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ORIGINAL ARTICLE

# Different *Arabidopsis thaliana* photosynthetic and defense responses to hemibiotrophic pathogen induced by local or distal inoculation of *Burkholderia phytofirmans*

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**Abstract** Pathogen infection of plant results in modification of photosynthesis and defense mechanisms. Beneficial microorganisms are known to improve plant tolerance to stresses. *Burkholderia phytofirmans* PsJN (Bp), a beneficial endophytic bacterium, promotes growth of a wide range of plants and induces plant resistance against abiotic and biotic stresses such as coldness and infection by a necrotrophic pathogen. However, mechanisms underlying its role in plant tolerance towards (hemi)biotrophic invaders is still lacking. We thus decipher photosynthetic and defense responses during the interaction between Arabidopsis, Bp and the hemibiotrophic bacterium *Pseudomonas syringae* pv. *tomato* DC3000 (Pst). Different Bp inoculations allowed analyzes at both systemic and local levels. Despite no direct antibacterial action, our results showed that only local presence of Bp alleviates Pst growth in planta during the early stage of infection. Molecular investigations showed that seed inoculation of Bp, leading to a restricted presence in the root system, transiently primed *PR1* expression after challenge with Pst but continuously primed *PDF1.2* expression. Bacterization with Bp reduced Y(ND) but had no impact on PSII activity or RuBisCO accumulation. Pst infection caused an increase of Y(NA) and a decrease in ΦPSI, ETRI and in PSII activity, showed by a decrease in Fv/Fm, Y(NPQ), ΦPSII, and ETRII values. Inoculation with both bacteria did not display any variation in photosynthetic activity compared to plants inoculated with only Pst. Our findings indicated that the role of Bp here is not multifaceted, and relies only on priming of defense mechanisms but not on improving photosynthetic activity.

**Keywords** PGPR · *Pseudomonas syringae* pv. *tomato* DC3000 · *Arabidopsis thaliana* · Photosystem I and II · Photosynthesis · Defense

**Abbreviations**

| | |
|---|---|
| ABA | Abscisic acid |
| Bp | *Burkholderia phytofirmans* strain PsJN |
| ET | Ethylene |
| ETRI | Electron transport rate of PSI |
| ETRII | Electron transport rate of PSII |
| $F_0$ | Minimal fluorescence yield of the dark-adapted state |
| $F_{0'}$ | Minimal fluorescence yield of the light-adapted state |
| $F_m$ | Maximal fluorescence yield of the dark-adapted state |
| $F_{m'}$ | Maximal fluorescence yield of the light-adapted state |
| $F_v/F_m$ | Maximum quantum yield of PSII photochemistry |
| JA | Jasmonic acid |
| MAMPs | Microbe-associated molecular patterns |
| PAMPs | Pathogen-associated molecular patterns |
| PGPR | Plant-growth-promoting rhizobacteria |
| PS | Photosystem |
| Pst | *Pseudomonas syringae* pv. *tomato* DC3000 |

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| | |
|---|---|
| RbcL | RuBisCO large subunit |
| RbcS | RuBisCO small subunit |
| RuBisCO | Ribulose-1,5 bisphosphate carboxylase/oxygenase |
| SA | Salicylic acid |
| YI | Efficient quantum yield of PSI |
| YII | Efficient quantum yield of PSII |
| Y(NA) | PSI acceptor side limitation |
| Y(ND) | PSI donor side limitation |
| Y(NO) | Quantum yield of non-regulated energy dissipation |
| Y(NPQ) | Quantum yield of regulated energy dissipation |

## Introduction

It is well established that pathogen infection triggers modifications in photosynthesis processes and consequently carbohydrate metabolism. On one side, the pathogen redirects sugars for its own benefit and on the other side, the plant shut down its primary metabolism to develop its defense strategy. Plants have to make trade-offs between stress defense responses and primary metabolism (Demmig-Adams et al. 2017; Foyer and Noctor 2005; Karpinski et al. 2003). The chloroplast, in which photosynthesis takes place, is now considered as a key defense organelle as it is able to perceive external signals occurring during biotic or abiotic stresses (Serrano et al. 2016). Some pathogen effectors are able to interfere with some chloroplastic functions to facilitate their multiplication (de Torres Zabala et al. 2015). It was recently demonstrated that *Sclerotinia* suppress host defense *via* antagonizing ABA biosynthesis by manipulating the xanthophyll cycle in early pathogenesis (Zhou et al. 2015), suggesting that photoprotective metabolites could be integrated into the defense responses. Oxylipin (OPDA and/or JA) levels are increased in photoprotection mutants either deficient in components involved in thermal dissipation and/or detoxification (Demmig-Adams et al. 2013). By using Arabidopsis over-expressing or lacking PsbS (a chlorophyll-binding protein of photosystem II), it was shown that differences in herbivore preferences towards plants were due to differences in the primary metabolism of these plants rather than in their contents of typical defence compounds (Johansson Jänkänpää et al. 2013). Similarly, PsbS-deficient rice plants were also more resistant to fungal and bacterial pathogens (Zulfugarov et al. 2016). Moreover, redox metabolism and related signaling are key players in tolerance to biotic stresses in plants (Munné-Bosch et al. 2013). The foliar hemibiotrophic bacterium *Pseudomonas syringae* pv. *tomato* strain DC3000 (Pst) is able to cause disease symptoms in a wide range of plant species, including Arabidopsis (Xin and He 2013). Arabidopsis recognizes type III virulence effectors of Pst (Shan et al. 2007; Xin and He 2013) and led to activation of several defense mechanisms, including stomatal closure (Boureau et al. 2002; Melotto et al. 2008), programmed cell death at the infection site (Lenz et al. 2011; Dong and Chen 2013) and activation of salicylic acid (SA) signaling pathway (Glazebrook 2005), which could lead to systemic resistance (Mishina and Zeier 2007). Moreover, two Pst effectors are imported into chloroplasts, meaning that this bacterium targets the chloroplast during its infection process (de Torres Zabala et al. 2015). Thilmony et al. (2006) and Cartieaux et al. (2008) showed an induction of defense-related genes correlated with a downregulation of photosynthetic genes. Infection of Arabidopsis with Pst also induces modifications on photosynthesis, carbon metabolism and carbohydrate distribution in tomato plants (Berger et al. 2004) and Arabidopsis (Bonfig et al. 2006; de Torres Zabala et al. 2015). However, repression of photosynthetic parameters was restricted at the site of infection (Berger et al. 2004; Bonfig et al. 2006). Infections with the bacterium inhibit photosynthetic $CO_2$ assimilation through disruption of photosystem II. Li et al. (2015) showed that elevated $CO_2$ concentration-induced stomatal closures not only reduce entry of Pst by controlling stomatal apertures, but also involve a stomata-independent pathway to resist against Pst.

Plants are sessile organisms and do not possess an adaptive immune system. To restrict pathogen infection, plants have evolved an array of defense mechanisms. The success of plant resistance firstly relies on the capacity of the plant to recognize its invader. Plants detect the presence of microorganisms by microbe- or pathogen-associated molecular patterns, such as bacterial lipopolysaccharides, flagellin, and fungal chitin (Boller and Felix 2009; Henry et al. 2012; Walters 2015). Pattern recognition by specific receptors located on the plant plasma membrane is required to trigger plant innate immune mechanisms (pattern-triggered immunity, PTI) (Bittel and Robatzek 2007; Walters 2015). Some pathogens are able to bypass this first line of plant defense by preventing host plant detection or by deleting PTI signals (Dodds and Rathjen 2010; Walters 2015). However, many plants could recognize specific effectors of pathogens, resulting in more powerful defense responses (effector–triggered immunity, ETI) (Dodds and Rathjen 2010). Activation of the PTI or the ETI limits the development of pathogens at the site of infection, and could trigger an induced resistance in intact tissues with one or more long-distance signals (Newton et al. 2014; Walters 2015). The plant hormones SA, jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) are key players in defense signaling network. In Arabidopsis, SA is involved in plant resistance against (hemi)biotrophic pathogens, whereas JA and ET are thought to be involved in resistance against necrotrophic pathogens (Thomma et al. 2001; Glazebrook 2005). Pathogen perception then initiates a large array of immune responses including modification of cell walls as well as the production of anti-microbial proteins

and metabolites like pathogenesis-related (PR) proteins (Schwessinger and Ronald 2012).

Several rhizobacteria, the so-called plant-growth-promoting rhizobacteria (PGPR), are capable to promote plant growth through uptake facilitation of some soil nutrients (Vessey 2003; Yadav et al. 2015), modification of phytohormones homeostasis (Castillo et al. 2015; Kumar et al. 2015), and/or improvement of photosynthesis efficiency (Stefan et al. 2013; Cohen et al. 2015). Some PGPR could also restrict pathogen developing in plants (Compant et al. 2005; Wang et al. 2015). PGPR could inhibit directly pathogen infection and propagation by their antagonistic activity, including production of antibiotics, siderophores, and hydrolytic enzymes (Vessey 2003; Somers et al. 2004; Pathma et al. 2011). Indirectly, PGPR application could activate plant defense mechanisms to lead plant in a systemically resistant state (induced systemic resistance, ISR) (van Loon et al. 1998; Kloepper et al. 2004; Chandler et al. 2008). The perception of flagellin from Bp by Arabidopsis cells induced defense responses, like production of reactive oxygen species and expression of defense-related genes (Trdá et al. 2014). Bp has been shown to protect plants against abiotic stresses such as cold (Su et al. 2015; Theocharis et al. 2012), drought (Naveed et al. 2014), or salt (Pinedo et al. 2015), or biotic stresses such as the necrotrophic pathogen *Botrytis cinerea* (Miotto-Vilanova et al. 2016). However, little is known about the impact of Bp inoculation on an attack by a (hemi)biotrophic pathogen (Sharma and Novak 1998). Furthermore, mechanisms underlying the induced resistance are still unclear. The present study thus aims to investigate if Bp could be efficient to protect plants against a hemibiotrophic pathogen. We first evaluated the eventual direct antibacterial effect of Bp on Pst growth. Since biotic stress and photosynthesis are intimately linked, photosynthetic parameters (PSI and PSII activity, RuBisCO levels) and defense-related gene expression were quantified when Arabidopsis plants were co-inoculated with the two bacteria. To decipher how the presence of Bp in the plant could modify tolerance to Pst attack, Bp was locally (leaf) or systemically (root) inoculated.

## Materials and methods

### Plant material and growth conditions

*Arabidopsis thaliana* ecotype Col-0 plants were grown in soil condition in a controlled chamber at 20/15 °C (day/night), with 60% of relative humidity and a 12-h photoperiod (photosynthetically active radiation, PAR = 120 µmol $m^{-2}$ $s^{-1}$). For all of the experiments, measurements were performed on mature leaves of 6-week-old plants. Three plants were used per treatment with three biological replicates.

### Bacterial strains and inoculation

PGPR *Burkholderia phytofirmans* strain PsJN (Bp) tagged with GFP and pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (Pst) were grown for 24 h at 28 °C at 180 rpm in King's B liquid medium supplemented with 50 µg $mL^{-1}$ antibiotics (kanamycin and cycloheximide or rifampicin for Bp or Pst, respectively). Bacteria were collected after centrifugation at 4500×*g* for 10 min and suspended in 10 mM $MgCl_2$. The concentration of bacterial inocula was adjusted by spectrophotometry at 600 nm (Pillay and Nowak 1997).

For direct antibacterial effect on growth assays, Pst ($10^3$ colony forming units per mL, cfu $mL^{-1}$) or Bp ($10^3$ cfu $mL^{-1}$) were inoculated alone or side by side on the King's B medium supplemented with kanamycin (50 µg $mL^{-1}$). Plates were then placed in a growth chamber at 28 °C. Colony growth of the two bacteria was observed under UV 365 nm to assess possible direct antagonism between these two bacteria.

Bp inoculum ($10^5$ cfu $mL^{-1}$) was infiltrated into Arabidopsis mature leaves using a needleless syringe (Bp). Control plants (Mock) were infiltrated with 10 mM $MgCl_2$. Arabidopsis seeds were immersed in bacterial inoculum of $5.10^8$ cfu $mL^{-1}$ (SBp) or PBS for 3 h at 4 °C (Mock). Pst infection was performed 8-h post Bp application by dipping Bp and Mock plants in Pst suspension ($10^8$ cfu $mL^{-1}$) supplemented with Silwet L77 0.02% (Bp + Pst and Pst, respectively), or 10 mM $MgCl_2$ Silwet L77 0.02% as Mock. 3 days after Bp and Pst inoculation, Pst symptoms were photographed on Pst and Bp + Pst inoculated plants.

### Protein extraction and western blotting analysis

Total proteins were extracted from 0.2 g of leaf with 500 µL cold extraction buffer (250 mM sorbitol, 50 mM Tris–HCl, pH 8.0, 2 mM EDTA, 7 g $L^{-1}$ PVPP, 5 mM DTT, 1 mM PMSF and 1/100 Halt Protease Inhibitor Cocktail-Thermo Scientific) and centrifuged at 10,000×*g* for 10 min at 4 °C. The supernatant was then collected and proteins were quantified by the Bradford method using bovine serum albumin as the standard (Bradford 1976). Protein samples (2 µg) were solubilized for 3 min at 95 °C in Laemmli buffer (Laemmli 1970) and separated by SDS-PAGE in 12% (w/v) polyacrylamide gels, using Mini-protean three Cell electrophoresis equipment (Bio-Rad). Proteins were electro-transferred onto a polyvinylidene difluoride (PVDF) membrane using iBlot system (Invitrogen). Western blotting was performed according to standard procedures using rabbit anti-RbcL or -RbcS antibodies (Agrisera; 1:10,000) and peroxidase-coupled anti-rabbit IgG antibodies (Cell signaling; 1:5000). Actin (Agrisera; 1:1000) was used as internal quantification control.

### RNA extraction and real-time RT-PCR

RNA extraction and real-time RT-PCR analysis were performed as described by Magnin-Robert et al. (2015). For each sample, 100 mg of leaves was ground in liquid nitrogen. Total RNA was isolated using Extract' All (Eurobio) and followed reverse transcription by using the Verso cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. The transcript levels were determined by qPCR using the CFX 96TM Real Time System (Bio-Rad, France) and the SYBR Green Master Mix PCR kit as recommended by the manufacturer (Applied Biosystems). PCR conditions were 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing/extension) for 40 cycles on CFX 96TM Real Time System (Bio-Rad, France). Traditional reference genes were evaluated with Bio-Rad CFX MANAGER software v.3.0 (Actin2, UBQ5, UBQ10, EF1α, and Tubulin2) to select a reference gene with a stable expression in all tested conditions (Hong et al. 2010). The expression stability geNorm M value of UBQ5 was below the critical value of 0.5 in Arabidopsis samples. Transcript level and Pst relative quantity were calculated using the standard curve method and normalized against UBQ5 gene as an internal control. The specific primers used in this study were listed in Table S1 in Supplementary Material.

### PSI and PSII photochemistry

Chlorophyll fluorescence parameters and the redox change of P700 were assessed simultaneously with a Dual-PAM-100 measuring system (dual-wavelength pulse-amplitude-modulated fluorescence monitoring system, Heinz Walz, Germany) on Arabidopsis leaves.

*PSII photochemistry*

Leaves were dark adapted for 30 min to determine the minimal level of fluorescence (F0) and the maximal fluorescence ($F_m$) after a saturating flash (1 s, 13,000 μmol $m^{-2}$ $s^{-1}$). The ratio of variable to maximal fluorescence [$F_v/F_m = (F_m - F_0)/F_m$] was calculated. Actinic illumination (216 μmol $m^{-2}$ $s^{-1}$) was applied and after fluorescence stabilization, a second saturating flash (1 s) was imposed to determine the maximal fluorescence ($F_{m'}$) of a light-adapted inflorescence. Removal of the actinic light and exposure to a short period of far-red light allowed measurement of the zero level of fluorescence ($F_{0'}$). In both dark- and light-adapted states, the fluorescence parameters were calculated according to Genty et al. (1989) and Schreiber et al. (1994). Quantum yields, designated by YII [$YII = (F_{m'} - F)/F_{m'}$] for photochemical energy utilization in PSII, YNPQ for regulated energy dissipation in PSII and YNO for non-regulated energy dissipation in PSII, were calculated according to Kramer et al. (2004). The electron flow through PSII (ETRII) was calculated according to Miyake et al. (2005) according to $ETRII = YII \times PPFD \times \alpha II$ [$\alpha II$ = fraction of the incident light absorbed by organ ($p$) × fraction of the absorbed light distributed to PSII ($d$II)].

*PSI photochemistry*

Together with fluorescence measurement, the saturation pulse method was used to determine P700 parameters following the method of Klughammer and Schreiber (1994, 2008). The P700+ signals may vary between a minimal (P700 fully reduced, closed) and a maximal level (P700 fully oxidized). P700 fully oxidized ($P_m$) was determined by application of a saturation pulse after far-red pre-illumination. YNA, the quantum yield of non-photochemical energy dissipation due to acceptor side limitation, was calculated based on a $P_{m'}$ determination at 216 μmol $m^{-2}$ $s^{-1}$ actinic light according to the following: $YNA = (P_m - P_{m'})/P_m$. YND, the non-photochemical quantum yield of PSI due to donor side limitation, was calculated according to the following: $YND = 1 - P700$ red. YI, the photochemical quantum yield of PSI, was defined by the fraction of overall P700, which is reduced and not limited by acceptor side. YI was calculated from the complementary PSI quantum yields of non-photochemical energy dissipation, YND, and YNA according to the following: $YI = 1 - YND - YNA$. The electron transport rate of PSI, ETRI, was calculated by Dual-PAM software.

### Statistical analyses

All experiments were repeated independently at least three times. Photosynthesis parameters considered to vary significantly between treatments were those with $P < 0.05$ using Student's $t$-tests.

## Results

### No direct antagonistic relationship between Bp and Pst in vitro

The direct antifungal effect of Bp could partially explain the protection of grapevine against *B. cinerea* (Miotto-Villanova et al. 2016). We thus analyzed the antibacterial effect of this PGPR on the hemibiotrophic bacterium Pst. First, Pst growth on King's B medium supplemented with either kanamycin or kanamycin and rifampicin was quantified. Pst growth was similar between the two media (supplemental figure S1), suggesting that removal of rifampicin from culture medium is not a stressful condition for Pst growth. Then, growth of Pst colonies with or without Bp was thus followed 2, 3, and

4 days after inoculation of the plate to assess in vitro a direct interaction between the two strains (Fig. 1). The strain Bp is labeled with GFP (green) and Pst produces a blue fluorescent pigment (Fackrell and Sinha 1983), allowing to distinguish between the two bacterium growth under UV light. In vitro, the presence of a strain does not inhibit the development of the other even after 4 days of co-culture. Bp has thus no direct antagonism against Pst or inversely.

### Only local presence of Bp alleviates Pst growth in planta during the early stage of infection

As previously demonstrated, Bp promotes Arabidopsis aerial plant growth when inoculated on seed or by root irrigation. Similarly to most of rhizobacteria, Bp is strictly localized to the root system independently of inoculation method (Su et al. 2015). In order to assess a potential ISR against Pst infection, Bp was seed inoculated. Moreover, in order to determine if Bp is able to protect locally Arabidopsis plants against Pst, Bp was also infiltrated in plant leaves (Su et al. 2016) prior to Pst infection. Bp-seed inoculated or -leaf infiltrated plants do not display symptoms (Fig. 2a, b). 3 days after infection, no difference between symptoms developed by Pst-infected plants subjected to leaf infiltration or seed inoculation with Bp was observed (Fig. 2a, b). Quantification of Pst was thus performed by qPCR. In seed bacterized plants, Pst growth was enhanced compared to non seed bacterized plants at 1 dpi (Fig. 2c; 26-fold). However, this growth stimulation was less induced at 3 dpi (Fig. 2c; 2.5-fold). Moreover, Pst only slightly grew between 1 and 3 dpi (×1.3-fold) in seed bacterized plants compared to non-bacterized plants (×14-fold). This suggested that Bp inoculation slightly slowed Pst spread when it is systemically present. Interestingly, Pst growth was drastically reduced in leaf

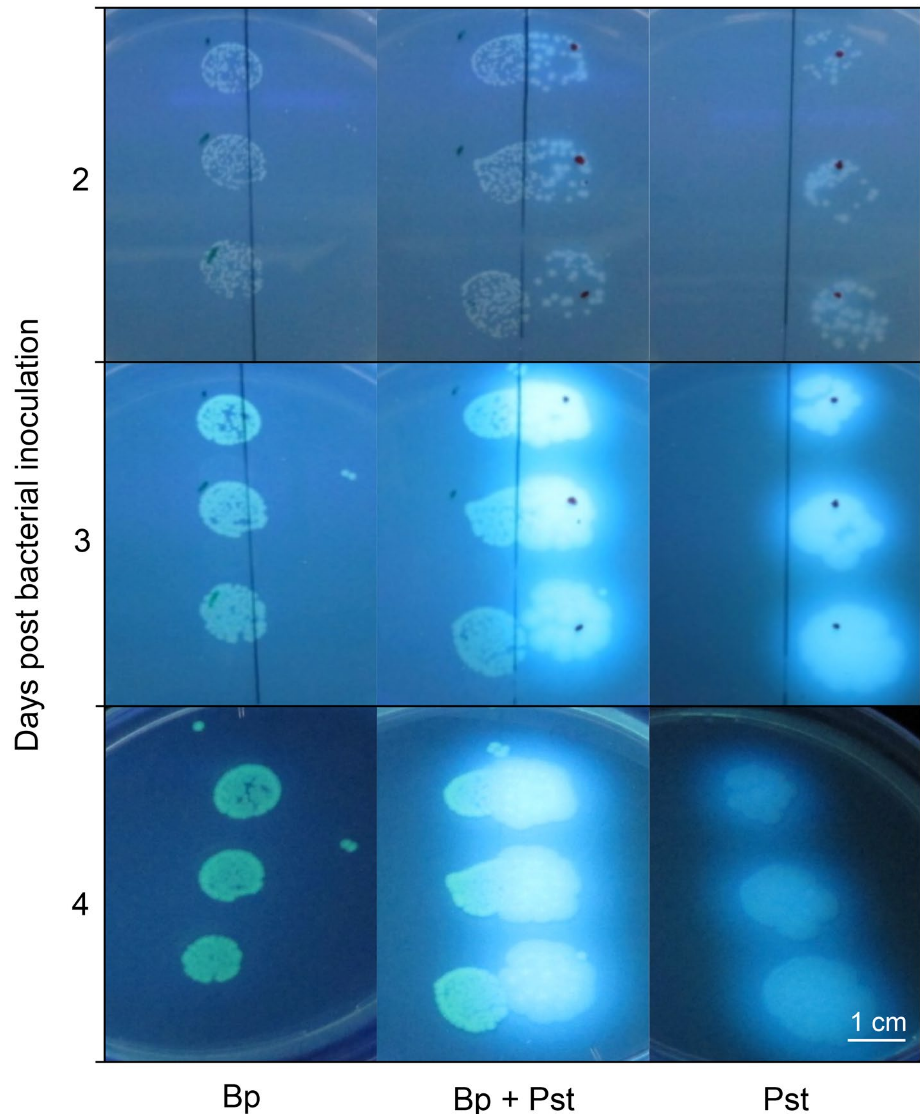


**Fig. 1** Co-culture of Bp and Pst in vitro. Pst ($10^3$ cfu mL$^{-1}$) was co-cultured with Bp ($10^3$ cfu mL$^{-1}$) in King's B medium supplemented with kanamycin (50 µg mL$^{-1}$) at 28 °C. Observations of two bacterial colonies were carried out under UV. Photographs display the results of one representative experiment among three independent repetitions

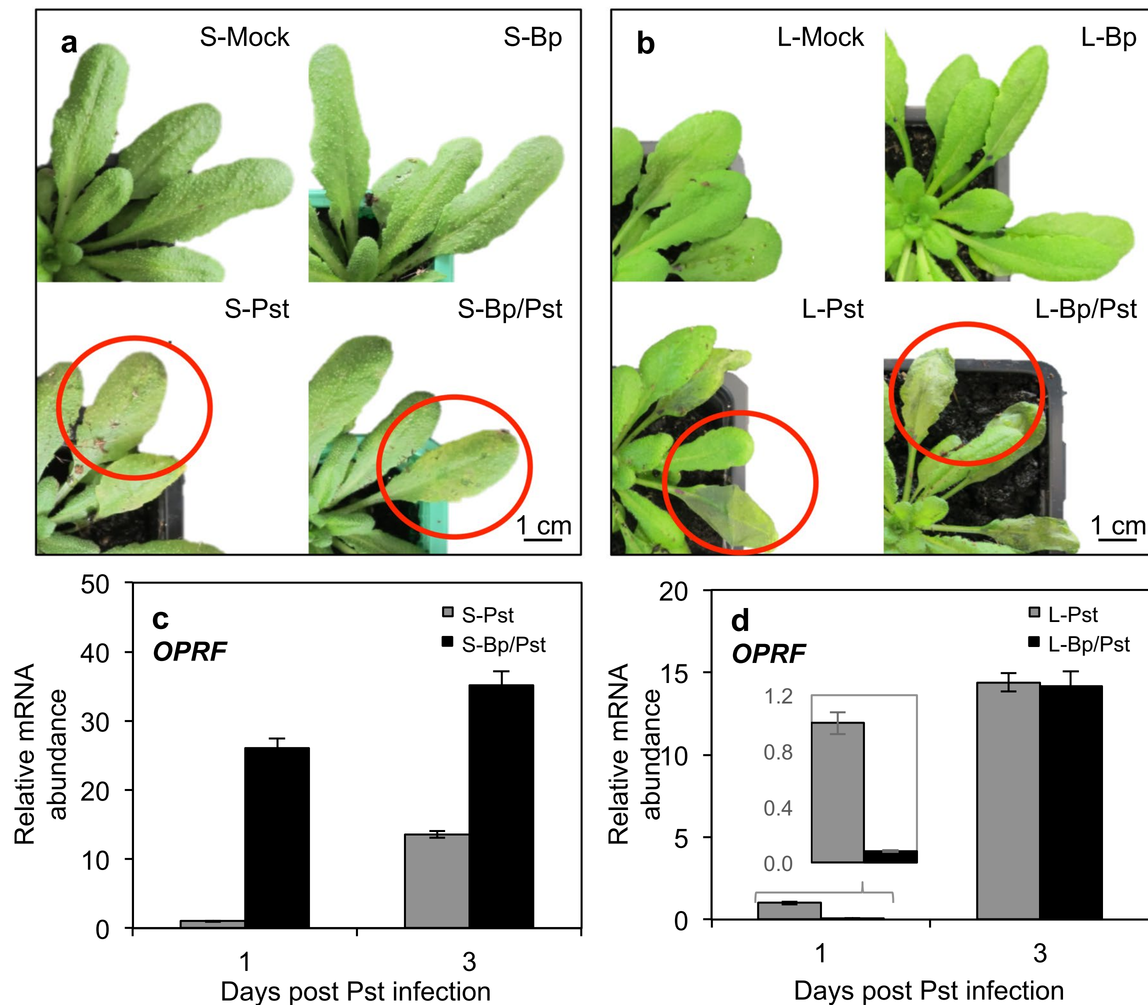


**Fig. 2** Pst development in Arabidopsis leaves. The plants bacterized by Bp on seeds (S-; **a, c**) or leaves (L-; **b, d**) were dipped in a suspension of Pst ($10^8$ cfu $mL^{-1}$) at the age of 6 weeks. Leaf symptoms caused by Pst (**a, b**) observed 3 days post infection. Photographs display the results of one representative experiment among three independent repetitions. Quantification of Pst (**c, d**) estimated by the abundance of Pst mRNA (*PsyrOPRF*) relative to the respective Pst sample on the first day post infection, referred to as the ×1 expression level. Values shown are mean ± SE of duplicate data from one representative experiment among three independent repetitions

bacterized plants at 1 dpi (Fig. 2d; 12-fold less). However, the presence of Bp at the same place than Pst has no influence on Pst growth 3 dpi.

### Priming of both SA- and JA-related genes by Bp

To determine whether the altered Pst growth in Bp-inoculated plants is linked to changes in the regulation of defense genes, the expression pattern of defense-related genes (*PR1* and *PDF1.2*) was monitored before and after challenge with Pst (Fig. 3). *PR1* is well-known SA-dependent defense marker gene and *PDF1.2* expression is regulated by JA and ET (Glazebrook 2005). Bp inoculation, either in roots or in leaves, did not significantly trigger *PR1* or *PDF1.2* expression, except when performed in leaves at 3 dpi for *PR1* (Fig. 3). As already described, colonization by Pst induced expression of *PR1* in control plants (Fig. 3a, b). *PDF1.2* expression is stimulated at the early stage of infection but this induction fell progressively down until the end of the time-course (Fig. 3c, d). In seed bacterized plants, induction of *PR1* is earlier and stronger than in plants infected only with Pst. However, *PR1* expression was similar in S-Pst and S-Bp/Pst plants at 3 dpi (Fig. 3a). In contrast, expression of *PDF1.2* is continuously enhanced in seed Bp-treated plants compared to S-Pst plants (Fig. 3c). Infiltration of Bp in leaves did not alter *PR1* or *PDF1.2* expression after challenge with Pst (Fig. 3b, d).

### Photosystem I and II activities

Infection of plants with pathogens results in photosynthetic parameter disturbance. To evaluate the impacts on

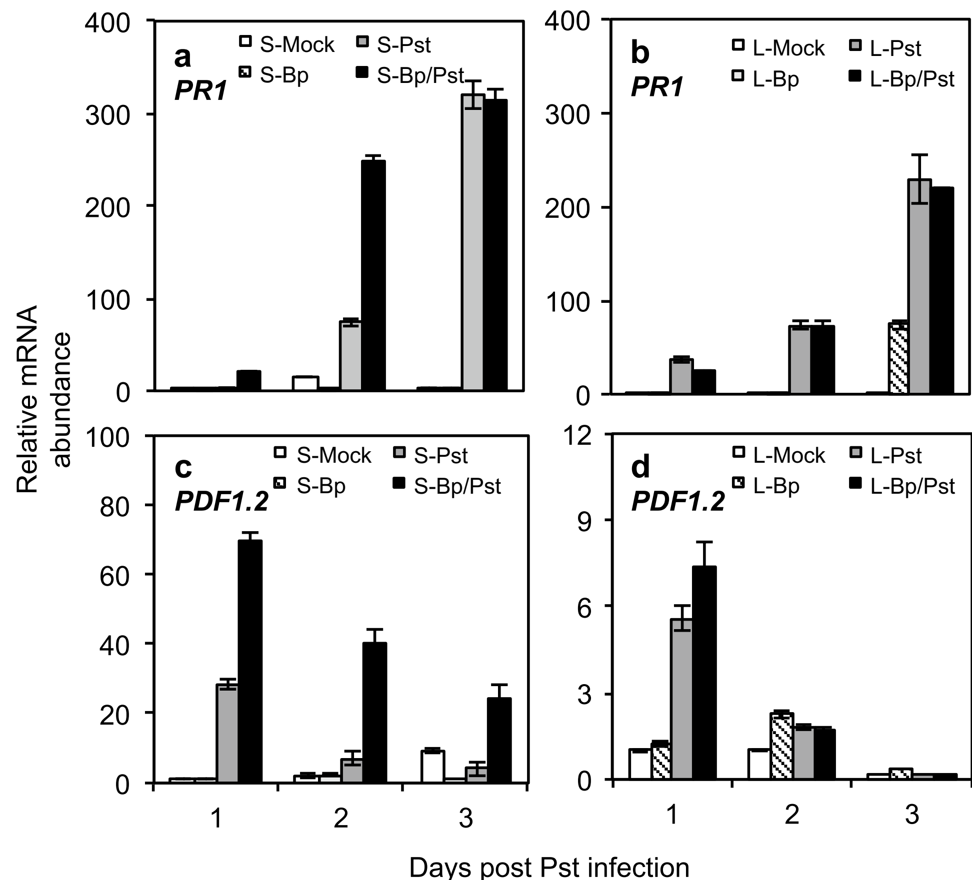


**Fig. 3** Expression levels of the defense-related genes *PR1* (**a, b**) and *PDF1.2* (**c, d**) in Arabidopsis leaves after seed inoculation (S-; **a, c**) or leaf infiltration (L-; **b, d**) with Bp, infection by Pst, or co-inoculation with the two bacterial strains. Data represent mean fold increases in mRNA levels relative to those of control plants (Mock on the first day), referred to as the ×1 expression level. Values shown are mean ± SE of duplicate data from one representative experiment among three independent repetitions

photosynthetic activity triggered by a PGPR, a virulent bacterium, or a co-inoculation with both bacteria, the regulation of the PSI and PSII activities was monitored.

*PSI photochemistry*

Excitation energy transferred to the PSI centers will result in photochemical charge separation with quantum yield ΦPSI or in non-photochemically conversion to heat (Nelson and Yocum 2006). The quantum yield of non-photochemical energy dissipation can be due to limitation of the acceptor-site Y(NA) or the donor-site Y(ND).

Seed inoculation of Bp altered neither the regulation of PSI (Y(NA), ΦPSI) nor the transport rate of electrons (ETRI) (Fig. 4a). A slight but significant decrease in non-photochemical efficiency due to the limitation of electron donor side, Y(ND), was measured in SBp at 3 dpi. At 3 dpi, infection with Pst caused an increase of Y(NA), which represents the limitation of the electron acceptor side, while ΦPSI and ETRI decreased (Fig. 4a). Infection with Pst in SBp increased Y(ND) at 2 dpi comparatively to the plant without Bp.

Leaf infiltration of Bp triggered a decrease in Y(ND) at 1 dpi, and an increase in ΦPSI and ETRI values at 3 dpi (Fig. 4b). Pst infection decreased ΦPSI and ETRI (2 dpi) (Fig. 4b). The presence of Bp did not modify the impact of Pst on PSI activity.

*PSII photochemistry*

The excitation energies absorbed by PSII centers are contributed in photochemical utilization (ΦPSII) or heat dissipation (Nelson and Yocum 2006). In PSII, the heat dissipation includes regulated and non-regulated energy dissipation [Y(NPQ) and Y(NO), respectively].

Seed inoculation by Bp did not provoke modifications of PSII parameters at all 3 days monitored (Fig. 5a). However, Pst invasion damaged PSII activity, presented by decreased Fv/Fm and Y(NPQ) values at 2 dpi, and ΦPSII and ETRII values at 3 dpi. Along with the decrease in ΦPSII, Pst-infected plants showed an increase in Y(NO) compared to mock-treated plants. A greater reduction of ΦPSII, Fv/Fm, and ETRII and a greater increase in Y(NO) at 2 dpi were observed in plants inoculated with Bp and infected by Pst compared to plants inoculated only with Pst.

Leaf infiltration of Bp did not induce modifications of PSII activity (Fig. 5b). Pst infection led to an increase of Y(NO) at 2 dpi and a decrease of ΦPSII, Y(NPQ), $F_v/F_m$,

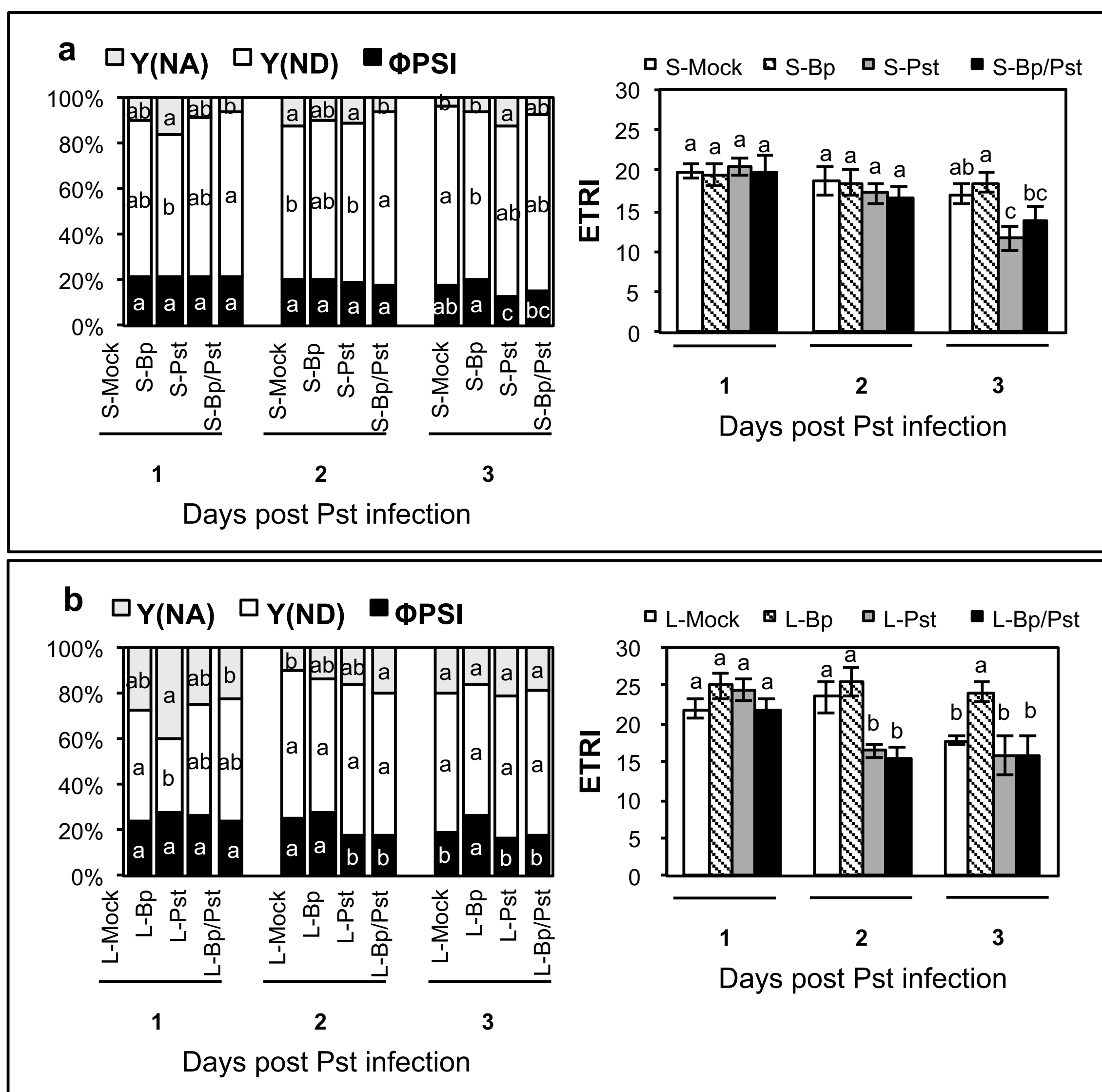


**Fig. 4** PSI photochemistry in Arabidopsis leaves after seed inoculation (S-; **a**) or leaf infiltration (L-; **b**) with Bp, infection by Pst, or co-inoculation with the two bacterial strains. PSI acceptor side limitation [Y(NA)], PSI donor side limitation [Y(ND)], efficient quantum yield of PSI (ΦPSI), and PSI electron transport rate (ETRI) are shown. Data are mean ± SE of three experimental replicates, each with three plants per treatment ($n=9$). *Same letters* indicate non-significant difference among all conditions on the same day (Student's *t*-test; $P<0.05$)

and ETRII. However, no change in PSII activity could be detected when plants were inoculated with Bp and infected by Pst compared to plants inoculated only with Pst.

*RuBisCO accumulation*

To complete photosynthetic activities, protein accumulation of two subunits of the RuBisCO (the nuclear-encoded RbcS and the chloroplast-encoded RbcL) was followed (Fig. 6). As previously shown (Su et al. 2015), Bp seed-inoculated plants did not modify RbcS or RbcL accumulation. However, no change in these protein accumulations was detected in bacterized or non-bacterized plants after challenge with Pst.

## Discussion

Previous studies demonstrated that Bp protected grapevine against the necrotrophic pathogen *Botrytis cinerea* (Ait Barka et al. 2006; Miotto-Vilanova et al. 2016). However, only few information is available on the capability of Bp to protect plants against an attack by a (hemi)biotrophic pathogen (Sharma and Novak 1998). Here, we described

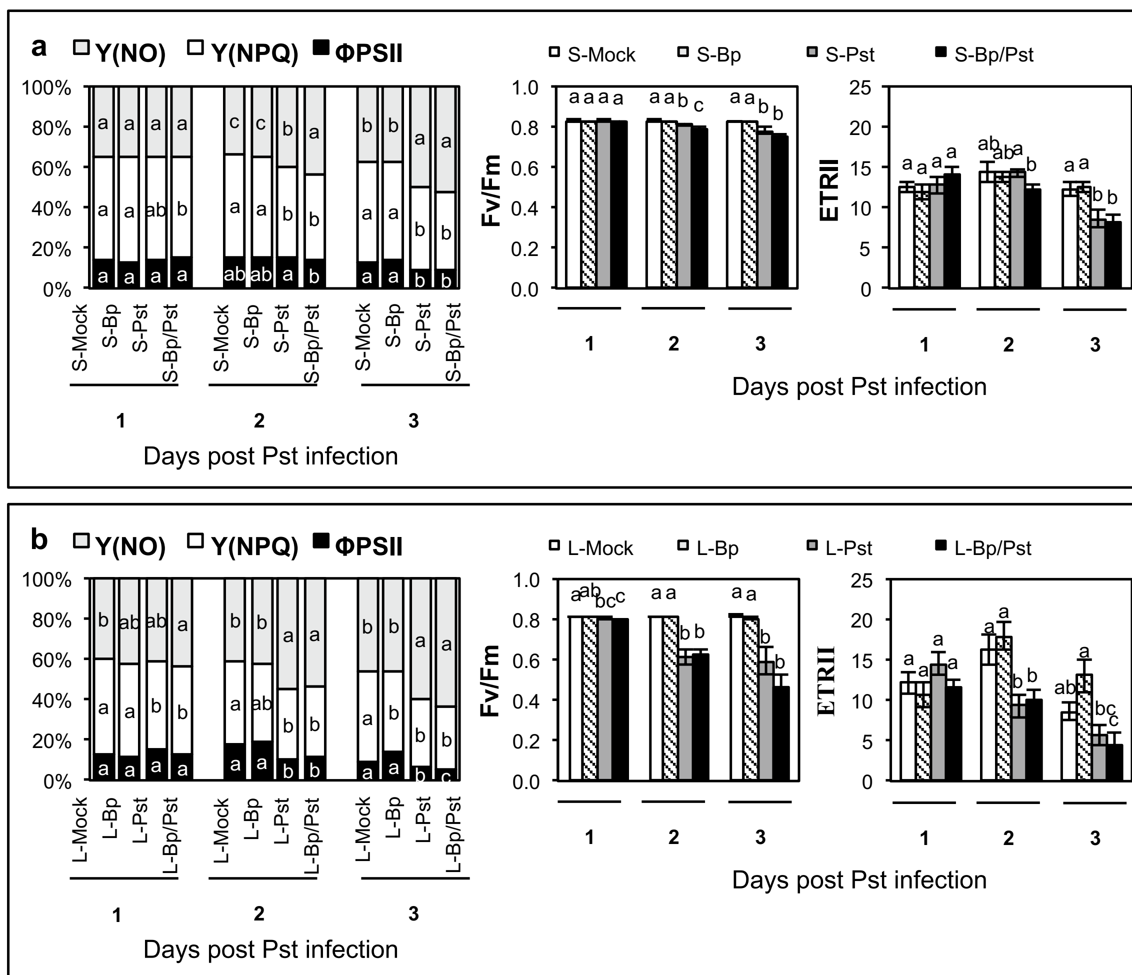


**Fig. 5** PSII photochemistry in Arabidopsis leaves after seed inoculation (S-; **a**) or leaf infiltration (L-; **b**) with Bp, infection by Pst, or co-inoculation with the two bacterial strains. Quantum yield of non-regulated energy dissipation in PSII [Y(NO)] and regulated energy dissipation in PSII [Y(NPQ)], efficient quantum yield of PSII (ΦPSI), maximum photochemical efficiency of PSII ($F_v/F_m$), and PSII electron transport rate (ETRII) are shown. Data are mean ± SE of three experimental replicates, each with three plants per treatment ($n = 9$). *Same letters* indicate non-significant difference among all conditions on the same day (Student's *t*-test; $P < 0.05$)

the mechanisms underlying the interaction between Bp and the hemibiotrophic bacterium Pst. We focused on photosynthetic parameters and on some defense-related processes underlying the link between photosynthesis and biotic stress.

## Defense mechanism regulation during infection with Bp and/or Pst

It is well known that PGPR could protect plants against pathogens by anti-microbial activities (Lugtenberg and Kamilova 2009). Recently, it was shown that Bp possesses antifungal activity against *B. cinerea* since it reduced its growth development (Miotto-Vilanova et al. 2016). This inhibition by Bp partially protect grapevine against *B. cinerea*. Here, co-culture of Bp and Pst did not display any antibiosis impact between these two bacteria.

Sur-expression of defense-related gene *PR1* normally relates to SA-mediated signaling pathway, and responses to (hemi)biotrophic pathogens infection in Arabidopsis (Reddy 2013; Vidhyasekaran 2015), whereas induction of *PDF1.2* expression is under control of JA and ET accumulation. Here, inoculation with Bp either on seeds or in leaves did not induce the accumulation of mRNA of *PR1*. In contrast to Poupin et al. (2013), inoculation of Bp on Arabidopsis plants did not induce *PDF1.2* expression as well. This discrepancy might be due probably to the different methods of bacterial inoculation and plant growth conditions. Plants can establish the so-called "primed" state (Ton et al. 2005; Conrath 2009) following root colonization by beneficial microbes or after chemical treatment by activating their defense responses faster or more strongly when subsequently challenged by microbial pathogens (Pozo et al. 2008; Conrath 2009), resulting in a better

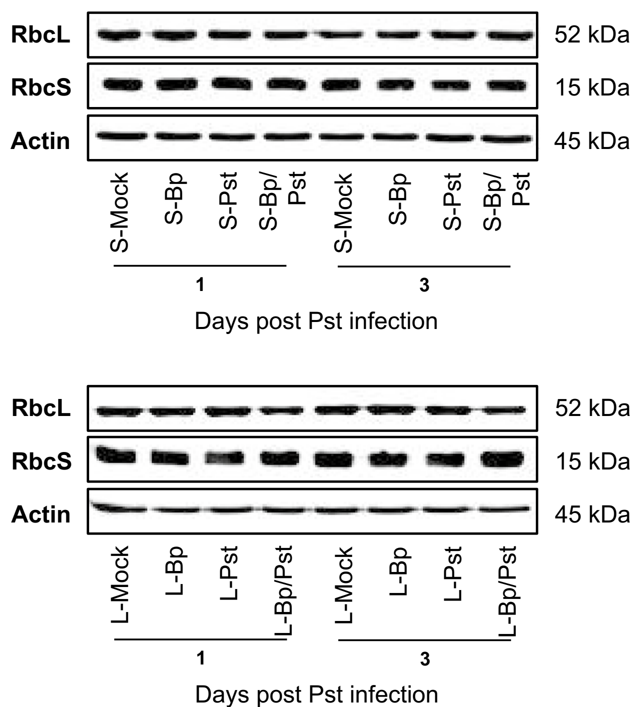


**Fig. 6** RbcL and RbcS accumulation in Arabidopsis leaves after seed inoculation (S-) or leaf infiltration (L-) with Bp, infection by Pst, or co-inoculation with the two bacterial strains. Normalization was carried out with Actin. *Numbers on the right indicate* molecular mass in kilodaltons. Data display the results of one representative experiment among three independent repetitions

tolerance towards this stress. The priming of *PR* gene expressions by Bp was described in grapevine in order to confer tolerance towards low non-freezing temperatures or *B. cinerea* (Theocharis et al. 2012; Miotto-Vilanova et al. 2016). Interestingly, Bp inoculation on Arabidopsis seeds conditioned the plant to produce *PR1* and *PDF1.2* mRNA more rapidly, suggesting that Bp potentiated the accumulation of *PR1* and *PDF1.2* mRNA during the first stage of virulent bacterium attack. Such a simultaneous activation of expressions of both SA- and JA-related genes has already been observed after inoculation with diverse PGPRs, including Bp (Conn et al. 2008; Niu et al. 2011; Brock et al. 2013; Miotto-Vilanova et al. 2016). All these data suggested that priming of *PR1* expression could significantly slowed Pst growth in S-Bp/Pst plants compared to S-Pst plants at 3 dpi. Whereas *PR1* potentiation is only transitory, *PDF1.2* potentiation is maintained at 3 dpi. It is well admitted in Arabidopsis that SA pathway has antagonistic effect on JA signaling and reciprocally (Thaler et al. 2002; Bostock 2005; Glazebrook 2005; Spoel et al. 2007; Derksen et al. 2013). This hypothesized that activation of JA signaling via the extended induction of *PDF1.2* expression could inhibit effect of *PR1* potentiation, resulting in an absence of tolerance. This also hypothesized that Bp protect plants against necrotrophic pathogen but not against (hemi)biotrophic invaders.

## Photosynthesis regulation during infection with Bp and/or Pst

In order to better understand the link existing between photosynthesis and biotic stress, different photosynthetic parameters were analyzed in response to BP and/or Pst infection. Our results showed small and transient modifications of PSI activity after Bp inoculation. A decrease in Y(ND) at 3 dpi (PSI) was recorded after seed bacterization. Leaf infiltration of Bp triggered a decrease in Y(ND) at 1 dpi, and an increase in ΦPSI and ETRI values at 3 dpi (PSI), without PSII modification. These data are in agreement with our previous results (Su et al. 2015). However, effects of PGPR are highly dependent on the plant–bacteria interaction (van Loon 2007). Ait Barka et al. (2006) reported that *Bp*-inoculated grapevine plantlets exhibit a higher photosynthetic activity compared to non-bacterized plantlets. Many PGPR strains, such as *Azospirillum brasilense* (Ruíz-Sánchez et al. 2011; Cohen et al. 2015), *Bacillus subtilis* GB03 (Zhang et al. 2008), *Pseudomonas fluorescens* (Rincón et al. 2008), and Bp (in maize) (Naveed et al. 2014), were reported to enhance PSII activities. *A. brasilense* (Cohen 2008, 2015) or *B. subtilis* GB03 (Zhang et al. 2008) also promote the accumulation of photosynthetic pigment contents. Zhang et al. (2008) found that PGPR strain *B. subtilis* GB03 increased chlorophyll content and photosynthetic efficiency of Arabidopsis by modulation of endogenous glucose concentration and abscisic acid signaling. Miotto-Vilanova et al. (2016) showed in grapevine no modification in Y(II), Y(NO), Y(NPQ) 3–6 days after root-bacterization with Bp. For the first time, we showed that there is also no variation in the regulation of energy at the PSI level.

The photosynthetic activity is due to the RuBisCO activity, enzyme able to fix carbon in the chloroplast. The protein is composed of two subunits: the nuclear-encoded *RbcS* gene and the chloroplast-encoded *RbcL* gene. Local or systemic presence of Bp in planta and of Pst in leaf did not modify the RuBisCO protein accumulation pattern. In Arabidopsis and tomato, many genes encoding chloroplast-localized proteins involved in photosynthesis and the Calvin cycle are repressed by Pst (Bonfig et al. 2006; Thilmony et al. 2006), but no data on protein accumulation were available.

Pathogen infection not only affects defense reactions but also leads to changes in carbohydrate metabolism (Ehness et al. 1997; Roitsch 1999). Several studies reported a decrease in photosynthesis after infection, which might be due to a downregulation of photosynthesis or damage of the photosynthetic apparatus (Chou et al. 2000; Berger et al. 2004). The damaged in PSII led a decreased of $F_v/F_m$, ΦPSII, ETRII, Y(NPQ), and an increase of Y(NO). The

results on PSII were in accordance with previous studies showing a decrease in $F_v/F_m$, ΦPSII after infiltration of Pst in Arabidopsis or in bean (Bonfig et al. 2006; Pérez-Bueno et al. 2015). The partitioning of absorbed excitation energy in PSII takes place into three fundamental pathways, (i) photochemical utilization, (ii) regulated heat dissipation (a loss process serving for protection) and (iii) non-regulated heat dissipation (a loss process due to PSII inactivity). A higher Y(NPQ) value than Y(NO) value indicates that excess excitation energy is safely dissipated at the antenna level, meaning that photosynthetic energy fluxes are well regulated. In variance, high values of Y(NO) signify that excess excitation energy is reaching the reaction centers, resulting in strong reduction of PSII acceptors and photodamage, e.g., via formation of reactive oxygen species. The impact on NPQ seems to be dependent on the studied pathosystem: an increase in Arabidopsis inoculated with Pst (Berger et al. 2007) but a decrease in bean plants inoculated with Pst or P. syringae pv. phaseolicola (Rodríguez-Moreno et al. 2008) was observed. A decrease in NPQ was also detected following the infection of tobacco with tobacco mosaic virus (Balachandran et al. 1994), *Abutilon striatum* with Abutilon mosaic virus (Lohaus et al. 2000), and oat leaves with *Puccinia* (Scholes and Rolfe 1996). In contrast, an increase was observed in response to the biotrophic fungus *Albugo candida* in Arabidopsis (Chou et al. 2000) and to the necrotrophic fungus *B. cinerea* in tomato (Berger et al. 2004). Indeed, stress and particularly disease can promote excess excitation energy even at light intensities that would not pose a problem under control conditions (Mullineaux and Karpinski 2002).

Photosystem II is considered to be more vulnerable than PSI when plants encounter stresses (Barth et al. 2001). Several researches have been conducted on PSII regulation after challenge with Pst. However, only few information are available on PSI activity after pathogen infection. Infection with Pst caused a modification in PSI and PSI activity and regulation. In PSI, we recorded an increase in the limitation of the electron acceptor side Y(NA) and a decrease of ΦPSI and ETRI values. Cheng et al. (2016) measured also a decrease of PSI activity after *P. syringae* pv. *tabaci* infection and indicated a degradation of PsbO, D1, and PsaA proteins in tobacco.

Overall, the presence of Bp did not modify the impact of Pst on PSI and PSII regulation and on RuBisCO contents, except an increased Y(ND) at 3 dpi in Pst/SBp infected plants.

## Conclusions

PGPR may prevent attack from pathogenic microorganisms (van Loon et al. 1998). The ability of the PGPR *Burkholderia phytofirmans* strain *PsJN* to induce a local or a systemic resistance on Arabidopsis against an infection with the hemibiotrophic bacterium *Pseudomonas syringae pv. tomato* DC3000 was evaluated. Since photosynthesis mechanisms are also involved in plant resistance to biotic stress, photosynthetic parameters and defense response were both quantified. No antibiosis between these two microorganisms was detected. Pre-inoculation of plant roots with Bp primed both *PR1* and *PDF1.2* expressions that led to a slower spread of Pst during the first stages after pathogen inoculation. However, such priming effect was not displayed when Bp was infiltrated in leaves. Infection with Pst decreased PSI and PSII activities. More precisely, the pathogen inoculation triggered a decrease in ΦPSI and ETRI and an increase in Y(NA). Additionally, photosynthetic parameters were not modified by a pre-inoculation with Bp. Barriuso et al. (2008) showed that infection with Pst of Arabidopsis plants pre-inoculated with different PGPR (*Staphylococcus, Bacillus, Curtobacterium*, or *Arthrobacter oxidanspathogen*) led to significantly higher values of $F_v/F_m$ than the pathogen control. The bacterium Bp induced plant tolerance against abiotic and biotic stresses. In *B. cinerea*-infected leaves of root-inoculated grapevine with Bp, an increase of Y(II) was observed in bacterized plantlets, suggesting the protective role of bacteria on grapevine photosynthetic apparatus during the first step of infection (without modification of Y(NO), Y(NPQ), Fv/Fm, or ETR) (Miotto-Vilanova et al. 2016). In response to abiotic stress, the results are also reversed. Beneficial effects triggered by PGPR colonization against cold are associated to photosynthesis, carbohydrates, and related metabolites (Ait Barka et al. 2006; Fernandez et al. 2012). But Su et al. (2015) showed that Bp inoculation did not affect PSII activity and gas exchange during and after one cold night at 0 or −1 °C but increased net photosynthesis after one night at −3 °C.

In conclusion, the role of Bp here is not multifaceted like in grapevine tolerance towards *B. cinerea*, and relies only on priming of defense mechanisms but not on improving photosynthetic activity.

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## References

Aït Barka E, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252. doi:10.1128/AEM.01047-06

Balachandran S, Osmond CB, Daley PF (1994) Diagnosis of the earliest strain-specific interactions between tobacco mosaic virus and chloroplasts of tobacco leaves in vivo by means of chlorophyll

fluorescence imaging. Plant Physiol 104:1059–1065. doi:10.1104/pp.104.3.1059

Barriuso J, Ramos Solano B, Gutiérrez Mañero FJ (2008) Protection against pathogen and salt stress by four plant growth-promoting rhizobacteria isolated from *Pinus* sp. on *Arabidopsis thaliana*. Phytopathology 98:666–672. doi:10.1094/PHYTO-98-6-0666

Barth C, Krause GH, Winter K (2001) Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves. Plant Cell Environ 24:163–176. doi:10.1111/j.1365-3040.2001.00673

Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T (2004) Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol Plant 122:419–428. doi:10.1111/j.1399-3054.2004.00433.x

Berger S, Benediktyova Z, Matous K, Bonfig K, Mueller MJ, Nedbal L, Roitsch T (2007) Visualization of dynamics of plant-pathogen interaction by novel combination of chlorophyll fluorescence imaging and statistical analysis: differential effects of virulent and avirulent strains of *P. syringae* and of oxylipins on *A. thaliana*. J Exp Bot 58:797–806. doi:10.1093/jxb/erl208

Bittel P, Robatzek S (2007) Microbe-associated molecular patterns (MAMPs) probe plant immunity. Curr Opin Plant Biol 10:335–341. doi:10.1016/j.pbi.2007.04.021

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379–406. doi:10.1146/annurev.arplant.57.032905.105346

Bonfig K, Schreiber U, Gabler A, Roitsch T, Berger S (2006) Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. Planta 225:1–12. doi:10.1007/s00425-006-0303-3

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol 43:545–580. doi:10.1146/annurev.phyto.41.052002.095505

Boureau T, Routtu J, Roine E, Taira S, Romantschuk M (2002) Localization of hrpA-induced *Pseudomonas syringae* pv. *tomato* DC3000 in infected tomato leaves. Mol Plant Pathol 3:451–460. doi:10.1046/j.1364-3703.2002.00139.x

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Brock AK, Berger B, Mewis I, Ruppel S (2013) Impact of the PGPB *Enterobacter radicincitans* DSM 16656 on growth, glucosinolate profile, and immune responses of *Arabidopsis thaliana*. Microb Ecol 65:661–670. doi:10.1007/s00248-012-0146-3

Cartieaux F, Contesto C, Gallou A, Desbrosses G, Kopka J, Taconnat L, Renou JP, Touraine B (2008) Simultaneous interaction of *Arabidopsis thaliana* with *Bradyrhizobium* sp. strain ORS278 and *Pseudomonas syringae* pv. *tomato* DC3000 leads to complex transcriptome changes. Mol Plant Microbe Interact 21:244–259. doi:10.1094/MPMI-21-2-0244

Castillo P, Molina R, Andrade A, Vigliocco A, Alemano S, Cassán FD (2015) Phytohormones and other plant growth regulators produced by PGPR: the Genus *Azospirillum* in Cassán FD. In: Okon Y, Creus CM (eds) Handbook for *Azospirillum*. Springer, Switzerland, pp 115–138. doi:10.1007/978-3-319-06542-7_7

Chandler D, Davidson G, Grant W, Greaves J, Tatchell G (2008) Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends Food Sci Tech 19:275–283. doi: 10.1016/j.tifs.2007.12.009

Cheng DD, Zhang ZS, Sun XB, Zhao M, Sun GY, Chow WS (2016) Photoinhibition and photoinhibition-like damage to the photosynthetic apparatus in tobacco leaves induced by *Pseudomonas syringae* pv. *tabaci* under light and dark conditions. BMC Plant Biol 16:29. doi:10.1186/s12870-016-0723-6

Chou H, Bundock N, Rolfe SA, Scholes JD (2000) Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. Mol Plant Pathol 1:99–113. doi:10.1046/j.1364-3703.2000.00013.x

Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in Arabidopsis plants. Plant Growth Regul 54:97–103. doi:10.1007/s10725-007-9232-9

Cohen AC, Bottini R, Pontin M, Berli FJ, Moreno D, Boccanlandro H, Travaglia CN, Piccoli PN (2015) *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153:79–90. doi:10.1111/ppl.12221

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959. doi:10.1128/aem.71.9.4951-4959.2005

Conn VM, Walker AR, Franco CMM (2008) Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. Mol Plant Microbe Interact 21:208–218. doi: 10.1094/MPMI-21-2-0208

Conrath U (2009) Priming of induced plant defense responses. In: Sauvion N, Thiéry D, Calatayud PA (eds) Insect-plant interactions in a crop protection perspective. Elsevier, New York, pp 361–395. doi:10.1016/S0065-2296(09)51009-9

de Torres Zabala M, Littlejohn G, Jayaraman S, Studholme D, Bailey T, Lawson T, Tillich M, Licht D, Bölter B, Delfino L, Truman W, Mansfield J, Smirnoff N, Grant M (2015) Chloroplasts play a central role in plant defence and are targeted by pathogen effectors. Nat Plants 1:15074–15083. doi:10.1038/nplants.2015.74

Demmig-Adams B, Cohu CM, Amiard V, Zadelhoff G, Veldink GA, Muller O, Adams WW (2013) Emerging trade-offs-impact of photoprotectants (PsbS, xanthophylls, and vitamin E) on oxylipins as regulators of development and defense. New Phytol 197:720–729. doi:10.1111/nph.12100

Demmig-Adams B, Stewart JJ, Adams WW (2017) Environmental regulation of intrinsic photosynthetic capacity: an integrated view. Curr Opin Plant Biol 37:34–41. doi:10.1016/j.pbi.2017.03.008

Derksen H, Rampitsch C, Daayf F (2013) Signaling cross-talk in plant disease resistance. Plant Sci 207:79–87. doi:10.1016/j.plantsci.2013.03.004

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548

Dong J, Chen W (2013). The role of autophagy in chloroplast degradation and chlorophagy in immune defenses during *Pst* DC3000 (AvrRps4) infection. PLoS ONE 8:e73091. doi:10.1371/journal.pone.0073091

Ehness R, Ecker M, Godt DE, Roitsch T (1997) Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. Plant Cell 9:1825–1841. doi: 10.1105/tpc.9.10.1825

Fackrell HB, Sinha RC (1983) Serological analysis of *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:178–181

Fernandez O, Theocharis A, Bordiec S, Feil R, Jacquens L, Clément C, Fontaine F, Aït Barka A (2012) *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. Mol Plant Microbe Interact 25:496–504. doi:10.1094/MPMI-09-11-0245

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875. doi:10.1105/tpc.105.033589

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching

of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92. doi:10.1016/S0304-4165(89)80016-9
Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227. doi:10.1146/annurev.phyto.43.040204.135923
Henry G, Thonart P, Ongena M (2012) PAMPs, MAMPs, DAMPs and others: an update on the diversity of plant immunity elicitors. Biotechnol Agron Soc Environ 16:257–268. http://popups.ulg.ac.be/1780-4507/index.php?id=8709
Hong SM, Bahn SC, Lyu A, Jung HS, Ahn JH (2010) Identification and testing of superior reference genes for a starting pool of transcript normalization in Arabidopsis. Plant Cell Physiol 51:1694–1706. doi: 10.1093/pcp/pcq128
Johansson Jänkänpää HJ, Frenkel M, Zulfugarov I, Reichelt M, Krieger-Liszkay A, Mishra Y, Gershenzon J, Moen J, Lee CH, Jansson S (2013) Non-photochemical quenching capacity in *Arabidopsis thaliana* affects herbivore behaviour. PLoS ONE 8:e53232. doi:10.1371/journal.pone.0053232
Karpinski S, Gabrys H, Mateo A, Karpinska B, Mullineaux PM (2003) Light perception in plant disease defence signalling. Curr Opin Plant Biol 6:390–396. doi:10.1016/S1369-5266(03)00061-X
Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94:1259–1266. doi:10.1094/phyto.2004.94.11.1259
Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via P700+-absorbance changes at 830 nm. Planta 192:261–268. doi:10.1007/BF01089043
Klughammer C, Schreiber U (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. PAM Appl Notes 1:27–35
Kramer DM, Avenson TJ, Edwards GE (2004) Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions. Trends Plant Sci 9:349–357. doi:10.1016/j.tplants.2004.05.001
Kumar S, Agarwal M, Dheeman S, Maheshwari DK (2015) Exploitation of phytohormone-producing PGPR in development of multispecies bioinoculant formulation. In: Maheshwari (ed) Bacterial metabolites in sustainable agroecosystem. sustainable development and biodiversity, vol 12. Springer, Switzerland, pp 297–317. doi:10.1007/978-3-319-24654-3_11
Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685. doi:10.1038/227680a0
Lenz HD, Haller E, Melzer E, Kober K, Wurster K, Stahl M, Bassham DC, Vierstra RD, Parker JE, Bautor J (2011) Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. Plant J 66:818–830. doi:10.1111/j.1365-313X.2011.04546.x
Li X, Sun Z, Shao S, Zhang S, Ahammed GJ, Zhang G, Jiang Y, Zhou J, Xia X, Zhou Y, Yu J, Shi K (2015) Tomato-*Pseudomonas syringae* interactions under elevated $CO_2$ concentration: the role of stomata. J Exp Bot 66:307–316. doi:10.1093/jxb/eru420
Lohaus G, Heldt HW, Osmond CB (2000) Infection with phloem limited Abutilon mosaic virus causes localized carbohydrate accumulation in leaves of *Abutilon striatum*: relationships to symptom development and effects on chlorophyll fluorescence quenching during photosynthetic induction. Plant Biol 2:161–167. doi:10.1055/s-2000-9461
Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556. doi:10.1146/annurev.micro.62.081307.162918
Magnin-Robert M, Le Bourse D, Markham J, Dorey S, Clément C, Baillieul F, Dhondt-Cordelier S (2015) Modifications of sphingolipid content affect tolerance to hemibiotrophic and necrotrophic pathogens by modulating plant defense responses in Arabidopsis. Plant Physiol 169:2255–2274. doi:10.1104/pp.15.01126
Melotto M, Underwood W, He SY (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. Annu Rev Phytopathol 46:101–122. doi:10.1146/annurev.phyto.121107.104959
Miotto-Vilanova L, Jacquard C, Courteaux B, Wortham L, Michel J, Clément C, Aït Barka E, Sanchez L (2016) *Burkholderia phytofirmans* PsJN confers grapevine resistance against *Botrytis cinerea via* a direct antimicrobial effect combined with a better resource mobilization. Front Plant Sci 7:1236. doi:10.3389/fpls.2016.01236
Mishina TE, Zeier J (2007) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. Plant J 50:500–513. doi:10.1111/j.1365-313X.2007.03067.x
Miyake C, Horigushi S, Makino A, Shinzaki Y, Yamamoto H, Tomizawa KI (2005) Effects of light intensity on cyclic electron flow around PSI and its relationship to non-photochemical quenching of Chl fluorescence in tobacco leaves. Plant Cell Physiol 46:1819–1830. doi:10.1093/pcp/pci197
Mullineaux P, Karpinski S (2002) Signal transduction in response to excess light: getting out of the chloroplast. Curr Opin Plant Biol 5:43–48. doi:10.1016/S1369-5266(03)00061-X
Munné-Bosch S, Queval G, Foyer CH (2013) The impact of global change factors on redox signaling underpinning stress tolerance. Plant Physiol 161:5–19. doi:10.1104/pp.112.205690
Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. Environ Exp Bot 97:30–39. doi:10.1016/j.envexpbot.2013.09.014
Nelson N, Yocum CF (2006) Structure and function of photosystem I and II. Annu Rev Plant Biol 57:521–565. doi:10.1146/annurev.arplant.57.032905.105350
Newton A, Holden N, De Vega Perez D, Gravouill C, Walters D (2014) Induced resistance in crop protection: an overview. IOBC-WPRS Bull 102:169–174. ISBN: 978-92-9067-283-8
Niu DD, Liu HX, Jiang CH, Wang YP, Wang QY, Jin HL, Guo JH (2011) The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. Mol Plant Microbe Interact 24:533–542. doi:10.1094/MPMI-09-10-0213
Pathma J, Rahul GR, Kamaraj KR, Subashri R, Sakthivel N (2011) Secondary metabolite production by bacterial antagonists. J Biol Control 25:165–181. doi:10.18311/jbc/2011/3716
Pérez-Bueno ML, Pineda M, Díaz-Casado E, Barón M (2015) Spatial and temporal dynamics of primary and secondary metabolism in *Phaseolus vulgaris* challenged by *Pseudomonas syringae*. Physiol Plant 153:161–174. doi:10.1111/ppl.12237
Pillay VK, Nowak J (1997) Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol 43:354–361. doi:10.1139/m97-049
Pinedo I, Ledger T, Greve M, Poupin MJ (2015) *Burkholderia phytofirmans* PsJN induces long-term metabolic and transcriptional changes involved in *Arabidopsis thaliana* salt tolerance. Front Plant Sci 6:466. doi:10.3389/fpls.2015.00466
Poupin JM, Timmermann T, Vega A, Zuniga A, Gonzalez B (2013) Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. PLoS ONE 8:e69435. doi:10.1371/journal.pone.0069435
Pozo MJ, Van Der Ent S, Van Loon L, Pieterse CMJ (2008) Transcription factor MYC2 is involved in priming for enhanced

defense during rhizobacteria induced systemic resistance in *Arabidopsis thaliana*. New Phytol 180:511–523. doi:10.1111/j.1469-8137.2008.02578.x

Reddy PP (2013) Pathogenesis-related proteins. In: Reddy PP (ed) Recent advances in crop protection. Springer, New Delhi, pp 245–252. doi:10.1007/978-81-322-0723-8_15

Rincón A, Valladares F, Gimeno TE, Pueyo JJ (2008) Water stress responses of two Mediterranean tree species influenced by native soil microorganisms and inoculation with a plant growth promoting rhizobacterium. Tree Physiol 28:1693–1701

Rodríguez-Moreno L, Pineda M, Soukupová J, Macho AP, Beuzón CR, Baron M, Ramós C (2008) Early detection of bean infection by *Pseudomonas syringae* in asymptomatic leaf areas using chlorophyll fluorescence imaging. Photosynth Res 96:27–35. doi:10.1007/s11120-007-9278-6

Roitsch T (1999) Source-sink regulation by sugar and stress. Curr Opin Plant Biol 2:198–206. doi:10.1016/S1369-5266(99)80036-3

Ruíz-Sánchez M, Armada E, Muñoz Y, de Salamone IEG, Aroca R, Ruíz-Lozano JM, Azcón R (2011) *Azospirillum* and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J Plant Physiol 168:1031–1037. doi:10.1016/j.jplph.2010.12.019

Scholes JD, Rolfe SA (1996) Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): quantitative imaging of chlorophyll fluorescence. Planta 199:573–582. doi:10.1007/BF00195189

Schreiber U (1994) New emitter-detector-cuvette assembly for measuring modulated chlorophyll fluorescence of highly diluted suspensions in conjunction with the standard PAM fluorometer. Z Naturforsch 49c:646–656. doi:10.1515/znc-1994-9-1016

Schwessinger B, Ronald PC (2012) Plant innate immunity: perception of conserved microbial signatures. Annu Rev Plant Biol 63:451–482. doi:10.1146/annurev-arplant-042811-105518

Serrano I, Audran C, Rivas S (2016) Chloroplasts at work during plant innate immunity. J Exp Bot 67:3845–3854. doi:10.1093/jxb/erw088

Shan L, He P, Sheen J (2007) Intercepting host MAPK signaling cascades by bacterial type III effectors. Cell Host Microbe 1:167–174. doi:10.1016/j.chom.2007.04.008

Sharma VK, Nowak J (1998) Enhancement of verticillium wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). Can J Microbiol 44:528–536. doi:10.1139/w98-017

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240. doi:10.1080/10408410490468786

Spoel SH, Johnson JS, Dong X (2007) Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc Natl Acad Sci USA 104:18842–18847. doi:10.1073/pnas.0708139104

Stefan M, Munteanu N, Stoleru V, Mihasan M, Hritcu L (2013) Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). Sci Hortic 151:22–29. doi:10.1016/j.scienta.2012.12.006

Su F, Jacquard C, Villaume S, Michel J, Rabenoelina F, Clément C, Aït Barka E, Dhondt-Cordelier S, Vaillant-Gaveau N (2015) *Burkholderia phytofirmans* PsJN reduces impact of freezing temperatures on photosynthesis in *Arabidopsis thaliana*. Front Plant Sci 6:810. doi:10.3389/fpls.2015.00810

Su F, Gilard F, Guérard F, Citerne S, Clément C, Vaillant-Gaveau N, Dhondt-Cordelier S (2016) Spatio-temporal responses of Arabidopsis leaves in photosynthetic performance and metabolite contents to *Burkholderia phytofirmans* PsJN. Front Plant Sci 7:403. doi:10.3389/fpls.2016.00403

Thaler JS, Fidanstef AL, Bostock RM (2002) Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. J Chem Ecol 28:1131–1159. doi:10.1023/A:1016225515936

Theocharis A, Clément C, Barka EA (2012) Physiological and molecular changes in plants grown at low temperatures. Planta 235:1091–1105. doi:10.1007/s00425-012-1641-y

Thilmony R, Underwood W, He SY (2006) Genome wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the human pathogen *Escherichia coli* O157: H7. Plant J 46:34–53. doi:10.1111/j.1365-313X.2006.02725.x

Thomma BP, Penninckx IA, Cammue BP, Broekaert WF (2001) The complexity of disease signaling in Arabidopsis. Curr Opin Immunol 13:63–68. doi:10.1016/S0952-7915(00)00183-7

Ton J, Jakab G, Toquin V, Flors V, Iavicoli A, Maeder MN, Métraux JP, Mauch-Mani B (2005) Dissecting the β-aminobutyric acid-induced priming phenomenon in Arabidopsis. Plant Cell 17:987–999. doi:10.1105/tpc.104.029728

Trdá L, Fernandez O, Boutrot F, Héloir MC, Kelloniemi J, Daire X, Adrian M, Clément C, Zipfel C, Dorey S, Poinssot B (2014) The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. New Phytol 201:1371–1384. doi:10.1111/nph.12592

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 199:243–254. doi:10.1007/s10658-007-9165-1

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483. doi:10.1146/annurev.phyto.36.1.453

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586. doi:10.1023/a:1026037216893

Vidhyasekaran P (2015) Salicylic acid signaling in plant innate immunity. Plant hormone signaling systems in plant innate immunity. Signaling and communication in plants 2, Springer, Dordrecht, pp 27–122. doi:10.1007/978-94-017-9285-1_2

Walters M (2015) The plant innate immune system. Endocytobiosis Cell Res 26:8–12. ISSN: 0256-1514

Wang X, Mavrodi DV, Ke L, Mavrodi OV, Yang M, Thomashow LS, Zheng N, Weller DM, Zhang J (2015) Biocontrol and plant growth-promoting activity of rhizobacteria from Chinese fields with contaminated soils. Microb Biotechnol 8:404–418. doi:10.1111/1751-7915.12158

Xin XF, He SY (2013) *Pseudomonas syringae* pv. *tomato* DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annu Rev Phytopathol 51:473–498. doi:10.1146/annurev-phyto-082712-102321

Yadav BK, Akhtar MS, Panwar J (2015) Rhizospheric plant-microbe interactions: key factors to soil fertility and plant nutrition. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, New Delhi pp 127–145. doi:10.1007/978-81-322-2068-8$46

Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Paré PW (2008) Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56:264–273. doi:10.1111/j.1365-313X.2008.03593.x

Zhou J, Zeng L, Liu J, Xing D (2015) Manipulation of the xanthophyll cycle increases plant susceptibility to *Sclerotinia sclerotiorum*. PLoS Pathog 11:e1004878. doi:10.1371/journal.ppat.1004878

Zulfugarov IS, Tovuu A, Kim CY, Vo KTX, Ko SY, Hall M, Soek HY, Kim YK, Moon YH, Jansson S, Jeon JS, Lee CH (2016) Enhanced resistance of PsbS-deficient rice (*Oryza sativa* L.) to fungal and bacterial pathogens. J Plant Biol 59:616–626. doi:10.1007/s12374-016-0068-6