enhanced the shoot and root biomass (278% and 473% increase, respectively), increased the lateral root length and number (452% and 456% increase,

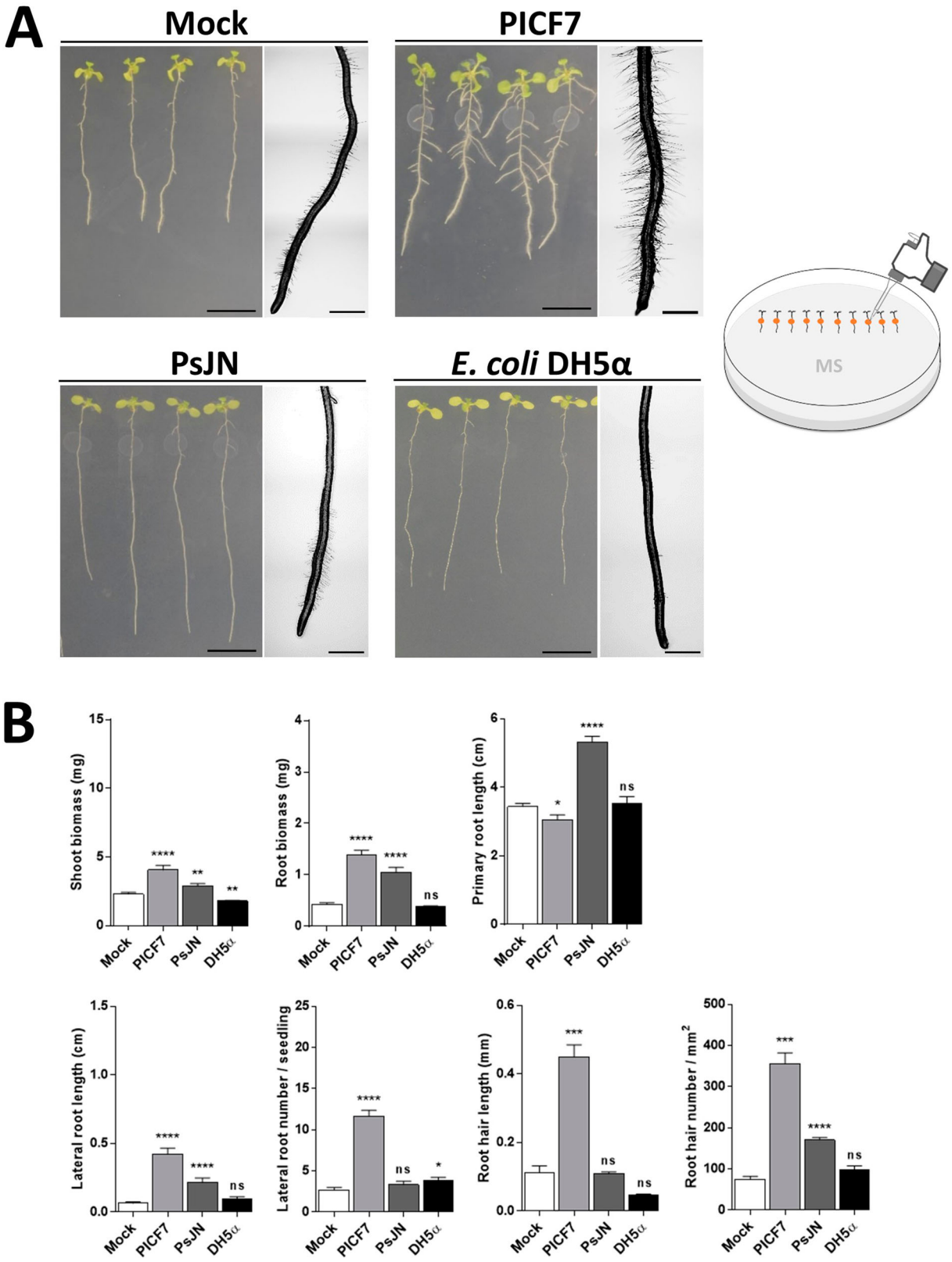

Figure 1. Phenotypic effects of *Pseudomonas simiae* PICF7, *Burkholoderia phytofirmans* PsJN and *Escherichia coli* DH5α on *Arabidopsis thaliana* Col-0 seedlings 7 days post inoculation (dpi). Five day old seedlings were either mock-treated or inoculated with 10 µl of PICF7, PsJN or DH5α at 2.10^6 CFU.ml^{-1} in MgS04 10 mM. **A.** Pictures of whole seedlings (scale bar, 1 cm) and macroscopy pictures of root tips (scale bar, 1 mm) at 7 dpi. **B.** Root and shoot biomasses of the seedlings and quantitative phenotypic analysis of their root system architecture at 7 dpi. Data are means ± SEM of 9 biological replicates (*n*) from 3 independent experiments. Stars indicate statistically significant differences with the mock according to a Mann–Whitney–Wilcoxon test (ns, non-significant; *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$).

respectively) of the seedlings, and significantly increased ($P < .05$) their root hair density (34% increase) in comparison to the control (Mock treatment). Lastly, all three strains emitted volatile compounds that significantly ($P < .0001$) enhanced the primary root length in comparison to the mock-treated condition (Figure 2 and Table S2).

2.3. The strains PICF7 and PsJN and E. coli DH5α transcriptionally regulate several plant sugar transporter genes

In a previous study in which genome-wide and targeted gene expression analyses (by RNA-sequencing and qRT-PCR)

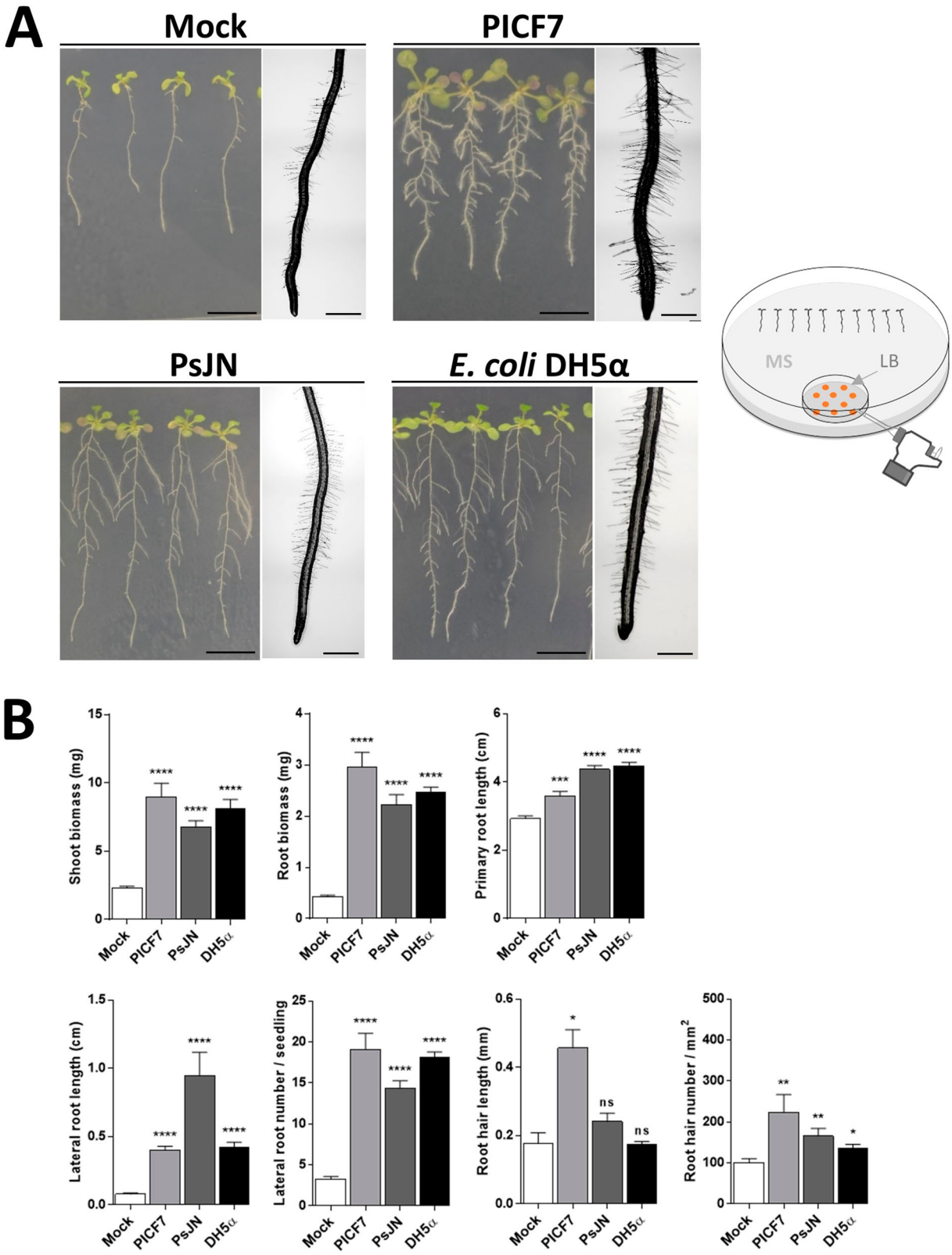

Figure 2. Phenotypic effects of *Pseudomonas simiae* PICF7, *Burkholoderia phytofirmans* PsJN and *Escherichia coli* DH5α volatile compounds on *Arabidopsis thaliana* Col-0 seedlings 7 days post inoculation (dpi). Five day old seedlings were either mock-treated or exposed to PICF7, PsJN or DH5α volatile compounds by spotting 100 µl of the inoculum at 2.10^6 CFU.mL^{-1} in MgSO4 10 mM on LB medium, per plate of 10 seedlings, and without physical contact with them. A. Pictures of whole seedlings (scale bar, 1 cm) and macroscopy pictures of root tips (scale bar, 1 mm) at 7 dpi. B. Root and shoot biomasses of the seedlings and quantitative phenotypic analysis of their root system architecture at 7 dpi. Data are means ± SEM of 9 biological replicates (*n*) from 3 independent experiments. Stars indicate statistically significant differences with the mock according to a Mann-Whitney-Wilcoxon test (ns, nonsignificant; **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001).

were performed, 14 sugar transporter genes (among the 79 ones present in the Arabidopsis genome) were found transcriptionally regulated 7 days post inoculation of five-day-old Arabidopsis seedlings with the PGPR PsWCS417r (Desrut et al. 2020). All these genes, except one (an inositol transporter gene, INT2) belong to the SWEET and ERD6-like gene families of sugar transporters. These genes were differentially expressed in the mock- and PGPR-treated conditions, either in roots (*SWEET3, SWEET11, SWEET12, ERD6-like13, ERD6-like15, and ERD6-like18*) or in shoots

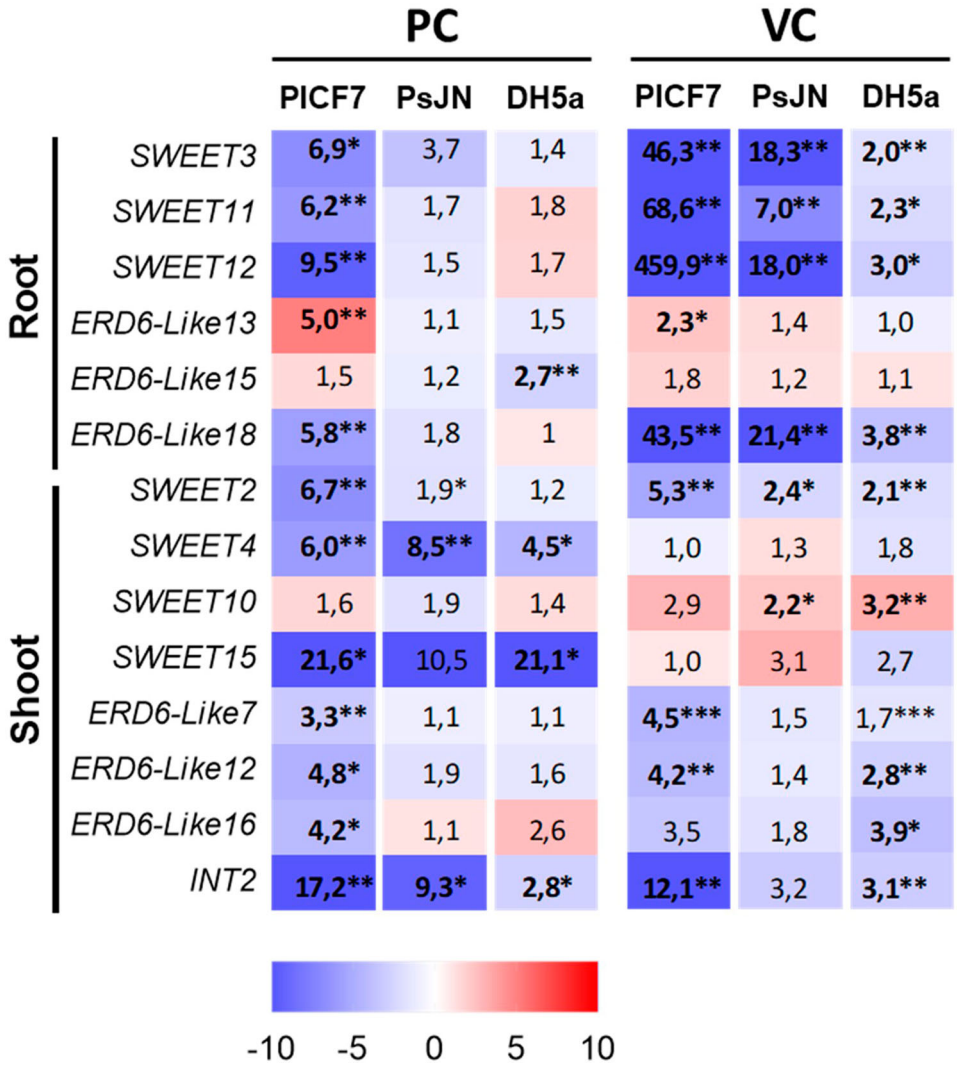

Figure 3. Effects of *Pseudomonas simiae* PICF7, *Burkholoderia phytofirmans* PsJN or *Escherichia coli* DH5α on the relative expression of genes coding for sugar transporters in *Arabidopsis thaliana* Col-0 seedlings roots and shoots. Heatmap of gene expression changes in roots and in shoots induced by PICF7, PsJN or DH5α in physical contact with the seedling roots ('PC') (a), or by PICF7, PsJN or DH5α volatile compounds ('VC') at 7 dpi. Five-day-old seedlings were either mock-treated or treated with bacterial inoculum. Seven days post inoculation, root and shoot tissues were harvested (at midday, 8h light) and transcript levels were quantified by qRT-PCR. The expression level of each gene was normalized to the reference gene At4g26410. Data are mean ± SEM of at least 4 biological replicates, each from an independent experiment. Fold changes (Fc) of gene expression are in red for induction and in blue for repression in comparison to the mock. Stars indicate statistically significant differences according to a Mann-Whitney-Wilcoxon test (ns, non-significant; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$).

(*SWEET2, SWEET4, SWEET10, SWEET15, ERD6-like7, ERD6-like12, ERD6-like16 and INT2*). Among them, only three genes (*ERD6-like13*, *ERD6-like15*and *SWEET10*) were found up-regulated, and all the others were down-regulated in response to inoculation with PsWCS417r.

In order to identify strain-specific and strain-conserved transcriptional changes, and help prioritize functional characterization studies, we carried out a gene expression analysis by qRT-PCR in both roots and shoots of Arabidopsis seedlings inoculated with PICF7, PsJN, and *E. coli* DH5α on all the candidate genes identified from a previous study in response to PsWCS417r (Desrut et al. 2020). Data from this analysis are presented in Table S3 and S4, and summarized in Figure 3 as well.

Our results reveal that several of these sugar transporter genes are up- or down-regulated by all three strains in addition to WCS417r, either in physical contact condition with the seedling roots (*SWEET4* and *INT2* in shoot), or via the emission of volatile compounds only (*SWEET3, SWEET11, SWEET12*, and *ERD6-like18* in root, and *SWEET2* in shoot), whereas the others display some strain-specific transcriptional regulation. Furthermore, the transcriptional regulation of the gene *SWEET4* in the seedling shoots was specific to the root inoculation condition for all the bacterial strains tested. Lastly, it is interesting to note that the strain PICF7 triggered changes in sugar transporter gene expression very similar to those obtained in response to PsWCS417r in both experimental conditions tested (Figure 3) (Desrut et al. 2020).

3. Discussion

PICF7 and PsJN are well characterized PGPR strains (Prieto and Mercado-Blanco 2008; Poupin et al. 2013; Zuniga et al. 2013; Maldonado-González et al. 2015; Pinedo et al. 2015; Mercado-Blanco et al. 2016; Zhao et al. 2016; Montes-Osuna et al. 2021). In our experimental conditions, PICF7 displayed marked plant growth-promoting activities, both in physical contact with seedling roots and via the production of volatile compounds only (Figures 1 and 2). Noteworthy, PICF7 grew better on LB medium than on MS medium (when placed in physical contact with the seedling

roots). Hence, a different blend and/or quantity of volatile compounds may be produced between the two experimental conditions. Nevertheless, highly similar PICF7-induced plant growth and development promoting activities were observed in both systems (Figures 1 and 2), suggesting the volatile compounds emitted by this PGPR strain contribute to a large extent to its plant growth-promoting activities. In contrast, the PGPR strain PsJN displayed relatively mild plant growth-promoting effects when the seedling roots were inoculated with this strain. In agreement with previous studies carried out on seed-inoculated Arabidopsis seedlings growing in vitro (Poupin et al. 2013; Zuniga et al. 2013), inoculation of the seedlings with PsJN mostly increased their primary root length in our experimental conditions (Figure 1). However, PsJN triggered strong positive effects on the root and shoot biomasses of the seedlings and their root architecture traits via the production of volatile compounds only (Figure 2). Lastly, the strain *E. coli* DH5α was used as non-PGPR control in physical contact condition in our study. As expected, only very minor changes in the seedling biomasses and root architecture traits were observed in response to inoculation of the seedling roots with this strain. In contrast, in the second experimental condition in which *E. coli* DH5α was physically separated from the seedlings so that only its volatile compounds could have an effect, strong plant growth-promoting effects were observed. These unexpected results are in agreement with a previous study demonstrating the phytostimulatory properties of *E. coli* volatile compounds on the biomass and root architecture system of Arabidopsis seedlings (Bailly et al. 2014). Importantly, the results of this study also revealed the importance of indole in the plant growth-promoting effects triggered by *E. coli*. Indeed, these effects were abolished in an *E. coli* mutant devoid of tryptophanase activity (*tnaA*), and thus unable to produce indole (Bailly et al. 2014). Some evidence also exists in the literature about the volatile compounds potentially implicated in the plant growth-promoting effects observed for PsJN. This PGPR strain was shown to produce the organic compounds 2-undecanone, 7-hexanol and 3-methylbutanol, and its plant growth-promoting effects could be mimicked with exposure to a blend of these three VOCs (Ledger et al. 2016). Overall, our results reveal both PsJN and *E. coli* DH5α mostly display beneficial effects on the seedling growth and development via the emission of volatile compounds only. Potentially, this might be due to: (i) dose-dependent response (the bacteria growth was better on LB than on MS); (ii) the presence of compounds necessary to produce the volatile substances only or at higher concentration in the LB medium than in the MS medium and/or via the root exudates; and (iii) a mix of beneficial and negative effects when the strain is in physical contact with the seedling roots.

To provide new avenues of investigation by identifying candidate genes transcriptionally regulated by these rhizobacterial strains and their volatile compounds, we also carried out a targeted gene expression analysis on a set of sugar transporter genes (essentially genes of the SWEET and ERD6-like families) earlier identified as being transcriptionally regulated by the PGPR strain PsWCS417r (Sharifi and Ryu 2018). Our results show that several of these genes are regulated by the three strains tested in this study (namely, *SWEET4* and *INT2* in 'physical contact' condition and *SWEET2*, *SWEET3*, *SWEET11*, *SWEET12*, and *ERD6-like18* in 'volatile compounds' condition), whereas other genes displayed a strain-specific transcriptional regulation (e.g. *SWEET3*, *SWEET11*, *SWEET12*, *ERD6-like13* and *ERD6-like18* in response to PICF7 in 'physical contact' condition).

It is unlikely that changes in sugar transporter gene expression are solely responsible for all the PGPR-triggered plant growth-promoting effects observed. Notably, regulation of the plant hormonal pathways may also be involved (Dahmani et al. 2020; Desrut et al. 2020). Nevertheless, our results show PICF7, PsJN and DH5α volatile compounds trigger rather similar transcriptional changes for the sugar transporter genes we analyzed (i.e. 5 out of the 14 candidate genes were significantly ($P < .05$) repressed by all three strains, albeit to a different extent) as well as strong plant growth-promoting effects. Our results also show that the growth-promoting effects of PsJN and DH5α are rather similar and minor, in agreement with the changes they induce in plant sugar transporter gene expression upon physical contact with the roots. Notably, *SWEET11* and *SWEET12*, two genes functionally involved in the efficiency of the interaction between PsWCS417r and Arabidopsis (Desrut et al. 2020) were significantly transcriptionally repressed in response to PICF7 in physical contact condition, and to the volatile compounds of the three strains PICF7, PsJN, and *E. coli* DH5α (Figure 3). Interestingly, these conditions are those in which the strongest positive effects of the bacterial strains on the seedling growth and development are observed (Figures 1 and 2). Similarly, the bacteria-triggered phytobeneficial effects observed are associated with the transcriptional down-regulation of the genes *SWEET3* and *ERD6-like18* in root and *SWEET2* in shoot (Figures 1–3), which therefore represent good candidate genes for future functional characterization studies in plant-PGPR interactions.

Very little is known regarding the function of *ERD6-like* genes. In contrast, many studies have focused on the function of *SWEET* genes in plant-microorganism interactions in the past few years (reviewed in Chandran et al. 2015, Julius et al. 2017). Sugar transporter up-regulation may be part of the microorganism strategy to gain sugar from the host plant, as demonstrated from some *SWEET* genes in plant-pathogen interactions (Chandran et al. 2015; Julius et al. 2017) and in mutualistic symbioses (Manck-Gotzenberger and Requena 2016). Unexpectedly, almost all the sugar transporter genes analyzed in our study were repressed by the PGPR inoculation or by exposure to their volatile compounds. The biological significance of this finding is unknown, but it is interesting to note that similar results have been obtained in another study exploring the implication of *SWEET* genes in plant-pathogen interaction (Asai et al. 2016). These authors have studied the expression of all 31 tomato *SlWEET* genes in response to infection with the necrotrophic fungal pathogen *Botrytis cinerea*. Among them, only one gene (*SlWSEET15*) was induced, and evidence suggests it may be exploited by the fungus to gain more sugars from the plant cells. Surprisingly, most of the other *SWEET* genes were repressed. The authors suggested these genes are positively involved in defense reactions and that their downregulation by pathogen effectors may suppress the plant host immunity (Asai et al. 2016). Alternatively, down-regulation of these *SWEET* genes might be part of the host defense responses aiming at reallocating

and retaining carbohydrates in plant cells to limit pathogen proliferation. Similar regulatory processes may be at play in beneficial plant-microorganism interactions. This hypothesis is supported by evidence in the literature demonstrating the importance of regulating the amount of carbohydrates available to the symbiont in arbuscular mycorrhizal (AM) symbiosis to achieve optimal benefits from the symbiosis for the host plant growth (Bitterlich et al. 2014). Besides, in a previous study profiling the expression of 35 SWEET genes in potatoes in response to inoculation with the AM fungus *Rhizophagus irregularis*, 10 of them (mostly belonging to the clade III SWEETs) were found repressed (Manck-Gotzenberger and Requena 2016). The authors suggested AM fungal colonization may be somewhat perceived as a stress for the plant that could be affecting the partitioning of sugars between roots and shoots via transcriptional regulation of these genes. Further investigations into the function of the *SWEET* genes repressed during plant-microorganism interactions are needed to test these hypotheses and establish whether their down-regulation affects sugar allocation to the symbiont/pathogen.

4. Materials and methods

4.1. Inoculation of Arabidopsis thaliana seedlings with the bacteria and in vitro co-cultivation assays

4.1.1. Plant material and growth conditions

Arabidopsis thaliana (Arabidopsis) ecotype Columbia (Col-0) was used as model plant species in this study. Arabidopsis seeds were surface sterilized and grown on half strength (0.5X) Murashige and Skoog (MS) medium (M0222, Duchefa Biochemie, Haarlem, The Netherlands), without sucrose, supplemented with 0.5% of MES (Morpholino-Ethane-Sulfonic acid monohydrate; MW=213.2 $gmol^{-1}$) (ACROS Organics™, 172591000) as previously described (Sharifi and Ryu 2018).

4.1.2. Bacterial strains

Pseudomonas simiae (originally designated *P. fluorescens*) PICF7 was isolated from olive roots (Prieto and Mercado-Blanco 2008). This strain is phylogenetically close to *P. simiae* (originally designated *P. fluorescens*) WCS417r (Gómez-Lama Cabánas et al. 2018; Montes-Osuna et al. 2021) and presents the following features: (i) a sequenced genome (Martínez-García et al. 2015); (ii) an endophytic lifestyle in olive roots and cultivated cereals, but it only colonizes the surface of Arabidopsis roots (Maldonado-González et al. 2015; Mercado-Blanco et al. 2016); and (iii) biocontrol activity against *Verticillium dahliae* in olive trees (Montes-Osuna et al. 2021), *Botrytis cinerea* in Arabidopsis (Maldonado-González et al. 2015), and *Fusarium oxysporum* f. sp. *cubense* in banana (Gómez-Lama Cabánas et al. 2021).

Burkholderia phytofirmans PsJN (originally designated as a *Pseudomonas sp.*, thereafter named PsJN) was isolated from onion roots and is well characterized regarding its plant growth-promoting properties in potatoes, vegetables and grapevines (Sessitsch et al. 2005). In addition, PsJN enhances the seedling primary root length, accelerates the growth rate, flowering time, and enhances salt tolerance in Arabidopsis (seed inoculation) (Poupin et al. 2013; Zuniga et al. 2013; Pinedo et al. 2015; Zhao et al. 2016). Besides, this strain is known to display a biocontrol activity against *B. cinerea*, the agent of gray mould disease in grapevine (Barka et al. 2000) and in Arabidopsis (Miotto-Vilanova et al. 2016). Lastly, its genome has been sequenced (Weilharter et al. 2011; Mitter et al. 2013).

The strain *Escherichia coli* DH5α (thereafter named *E. coli* DH5α) was used as non-PGPR control for our assays with the bacteria in physical contact with the seedling roots. Unexpectedly, however, *E. coli* has been shown to display phytostimulatory effects on maize seedlings from inoculated seeds growing in non-sterile soil (Walker et al. 2013) as well as on in vitro growing Arabidopsis seedlings exposed to its volatile compounds (Bailly et al. 2014).

4.1.3. Preparation of inoculum and inoculation treatment

For preparation of the inoculum, an aliquot of glycerol stock of the bacteria was streaked on solid KB medium (20 $g.L^{-1}$ Bacto™ Peptone, 1.5 $g.L^{-1}$ Dipotassium Phosphate, 1.5 $g.L^{-1}$ Magnesium Sulfate, 15 $g.L^{-1}$ Bacteriological agar type E, pH 7) for the strains PICF7 and PsJN, and on solid LB medium (10 g.L1 Bacto-tryptone, 5 $g.L^{-1}$ Yeast Extract, 5 $g.L^{-1}$ NaCl, 15 $g.L^{-1}$ Agar, pH 7) for the strain *E. coli* DH5α. After 24 h at 28°C, bacterial cells were collected in 10 mM MgSO4, washed twice with 50 mL of 10 mM MgSO4 by centrifugation for 5 min at 5000 g, and resuspended in 50 mL of 10 mM MgSO4. The bacterial titer was adjusted to an OD600 nm of 0.002 for PICF7 and E. coli DH5α, and 0.01 for PsJN, in order to obtain an inoculum with a bacterial density of 2×10^6 Colony-Forming Units.mL^{-1} (CFU.mL^{-1}). For all experiments, this bacterial density was confirmed by counting the number of CFU on LB medium.

Co-cultivation experiments of *Arabidopsis thaliana* with the different bacterial strains either in physical contact with the seedling roots and those involving solely the effects of their volatile compounds were performed as detailed in our previous studies (Desrut et al. 2020; Dahmani et al. 2020).

4.1.4. Phenotypic analyses

Shoot and root fresh weight were measured on an analytical balance. Primary, lateral root, and root hair analyses were carried out using the plugin SmartRoot (Lobet et al. 2011) of the ImageJ software (Schneider et al. 2012).

4.2. Gene expression profiling

4.2.1. Total RNA extraction

Plant samples for gene expression analysis were harvested at mid-day (8 h of light, 16 h photoperiod), 7 days post inoculation. Roots and shoots of Arabidopsis seedlings were harvested separately by sectioning the root-shoot junction, immediately frozen in liquid nitrogen, and stored at −80° C. Total RNA was extracted from 25 to 100 mg of shoot and root tissues using a phenol/chloroform extraction procedure adapted from (Box et al. 2011) as previously described (Desrut et al. 2020).

4.2.2. Relative gene expression analysis by real-time quantitative RT–PCR (qRT-PCR)

Primers for qRT-PCR were designed using the NCBI Primer-Blast software (Ye et al. 2012), ideally with the following criteria: a primer size comprised between 18 and 25 bp, a GC %

of 45–60%, a melting temperature (Tm) between 58°C and 63°C, and a PCR product size of 50–200 bp. Moreover, preferences were given for primer pairs that were exon-exon shuffling or intron spanning. Sequences of the primers used in this study and efficiency of the primers for the selected candidate genes are listed in Table S5.

Gene expression analyses were performed by qRT-PCR using the GoTaq qPCR MasterMix (Promega) according to the manufacturer instructions (1X GoTaq® qPCR Master Mix, 0.33 µM of forward and reverse primer, and 5 µL of 10-fold diluted cDNA per well) and as described in a previous study (Desrut et al. 2020). Target gene expression was normalized using the reference gene At4g26410 (Czechowski et al. 2005; Lemonnier et al. 2014) whose expression remained stable in all conditions evaluated (in the different tissues, time points, and following inoculation with the PGPR strain) according to the results obtained with a second reference gene: AtUPL7 (At3g53090). Results were expressed as relative gene expression values using the $2^{-\Delta\Delta Ct}$ method (Schneider et al. 2012).

4.3. Statistical analysis

Statistical analyses of differences for morphological traits and relative gene expressions were carried out using a non-parametric Mann–Whitney–Wilcoxon test ($n < 30$) unless otherwise indicated. Tests were performed using the software GraphPad Prism® version 7.0.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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