



# Phenotypic and molecular responses of rice to Burkholderia s.l. species

Eoghan King

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# THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Biologie des Systèmes

École doctorale GAIA

Unité de recherche UMR IPME

**Caractérisation phénotypique et moléculaire  
de la réponse du riz au cours de l'interaction  
avec des espèces de *Burkholderia* s.l.**

Présentée par Eoghan KING  
Le 11 décembre 2019

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UNIVERSITÉ  
DE MONTPELLIER



« Ceci est le véritable secret de la vie :  
Etre complètement engagé avec ce que vous faites dans l'ici et maintenant.  
Et au lieu d'appeler cela travailler, réaliser que c'est jouer »  
Allan Watts

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## Abbréviations

ABA : Acide Abscissique	KEGG : Kyoto Encyclopedia of Genes and Genomes	PR : Pathogenesis Related
ACC : Acide 1-aminocyclopropane-1-carboxylic acid	LRR : Leucin Rich Repeat	PTI : PAMP-Triggered Immunity
ACO : ACC oxidase	LRR-RLK : Leucin Rich Repeat-Receptor Like Kinase	qPCR : Quantitative Polymerase Chain Reaction
ACS : Acide 1-aminocyclopropane Synthase	LysM : Lysin Motif	RT-qPCR : Reverse Transcription Quantitative Polymerase Chain Reaction
AM : Arbuscular Mycorrhiza	MAMP : Microbe-Associated Molecular Pattern	RLK : Receptor-Like Kinase
AOS : Allene Oxide Synthase	MAPK : Mitogen-Activated Protein Kinase	RLP : Receptor-like protein
IAA : Indole-3-Acetic Acid	MPKK : Mitogen-activated Protein Kinase Kinase	RNA : Ribonucleic Acid
ARF : Auxin Response Factor	MTI : MAMP-Triggered Immunity	RNA-Seq : RNA Sequencing
BR : Brassinosteroid	NB-ARC : Nucleotide Binding-APAF-1, R proteins, and CED-4	ROS : Reactive Oxygen Species
CFU : Colony Forming Unit	NBS-LRR : Nucleotide-Binding Site-Leucine Rich Repeat	RR : Response Regulator
CK : Cytokinine	NGS: Next-Generation Sequencing	SA : Salicylic Acid
DEG : Differentially Expressed Gene	NLR : Nucleotide-binding domain, leucine-rich repeat containing	SAR : Systemic Acquired Resistance
DNA : Desoxyribonucleic Acid	PAL : Phenylalanine Ammonia-Lyase	SL : Strigolactone
ERF : Ethylene Responsive Factors	PAMP : Pathogen-Associated Molecular Pattern	WAK : Wall-Associated Kinase
ETI : Effector-Triggered Immunity	PBZ : Probenazole Inducible Protein	
ETS : Effector-Triggered Susceptibility	PIN : pin Formed	
FDR : False Discovery Rate	PGPF : Plant Growth-Promoting Fungi	
Flg22 : Flagellin 22	PGPM : Plant Growth-Promoting Microbe	
FLS2 : Flg sensing 2	PGPR : Plant Growth-Promoting Rhizobacteria	
GA : Gibberellin	PRR : Pattern Recognition Receptor	
GO : Gene Ontology		
HR : Hypersensitive Response		
ISR : Induced Systemic Resistance		
JA : Jasmonic Acid		
JAZ : Jasmonate ZIM (zinc-finger inflorescence meristem) domain		

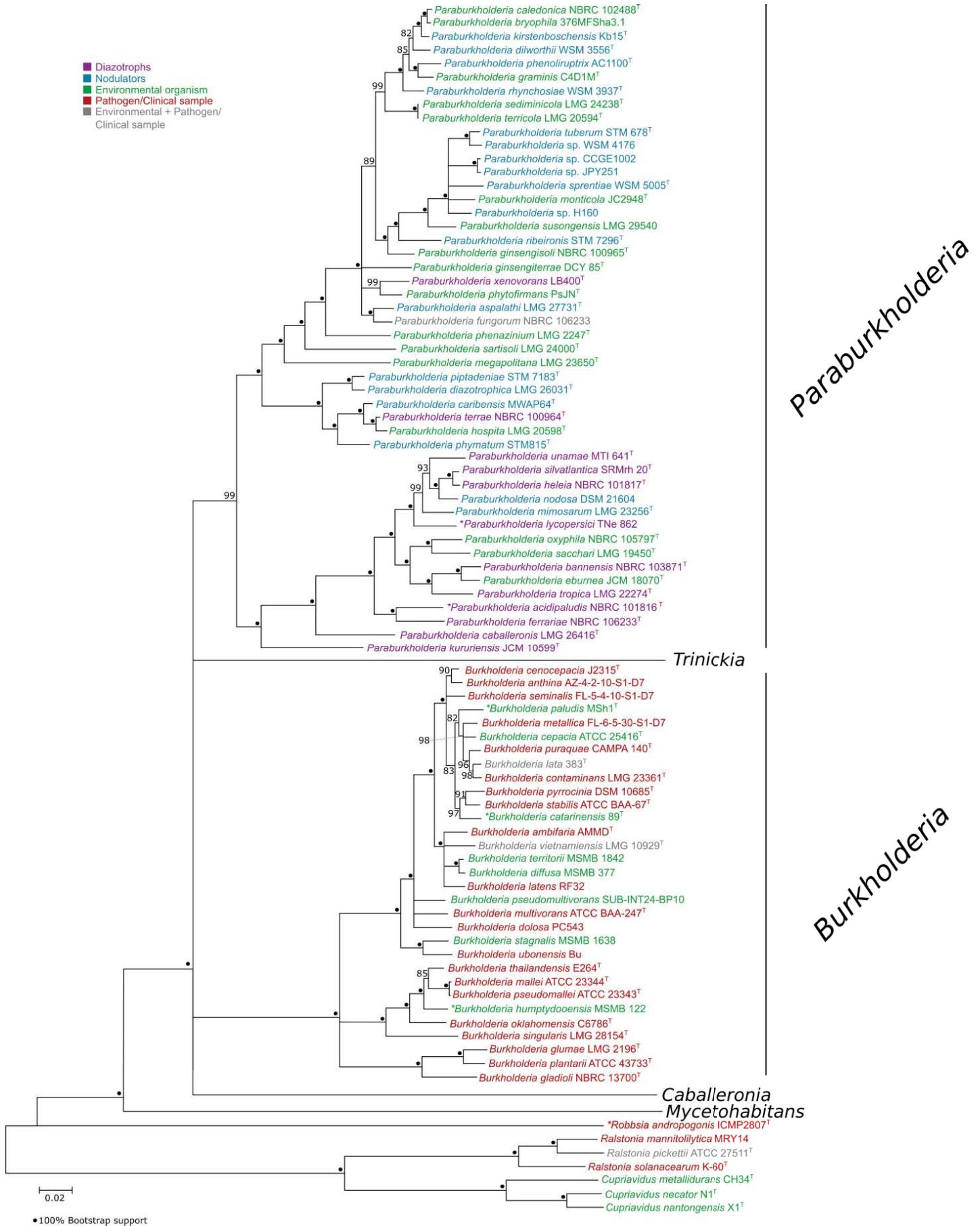




## **Introduction générale**

L'une des caractéristiques fondamentales des végétaux est leur régime énergétique autotrophe: en transformant l'énergie provenant du rayonnement solaire en énergie chimique utilisée pour la fixation du CO<sub>2</sub>, les plantes alimentent l'ensemble des réseaux trophiques continentaux en ressources organiques exploitables. Ayant par conséquent un rôle central dans le fonctionnement des écosystèmes terrestres, les plantes sont très attractives pour une grande diversité d'organismes vivants. De ce fait les plantes sont continuellement confrontées à une abondance de microorganismes regroupée sous le terme de « microbiote ». On retrouve au sein de ces communautés des microorganismes entretenant des interactions très diverses, pouvant être bénéfique, neutre ou délétère, pour la plante hôte. Quelque soit le type d'interaction mis en place entre une plante et un microorganisme donné, des mécanismes moléculaires complexes sont mis en jeu. En particulier, les plantes sont capables de reconnaître des motifs moléculaires microbiens conservés qui induisent l'activation d'une réponse immunitaire dite « non-hôte ». Cette réponse immunitaire basale a été largement étudiée dans des systèmes expérimentaux impliquant des microorganismes mutualistes et pathogènes. En effet, cette réponse immunitaire peut-être évitée, inhibée ou modulée par les microorganismes via différents mécanismes moléculaires.

Néanmoins, dans le contexte d'interactions facultatives appelées « symbioses associatives » entre les plantes et des microorganismes bénéfiques (appelées aussi PGPM, pour Plant Growth Promoting Microbe), qui n'impliquent pas la formation d'organes spécialisés, les processus de régulation immunitaire ont très peu été étudiés. Ces associations améliorent la croissance des plantes par différents mécanismes pouvant être hormonaux, ou passer par des apports nutritionnels supplémentaires ou encore en augmentant la tolérance des plantes à des bioagresseurs. Ainsi, dans le contexte actuel de transition vers une agriculture visant à limiter l'utilisation d'intrants chimiques comme engrais et pesticides, ce type d'interactions, plante-microorganismes bénéfiques, émerge comme une solution prometteuse. Cependant afin de traduire ce potentiel en réelle application agronomique, une profonde compréhension du fonctionnement de ces symbioses est nécessaire afin d'en tirer le meilleur parti. En effet, le transfert d'effets bénéfiques mesurés dans des conditions contrôlées de laboratoire vers le champ se solde souvent par des échecs. L'une des questions fondamentales actuelles dans ce champ de recherche est de déterminer comment les plantes sont capables de reconnaître et de favoriser des microorganismes bénéfiques tout en empêchant la colonisation de ses tissus par des microorganismes délétères. Pour se faire, étudier la réponse des plantes face à des microorganismes entretenant avec leur hôte des interactions variées est nécessaire. Cependant,



### Phylogénie des *Burkholderia* s.l. adapté de Estrada-de los Santos et al., 2018

Phylogénie basé sur les séquences d'acides aminés de 106 gènes concaténés de 122 souches de *Burkholderia* s.l. parmi les génomes disponibles selon la méthode du maximum de vraisemblance. Les couleurs indiquent les environnements d'isolement et les niches écologiques générales des différentes souches. La barre d'échelle indique le nombre de substitution par site.

comparer l'interaction entre une plante donnée et des microorganismes très éloignés au niveau phylogénétique pourrait masquer l'effet du type d'interaction mis en place. Néanmoins, il existe quelques taxons regroupant des espèces bénéfiques et pathogènes de plantes (*Pseudomonas*, *Stenotrophomonas*, *Pantoae*, *Burkholderia*, *Herbaspirillum* entre autres) permettant ainsi de comparer la réponse des plantes à des espèces relativement proches évolutivement.

Parmi ces taxons, le genre *Burkholderia* *sensu lato* (*s.l.*), une sous-classe des bétaprotéobactéries, présente des caractéristiques intéressantes. Premièrement, c'est un genre ubiquiste, on retrouve des membres de ce genre dans une grande diversité d'environnements, interagissant avec de nombreux hôtes, en tant que pathogènes, symbiotes bénéfiques ou commensaux (Coenye et Vandamme, 2003). Des études phylogénétiques récentes ont amené le genre *Burkholderia* *s.l.* à être divisé en plusieurs genres. En particulier, ces études ont amené à la création de deux genres regroupant des espèces bactériennes entretenant avec les plantes des types d'interactions opposées (Beukes *et al.*, 2017; Estrada-de los Santos *et al.* 2016; Sawana *et al.* 2014). Le genre *Paraburkholderia* regroupe des espèces environnementales et symbiotiques de plantes (Kaur *et al.*, 2017) dont certains membres ont montré des effets bénéfiques sur la croissance de leur hôte soit par la formation de nodules fixateurs d'azote (Gyaneshwar *et al.*, 2011) soit en formant des symbioses associatives (Voir phylogénie des *Burkholderia* *s.l.*). Tandis que le genre *Burkholderia* *sensu stricto* (*s.s.*) regroupe des espèces bactériennes opportunistes pour l'homme, en particulier le complexe *B. cepacia* (Vial *et al.*, 2011), ainsi que pathogènes de mammifères et de plantes (Eberl et Vandamme, 2016). De plus, on retrouve des membres des deux clades naturellement associés aux racines de plantes, indiquant que la capacité de colonisation de l'environnement "plante" est partagée par ces deux lignées bactériennes (Manna et *al.*, 2018). D'autres genres bactériens ont été récemment définis, plus anecdotiques en termes de nombre de taxons les composant, ils regroupent également des espèces avec des écologies particulières (Estrada-de los Santos *et al.*, 2018). Le genre *Mycetohabitans* regroupe des endosymbiotes de champignons, le genre *Caballeronia* des espèces dites environnementales, le genre *Trinickia* des espèces retrouvées dans le sol et associées aux plantes, enfin, le genre *Robbsia* permet d'accueillir phylogénétiquement une espèce pathogène, *R. andropogonis* (Lopes-Santos *et al.*, 2017). Les genres *Burkholderia* *s.s.* et *Paraburkholderia* regroupent des espèces capables de coloniser la zone proche des racines, la rhizosphère, ainsi que leur surface, le rhizoplan, mais également les tissus internes des plantes, l'endosphère. En particulier, l'espèce *P. phytofirmans* est considéré comme un modèle d'endophyte bactérien, elle très étudié au vu de ses effets

bénéfiques pour les plantes (Esmaeel *et al.*, 2018). En effet, cette espèce bactérienne est capable de coloniser l'endosphère d'une grande diversité de plantes, allant jusqu'à coloniser les fruits de la vigne (*Vitis vinifera*) et les tissus des plantes des générations suivantes (Compan *et al.*, 2008). Au vu de ces particularités, le genre *Burkholderia s.l.* est un modèle de choix pour procéder à des études comparatives afin de décrire les facteurs biologiques discriminant les interactions bénéfiques et délétères dans les interactions plante-bactéries.

Du côté du modèle végétal, le riz (*Oryza sativa*) émerge comme une plante hôte privilégiée pour étudier cette frontière entre symbiose bénéfique et pathogénie au sein de ce taxon. En effet, plusieurs espèces de *Burkholderia s.s.* sont des bactéries phytopathogènes du riz comme *B. glumae*, *B. plantarii* et *B. gladioli* (Maeda *et al.*, 2006). De plus, deux souches de *Burkholderia s.l.*, capables de fixer l'azote atmosphérique ont été isolées du riz. *Paraburkholderia kururiensis* M130, une souche endophyte du riz (Baldani *et al.*, 1997) et la souche *Burkholderia vietnamiensis* TVV75<sup>T</sup> (Van *et al.*, 1996), qui appartient au complexe *B. cepacia*. Ces deux souches ont montré des effets bénéfiques sur la croissance et le rendement du riz lorsqu'elle sont inoculées sur les racines de cette plante (Govindarajan *et al.*, 2008; Mattos *et al.*, 2008). Ainsi, l'étude comparative de ces deux modèles permettrait d'étudier comment des interactions bénéfiques peuvent émerger de contextes phylogénétiques différents en terme d'écologie. Finalement, le riz en plus d'être la céréale la plus importante en termes de consommation humaine -elle est la ressource nutritionnelle principale pour plus de la moitié de la population mondiale (Muthayya *et al.*, 2014)-, est la monocotylédone modèle de la communauté scientifique. C'est la première plante d'intérêt agronomique à avoir vu son génome séquencé par un consortium international au début des années 2000 et ce pour les deux sous-espèces d'*Oryza sativa*: respectivement les sous-espèces *japonica* (Goff *et al.*, 2002) et *indica* (Yu *et al.*, 2002). De plus, de nombreuses ressources génétiques et génomiques ont depuis été développées pour l'étude de cette plante (Delseny *et al.*, 2001; Huang *et al.*, 2013; Kawahara *et al.*, 2013; Li *et al.*, 2017; Sakai *et al.*, 2013). Enfin, le riz est également un modèle pour l'étude des interactions plante-microorganismes. En effet, l'étude de certains des pathogènes du riz, comme le champignon *Magnaporthe oryzae* ou les bactéries *Xanthomonas oryzae*, ont permis l'établissement de pathosystèmes de première importance au sein de la communauté scientifique (Niño-Liu *et al.*, 2006; Wilson et Talbot, 2009).

En tirant partie de ce modèle expérimental **riz-*Burkholderia***, ces travaux de thèse ont eu pour objectif de **décrire les réponses physiologiques et transcriptionnelles du riz lors de l'interaction avec des bactéries aux contextes phylogénétiques différents entretenant**

**avec le riz des types d'interactions variés.** Pour ce faire, cela a nécessité la description des différents types d'interactions, en particulier en termes de colonisation des tissus racinaires. Par la suite, des analyses transcriptomiques des feuilles et des racines de riz ont permis de mettre en évidence les processus physiologiques régulés au cours de la mise en place des différentes interactions. Par ces approches, ces travaux de thèse cherchent à répondre aux questions suivantes :

1. Quelle sont les régulations transcriptionnelles du riz au cours de l'interaction avec des bactéries endophytes bénéfiques ayant des contextes phylogénétiques contrastés ?
2. Quelle est la réponse du riz à une bactérie endophyte modèle par rapport à des endophytes lui étant naturellement associé ?
3. Quelle est la réponse du riz à une diversité de souches ayant des écologies contrastées avec leur hôte ?

Ce manuscrit se décline en 5 chapitres. Le premier chapitre est une synthèse bibliographique qui a pour but de réunir les connaissances actuelles sur l'importance du microbiote des plantes ainsi que le changement de paradigme que ces nouvelles connaissances amènent dans la compréhension du fonctionnement de l'interaction entre les végétaux et leur environnement biotique. Ce chapitre traite (1) des caractéristiques du microbiote des plantes, (2) de la réponse des plantes aux différents types d'interactions plante-microorganismes, (3) de l'état des connaissances dans l'étude des régulations physiologiques induites par les plantes au cours de symbioses associatives et en particulier les régulations transcriptionnelles des monocotylédones au cours de l'interaction avec des bactéries PGPR et enfin (4) du potentiel du microbiote ainsi que les perspectives d'applications agronomiques.

Le deuxième chapitre traite de l'analyse comparative de la réponse transcriptionnelle du riz lors de l'interaction avec deux souches bénéfiques de *Burkholderia* *s.l.* aux contextes phylogénétiques opposés en terme d'écologie: *Paraburkholderia kururiensis* M130 et *Burkholderia vietnamensis* TVV75<sup>T</sup>. Le troisième chapitre traite des réponses physiologiques et transcriptionnelle du riz lors de l'interaction avec une bactérie endophyte à large spectre d'hôte: *Paraburkholderia phytofirmans* PsJN<sup>T</sup>, en comparaison avec les souches isolées du riz. Le quatrième chapitre traite de la réponse transcriptionnelle du riz face à une diversité de souches de *Burkholderia* *s.l.* isolées du riz, entretenant avec leur hôte des interactions allant de la symbiose bénéfique à la pathogénie. Enfin, la cinquième partie correspond à une discussion générale de l'ensemble des résultats, accompagnée des perspectives découlant de ces travaux de thèse.

Les chapitres 2, 3 et 4 sont rédigés sous forme d'articles scientifiques et sont par conséquent en langue anglaise. De même, en vue d'une publication d'article de revue, le premier chapitre également est rédigé en anglais. Néanmoins, les méthodes appliquées ainsi que les principaux résultats de chaque article sont résumés en préambule des chapitres 2, 3 et 4 en langue française.

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# **CHAPITRE 1**

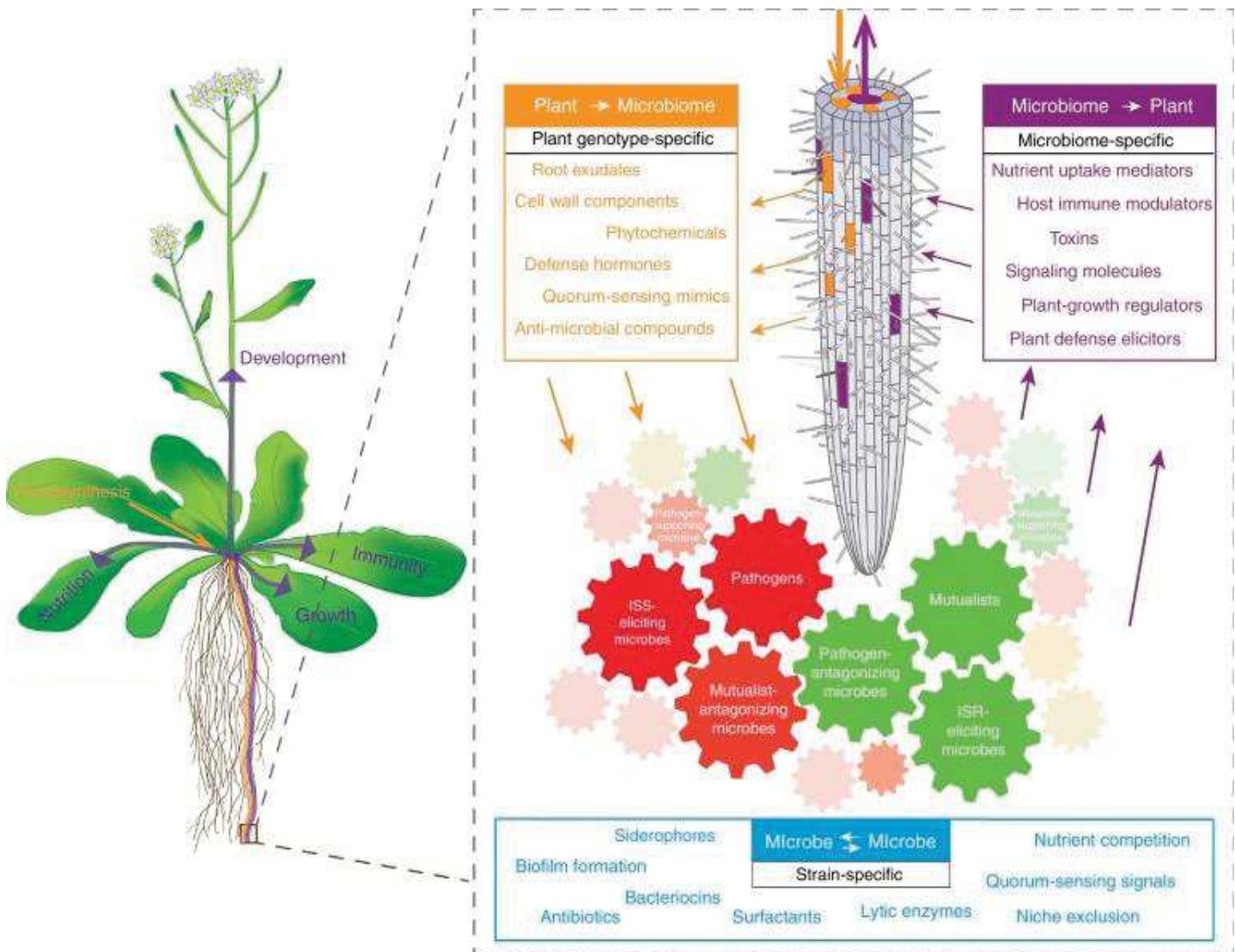
## **SYNTHÈSE BIBLIOGRAPHIQUE**

## **Multicellular organisms as metaorganisms**

The vast majority of living organisms on earth are microorganisms belonging to the three kingdoms of life: Bacteria, Archaea and Eukaryotes. Indeed, microbial life forms represent 5-15% of earth carbon biomass (Bar-On *et al.*, 2018; Kallmeyer *et al.*, 2012; Whitman *et al.*, 1998) and have virtually colonized all habitats. In consequence, since their apparition, multicellular life forms have been confronted to the presence of microorganisms. Indeed, multicellularity creates nutrient niches for microbial colonization. Thus formed niches can be colonized by numerous and highly diverse microorganisms. These microbial communities are regrouped under the term "microbiome", defined as "the ecological communities of commensal, symbiotic and pathogenic microorganisms associated to the tissues of a host". However, as this concept is not just limited to human-associated microbes, it has been extended to all kinds of environments -other hosts but not only-. Nonetheless, the term evolved to refer to the collection of genomes of all the microorganisms and the host, therefore leading to the concept of "second genome" of hosts. The term "microbiota" is used to refer to the microbial communities associated to a host.

## **Animal and plant microbiome commonalities**

Although the fact that the human body is mostly composed of microbial cells was known for a long time (Savage, 2003), the two past decades revolutionized the way we conceive the evolution and the functioning of multicellular organisms. Indeed, recent technological advances of next-generation sequencing (NGS) enabled to unravel the taxonomic diversity of the microbiota through culture-independent approaches. But most importantly, the role of the microbiome on hosts' functions such as nutrition or health was discovered especially for vertebrates (Chung *et al.*, 2012) and plants (Müller *et al.*, 2016). Interestingly, these two clades have in common the fact that their respective organs dedicated to nutrient acquisition - the gut and roots - are highly colonized by a wide diversity of microbes (Mendes and Raaijmakers, 2015). While the both organs are dominated by the same bacterial phyla (Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria), lower taxonomic levels shows distinct bacterial microbiota compositions for each organ (Hacquard *et al.*, 2015). However, distinct microbiota composed of different taxa can exhibit functional redundancy; this was



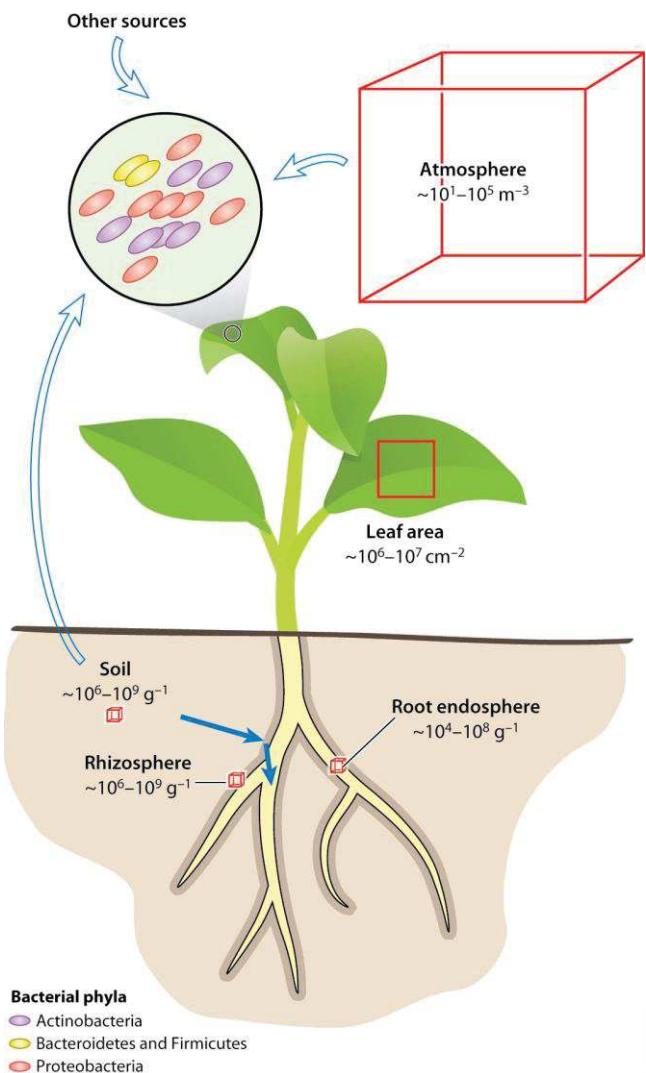
**Figure 1: Interplay between host and microbes in plant-microbiome interactions (Adapted from Pieterse et al., 2016)**

Plants invest a proportion of their photosynthetically fixed carbon sources in maintaining and shaping the microbial community in and around their roots. In turn, plants profit from microbiome-encoded functions, resulting in improved growth, development, nutrition, and/or immunity. The plant genotype affects root metabolism, immune system functioning, and root exudate composition (orange box and arrows), which in turn influences the activity and structure of the root microbiome. The relative success of soil-borne pathogens and mutualists in their respective association with plant roots is affected by the degree of activity of microbes that antagonize or support them. Such microbe-microbe interactions (blue box) are mediated via strain-specific production and perception of molecules with dedicated antimicrobial or probiotic activities that selectively inhibit or support microbial growth. Collectively, microbiota in and around the root provide important services to the plant, such as improved root architecture, enhanced nutrient uptake, promotion of plant growth, activation of induced systemic resistance (ISR), and suppression of soil-borne pathogens and microbes that stimulate induced systemic susceptibility (ISS) (purple box and arrows).

firstly discovered for humans (Huttenhower *et al.*, 2012) and was also observed for plants (Ofek-Lalzar *et al.*, 2014). Although these two organs are not enriched in the same microbial functions, root-associated microbiota was proposed to be a human opportunistic pathogens reservoir (Van Overbeek *et al.*, 2014) as the functions needed for both environments colonization are shared (Chemotaxis, antibiotic resistance, competition for colonization niches and nutrients among others) (Berg *et al.*, 2005).

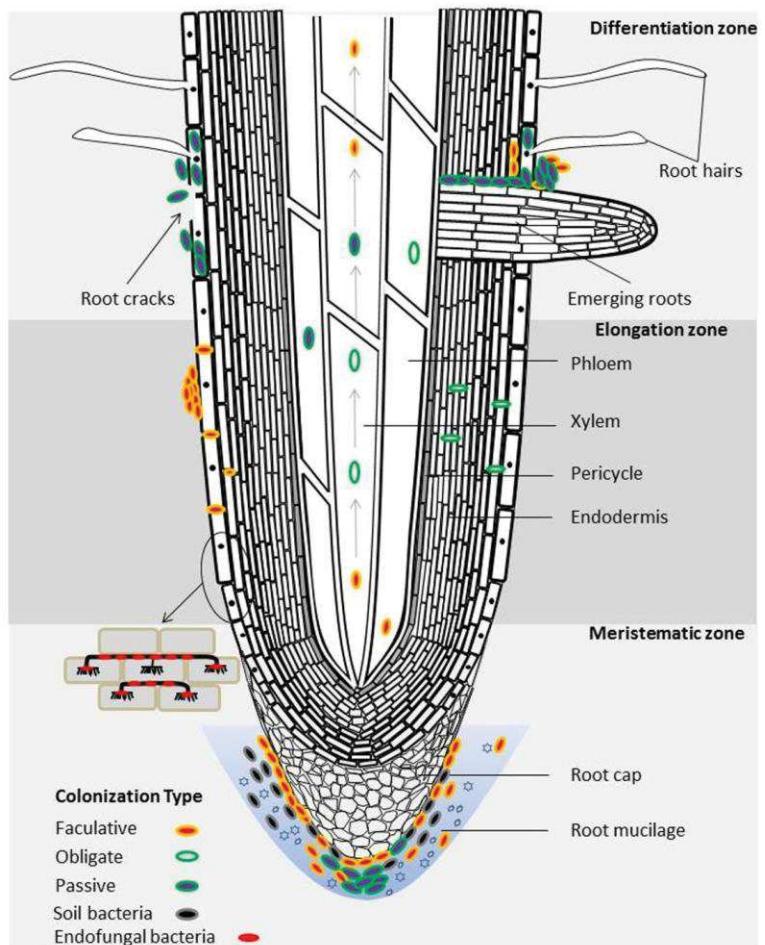
### **Plant microbiome specificities**

In spite of all of these commonalities between plants' and animals' microbiomes, a major physiological difference is the distinct energy production strategies of each host. While animals are heterotrophs and therefore entirely rely on the energy originally captured by other organisms, plants are autotrophs, producing their energy through photosynthesis. This fact has two main consequences: (i) Nutrient acquisition by roots is almost exclusively limited to mineral ions and water uptake (ii) The long-distance transport of photo-assimilates from leaves to the “sink” organs drives plants’ attractiveness for other organisms including microorganisms. This latest is even more accurate for roots, given that 15-60% of plants total photosynthesized C is transferred to the roots (Kuzyakov and Domanski, 2000) of which 40-90% (Lynch and Whipps, 1990) are transferred to the soil through the active process of rhizodeposition of a wide range of molecules (sugars, amino acids, small-molecular weight metabolites, enzymes for example) which influence the interactions between plants and other surrounding organisms (Bais *et al.*, 2006). This has major consequences on the soil microbiota as it provides a high concentration of nutrients for microbes in the surrounding of the root. Consequently, the composition and/or the abundance of root-associated microbiota are different from the soil microbiota which is considered as the main inoculum for root-associated microbes (Bulgarelli *et al.*, 2012). This is called the “rhizosphere effect” and has been described in a number of plants (Grayston *et al.*, 1997; Obraztsova, 1936; Timonin, 1939), the rhizosphere being defined as “the zone of soil under plants’ roots influence” by Lorentz Hiltner (1904) (Hiltner, 1904). This fact led to the idea that plants are able to “shape” their associated microbiota particularly through rhizodeposition (Hartmann *et al.*, 2009; Sasse *et al.*, 2018). Undoubtedly, numerous factors dynamically influence plant microbiota such as: the host species (Fitzpatrick *et al.*, 2018; Schlaeppi *et al.*, 2014), abiotic and biotic stresses (Berendsen *et al.*, 2012; Caddell *et al.*, 2019), the nutritional status (Castrillo *et al.*, 2017; Pii *et al.*, 2016) and the development stage (Dombrowski *et al.*, 2017; Edwards *et al.*, 2018) among others (Figure 1). The current trending idea is that rhizodeposition is modulated by



**Figure 2: Plant microbiota and origins of plant-associated microbes (from Bulgarelli *et al.*, 2013)**

Bacterial population sizes and phyllosphere community composition. Numbers correspond to estimations of number of bacterial cells in phyllosphere, atmosphere, rhizosphere, and root and soil bacterial communities. Open and solid arrows represent inoculation routes for the phyllosphere and root microbiota, respectively.



**Figure 3: Microbial root colonization hotspots (From Liu *et al.*, 2017)**

Schematic representation of the bacterial distribution and colonization patterns of the endosphere of a plant root. The emerging sites of lateral roots are among the hotspots of bacterial colonization. Arrows represent the translocation of bacteria inside the xylem and phloem. Endophytic bacteria may engage in different life styles as depicted by different colored ovals.

these factors, so that plants recruit microbes from the soil to provide microbial functions beneficial in different contexts (Bakker *et al.*, 2018).

### **Plant-associated microbial niches**

Nonetheless, plants' roots and their vicinity are not the only plant-associated niches for microbes to colonize. In fact, as humans, virtually all plants' tissues are colonized by microbes. Consequently, several plant-associated microbial niches have been defined (Turner *et al.*, 2013) (Figure 2). The most studied niche is the rhizosphere (as defined in previous paragraph) but the surface of plants' tissues are also colonized, root surface is called the rhizoplane (Foster, 2003) while leaves' surface is referred as the phyllosphere (Lindow and Brandl, 2003; Vorholt, 2012). Microorganisms colonizing the surface of plants are defined as epiphytes in opposition to endophytes which are able to colonize the interior of plants tissues, referred to as the endosphere. The concept of endophytes is a rather old one as it was firstly proposed in 1809 (Link, 1809), endophytic microbes were termed "entophytæ" at this time. The first mention of endophytes is attributed to M.L.V. Galippe in 1887 (Compant *et al.*, 2012), which proposed the pioneering idea that endophytes are soil-derived microorganisms. His work was extensively criticized in the 19th century following Pasteur's view that a healthy organism is one free of any microbial colonization. This idea was since largely rejected (Partida-Martínez and Heil, 2011) as microbe-free or "axenic" organisms exhibit abnormal physiological functioning especially for nutrient uptake and immunity. Also, misleading ways to define endophytes have been used, in particular the fact that endophytes "are able to colonize inner tissues without causing any harm" (Wilson, 2006). In fact, endophytes can be pathogenic as well as non-pathogenic and each category harbor major similarities in terms of colonization processes and genomic repertoires (Brader *et al.*, 2017). Among the diversity of endophytes, some are restricted to the interior of roots ("endorhizosphere") while others are able to systemically colonize plants in the stems and leaves particularly by colonizing the xylem and being transported following the transpiration flow (Afzal *et al.*, 2019; Compant *et al.*, 2010) (Table 1, Figure 3).

Finally, the most recently defined niche is the spermosphere: indeed, some microbes are able to colonize seeds -both on the surface and the interior- and can also be vertically transmitted (Shade *et al.*, 2017). The microbial diversity of these different niches has been studied through the defining of the core root microbiome of several accessions of *Arabidopsis thaliana* (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012) and rice (*Oryza sativa*) (Edwards *et*

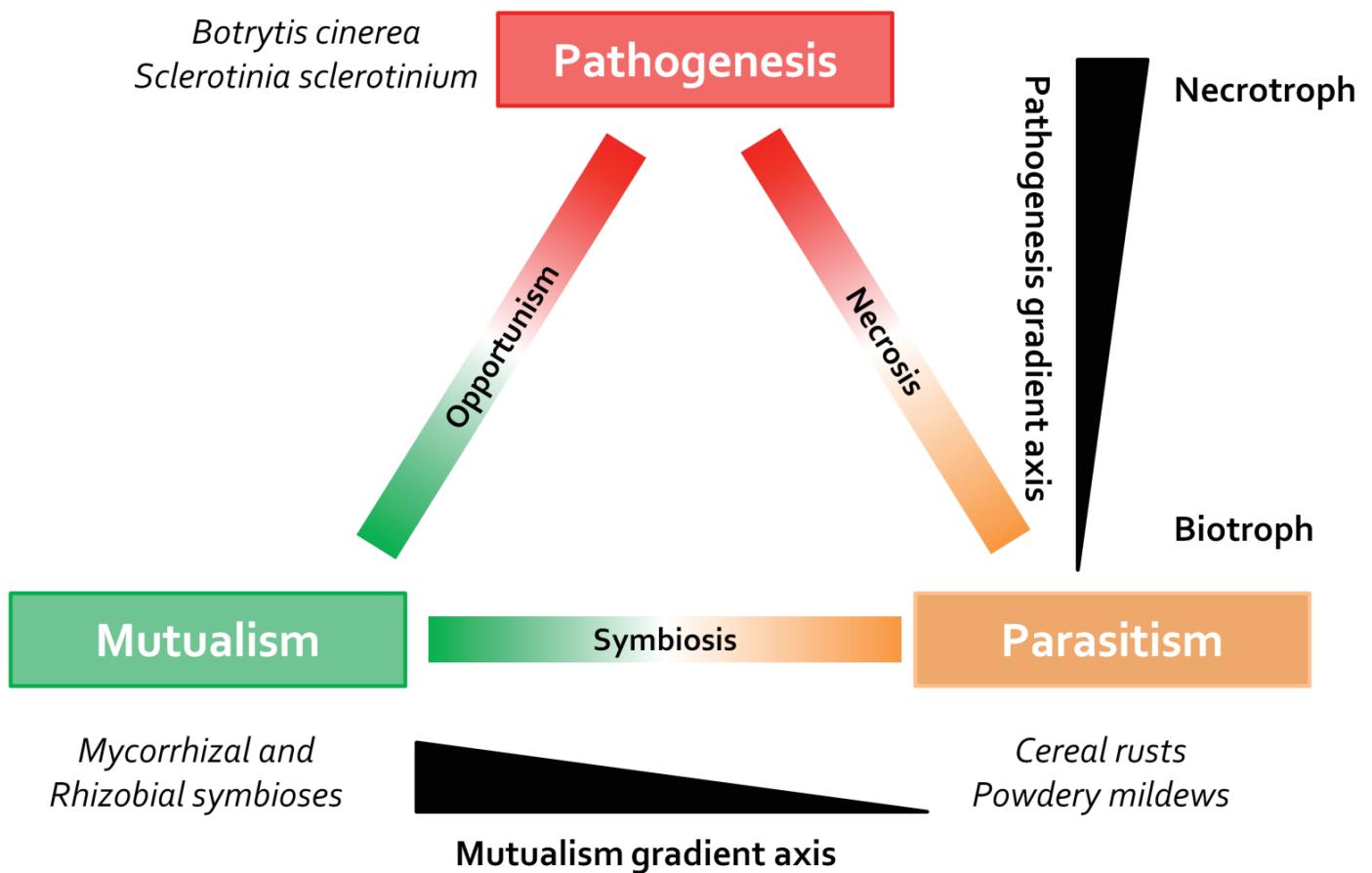
Table 1: Non exhaustive list of bacterial and fungal endophytic strains with published genome

Strain	Host plants	PGP activities	Reference
<b>Bacterial endophytes</b>			
<i>Azoarcus sp.</i> BH72	Rice	Nitrogen fixation	Krause <i>et al.</i> (2006)
<i>Azospirillum lipoferum</i> 4B	Rice, maize, wheat	Nitrogen fixation, phytohormone secretion	Wisniewski-Dyé <i>et al.</i> (2011)
<i>Azospirillum sp.</i> B510	Rice	Nitrogen fixation, phytohormone secretion	Kaneko <i>et al.</i> (2010)
<i>Burkholderia phytofirmans</i> PsJN	Potato, tomato, maize, barley, onion, canola, grapevine	IAA synthesis, ACC deaminase	Weilharter <i>et al.</i> (2011)
<i>Burkholderia spp.</i> KJ006	Rice	ACC deaminase, <i>nif</i> gene cluster, antifungal action (indirect PGP)	Kwak <i>et al.</i> (2012)
<i>Enterobacter cloacae</i> ENHKU01	Pepper	Unkwon role in PGP	Liu <i>et al.</i> (2012)
<i>Enterobacter sp.</i> 638	Poplar	Siderophore, IAA, Biocontrol	Taghavi <i>et al.</i> (2009)
<i>Gluconacetobacter diazotrophicus</i> PaI5	Sugarcane, rice, <i>Coffea</i> , <i>Camellia</i>	Nitrogen fixation, auxin synthesis	Bertalan <i>et al.</i> (2009)
<i>Herbaspirillum seropedicae</i> SmR1	Rice	Nitrogen fixation, IAA synthesis, ACC deaminase	Pedrosa <i>et al.</i> (2011)
<i>Klebsiella pneumoniae</i> 342	Maize, wheat	Nitrogen fixation	Fouts <i>et al.</i> (2008)
<i>Paraburkholderia kururiensis</i> M130	Rice	Nitrogen fixation, IAA synthesis, ACC deaminase	Coutinho et al (2013)
<i>Paraburkholderia tropica</i> Ppe8	Sugarcane	Nitrogen fixation	da Silva <i>et al.</i> (2018)
<i>Pseudomonas putida</i> W619	Poplar	IAA synthesis, ACC deaminase	Taghavi <i>et al.</i> (2009)
<i>Pseudomonas stutzeri</i> A1501	Rice	Nitrogen fixation	Yan <i>et al.</i> (2008)
<i>Serratia proteamaculans</i> 568	Soybean	IAA synthesis, ACC deaminase, Biocontrol	Taghavi <i>et al.</i> (2009)
<i>Stenotrophomonas maltophilia</i> R551-3	Poplar	IAA synthesis, ACC deaminase	Taghavi <i>et al.</i> (2009)
<i>Paenibacillus polymyxa</i> M1	Wheat	NRPS and lipopeptide synthesis	Eastman <i>et al.</i> (2014)
<b>Fungal endophytes</b>			
<i>Epichloë festucae</i>	<i>Pooideae</i> grasses	Alkaloids synthesis	Winter <i>et al.</i> (2018)
<i>Piriformospora indica</i>	<i>Brassicaceae</i> , Barley	Stress tolerance	Zuccaro <i>et al.</i> (2011)
<i>Gaeumannomyces</i> sp. JS-464	<i>Phragmites communis</i>	Secondary metabolites synthesis	Kim <i>et al.</i> (2017)
<i>Trichoderma</i> sp.	Various plants	Biocontrol	Mukherjee <i>et al.</i> (2013)

*al.*, 2015). The analysis of the relative abundance of core taxa revealed a bottleneck in the global species richness from the rhizosphere to the endosphere. This observation was attributed to an increasing selective gradient from the rhizosphere to the endosphere (Bulgarelli *et al.*, 2013) for microbes holding at the same time rhizoplane, rhizosphere and endosphere-competences. This can be extrapolated to the microbes able to colonize the interior of the above-ground part of the plants.

### Different types of plant-microbe interactions

Within the different microbial niches previously introduced, microorganisms exhibiting various ecological interactions with plants can be found. Classically, three extreme categories of plant-associated microorganisms are defined: “pathogens”, “parasites” and “mutualists”. Also, we will here use the term “symbiosis” as defined by “the living together of two dissimilar organisms in intimate association or close union”. Therefore, in the broad sense, interactions ranging from mutualism, where both plant and microbe benefits, commensalism, where the microbe benefits while not negatively impacting the plant, to parasitism, where the microbes benefits at host’ expense, can all be considered as symbiotic (Figure 4) (Newton *et al.*, 2010). Another distinction can be established between parasites and pathogens, while the latter can actively damage tissues and hijack its host physiology for their own trophic benefit, one could consider that parasites indirectly negatively impact their host by exploiting the niche of hosts’ tissues (Newton *et al.*, 2010). While these definitions are useful, they are limited to have broad sense in the diversity of host-microbes interactions, particularly in the case of interactions changing categories throughout the microbe life cycle (Newton *et al.*, 2010). Therefore, a “continuum” vision of plant-microbe interactions from detrimental to beneficial was proposed to accommodate recent findings and particular study cases (Hirsch, 2004). This statement has a special relevance in the light of the recent unraveling of the plant microbiome composition. Indeed, the concept of commensalism is central to the “metaorganism” vision of plant microbiome (Berg *et al.*, 2016; Bosch and McFall-Ngai, 2011). Effectively, commensals can have direct or indirect effects in particular environmental conditions impacting host fitness consequently not being commensals anymore. The previously introduced idea that plants are in fact metaorganisms led to the concept of holobiont, referring as the unit of selection in evolution (Zilber-Rosenberg and Rosenberg, 2008). Historically, the understanding of plant-microbe interactions came from the analysis of plant-pathogen (Gabriel and Rolfe, 1990; Mansfield *et al.*, 2012) and plant-mutualist models (Bonfante and Genre, 2010; Douglas Cook, 1999). This can be due to the economic



**Figure 4: Trophic space occupied by microorganisms in association with plants (adapted from Newton *et al.*, 2010)**

The range of trophic relationships of example microbe–plant associations is represented as space occupied between the three key trophic states of pathogenicity, mutualism and parasitism at the corners of the triangle. The vertical axis represents a gradient of pathogenesis from necrotrophy to biotrophy. The horizontal axis represents a gradient from mutualism to parasitism for symbiotic relationships.

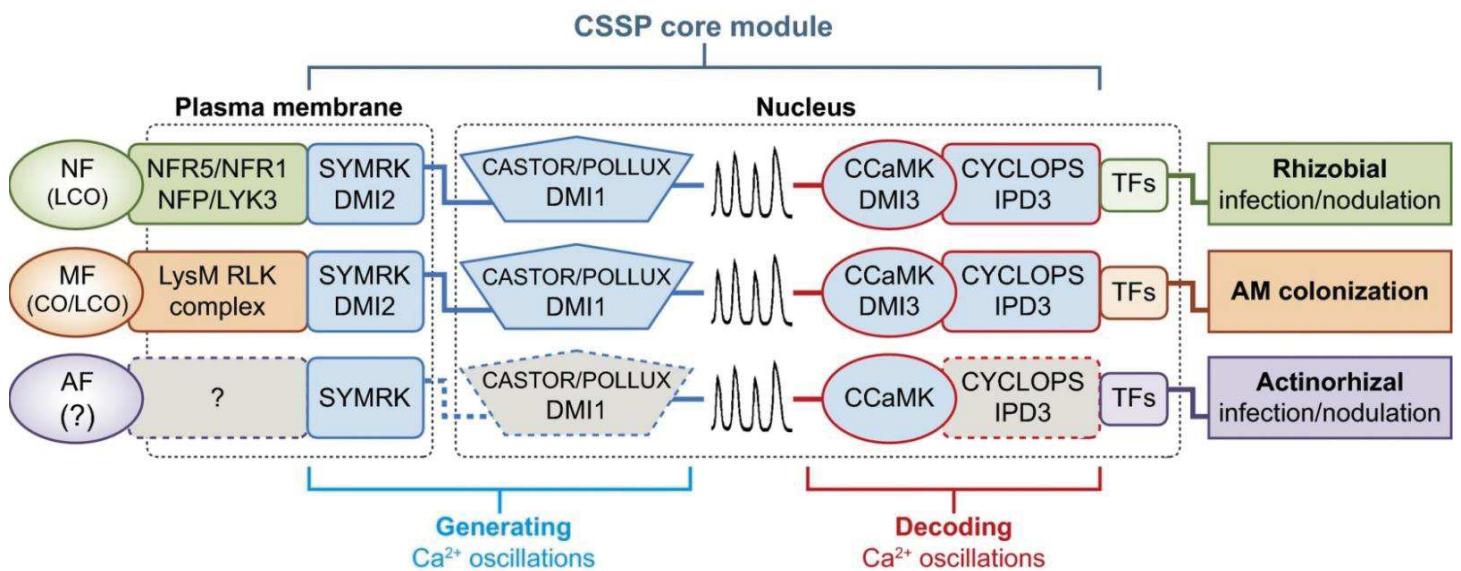
importance of such interactions -in particular for plant diseases-. Another major reason probably is the strong phenotypes that can be relatively easily analyzed in such models. Indeed, this statement can be argued in opposition to the discrete and highly context-dependent phenotypes driven by other members of the plant microbiome.

## **Plant-beneficial microbial interactions**

### Main plant-mutualist models overview

As previously stated, the first extensively models used to study the interactions between plant and beneficial microbes are mutualistic ones. The most important, in terms of evolutionary, ecological and economical relevance is the plant-mycorrhiza model. The name of this symbiosis (from the Greek “mycos”, meaning fungus and “rhiza” meaning root) clearly states that this association give rise to a new organism composed of both the fungus and the root. It is, indeed, the most widespread plant-microbe symbiosis as 70-90% of land plants are associated to mycorrhiza (Parniske, 2008) and also the most ancient, as land plants ancestors were apparently associated with similar fungal structures (Remy *et al.*, 1994). This is most likely due to the fact that such association was needed for land colonization by plants (Selosse and Le Tacon, 1998), indeed these fungi provide additional potential of nutrients and water uptake for land plants (Harley, 1989). Probably the most crucial role of mycorrhiza for plants is their help for the uptake of phosphorus (P) from soil. This element is an essential plant nutrient and one of the most limiting, especially in soil. Indeed, roots can only uptake inorganic P directly while this form is usually in minority in soils and therefore not available for plants (Hinsinger, 2001). As they can transform P in chemical forms available for plants, mycorrhiza are highly crucial for plant nutrition (López-Arredondo *et al.*, 2014). Two types of mycorrhiza can be distinguished: (i) Ectomycorrhiza, mainly found in association with trees in temperate forests, remains outside of plant cells. (ii) Endomycorrhiza, for which, part of the fungal hyphae is endocellular although a double membrane is formed by the invagination of plant cell membrane (Parniske, 2008). In the case of the most largely distributed endomycorrhiza, arbuscular mycorrhiza (AM), the formation of so-called “arbuscules” enables efficient nutrient exchanges between host and symbiont.

The second most extensively studied plant-mutualist model, the legume-rhizobium symbiosis, also implies endocellular colonization and improves nutrient availability for plants (Oldroyd *et al.*, 2011). In this case, the symbionts are Proteobacteria (from alpha and beta-subclasses) (Gyaneshwar *et al.*, 2011; Shamseldin *et al.*, 2017) which improve legumes nitrogen (N)



**Figure 5: Schema illustrating the common symbiosis signalling pathway (CSSP) (From Barker *et al.*, 2017)**

A number of plant genes and secondary messengers are required for the successful functioning of the conserved CSSP core module, first discovered in the model legume species *Lotus japonicus* (Lj) and *Medicago truncatula* (Mt), and now extended to plant hosts of all known root endosymbiotic associations including the rhizobial/legume, arbuscular mycorrhizal (AM) and actinorhizal symbioses. In the case of the rhizobial and AM symbioses the CSSP is activated following symbiotic signal perception (NF, Nod factors; MF, Myc factors) by plasma membrane (PM) localized LysM-RLK receptors, most probably part of a larger complex including LjSYMRK/MtDMI2. Following signal transduction from the PM to the nucleus, nuclear membrane cation channels known as LjCASTOR/LjPOLLUX/MtDMI1, likely in association with cyclic nucleotide gated-calcium channel complex (CNGC) required for rapid  $\text{Ca}^{2+}$  release and the initiation of nucleoplasmic  $\text{Ca}^{2+}$  spiking. The subsequent decoding of the intranuclear  $\text{Ca}^{2+}$  oscillatory response involves two key associated components (LjCCaMK/MtDMI3 and LjCYCLOPS/MtIPD3). Binding of  $\text{Ca}^{2+}$  to the calcium and calmodulin-dependent kinase CCaMK leads to phosphorylation of the coiled-coil protein CYCLOPS. Finally, the activation of a downstream signalling cascade via a repertoire of GRAS/ERF transcription factors results in the synthesis of the suite of proteins required for the transcriptional re-modelling of the epidermal cell in preparation for apoplastic infection. In the case of the N-fixing actinorhizal association, the nature of both the *Frankia* signal (AF, actinorhizal factor) and the corresponding host receptor remain to be determined, and that direct evidence for an essential role in microbial/host signalling has only been demonstrated so far for CgSYMRK and CgCCaMK, although orthologues for both CASTOR/POLLUX/DMI1 and CYCLOPS/IPD3 have been found in *Casuarina glauca*.

nutrition. This implies the formation of specialized organs, called “nodules”, which are inhabited by the rhizobial cells (Brewin, 1991). This organ provides the required environment for the so-called “fixation of atmospheric N<sub>2</sub>” by the bacterial population.

Extensive years of research enabled to deeply appreciate the underlying molecular and developmental processes implicated in the establishment of these two mutualistic symbioses (Oldroyd, 2013) (Figure 5). The first step is the perception by microbes of plant-secreted flavonoids (Abdel-Lateif *et al.*, 2012) which will trigger the production of symbiotic signals by microbes. So called Nod and Myc factors which are (Lipo-)chitooligosaccharides derivatives (Gough and Cullimore, 2011) will be in turn recognize by the plant through receptor-like kinases receptor molecules of the LysM and SYMRK family (Gust *et al.*, 2012; Oldroyd, 2013). This molecular interaction will lead, through yet unclear mechanisms, to an essential symbiotic nuclear calcium oscillation dependent on cation channels located on the nuclear membranes. This will trigger transcriptional reprogramming leading to developmental processes which enable the symbionts endocellular accommodation through the formation of specialized organs: nodules, arbuscules and actinorhizal roots. Of particular interest is the fact that a large part of the genes implicated in the previously described molecular events are highly conserved among plant-microbe symbiosis (Figure 5). This symbiotic "toolkit" is referred as the common symbiosis signaling pathway. Some of these genes were even already present, for the early signaling steps, in the algal ancestor of land plants (Delaux *et al.*, 2015).

### Plant-microbe associative symbioses

These mutualistic plant-microbe symbioses can be considered as highly “advanced” ones, as undoubtedly, it is the result of a long-term co-evolution process (Martínez-Romero, 2009). This could not have happened if very important benefits were not shared by both partners. Although it is less clear for the mycorrhizal symbiosis, as P is commonly not accessible to land plants, it is very clear for rhizobial symbiosis, which appears to be an “extreme example”. Indeed, the biological nitrogen fixation process is very costly in terms of required energy (Atkins, 1984), therefore, if plants can fulfill their N nutrition “on their own”, they would have “no interest” in the investment needed for the establishment of an “efficient symbiosis”. Another fact supporting this view is that the availability of N negatively impacts the beneficial effects of this mutualistic association for the plant (Heath and Tiffin, 2007). However, in contrast to these two classical cases, numerous other examples of beneficial plant-microbe examples exist and have also been extensively studied.

Table 2 : Non-exhaustive list of microbial taxons comprising Plant Growth Promoting Microbes (From Ahemad *et al.*, 2014, Hayat *et al.*, 2010, Hossain *et al.*, 2017 and Jousset, 2017)

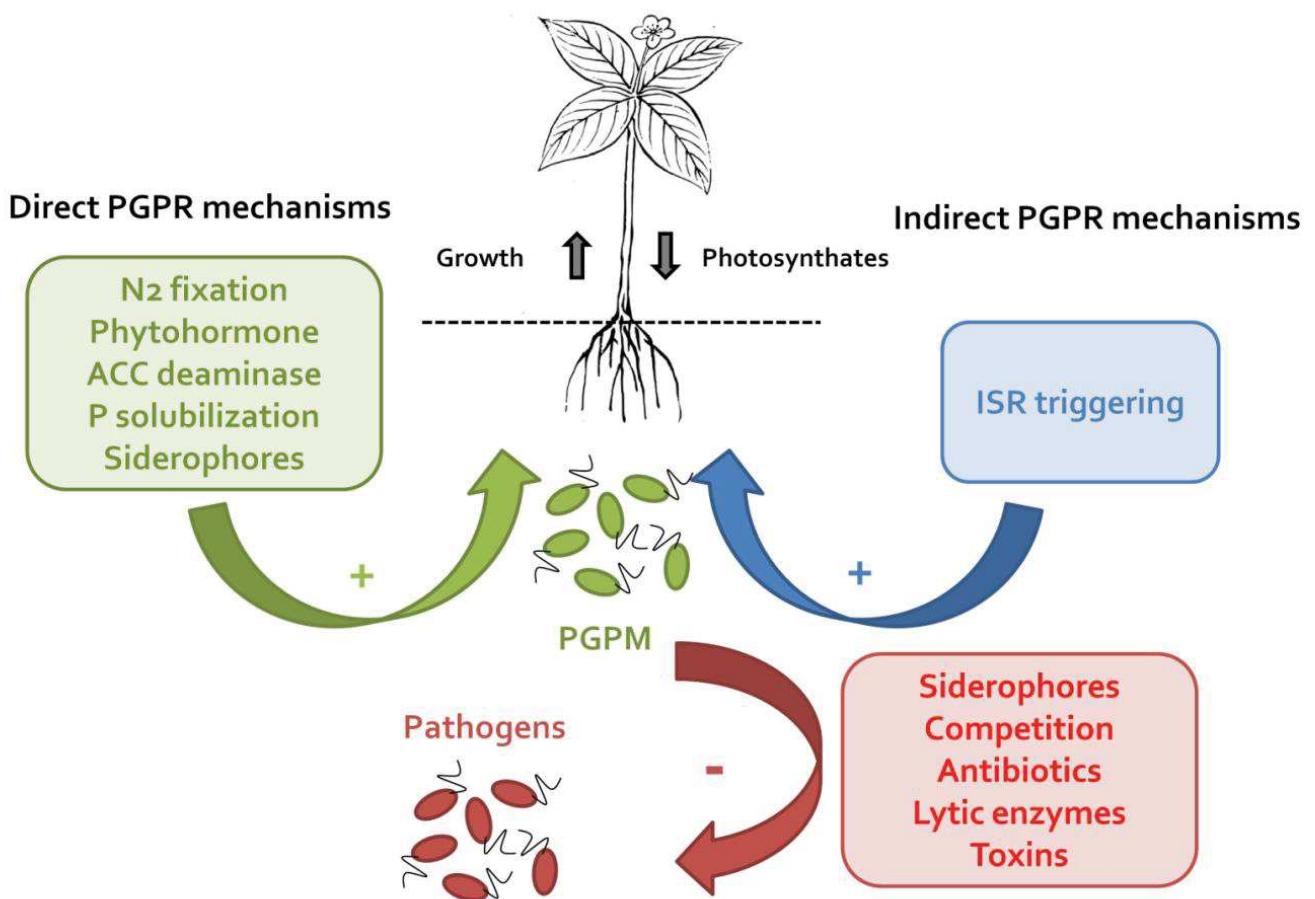
PGPR taxons	Genus	PGPF taxon	Genus	Protists taxon	Genus
<i>Proteobacteria</i>	<i>Acetobacter</i>	<i>Ascomycota</i>	<i>Aspergillus</i>	<i>Alveolata</i>	<i>Tetrahymena</i>
	<i>Achromobacter</i>		<i>Aureobasidium</i>	<i>Rhizaria</i>	<i>Cercomonas</i>
	<i>Acinetobacter</i>		<i>Chaetomium</i>		<i>Heteromita</i>
	<i>Azoarcus</i>		<i>Cladosporium</i>	<i>Euglenozoa</i>	<i>Bodo</i>
	<i>Azomonas</i>		<i>Colletotrichum</i>	<i>Excavata</i>	<i>Jakoba</i>
	<i>Azospirillum</i>		<i>Exophiala</i>	<i>Amoebozoa</i>	<i>Acanthamoeba</i>
	<i>Bradyrhizobium</i>		<i>Penicillium</i>	<i>Oomycota</i>	<i>Phytophthora</i>
	<i>Brevundimonas</i>		<i>Trichoderma</i>		<i>Pythium</i>
	<i>Burkholderia</i>		<i>Fusarium</i>		
	<i>Citrobacter</i>		<i>Gliocladium</i>		
	<i>Delftia</i>		<i>Phoma</i>		
	<i>Enterobacter</i>		<i>Phomopsis</i>		
	<i>Erwinia</i>		<i>Purpureocillium</i>		
	<i>Gluconacetobacter</i>		<i>Talaromyces</i>		
	<i>Herbaspirillum</i>	<i>Basidiomycota</i>	<i>Limonomycetes</i>		
	<i>Klebsiella</i>		<i>Rhodotorula</i>		
	<i>Kluyvera</i>		<i>Rhizoctonia</i>		
	<i>Mesorhizobium</i>		<i>Piriformospora</i>		
	<i>Ochrobactrum</i>	<i>Zygomycota</i>	<i>Mucor</i>		
	<i>Pantoae</i>		<i>Rhizopus</i>		
	<i>Paraburkholderia</i>				
	<i>Phyllobacterium</i>				
	<i>Pseudomonas</i>				
	<i>Psychrobacter</i>				
	<i>Rahnella</i>				
	<i>Rhizobium</i>				
	<i>Serratia</i>				
	<i>Sinorhizobium</i>				
	<i>Sphingomonas</i>				
	<i>Stenotrophomonas</i>				
	<i>Variovorax</i>				
	<i>Xanthomonas</i>				
<i>Bacteroidetes</i>	<i>Flavobacterium</i>				
<i>Actinobacteria</i>	<i>Arthrobacter</i>				
	<i>Cellulomonas</i>				
	<i>Mycobacterium</i>				
	<i>Rhodococcus</i>				
<i>Firmicutes</i>	<i>Bacillus</i>				
	<i>Brevibacillus</i>				
	<i>Paenibacillus</i>				

All of them have been regrouped under the name of associative symbioses or cooperations (Barea *et al.*, 2005). These interactions are defined as facultative, exhibit large host and symbionts spectrum and do not require the formation of specialized organs (Hayat *et al.*, 2010) in clear opposition to the previously introduced plant-microbe mutualistic interactions. In terms of phylums, multiple clades of microbes can be implicated in such interactions : The most studied ones are bacteria (Hayat *et al.*, 2010), fungi (Hossain *et al.*, 2017) and protists (Gao *et al.*, 2019) (Table 2). However, the possibility to identify plant-beneficial oomycetes, archeabacteria and even viruses can't be rule out as such organisms are also important parts of the plant microbiome. Although facultative beneficial microbes can inhabit the phyllosphere, most studies focused on root and rhizosphere microbiome. In order to exhibit beneficial effects while interacting with plants, microbes must firstly harbor specific traits to interact with plants. A number of functions have been linked to rhizosphere competence, for example: motility, attachment, stress resistance, production of secondary metabolites (Barret *et al.*, 2011; Compant *et al.*, 2010). However harboring these functions is not sufficient to exhibit strong beneficial effects in plants. A wide diversity of functions and activities has been described as "plant-growth promoting traits". Their identification mainly emanates from the study of so-called Plant-Growth Promoting Rhizobacteria (PGPR), an expression coined by J.W. Kloepper in the late 1970s (Kloepper and Schroth, 1978; Suslow *et al.*, 1979). Other terms followed this first one: Plant-Growth Promoting Bacteria (PGPB), Plant-Growth Promoting Fungi (PGPF). It is, however, interesting to recognize that the concept of "bacterial fertilization" have already been extensively studied and applied in the soviet union in the 1950s (Brown, 1974).

### **Plant Growth-Promoting traits**

PGP traits can be defined as any biochemical activity known to improve plant growth also encompassing the increased tolerance to diseases (Glick, 2012; Lugtenberg and Kamilova, 2009). Two main categories of PGP traits can be distinguished: Those having a direct impact on plant growth and those having indirect ones. This distinction is based on the fact that the indirect mechanisms exhibit growth promotion exclusively in the context of detrimental biotic interactions (pathogens, parasites, herbivores for example) (Figure 6).

The most studied process is probably the direct improvement of plant nutrition especially for the uptake of N, P and Fe although the nutrition in other (micro-)nutrients can also be



**Figure 6: Mechanisms of Plant Growth Promoting Microbes in enhancing plant growth (Adapted from Nazir *et al.*, 2018)**

Schematic representation of plant growth promoting traits separated between direct (green box) and indirect effects (blue and red boxes), the latter being dependent on the presence of pathogens. In exchange to these growth promotions microbial functions, plants provide photosynthates typically through root exudation. ACC: 1-aminocyclopropane-1-carboxylate, P: Phosphate, ISR : Induced Systemic Resistance

microbe-driven such as Zn, Mn, Cu (Adak *et al.*, 2016). The case of nitrogen is the most extensively studied, indeed so-called "free-living diazotrophs" -mainly bacteria- are able to fix atmospheric N and organic forms of N can be transferred to the plant (Bashan and Levanony, 1990; James *et al.*, 2000). The main studied models are part of the following phylums: *Acetobacter*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Rhizobium* (Kennedy *et al.*, 2004). In second place in terms of importance, comes the inorganic phosphate solubilization (Sharma *et al.*, 2013), this time being performed by both bacteria - main phylums being *Pseudomonas*, *Bacillus*, and *Rhizobium*- (Rodríguez and Fraga, 1999) and fungi - main phylums being *Aspergillus* and *Penicillium*- (Whitelaw, 1999). The underlying mechanisms encompass inorganic and enzymatic processes. Finally, iron availability for plants is also crucially dependent on different microbial activities. Main examples are soil acidification and iron sequestration by microbial siderophores that can be further on uptaked by plant roots when complexed with iron ions (Mimmo *et al.*, 2014).

Other major PGP traits are related to the hormonal regulation of plant physiology. Indeed the production of phytohormones -or analogs- as well as the modulation of the hormonal signaling is widespread among Plant-Growth Promoting Microbes (PGPM). Most literature about these processes focused on rhizobacteria (Dodd *et al.*, 2010), however plant-associated fungi -both beneficial and detrimental- are responsive and/or can modulate plant hormonal status (Chanclud and Morel, 2016; Hermosa *et al.*, 2012). The most studied hormonal pathways studied in this context are: abscisic acid (ABA), cytokinin(s) (CKs), ethylene, gibberellins (GAs), auxins (IAA) which are commonly referred as developmental hormones. But also defense-related hormones such as salicylic acid (SA) and jasmonic acid (JA) can be modulated by the interactions with PGPM (Dodd *et al.*, 2010). Two other classes of hormones are emerging in broad plant biology and will most likely be shown to be implicated in plant-PGPM interactions: Strigolactones (SLs) (Foo and Reid, 2013) and Brassinosteroids (BRs) (Kim and Wang, 2010). The molecular mechanisms underlying plant hormonal status modulation by PGPM will be discussed later on.

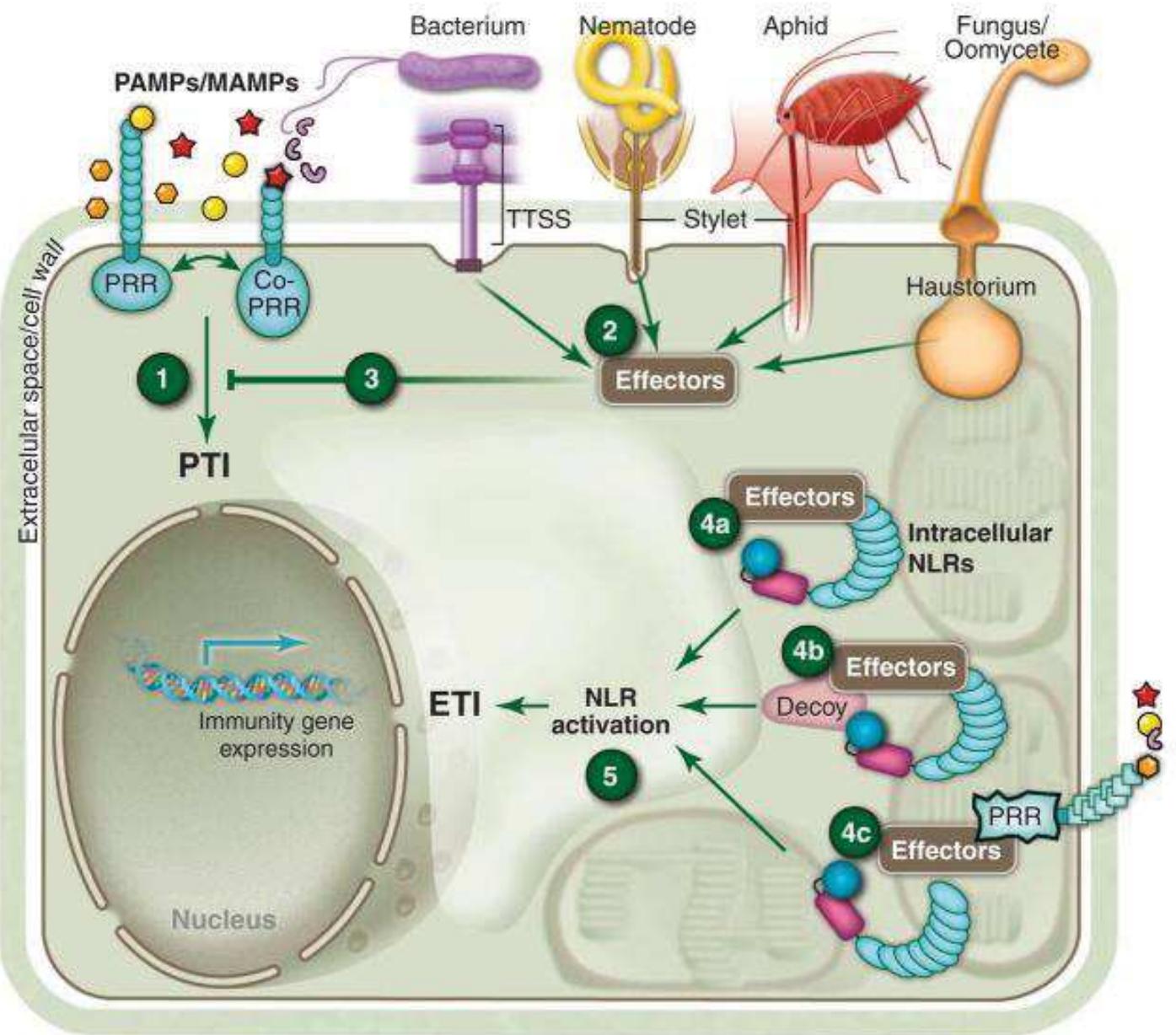
Another microbe-driven PGP activity is phytoremediation, indeed the decontamination of toxic trace elements by members of the rhizosphere can eventually lead to a plant growth promotion (Khan, 2005). The last direct mechanisms by which associative symbioses promote plant growth is the production of secondary metabolites (Brader *et al.*, 2014). This has been extensively studied for endophytes interacting with "niche plant models" such as medicinal plants (Kaul *et al.*, 2012) and grasses (Bush *et al.*, 1997). The microbe-driven synthesis of



secondary metabolites can enhance the potential of acclimation of plants to environmental stresses (Kuldau and Bacon, 2008; Torres *et al.*, 2012) as well as plant adaptation (Lane *et al.*, 2000).

For the indirect mechanisms of plant growth promotion, the ones having beneficial effects in the context of pathogen presence, two categories can be distinguished: The processes that rely on microbe-microbe interactions and those relying on microbe-triggered plant response (which will be discussed further on). The first category is based on the fact that important microbe-microbe interactions occur in the plant environment and shapes the outcoming plant productivity (Figures 1 and 6) (Andrews, 1992; Hassani *et al.*, 2018; Raaijmakers *et al.*, 2009). Antagonism between microbes is the most studied process of this category (Beneduzi *et al.*, 2012). Several molecular processes can lead to this outcome: (i) Antibiosis through the synthesis of antibiotics, enzymes and volatiles (Raaijmakers and Mazzola, 2012) (ii) Competition for the niche, the most prominent example being the competition for iron through the secretion of siderophores (Höfte and Bakker, 2007; Kloepper *et al.*, 1980) (iii) Interference of the quorum sensing (Dutta and Podile, 2010) (iv) Predation (Gao *et al.*, 2019).

A number of PGP mechanisms are dependent on the modulation of plant physiology. (i) Alteration of plant phenology by accelerating or shortening parts of their life cycle (Bresson *et al.*, 2013; Poupin *et al.*, 2013) (ii) Impacting plant development, particularly by hijacking plant hormonal synthesis and/or signaling (Vacheron *et al.*, 2013; Verbon and Liberman, 2016). For example, root system architecture can be impacted by PGPM. One of the typical responses of root system to PGPM is the reduction of root elongation and the increase of ramification, leading to a more efficient exploration of soil for nutrients. This process is dependent on auxin signaling and relies on the IAA/CK balance which is central to the development of roots (Contesto *et al.*, 2010). (iii) Plant-dependent recruitment of beneficial microbes. Indeed, very recently, the idea that infected plants were able to enrich microbes triggering an enhanced tolerance to pathogen was experimentally proven (Berendsen *et al.*, 2018). (iv) Increase of plants' tolerance to stresses. One of the main mechanisms underlying this effect is the production of l-aminocyclopropane-l-carboxylate (ACC) deaminase by microbes (Glick *et al.*, 2007). This enzyme will decrease the plant ethylene levels, considered as a "stress-related" hormone, by degrading ACC, a precursor of ethylene biosynthesis. Consequently, ACC deaminase-producing microbes can basically counteract stress response



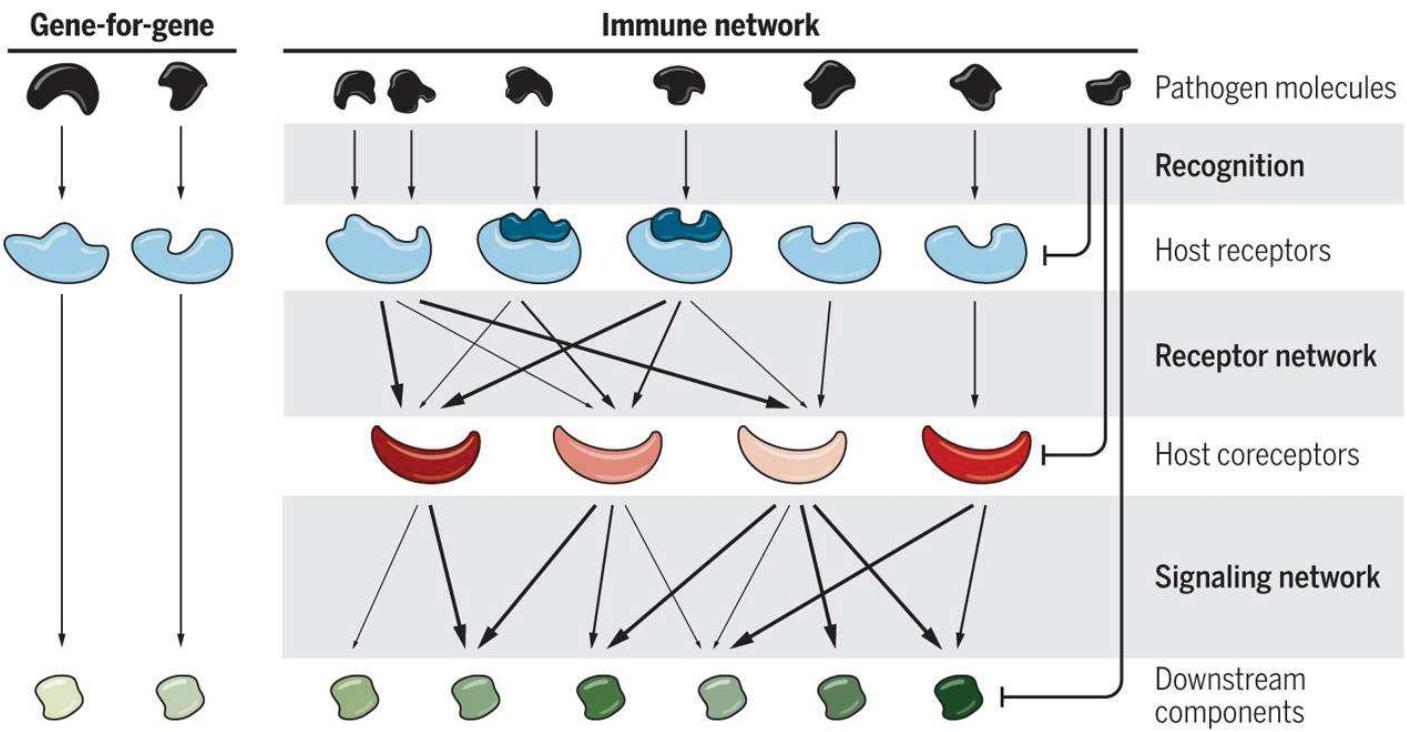
**Figure 7: Plant innate immunity overview (From Dangl *et al.*, 2013)**

Pathogens of all lifestyle classes express PAMPs and MAMPs as they colonize plants. Plants perceive these via extracellular PRRs and initiate PRR-mediated immunity (PTI; step 1). Pathogens deliver virulence effectors to both the plant cell apoplast to block PAMP/MAMP perception (not shown) and to the plant cell interior (step 2). These effectors are addressed to specific subcellular locations where they can suppress PTI and facilitate virulence (step 3). Intracellular NLR receptors can sense effectors in three principal ways: first, by direct receptor ligand interaction (step 4a); second, by sensing effector-mediated alteration in a decoy protein that structurally mimics an effector target, but has no other function in the plant cell (step 4b); and third, by sensing effector-mediated alteration of a host virulence target, like the cytosolic domain of a PRR (step 4c). It is not yet clear whether each of these activation modes proceeds by the same molecular mechanism, nor is it clear how, or where, each results in NLR-dependent effector-triggered immunity (ETI).

in plants, which will maintain growth in stressful conditions. The second process and probably the most studied one is "induced resistance". It relies on the potentiation of immunity upon a pathogen attack following the interaction with beneficial microbes -mainly soil-dwelling ones-. The underlying mechanisms will be discussed later.

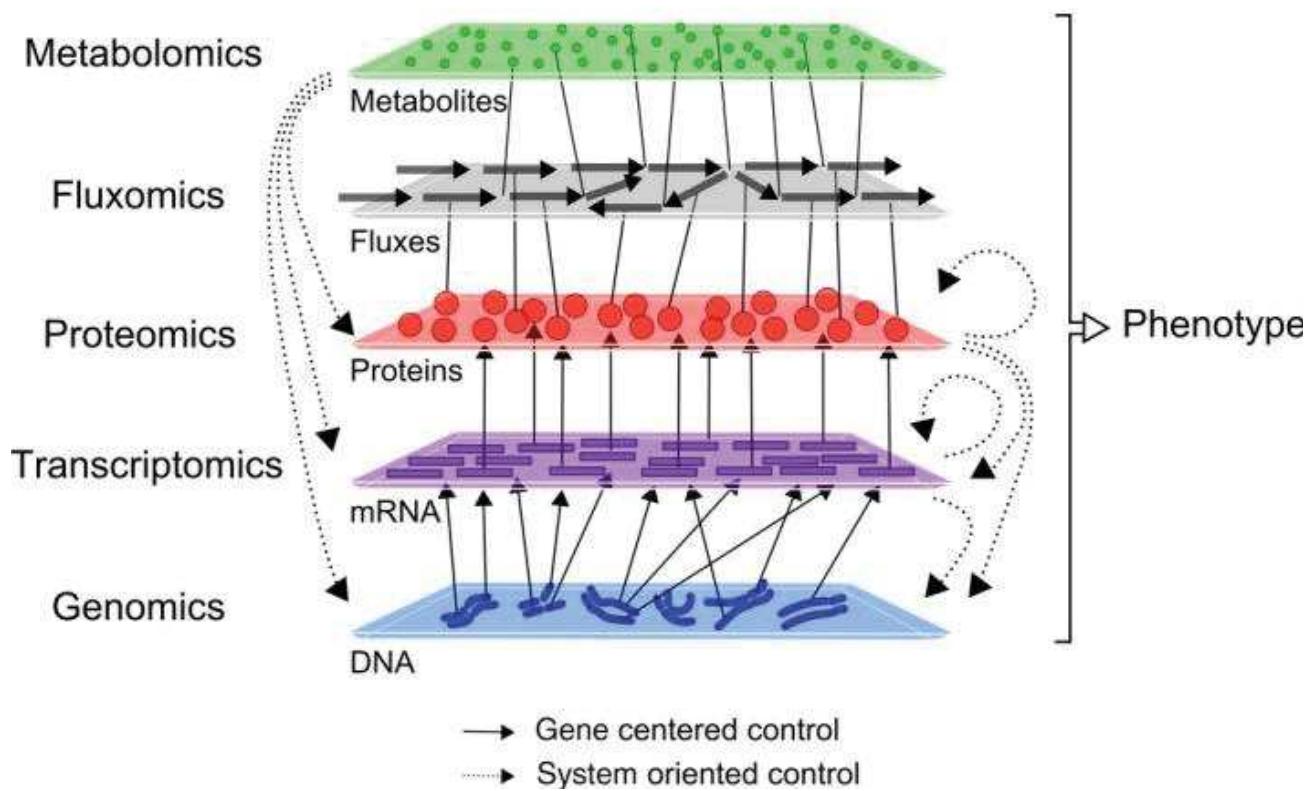
### The immune system of plants

Regarding the plethora of microorganisms interacting with plants, these organisms have evolved a highly sophisticated and dynamic innate immune system (Figure 7) (Cook *et al.*, 2015; Jones and Dangl, 2006). It mainly relies on the recognition of microbe-associated molecular patterns (MAMPs) which usually are molecules conserved among a wide diversity of microbes such as flagellin and chitin (Boller and Felix, 2009). Cell surface-localized pattern recognition receptors (PRRs) are able to physically interact with MAMPs and recruit regulatory receptor kinases. Thus formed complexes activates downstream signaling cascades through receptor-like cytoplasmic kinases which will eventually lead to MAMPs-Triggered Immunity (MTI) (Macho and Zipfel, 2014). A large diversity of PRRs have been identified so far with various specificity to specific MAMPs (Boutrot and Zipfel, 2017) however they have been shown to trigger convergent cellular signaling pathways. This observation recently led to the proposal of an integrated view of this system (Figure 8) (Wu *et al.*, 2018) where downstream co-receptors integrates the signals of the sensors (MAMPs-interacting PRRs) to provide a highly robust immune system to plants with high evolvability potential. PRRs are divided in two classes: (i) Cell surface receptors, which encompasses receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (ii) Intracellular receptors of the nucleotide-binding domain and leucine-rich repeat domain family (NLRs). In plants, the latter are mainly known to interact with so-called effectors or recognize effector-induced modifications of host proteins. Effectors are molecules secreted by microbes through microbial secretion systems, mainly type III (T3SS), to subvert MTI (Göhre and Robatzek, 2008; McCann and Guttmann, 2007) which will lead to effector-triggered susceptibility (ETS). The recognition of effectors by NLRs leads to effector-triggered immunity (ETI) -therefore defined as a Resistance protein- which is characterized by a strong and rapid immune response which usually leads to a programmed cell death, called hypersensitive response (HR). This localized cell death is thought to confine the microbial colonization in order to restrict its propagation (Morel and Dangl, 1997). This classical view of the plant immune system is however more and more questioned consequently to the rise and the democratization of 'omics' approaches.



**Figure 8: Plant receptor networks (From Wu *et al.*, 2018)**

There are three mechanistic layers that contribute to immune complexity; each layer is represented by network nodes that may display functional redundancy, differential dependency (represented by arrow intensity), and specificity. These layers may also be targeted by pathogen effectors.



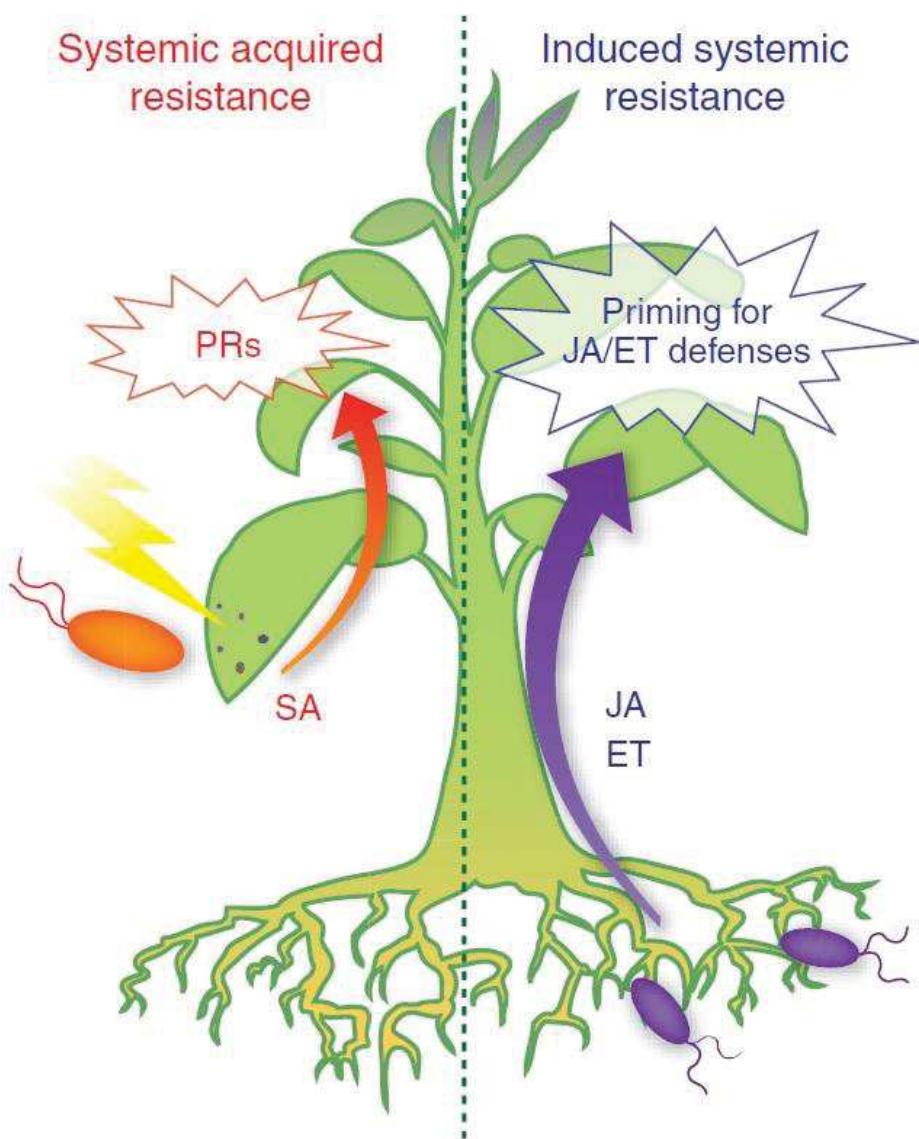
**Figure 9: Biochemical networks interactions drive phenotypic output (From Peyraud *et al.*, 2017)**

The cell organization links genes to transcripts to proteins to fluxes of metabolites and backward regulation of the gene expression via biochemical relationships. Each network layer can be studied using a corresponding methodology (Left)

In this context, systems biology approaches and the conceptual views it brings up, appears very promising to tackle the complexity of molecular plant-microbe interactions (Peyraud *et al.*, 2017; Rodriguez *et al.*, 2019). Systems biology can be defined as all the approaches that aims at understanding the properties of living organisms which emerge at the network (or system) level, particularly through the integration of multiple layers of quantitative data (cells to populations, genomes to metabolites), in order to characterize emerging properties (Figure 9). Such approaches have for example enable to identify a very restricted repertoire of plant immune hubs targeted by both bacterial and oomycetes effectors (Mukhtar *et al.*, 2011). This has consequences in the way we understand plant-pathogen evolution and also supports the previously described robustness of the plant immune system.

The molecular events occurring directly downstream of microbes recognition are mainly signaling processes. Several signaling pathways are implicated in plant immunity: the activation of mitogen-activated protein kinase (MAPK) cascades (Meng and Zhang, 2013), activation of calcium-dependent protein kinases (CDPKs) (Zhang *et al.*, 2014) and modulation of host gene transcription. The latter is achieved through a diverse repertoire of transcriptional regulators such as transcription factors of the AP2/ERF, MYB, NAC, WRKY, MYC/bHLH and TGA/bZIP families (Buscaill and Rivas, 2014). Recent evidences also provide insights in the role of chromatin states in the regulation of plant immunity (Ramirez-Prado *et al.*, 2018). The most particularly well described genes transcriptionally activated during plant immune response, especially HR, are the so-called pathogenesis-related (PRs) genes (Van Loon and Van Strien, 1999). Their transcription leads to the accumulation of antimicrobial proteins which are currently classified in 17 different families (Ebrahim *et al.*, 2011). Additionally, small peptides with antimicrobial activities -such as thionins, defensins and lipid transfer proteins (LTP)- are part of plants defense response (Broekaert *et al.*, 1997)

The other outcomes of defense signaling, at the cellular level, include a number of molecular processes: depolarization of the plasma membrane, ion channels activity modulation, production of reactive oxygen species (ROS), synthesis of antimicrobial compounds, deposition of lignin and callose at the plant cell wall. Among these, a large contribution of the defense response is attributed to metabolic reprogramming, in particular of the phenylpropanoid and oxylipin pathways (La Camera *et al.*, 2004). Indeed, these two pathways lead to the production of compounds having direct anti-microbial effects and some having signaling properties in plants. SA is synthesized through the phenylpropanoid pathway and JA

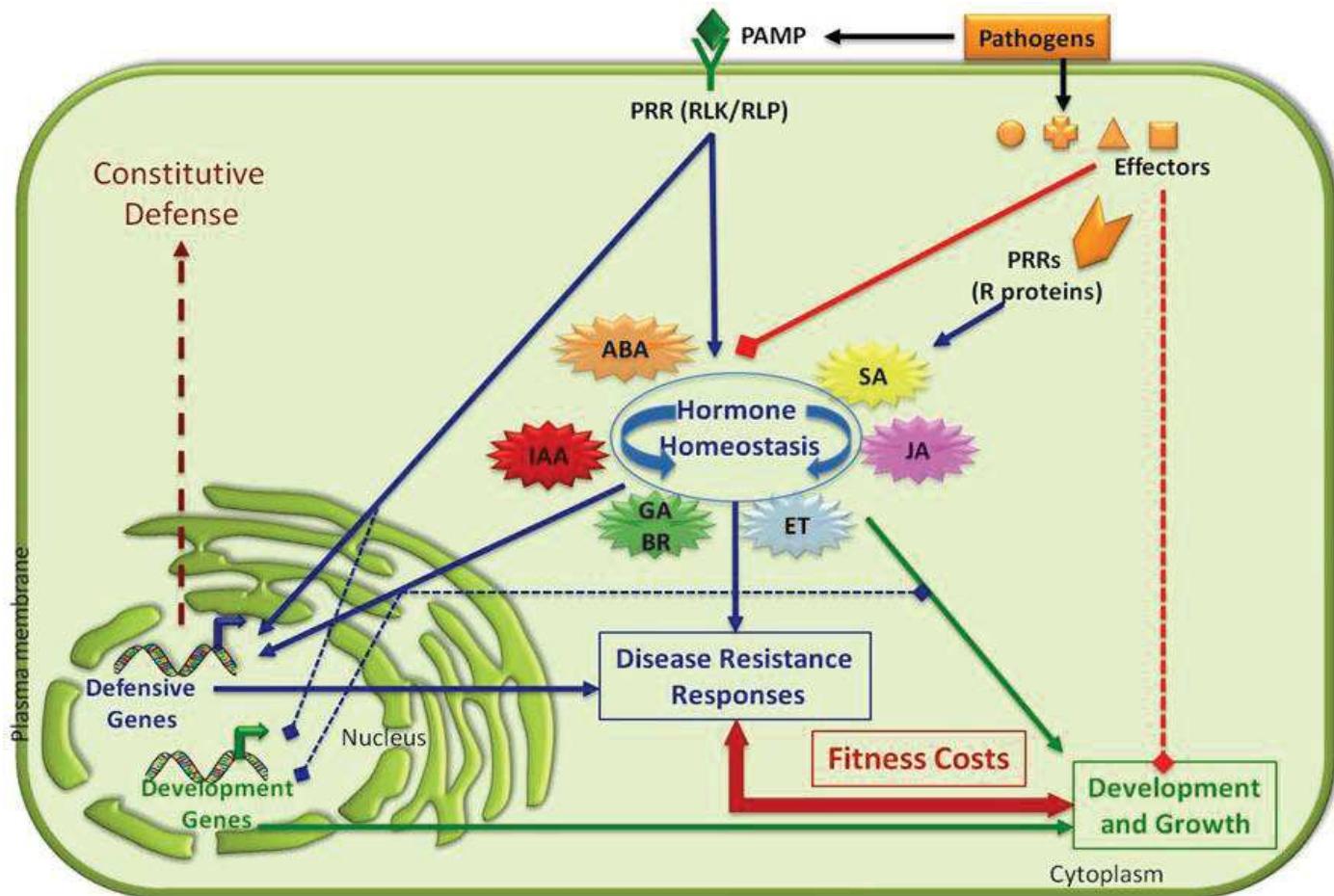


**Figure 10: Schematic representation of systemically induced immune responses (From Pieterse *et al.*, 2009)**

Systemic acquired resistance (SAR) is typically activated in healthy systemic tissues typically following local pathogen detection by an infected leaf. Upon pathogen infection, a mobile signal travels through the vascular system to activate defense responses in distal tissues. Salicylic acid (SA) is an essential signal molecule for the onset of SAR, as it is required for the activation of a large set of genes that encode pathogenesis-related proteins (PRs) with antimicrobial properties. Induced systemic resistance (ISR) is typically activated upon colonization of plant roots by beneficial microorganisms. Like SAR, a long-distance signal travels through the vascular system to activate systemic immunity in above-ground plant parts. ISR is commonly regulated by jasmonic acid (JA)- and ethylene (ET)-dependent signaling pathways and is typically not associated with the direct activation of PR genes. Instead, ISR-expressing plants are primed for accelerated JA- and ET-dependent gene expression, which becomes evident only after pathogen attack. Both SAR and ISR are effective against a broad spectrum of virulent plant pathogens.

through the oxylipin pathway. These two phytohormones have multiple roles in plant development, growth and reproduction but are mainly known to be the immune system signaling "backbone" (Pieterse *et al.*, 2012). The classical view states that SA signaling is effective against biotrophic pathogens while JA signaling against necrotrophic ones and that these two pathways antagonize each other. However, it is now clear that it is a highly simplified representation of hormone signaling in plant defense as other hormones plays important roles in this process: For example, Ethylene, IAAAs, ABA, CKs and BRs (Robert-Seilantianz *et al.*, 2011). This is even truer when stepping out of the *Arabidopsis* and dicot plants models. In rice, SA basal levels are high relatively to dicots and its concentration is not significantly changed upon pathogen attack although SA signaling does support disease resistance. Furthermore, while SA-JA antagonism can be observed in some cases, it appears that these two pathways can trigger a largely common defense response effective against both biotrophic and necrotrophic pathogens (De Vleesschauwer *et al.*, 2014).

While hormones have primordial roles in the establishment of local defense, they are also implicated in the regulation of systemic responses. Indeed, in plants, which lack circulatory systems and specialized immune cells, the perception of microbes can lead to enhance resistance of distal tissues (Figure 10) (Spoel and Dong, 2012). The first example is called systemic acquired resistance (SAR); it is usually triggered by HR and protects unattacked tissues from a wide range of pathogens. This process is dependent on SA synthesis and PR proteins production in distal tissues through the master regulator of SA signaling, NPR1 (Durrant and Dong, 2004). The second immune-related systemic response is called induced systemic resistance (ISR). It is triggered by the roots' perception of beneficial microbes and like SAR, it provides a broad-spectrum resistance to above-ground tissues and is NPR1-dependent (Pieterse *et al.*, 1998, 2014). However, there are major differences between these two systemic responses. Firstly, ISR is JA and ethylene-mediated while SA-independent (Pieterse *et al.*, 1996). Also, unlike SAR, ISR is not accompanied with hormones and PR proteins accumulation (Pieterse *et al.*, 1996, 2000). The enhanced resistance associated with ISR is rather related to a potentiation of defenses, in other words, in the context of a pathogen attack, tissues in the ISR state will trigger a faster and a stronger defense response. This phenomenon is referred as priming and is based on the accumulation of dormant MAPKs, chromatin modifications and alterations of the primary metabolism (Conrath, 2011). This has two major consequences: Firstly, there are no clear ISR marker genes but rather genes whose expression is enhanced upon pathogen subsequent attack, most notably JA-responsive genes



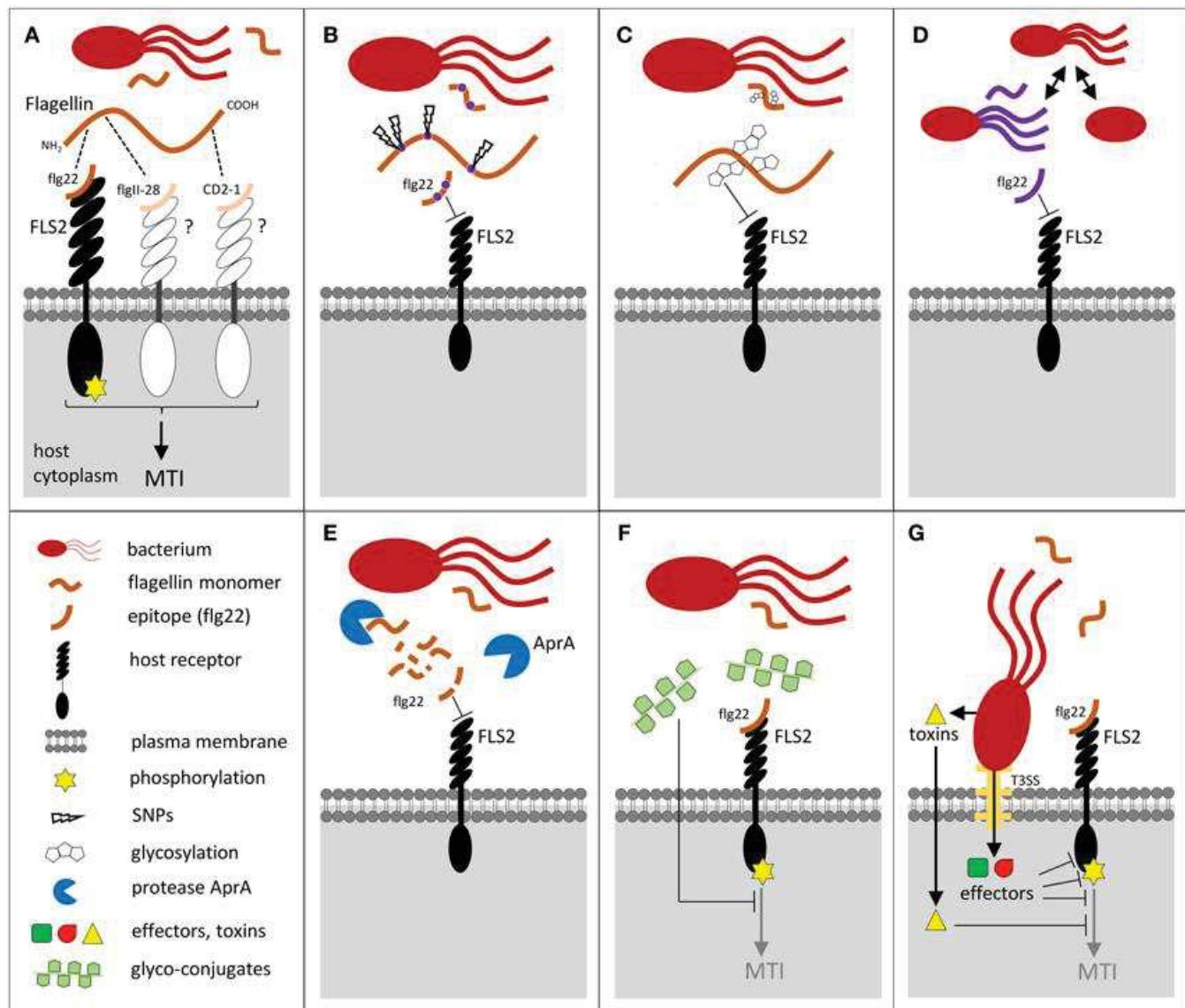
**Figure 11: Balancing plant immune responses and fitness costs (From Denancé *et al.*, 2013)**

Plant disease resistance responses are induced upon recognition of PAMPs/effectors from pathogens and pests by plant PRR proteins. This recognition modulates plant hormonal homeostasis and transcriptional reprogramming of defensive genes. The activation of these inducible resistance responses (PTI and/or ETI) negatively regulates the expression of developmental-associated genes impacting on plant fitness costs. Effectors from pathogens interfere with hormonal balance and the activation of PTI and ETI. Positive and negative interactions are indicated by arrows and squares, respectively. ABA: Abscisic acid, IAA: Auxins, ET: Ethylene, JA: Jasmonic acid, SA: Salicylic acid, GA: Gibberellic acid, BR: Brassinosteroids.

(Wees *et al.*, 1999). Secondly, the establishment of ISR does not imply an important energetic and metabolic investment compared to SAR. This has major consequences in the context of the trade-off that exists between growth and defense in plants.

### The growth–defense trade-off in plants

This concept emanates from the study of ecological lifestyles where organisms with short life cycles invest less energy in defense, in comparison to growth and development. Oppositely, organisms which have to bear with environments fluctuations throughout a longer life cycle have to invest more in stress tolerance processes. Recently, a number of molecular processes have been identified as implicated in this balance to optimize plants fitness (Huot *et al.*, 2014). In particular, hormone signaling and crosstalks between the different hormonal pathways appear to have a central role in this trade-off (Figure 11). Namely, major hormonal signaling pathways implicated in this process are CKs (Albrecht and Argueso, 2017), JA (Guo *et al.*, 2018), BRs (Lozano-Durán and Zipfel, 2015) and GAs (Yang *et al.*, 2012). A parallel view of crosstalk emerges in the study of the abiotic environment's impact on plant immunity. Indeed, as stated before, the plant immune system is highly dynamically tunable so that these sessile organisms can quickly acclimate to changing environmental conditions -both biotic and abiotic as well as their interaction- (Nobori and Tsuda, 2019). Indeed factors such as light, temperature, humidity, salinity and nutrient availability significantly impact the regulation of the plant immune system (Hua, 2013). For this latest factor, several examples have already been cited previously, such as the impact of nitrogen availability in the rhizobial symbiosis and phosphate starvation response on plant immunity (Castrillo *et al.*, 2017). Additionally, iron nutrition and homeostasis are emerging as being important in a wide range of plant immune processes. The impact of the availability of this micro-nutrient in planta and its surroundings was already known to be important in the outcome of plant-microbes interactions (Aznar *et al.*, 2015; Lemanceau *et al.*, 2009). Furthermore, recently, the molecular bases of a well-known link between iron nutrition and ISR (Leeman *et al.*, 1996) are starting to be unraveled. Indeed, an important overlap in the transcriptional regulations associated to ISR state and Fe deficiency response as well as identified common regulators were recently described (Romera *et al.*, 2019; Verbon *et al.*, 2017). These statements reinforce the idea of an important link between abiotic conditions and immunity as well as commonalities in the mechanisms influenced and exploited by pathogenic and beneficial microbes during their interaction with plants.



**Figure 12: Potential bacterial strategies employed to evade flagellin recognition upon bacterial invasion in plant tissues. (From Trda *et al.*, 2015)**

(A) Flagellin monomers are typically recognized by FLS2 *via* the flg22 epitope or possibly by other putative receptors detecting the epitopes flgII-28 and CD2-1. The ligand binding triggers receptor kinase phosphorylation and activates defense responses leading to MAMP-triggered immunity (MTI). (B–G) Evasion strategies that prevent FLS2 recognition: (B) Single Nucleotide Polymorphisms within the gene encoding the flagellin epitopes, (C) flagellin post-translational modifications such as glycosylation, (D) several bacterial pathogens are aflagellated, loose flagellin upon colonization, or express alternative flagellins, (E) alkaline protease AprA degrades flagellin. (F) Flagellin-mediated MTI is also inhibited by glyco-conjugates *via* yet poorly understood mechanisms, or (G) by bacterial effectors injected to plant cell by Type-III secretion system (T3SS), or by toxins.

## **Commonalities in pathogenic and beneficial microbes interacting with plants**

Several facts support this view, firstly the role of innate immunity in the establishment and the maintenance of mutualistic symbiosis is increasingly being supported as a crucial one (Gourion *et al.*, 2015). In a wider context, MAMPs have this characteristic of being widely conserved among microbes, encompassing therefore beneficial and detrimental ones (Pel and Pieterse, 2013). Therefore in order to avoid being perceived by plant cells, microbes can degrade, hide or modify their MAMPs (Figure 12) (Trdá *et al.*, 2015). This latest strategy can be exemplified by the mutualistic LCOs which are the result of co-evolution and which perception not only lowers plant defense but also triggers regulations to enable symbiosis establishment (Zipfel and Oldroyd, 2017). Also, the perception of both symbiotic and pathogenic MAMPs is carried by the same classes of receptors, RLKs, among which the most studied are LysM-RLK (Antolín-Llovera *et al.*, 2014; Gust *et al.*, 2012). The particular case of the rice gene *CERK1* is of particular interest as it acts as a pivotal point between immunity and symbiosis. This occurs through the co-receptors it interacts with: When it is associated with CEBIP it will recognize chitin and lead to a defense response, while when associated with NFR5 it will perceive Myc factors and trigger the symbiotic response (Miyata *et al.*, 2014; Zhang *et al.*, 2015). Another major strategy common to symbionts and pathogens to circumvent plant immunity is the injection of effectors in the host cell (Soto *et al.*, 2006) using secretion systems (Deakin and Broughton, 2009). They can act as suppressor of defenses, particularly by interfering with hormone signaling, but also as symbiotic host range specificity determinants (Fauvert and Michiels, 2008). Similar strategies as the one previously mentioned are also implicated in associative symbiosis (Zamioudis and Pieterse, 2012).

## **Plant perception of pathogenic and beneficial microbes**

The commonalities between pathogenic and beneficial microbes raise the question of how plants decipher between the two categories. This is even more crucial for roots which intimately interact with soil microbial communities, among the most dense and diversified microbial habitat on earth (Fierer and Jackson, 2006). Yu and collaborators very recently reviewed the specificities of root immunity with a focus on the interaction with beneficial microbes (2019). It appears that MTI is commonly triggered in roots by beneficial and pathogenic microbes if not escaped through the previously described mechanisms. However, this response is only transitory as it is subsequently suppressed by beneficial microbes. Recent works on rhizospheric *Pseudomonas fluorescens* showed that it is an active microbial process

(Stringlis *et al.*, 2018). For more invasive beneficial interactions, it has been proven that effectors, symbiotic signals or modified MAMPs can counteract different levels of plant immune signaling, such as the ROS burst, MAPK cascades and hormone signaling. For this latest example, several examples points to the suppression of JA signaling or processes dependent on this pathway with examples from ectomycorrhizal fungi (Plett *et al.*, 2014), PGPF (Jacobs *et al.*, 2011) and PGPR (Lakshmanan *et al.*, 2012). Also, the fact that there is a tissue- and cell type-specific expression of the *FLS2* gene in roots (Beck *et al.*, 2014) suggest that root "desensitize" their epidermis to prevent constitutive defense response in this cell layer regarding the microbe-rich environment that is soil. Finally, root are not only able to control the colonizing microbes but also shapes the rhizosphere microbiota through the exudation of coumarins, benzoxazinoids, triterpenes and camalexins (Yu *et al.*, 2019).

The vast majority of the previously described findings have been studied under laboratory settings, in the context of binary interactions between a pathogenic or symbiotic species and a host plant. However, has stated before, in natural conditions healthy plants live in association with a multitude of microorganisms. Therefore, although our current understanding of the interplay between innate immunity and plant microbiota remains sparse, pioneering research efforts are undergoing to propose a broad framework of their interconnection (Hacquard *et al.*, 2017). Several examples of the conceptual consequences of this change of scale will be hereafter listed.

Although MAMPs are considered highly conserved among microbes, notable genetic variations can be found in the epitopes residues -at the species or even strain level- which are not impacting the structure and the function of the whole macromolecules (Sun *et al.*, 2006). Also, a given MAMPs can induce variable defense response intensity depending on the interacting host plant (Vetter *et al.*, 2012). Similarly, although the detection of bacterial flagellin epitope flg22 is carried out among many species of angiosperms and gymnosperms by the widely distributed FLS2 receptor (Albert *et al.*, 2010), most known PRRs are restricted to a limited range of plant families or species (Boller and Felix, 2009). These two facts support the idea that plants evolved PRRs repertoires in relation to a given MAMPs diversity within their associated microbiome at the local scale to optimize overall fitness. Also, it suggests that the PRRs repertoires can act as host range determinants of successful microbial colonizers contributing therefore to the differences in the community profiles when comparing ecotypes and species (Schlaeppi *et al.*, 2014). Furthermore, this could make plants less vulnerable to an eventual MTI suppression by a unique member of the microbiota.

Indeed, as MTI signaling downstream of the previously described sensors is carried out by integration hubs, one event of MTI suppression could "open the gate" to the rest of the microbial community.

To this regard, taking apart all the previously described microbial strategies to avoid PRRs-driven detection, it appears that commensals and beneficial members of the microbiota do activate MTI (Vogel *et al.*, 2016). In addition, the fact that T3SS-lacking pathogens are able to colonize the apoplast without causing symptoms (Hanemian *et al.*, 2013) enables to propose that MTI act as a mechanism to control microbial abundance within plants tissues. Therefore, in the same way pathogens and mutualistic microbes are able to actively suppress MTI using effectors; it is probable that abundant microbiota members, and especially endophytes, apply the same strategy. However, the existence of alternative strategies can't be excluded. This has major consequences regarding the selective pressure on NLRs -or NLR-like immune receptors-, which have to enable pathogens and beneficial symbionts recognition at the same time with opposite outcomes in terms of plant fitness. Finally, defense signaling mediated by the hormones SA, JA and ethylene are important drivers of plant microbiota composition (Lebeis *et al.*, 2015) contrarily to MTI which could act as a general mechanism controlling potential microbial overload.

### **Omics to unravel the complexity of plant-microbiome interactions**

The democratization of next-generation sequencing and associated high-throughput technologies enables to study the complexity of plant-microbiome interaction. Indeed, these techniques enable to analyze without preconceptions and can provide totally novel knowledge. Multiple "-Omics" approaches enable to accumulate data at different level of regulation and resolution: Genomics, transcriptomics, proteomics and metabolomics as well as these same approaches in complex microbial communities therefore having the "meta-" prefix (Knief, 2014). Several publications have listed the potential of such analyses for several plant-microbe models (Kaul *et al.*, 2016; Kroll *et al.*, 2017; Lorito *et al.*, 2010; Salvioli and Bonfante, 2013).

These approaches have been applied to understand plant response during their interaction with microbes: i) for plant-pathogens, in particular the comparison of the response of susceptible and resistant varieties (AbuQamar *et al.*, 2016; Eulgem, 2005); ii) for plant-mutualists interactions, in particular the molecular regulations induced during the development of endosymbiosis were analyzed (Manthey *et al.*, 2004; Siciliano *et al.*, 2007). Also, more

Table 3: Studies analyzing transcriptomic response of *Arabidopsis thaliana* to bacterial associative symbiosis

<b>Ecotype</b>	<b>Bacterial strain</b>	<b>Phenotype</b>	<b>Method</b>	<b>Tissues</b>	<b>Reference</b>
<i>A. thaliana</i> C24	<i>Paenibacillus polymyxa</i> B2	ISR + drought tolerance	RNA-DD	Leaves	Timimusk and Wagner, 1999
<i>A. thaliana</i> WS-0	<i>Pseudomonas thivervalensis</i> MLG45	ISR	Microarray	Leaves and roots	Cartieaux <i>et al.</i> , 2003
<i>A. thaliana</i> Col-0	<i>Pseudomonas fluorescens</i> WCS417	ISR	Microarray	Leaves and roots	Verhagen <i>et al.</i> , 2004
<i>A. thaliana</i> Col-0	<i>Pseudomonas fluorescens</i> FPT9601-T5	ISR	Microarray	Leaves	Wang <i>et al.</i> , 2005
<i>A. thaliana</i> Col-0	<i>Bradyrhizobium</i> sp. ORS278	ISR	Microarray	Leaves	Cartieaux <i>et al.</i> , 2008
<i>A. thaliana</i> Col-0	<i>Pseudomonas fluorescens</i> Pf-5 and GM30	ISR	Microarray	Leaves and roots	Weston <i>et al.</i> , 2012
<i>A. thaliana</i> Col-0	<i>Pseudomonas fluorescens</i> SS101	ISR	Microarray	Leaves and roots	van de Mortel <i>et al.</i> , 2012
<i>A. thaliana</i> Col-0	<i>Bacillus subtilis</i> FB17	ISR	Microarray	Roots	Lakshmanan <i>et al.</i> , 2013
<i>A. thaliana</i> Col-0	<i>Pseudomonas fluorescens</i> WCS417	ISR	RNA-Seq	Roots	Stringlis <i>et al.</i> , 2017
<i>A. thaliana</i> Col-0	<i>Bacillus amyloliquefaciens</i> FZB42	Salt tolerance	RNA-seq	Shoots	Liu <i>et al.</i> , 2017
<i>A. thaliana</i> Col-0	<i>Paraburkholderia phytofirmans</i>	Salt tolerance	Microarray	Whole plants	Poupin <i>et al.</i> , 2013
<i>A. thaliana</i> Col-0	<i>Pseudomonas</i> sp. G62	Growth promotion	Microarray	Whole plants	Schwachtje <i>et al.</i> , 2011

Table 4: Studies analyzing transcriptomic response of monocot model species to bacterial associative symbiosis

<b>Plant</b>	<b>Bacterial strain</b>	<b>Phenotype</b>	<b>Method</b>	<b>Tissues</b>	<b>Ref</b>
<i>Oryza sativa</i> cv. 9311	<i>Bacillus amyloliquefaciens</i> FZB42	ISR	RNA-seq	Leaves and roots	Xie <i>et al.</i> (2017)
<i>Oryza sativa</i> cv. Nipponbare	<i>Herbaspirillum seropedicae</i> SmR1	Diazotrophic endophyte	RNA-Seq	Roots	Brusamarello-Santos <i>et al.</i> (2019)
<i>Oryza sativa</i> cv. Cigalon and Nipponbare	<i>Azospirillum lipoferum</i> 4B or sp. B510	Growth promotion	microarrays	Roots	Drogue <i>et al.</i> (2014)
<i>Oryza sativa</i> cv. Nipponbare	<i>Azospirillum brasilense</i> Sp245	Growth promotion	RNA-Seq	Roots	Thomas <i>et al.</i> (2019)
<i>Oryza sativa</i> cv. TKM9	<i>Bacillus subtilis</i> RR4	Growth promotion	RNA-Seq	Roots	Rekha <i>et al.</i> (2018)
<i>Triticum aestivum</i> var. CD104	<i>A. brasiliense</i> FP2	PGPR	dual RNA-Seq	Roots	Camilios-Neto <i>et al.</i> (2014)
<i>Saccharum officinale</i> cv. Q208	<i>Burkholderia</i> strain Q208	Diazotrophic endophyte	dual RNA-Seq	Roots	Paungfoo-Lonhienne <i>et al.</i> (2016)
<i>Oryza sativa</i> cv. IR36	<i>Azoarcus</i> sp. BH72	Diazotrophic endophyte	SSH + qPCR	Roots	Chen <i>et al.</i> (2015)

recently, studies provided comparative analysis of pathogenic and beneficial plant-microbe interactions (Giovannetti *et al.*, 2015; Guimil *et al.*, 2005; Kelly *et al.*, 2018) reviewed by Plett and Martin (2018). In addition to the fact that these approaches provide deeper and more dynamic understanding of well-established plant-microbe models, they enabled to study more efficiently discrete plant-microbe interactions such as commensalism and associative symbiosis. This latest category of interaction is of particular interest as it provides new possibilities for sustainable agricultural practices, especially for crops. However, this symbiotic relationship needs to be thoroughly understood for its potential to be well exploited.

The first studies focusing on the transcriptional response of plants to associative symbiosis were performed with *A. thaliana* in the context of induced systemic resistance (ISR) in order to identify marker genes (Table 3). This was reviewed by van Loon (2007) which showed that relatively few genes were transcriptionally regulated in the ISR state. Cartieaux and collaborators (2003) also reviewed the hormonal pathways implicated in plant growth promotion by rhizobacteria. Finally, more recently, Carvalho and collaborators (2016) reviewed the genetic and chromatin-mediated regulations implicated in the perception of rhizospheric and endophytic microbes with a focus on diazotrophic bacteria by non-leguminous plants.

Here we will review the physiological processes transcriptionally regulated in response to bacterial associative symbiosis revealed by genome-wide analysis, with a focus on monocots (Table 4). Indeed, while other genome-wide analysis approaches focused on the proteome (Chinnasamy, 2005; Kwon *et al.*, 2016), metabolome (Chamam *et al.*, 2013; Valette *et al.*, 2019; Walker *et al.*, 2011), association genetics (Remans *et al.*, 2008; Valente, 2018; Wintermans *et al.*, 2016) in the context of beneficial associative symbiosis, transcriptomic analysis have been applied in more models and addressing various fundamental questions (Table 5).

### **Review of transcriptomic responses of monocots to bacterial associative symbiosis**

Many recent studies focused their analysis on the regulation of defense-related genes in roots following inoculation. Indeed, previous studies in mutualistic symbiotic models reported the down-regulation of defense-related genes by the host plant. This is consistent with the idea that host plants, in order to accommodate their beneficial symbionts must induce an immune tolerance. And, indeed, Pathogenesis-Related genes, known to be transcriptional markers of plant disease resistance, appear to be down-regulated during the colonization of roots by

Table 5: Differentially expressed plant genes in response to bacterial associative symbiosis revealed by transcriptomic approaches

Host plant	Physiological process	Transcriptionally-regulated genes in roots		Reference
		Up	Down	
<b>Defense</b>				
Rice	<i>Bacillus amyloliquefaciens</i> FZB42	<i>FLS2, BAK1, EFR</i> , Resistance genes	<i>WRKY29, MYC2, WAK22, 5 PRs, Thionins genes, phytoalexine synthesis genes, WRKY46</i>	Xie <i>et al.</i> (2017)
Rice	<i>Herbaspirillum seropedicae SmR1</i>	<i>EDS1, WRKY51</i>		Brusamarello-Santos <i>et al.</i> (2019)
Rice	<i>Azospirillum sp.</i>	PR genes, Defense signaling, receptors	<i>WRKY62, WRKY8, WAK, Nramp6, PRs LysM-RLK</i>	Drogue <i>et al.</i> (2014)
Rice	<i>Azospirillum brasilense</i> Sp245		<i>Chitinases, PRs, Thionins</i>	Thomas <i>et al.</i> (2019)
Rice	<i>Bacillus subtilis</i> RR4		<i>PRs, Thionin</i>	Rekha <i>et al.</i> (2018)
Wheat	<i>A. brasiliense</i> FP2		<i>Resistance genes, WAK1, WRKY45</i>	Camilos-Neto <i>et al.</i> (2014)
Sugarcane	<i>Burkholderia Q208</i>	<i>FLS2, MPKK</i>	<i>BAK1, MPKK</i>	Paungfoo-Lonhienne <i>et al.</i> (2016)
Rice	<i>Azoarcus sp.</i> BH72		<i>Thionin</i>	Chen <i>et al.</i> (2015)
Rice	<i>Bacillus amyloliquefaciens</i> FZB42	JA	<i>AOS</i>	Xie <i>et al.</i> (2017)
Rice	<i>Bacillus subtilis</i> RR4		<i>LOX, JA-responsive Myb</i>	Rekha <i>et al.</i> (2018)
<b>Hormonal signaling</b>				
Rice	<i>Bacillus amyloliquefaciens</i> FZB42	Auxin	<i>SAUR, YUCCA</i>	Xie <i>et al.</i> (2017)
Rice	<i>Herbaspirillum seropedicae SmR1</i>		<i>Auxin responsive genes</i>	Brusamarello-Santos <i>et al.</i> (2019)
Rice	<i>Azospirillum sp.</i>		<i>IAA12</i>	Drogue <i>et al.</i> (2014)
Rice	<i>Azospirillum brasilense</i> Sp245		<i>ARF, Auxin efflux carrier, SAUR, Auxin-responsive genes</i>	Thomas <i>et al.</i> (2019)
Rice	<i>Bacillus subtilis</i> RR4		<i>IAA20, SAURI</i>	Rekha <i>et al.</i> (2018)

Wheat	<i>A. brasiliense</i> FP2	<i>SAUR</i>	<i>ARF3</i> , Auxin induced	Camilios-Neto <i>et al.</i> (2014)
Rice	<i>Azoarcus sp.</i> BH72		<i>IAA17, ARF6</i>	Chen <i>et al.</i> (2015)
Rice	<i>Bacillus amyloliquefaciens</i> FZB42		<i>ERF, ACO</i>	Xie <i>et al.</i> (2017)
Rice	<i>Herbaspirillum seropedicae SmR1</i>	Ethylene	<i>ACC synthase, ERF</i>	Brusamarello-Santos <i>et al.</i> (2019)
Rice	<i>Azospirillum brasilense</i> Sp245		<i>ACO</i>	Thomas <i>et al.</i> (2019)
Rice	<i>Bacillus subtilis</i> RR4		<i>ERF3</i>	Rekha <i>et al.</i> (2018)
Wheat	<i>A. brasiliense</i> FP2		<i>ERF5</i>	Camilios-Neto <i>et al.</i> (2014)
Sugarcane	<i>Burkholderia Q208</i>		<i>ACO</i>	Paungfoo-Lonhienne <i>et al.</i> (2016)
Rice	<i>Bacillus amyloliquefaciens</i> FZB42	ABA	<i>ACS, ACO</i>	Xie <i>et al.</i> (2017)
Rice	<i>Azoarcus sp.</i> BH72		Zeaxanthin epoxidase,	
Rice	<i>Bacillus amyloliquefaciens</i> FZB42		ABA hydroxylase	Xie <i>et al.</i> (2017)
Rice	<i>Azospirillum sp.</i>		<i>SAPK9</i> (response to abscisic acid stimulus)	Chen <i>et al.</i> (2015)
Rice	<i>Bacillus subtilis</i> RR4	CK	Cytokinin	Xie <i>et al.</i> (2017)
Rice	<i>Bacillus amyloliquefaciens</i> FZB42		glucosyltransferase	Drogue <i>et al.</i> (2014)
Rice	<i>Bacillus subtilis</i> RR4		<i>RRA</i>	Rekha <i>et al.</i> (2018)
Rice	<i>Bacillus amyloliquefaciens</i> FZB42	Other hormones	<i>BR11</i> , BR synthesis	Xie <i>et al.</i> (2017)
Rice	<i>Bacillus subtilis</i> RR4		GA-regulated gene	Rekha <i>et al.</i> (2018)
Rice	<i>Azoarcus sp.</i> BH72		<i>GAST1</i> (response to GA stimulus)	Chen <i>et al.</i> (2015)

### Nutrient homeostasis

Rice	<i>Herbaspirillum seropedicae SmR1</i>	Iron	Nicotianamine synthase, Transporters ( <i>YSL, NRamp6, IRT1, IRT2, TOM1, ENA1</i> )	<i>VIT</i> (Vacuolar iron transporter)	Brusamarello-Santos <i>et al.</i> (2019)
Rice	<i>Azospirillum sp.</i>		Nicotianamine synthase		Drogue <i>et al.</i> (2014)
Rice	<i>Bacillus subtilis</i> RR4		<i>Nramp6</i>		Rekha <i>et al.</i> (2018)
Rice	<i>Azospirillum brasilense</i> Sp245	Nitrogen	Nitrate and ammonium transporters	Ammonium transporters	Thomas <i>et al.</i> (2019)
Rice	<i>Bacillus subtilis</i> RR4			Amino acids/polyamine transporter	Rekha <i>et al.</i> (2018)

Rice	<i>Azoarcus</i> sp. BH72	<i>NRT1/PTR2</i> Nitrate efflux <i>NAXT</i> , <i>NRT1/PTR2</i> , Glutamine synthetase ( <i>GSI</i> )	Chen <i>et al.</i> (2015)
Wheat	<i>A. brasiliense</i> FP2		Camilos-Neto <i>et al.</i> (2014)
Rice	<i>Azospirillum brasiliense</i> Sp245	Sugars	Sugar transporter
Rice	<i>Bacillus subtilis</i> RR4		Sugar transporter
Sugarcane	<i>Burkholderia</i> Q208		Sucrose synthase
Rice	<i>Azoarcus</i> sp. BH72		Sucrose synthase, Sugar transporter
<b>Developmental process</b>			
Rice	<i>Herbaspirillum seropedicae</i> SmR1	$\beta$ -D-xylosidase, <i>expansin11</i>	Polygalacturonase
Rice	<i>Bacillus subtilis</i> RR4	23 genes coding for cell wall-loosening enzymes	Rekha <i>et al.</i> (2018)
Wheat	<i>A. brasiliense</i> FP2	24 genes implicated in cell cycle regulations Aerenchyma formation : <i>AP2/ERF</i> , prolyl 4-hydroxylase alpha (role in hypoxia stress)	Camilos-Neto <i>et al.</i> (2014)
Sugarcane	<i>Burkholderia</i> Q208		Paungfoo-Lonhienne <i>et al.</i> (2016)

rhizobacteria (Brusamarello-Santos *et al.*, 2019; Rekha *et al.*, 2018; Thomas *et al.*, 2019). This is also the case for genes related to the synthesis of antimicrobial compounds such as phytoalexines and thionins (Brusamarello-Santos *et al.*, 2019). This suppression of defense is not the only immune regulation triggered by the interaction with beneficial rhizobacteria. Indeed, transcriptional regulations of disease resistance genes and membrane-bound receptors occur in the inoculated tissues but also in distal tissues. The transcriptional regulation of disease resistance genes can alter the outcome of subsequent pathogen attack, possibly implicated in the ISR phenotype largely studied from the beginning of the 2000s. Also genes encoding for receptor-like kinases have been reported to be transcriptionally regulated. Particularly, receptors well known for their role in the disease resistance, such as WAKs (Brusamarello-Santos *et al.*, 2019), and mutualistic symbiosis, namely LysM-RLKs (Thomas *et al.*, 2019). However, a total suppression of plant defense could lead to uncontrolled root colonization by rhizobacteria. In accordance with this, defense responses are also observed in response to beneficial rhizobacteria root colonization. This process has been proposed to be phytohormone-mediated, in particular through JA (Miché *et al.*, 2006) and SA-mediated defense (Rekha *et al.*, 2018). Downstream transcriptional factors of the WRKY and MYB families have also been reported to be transcriptionally regulated (Brusamarello-Santos *et al.*, 2019; Camilios-Neto *et al.*, 2014; Drogue *et al.*, 2014; Rekha *et al.*, 2018; Xie *et al.*, 2017).

However, SA and JA are not the only hormone signaling pathways transcriptionally regulated by beneficial rhizobacteria. Indeed, as stated before, plants are able to modulate hormone signaling in plants. In particular, two hormonal pathways are largely studied in plant-rhizobacteria interactions: auxins and ethylene-mediated. The first can be synthesized by rhizobacteria and the second is modulated through the degradation of one of its biochemical precursors: ACC. Consequently, these two pathways are transcriptionally regulated in response to rhizobacteria colonization. For auxins, auxin transporters-encoding genes as well as auxin-responsive genes (*SAUR*, *Aux/IAA*) were transcriptionally regulated in response to *Azospirillum*, *Bacillus* and *Herbaspirillum* (Brusamarello-Santos *et al.*, 2019; Drogue *et al.*, 2014; Rekha *et al.*, 2018; Thomas *et al.*, 2019). This fact has been proposed to be related to rhizobacteria-triggered root development and defense as auxin signaling pathway is also linked to defense-related processes. Similarly, genes implicated in ethylene signaling (*ERF*) and ethylene synthesis are transcriptionally regulated in response to *Azospirillum*, *Bacillus* and *Herbaspirillum* (Brusamarello-Santos *et al.*, 2019; Camilios-Neto *et al.*, 2014; Drogue *et al.*, 2014; Rekha *et al.*, 2018; Thomas *et al.*, 2019). The case of *ACO* genes is of particular

interest, indeed most of differentially expressed *ACO* genes were down-regulated in response to *Azospirillum*, *Bacillus* and *Herbaspirillum* beneficial strains. On the other hand, an *Herbaspirillum rubrisubalbicans* pathogenic strain induced the upregulation of an *ACO* and the probable consequent accumulation of ethylene was proposed to lead a decrease of plant growth (Valdameri *et al.*, 2017). Also CK-related genes were reported to be transcriptionally regulated in response *Azospirillum* (Drogue *et al.*, 2014; Thomas *et al.*, 2019) and *Bacillus* (Rekha *et al.*, 2018). In general, the down-regulation of CK signaling and the up-regulation of CK degrading enzymes suggest a repression of this hormonal pathway. Few genes implicated in the GA hormonal pathway have also been reported to be transcriptionally regulated in rice in response to *Azospirillum* and *Bacillus subtilis* (Drogue *et al.*, 2014; Rekha *et al.*, 2018).

Many genes implicated in nutritional homeostasis are also transcriptionally regulated during associative symbiosis with PGPR. As most microbial models are diazotrophs, several studies focused on N metabolism. Indeed several nitrate and ammonium transporters are differentially expressed in wheat and rice roots inoculated with *Azospirillum* strains (Thomas *et al.*, 2019). Genes encoding for glutamine synthetase have also been reported to be transcriptionally regulated (Camillos-Neto *et al.*, 2014) therefore suggesting that associative symbiosis modulates the assimilation of N in plants. Iron homeostasis appears also to be modulated during the interaction with beneficial rhizobacteria as illustrated by Brusamarello-santos and collaborators (2019). Their transcriptomic analysis revealed that following *Herbaspirillum* inoculation a global up-regulation of genes implicated in the synthesis, efflux and assimilation of phytosiderophores occur in rice roots. These transcriptional regulations are characteristic of the iron deficiency response in plants and could be related to the capacity of rhizobacteria to chelate iron using their bacterial siderophores. Similar regulations are also observed in the context of ISR (Stringlis *et al.*, 2018). Finally, the impact of these symbioses on primary metabolism was also studied regarding the idea that plants feed their beneficial PGPR similarly to mycorrhizae and rhizobia. Regarding sucrose, the interaction with *B. subtilis* didn't change the expression of genes encoding for sucrose synthase in rice while the transcription of genes implicated in organic acid and amino acid metabolism was altered (Rekha *et al.*, 2018). On the other hand, *Burkholderia* Q208 triggered the up-regulation of two genes encoding for sucrose synthase in sugarcane (Paungfoo-Lonhienne *et al.*, 2016). Also, genes encoding for sugar transporters are up-regulated in response to *Azospirillum* in rice (Thomas *et al.*, 2019).

As the interaction with PGPR often leads to the modification of root architecture, some studies focused on the transcriptional regulation of genes related to development. Particularly, the interaction of rice with *Herbaspirillum* and *B. subtilis* led to transcriptional regulation of genes implicated in cell wall modeling (Brusamarello-Santos *et al.*, 2019; Rekha *et al.*, 2018). Also, Camilios-Neto and collaborators (2014) proposed that the up-regulation of genes implicated in cell cycle in wheat roots in response to *Azospirillum* was a transcriptional marker of an enhanced growth in roots as it suggests an increased rate of cell replication. Also, in this context, the study conducted by Paungfoo-Lonhienne and collaborators (2016) is of particular interest. Indeed, they produced a dual-RNA-Seq data of both sugarcane and the population of inoculated *Burkholderia* Q208. They proposed a model where the interaction led to an increased volume of aerenchyma caused by the oxygen consumption of endophytic bacterial biofilms likely to be due to nitrogen fixation.

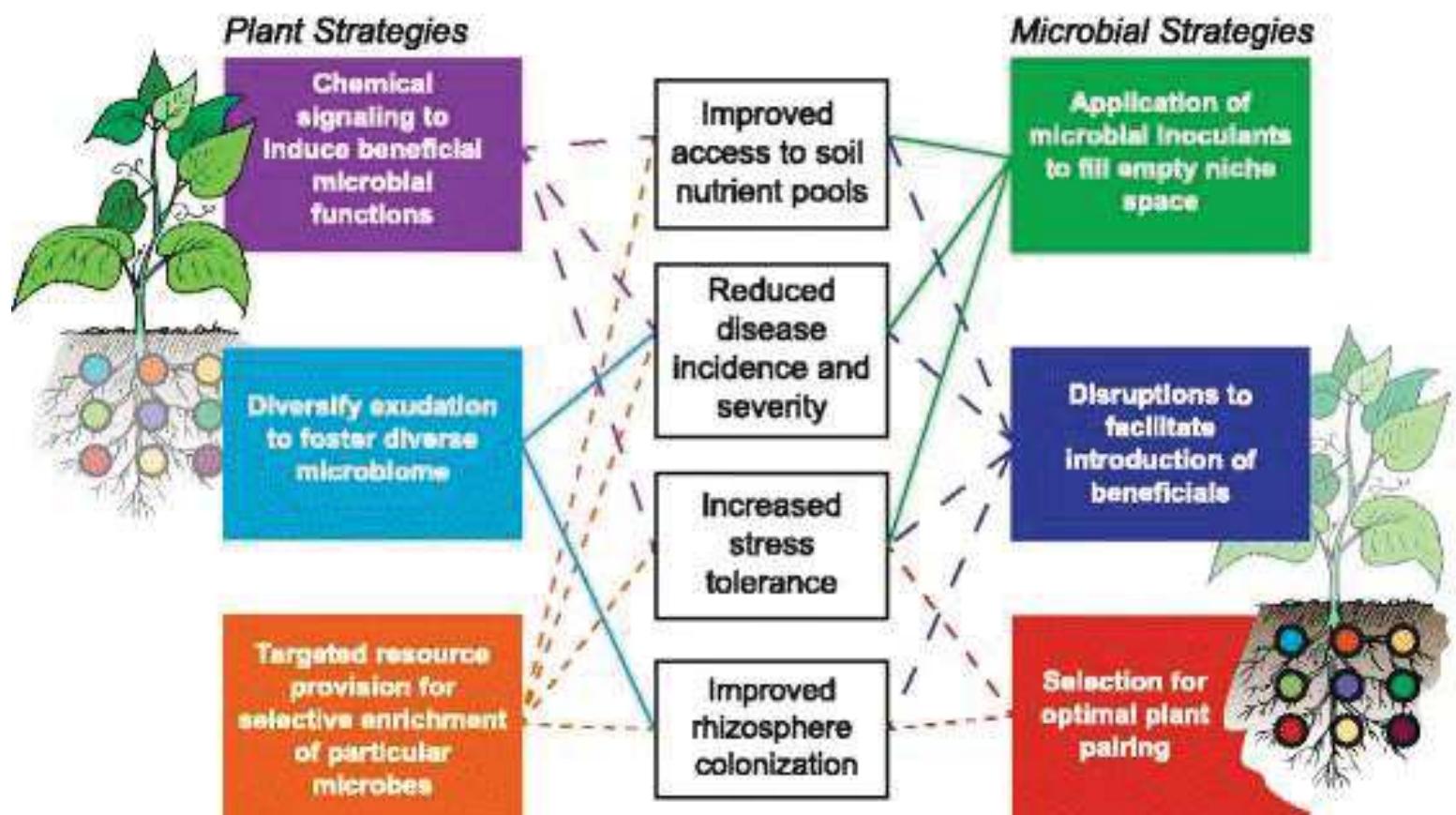
To conclude, transcriptomic analyses of plant response to beneficial microbes can also address fundamental questions. Such as Drogue and collaborators (Drogue *et al.*, 2014) who revealed a strain-dependent response of two cultivars of rice inoculated with two different strains of *Azospirillum*. Also, the analysis of the transcriptome dynamics of *Arabidopsis* inoculated with *Pseudomonas simiae* WCS417 compared to its MAMP flg22<sup>417</sup> revealed transcriptional pathways actively regulated by the bacterial cells to enable the establishment of the interaction (Stringlis *et al.*, 2018). The ultimate goal of these approaches is to identify the genes and transcriptional networks implicated in beneficial interactions to breed for crops more likely to get involved and take advantage of such interactions.

### **Applications of plant associative symbioses**

The current context of an ever growing global human population, climatic changes and the major environmental side-effects of post-green revolution agricultural practices are huge challenges for food production. Indeed, while the need to optimize resource use is crucial, food production still needs to increase in a sustainable way. To tackle these issues, the integration of beneficial plant microbiomes and the exploitation of its associated ecosystemic services into agricultural production appear to be very promising. Several ways to do so are recently emerging with their associated limitations in terms of application (Figure 13).

The first one and probably the most obvious one is the use of PGPM as biofertilizers, plant strengtheners and biocontrol agents. As previously described, PGPM can have tremendous effects on multiple plant traits. However, major limitations are observed when trying to

## Reducing chemical inputs and increasing yields



**Figure 13: Strategies to reduce chemical inputs and increasing yields from plant and microbial side (From Bakker *et al.*, 2012)**

A variety of strategies could be used to promote beneficial services provided by soil microbial communities, with the aim of reducing chemical inputs while sustaining or improving crop yields. Manipulating plant traits that are related to interactions with microbes (left side), or manipulating soil microbial communities directly (right side), could improve conditions for plant productivity (center mechanisms)

translate phenotypes described in the lab to the field conditions. Multiple aspects can explain this: The fact that lab phenotypes are often observed using sterilized soil substrates, the presence of the indigenous agricultural soil microbiota which can counteract the beneficial effects of introduced PGPM and also the impact of plant-microbiome co-adaptation (Bakker *et al.*, 2012). This implies that there is no universal PGPM for every agricultural context (Compant *et al.*, 2005). Therefore, highly specific usage of PGPM must be applied regarding host genotypes, individual soil environments as well as abiotic conditions (Schlaeppi and Bulgarelli, 2015).

Another application which tackles some of these limitations is the usage of complex microbial consortia to increase the potential of niche occupancy for microbes to exhibit their beneficial traits for plants. Also, in the case of endophytes, direct introduction of beneficial bacteria in the flowers has been proposed, this in order for them to be directly carried by the internal tissues of seeds to the progeny (Mitter *et al.*, 2017). Microbiome engineering is also a promising application, meaning using any leverage possible to optimize the beneficial functions provided to the plant (Orozco-Mosqueda *et al.*, 2018). This can be done through management practices (Chaparro *et al.*, 2012), the use of probiotics (Hu *et al.*, 2016) and host-mediated microbiome selection (Mueller and Sachs, 2015) for example. Finally, a last leverage to take advantage of plant-associated microbes is through breeding. Traditionally, the varieties were bred without considering their global biotic environment, with the exception of diseases. It has therefore been proposed to breed for microbe-driven plant phenotypes (Wei and Jousset, 2017). To do so, a deep understanding of the molecular bases of plant-microbe interactions is needed, particularly on the plant side.

In conclusion, the potential of plant-associated microbiota for agriculture have long been known but potentially neglected due to the complexity to apprehend soil microbial ecology. However, the emergence of novel technologies and methods, such as the use of synthetic communities (Finkel *et al.*, 2017), enables to tackle fundamental questions in plant and agricultural microbiome research (Busby *et al.*, 2017). More largely the importance of microbiomes also changed the vision of plants in the agricultural context giving birth to the "phytobiome" concept. This idea is to take into account all the factors interacting with plants (climate, animals, soils, microbes) and therefore encouraging scientists of all these disciplines to work together to understand the network of interactions in which plants are embedded.

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# **CHAPITRE 2**

Le chapitre 2 traite de l'analyse comparative de la réponse transcriptomique du riz à deux souches endophytes de *Burkholderia* s.l., *Paraburkholderia kururiensis* M130 et *Burkholderia vietnamiensis* TVV75, toutes deux précédemment décrites comme capable d'induire des effets bénéfiques sur la croissance et le rendement du riz.

Une analyse de la colonisation des racines du riz par les deux souches, ont mis en évidence des profils de colonisation contrastés. La souche *Burkholderia vietnamiensis* TVV75 a montré une capacité de colonisation plus invasive en particulier des événements de colonisation intracellulaire des cellules de l'épiderme racinaire. La souche *Paraburkholderia kururiensis* M130 se limite à une colonisation intercellulaire caractéristique des profils de colonisation d'autres souches endophytes bénéfiques. L'analyse transcriptomique par RNA-Seq de plantes inoculées avec ces deux souches a en particulier montré des différences, en fonction de la souche inoculée, dans la réponse immunitaire et hormonale des tissus racinaires et des feuilles. Par la suite, une analyse transcriptionnelle cinétique a mis en évidence une augmentation transitoire de l'expression d'un réseau de gènes lié à la signalisation et la synthèse de l'acide jasmonique, décalée dans le temps en fonction de la souche inoculée. La colonisation des tissus racinaires par *B. vietnamiensis* induisant un signal JA dans les feuilles à court terme, 6 heures post-inoculation, tandis qu'un même signal est mesuré à 7 jours post-inoculation en réponse à *P. kururiensis*.

Ces travaux ont été publiés dans la revue *Frontiers in Plant Science*. Pour des raisons de place, les tableaux supplémentaires 7 et 8, ne sont pas présent dans ce chapitre. Ils sont disponibles en ligne à l'url suivante: <https://doi.org/10.3389/fpls.2019.01141>

# **Monitoring of rice transcriptional responses to contrasted colonizing patterns of phytobeneficial *Burkholderia* s.l. reveals a temporal shift in JA systemic response**

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## **• Introduction**

Plant microbiome is nowadays extensively studied as it represents a huge potential for agriculture. Numerous studies describe the importance of microbes for plant's nutrient supply and resistance to diseases and pests (Finkel *et al.*, 2017). Therefore, microbes are potential solutions to do the transition to a sustainable agriculture while maintaining yield (Busby *et al.*, 2017). Especially, some rhizobacteria have been shown to have tremendous beneficial effects on plant growth (Hayat *et al.*, 2010) and resistance to pathogens (Beneduzi *et al.*, 2012). These beneficial effects are induced through hormonal modulations following the colonization of plant roots (Vacheron *et al.*, 2013) and inner tissues for endophytes (Hardoim *et al.*, 2008) as well as systemic regulations of immunity (Pieterse *et al.*, 2014). However, the perception of Microbe Associated Molecular Patterns (MAMPs) generally leads to an immune response called MAMPs Triggered Immunity (MTI) characterized by the synthesis of antimicrobial compounds (Jones and Dangl, 2006). In the same way, pathogens suppress plant immunity, beneficial microbes are able to escape (Trda *et al.*, 2015) or modulate (Zamioudis and Pieterse, 2012) the immune response of plants cells. Interestingly, the suppression of MTI by both pathogenic bacteria (Millet *et al.*, 2010) and beneficial fungi (Jacobs *et al.*, 2011) is commonly mediated via the Jasmonic Acid (JA) signaling pathway. However, the global physiological response and especially the transcriptional regulations induced by plants during the interaction with beneficial bacteria are not well described.

The interaction between plants and rhizospheric or endophytic bacteria forming associative symbioses, has been studied in several species belonging to the genera *Azoarcus*, *Azospirillum*, *Herbaspirillum*, *Acetobacter*, *Gluconacetobacter*, *Bacillus*, *Phyllobacterium*, *Pseudomonas* and (*Para-*)*Burkholderia* (Ahmed and Kibret, 2014). Most studies on these models focused on the bacterial response to the interaction with its host plant (Coutinho *et al.*, 2015; Sheibani-Tezerji *et al.*, 2015; Shidore *et al.*, 2012) while relatively few studies analyzed

the transcriptional response of plants. Nonetheless, several studies described plants' transcriptional regulations including lowering of defense (Bordiec *et al.*, 2011; Rekha *et al.*, 2018), hormonal signaling (Drogue *et al.*, 2014; Rekha *et al.*, 2018), developmental reprogramming (Paungfoo-Lonhienne *et al.*, 2016) as well as iron homeostasis (Brusamarello-Santos *et al.*, 2018; Stringlis *et al.*, 2018).

Within the diversity of bacteria interacting with plants, the *Burkholderia* *sensu lato* (*s.l.*) genus of betaproteobacteria stands out for several reasons. It contains plant pathogenic species (*B. glumae*, *B. gladioli*, *B. plantarii*) (Maeda *et al.*, 2006), N<sub>2</sub>-fixing nodule-forming species in association with tropical legumes (Gyaneshwar *et al.*, 2011) as well as species forming associative symbiosis particularly with cereals such as rice (Coutinho *et al.*, 2013; Govindarajan *et al.*, 2008). Also, a phylogenetic separation discriminates the *Burkholderia* *sensu stricto* (*s.s.*) genus which contains animal or plants pathogens as well as human opportunists and the *Paraburkholderia* genus which contains mainly plant-associated and environmental species (Estrada-de los Santos *et al.*, 2016; Sawana *et al.*, 2014). Also, most recent phylogenetic refinements of *Burkholderia* *s.l.* taxa defined other genera: *Caballeronia*, *Robbsia*, *Trinickia* and *Mycetohabitans* which are supported by both differences in genomic and ecological features (Estrada-de los Santos *et al.*, 2018).

Interestingly, two strains of the *Burkholderia* *s.l.* genus able to fix N<sub>2</sub> have been described as growth-promoters on rice. *Paraburkholderia kururiensis* M130 (hereafter *Pk*) is a beneficial rice endophyte (Mattos *et al.*, 2008) related to environmental and plant-beneficial strains (Kaur *et al.*, 2017). *Burkholderia vietnamiensis* TVV75<sup>T</sup> (hereafter *Bv*) is a rice-associated species having positive effect on yield (Govindarajan *et al.*, 2008; Trân Van *et al.*, 2000) which belongs to the *B. cepacia* complex, a complex of species that can cause serious risks to cystic fibrosis patients (Vial *et al.*, 2011). In order to decipher how rice perceives these two beneficial strains belonging to genera with contrasted ecologic backgrounds, we studied the transcriptional responses of rice during the establishment of the interaction. Our aim was to identify plant physiological processes and potentially key genes involved in beneficial rice-rhizobacteria interactions and also differentially regulated by each strain. We first analyzed the colonization patterns of *Pk* and *Bv* on the *Oryza sativa* Nipponbare genotype. We then analyzed the roots and leaves transcriptional responses to the bacterial colonization by RNA-Seq. This led us to the identification of a co-expression JA-related network. Therefore, we monitored, throughout the establishment of the interaction, the expression of JA-related genes by RT-qPCR.

- **Material & Methods**

### **Plants & bacterial cultivation**

*Oryza sativa L. ssp. japonica* cv Nipponbare was used in this study. For all experiments, seeds were dehusked and sterilized as follows: 70% ethanol for 10 minutes and 9.6% NaClO supplemented with 1% Tween 20 for 30 min. Treated seeds were rinsed twice with sterile distilled water, twice with 2% thiosulfate solution and finally four times with sterile distilled water. 100 µL of the last rinsing solution were plated on TSA medium (Sigma-Aldrich) to confirm surface sterilization. Seeds were then put in sterile distilled water at 28°C for 24 h and transferred on 8% H<sub>2</sub>O agar plate for 30 h. Homogeneously germinated seeds were transferred to sterile magenta boxes (SPL Lifesciences Co. Ltd) containing 150 mL of autoclaved perlite and 200 mL of sterile hydroponic medium (Recipe in Supplementary Table 1). Plants were grown in a growth chamber (16 h light; 8 h dark; 28°C; 70% humidity).

All bacterial strains (listed in Supplementary Table 2) were cultured as follows: Glycerol stocks (20%) of bacterial cells conserved at -80°C were plated on low salt LB (Sigma-Aldrich) agar plates and incubated for 72h at 28°C. 20 mL Liquid LBm medium were then inoculated in 50 mL falcon tubes and incubated for 16h under agitation (180 rpm) at 28°C. 500 µL of overnight culture were inoculated in fresh liquid medium for 2h. Bacterial cells were then centrifuged for 5 min at 4000 rpm and resuspended in sterile distilled water. Each plantlet was inoculated with 10<sup>7</sup> bacterial cells 4 days after sowing in hydroponic system.

### **Bacterial transformation**

*Paraburkholderia kururiensis* M130 and *Burkholderia vietnamiensis* TVV75 cells were transformed by electroporation with the pIN29 plasmid (Vergunst *et al.*, 2010). The plasmid chosen to transform the strains, pIN29 comprises a chloramphenicol resistance gene as well as the DsRed gene under the control of a constitutive TAC promoter. After 24 hours of incubation on selective medium LBm Cm (200 µg.mL<sup>-1</sup>) at 28 ° C, the most fluorescent colonies were selected.

### **Rice root colonization assays**

The roots of plants were harvested at 1, 7 and 14 days post inoculation (dpi), weighted and grinded in sterile water with a sterile ceramic bead using a FastPrep-24™ 5G at 6m.s<sup>-1</sup> for 40 seconds. The solution was then diluted and inoculated on low salt LB selective medium containing 200 µg.mL<sup>-1</sup> of chloramphenicol and incubated at 28°C for 24h. Colony forming unit were then enumerated. The size of the root-associated bacterial population was measured during two independent experiments each comprising 9 plants. In order to measure the size of

the endophytic population, the inoculated rice roots were surface-disinfected for 1 minute using a solution of 1% Chloramine T (Sigma-Aldrich) supplemented with 0,1% Tween 20. Roots were then rinsed 6 times with sterile water. Controls of disinfection were performed by plating rinsing water on TSA medium (Sigma-Aldrich) overnight. Surface-disinfected roots were then treated as previously described. The size of the endophytic population was measured on 5 plants.

### **Microscopy**

All microscopic observations of the bacterial colonization were restricted to the primary root in order to compare the colonization patterns on roots which have been in contact with the bacterial population for the same amount of time. Primary roots were harvested at 7 and 14 dpi and mounted between slide and slips cover and directly examined with the microscopes. Epifluorescence observations were performed using a Nikon Eclipse Ni-E microscope. Confocal Laser Scanning observations were performed using a Zeiss LSM880 confocal microscope.

### **RNA extraction**

For the analysis of roots and leaves transcriptional profiles, both plants' tissues were harvested at 6 hours post inoculation (hpi), 1 dpi, 7 dpi or 14 dpi with live bacterial cells. Each biological replicates consisted of five pooled root system or five pooled last mature leaves harvested from a single hydroponic system. For each time point and each inoculated strain, three biological replicates were harvested. Roots and leaves of untreated plants were collected at the same time points. After harvest, samples were snap-frozen in liquid nitrogen and stored at -80°C.

Rice roots were homogenized in liquid nitrogen using cooled mortar and pestles. Rice leaves were grinded using a TissueLyser II (Retsch) set to 30 Hz for 30 sec. Total RNA extraction using TRI-reagent (Sigma) was performed according to manufacturer's instructions. All samples were treated with DNase I (Ambion) and purified using the RNA Clean & Concentrator kit (Zymo) according to manufacturer's instructions. The integrity and quality of the total RNA was confirmed using a Nanodrop<sup>TM</sup> 1000 spectrophotometer (ThermoFisher) and a 2100 BioAnalyzer (Agilent).

### **RNA sequencing & mapping of reads**

Quality of RNA was checked by determining the RNA Integrity Number (RIN) with a Fragment Analyzer (Agilent). For the library preparation, samples with a RIN value > 6 were used. 18 RNA libraries were prepared using an Illumina TruSeq stranded mRNA sample preparation kit by MGX-Montpellier GenomiX core facility (MGX) France

(<https://www.mgx.cnrs.fr/>). Library construction and sequencing was performed as described in Kamakar *et al.* (2019) on an Illumina HiSeq 2500. The quantitative and qualitative validation of the library was performed by qPCR, ROCHE Light Cycler 480 and with a Fragment Analyser (Agilent) using a Standard Sensitivity NGS kit. Quality control and assessment of raw Illumina reads in FASTQ format were done by FastQC software (Version 0.11.5) to obtain per base quality, GC content and sequence length distribution. Clean reads were obtained by removing the low quality reads, adapters, poly-N containing reads by using Trimmomatic v0.36 software (Bolger *et al.*, 2014). RNA-Seq reads were aligned to the IRGSP 1.0 version of the rice genome using HISAT2 v2.0.5.1 (Kim *et al.*, 2015). The number of reads mapped to each gene locus was counted using HTSeq-count v0.6.0 (Anders *et al.*, 2015).

#### **Differential gene expression & Gene Ontology (GO) term enrichment analysis**

DESeq2 v3.7 (Love *et al.*, 2014) was used to calculate differential gene expression between non-inoculated and inoculated conditions. All genes having an adjusted p-value inferior to 0.01 were considered as significantly differentially expressed. All functional enrichment analysis was performed using g:Profiler (version e95\_eg42\_p13\_f6e58b9) with g:SCS multiple testing correction method applying significance threshold of 0.05 (Raudvere *et al.*, 2019; Reimand *et al.*, 2007).

#### **Quantification of mRNA levels using RT-qPCR**

cDNA was produced from 350 ng of DNase-treated total RNA using the SuperScript III Reverse Transcriptase (Thermo Fisher Scientific). The cDNA reaction was diluted 5 times before quantitative PCR using MESA BLUE qPCR Master Mix for SYBR® assay (Eurogentec) on an Mx3005P qPCR system (Agilent Technologies). The relative expression level was calculated according to Pfaffl (2001). Three independent samples were analyzed for each condition and each sample was assayed in triplicate. Primers used are listed in Supplementary Table 3.

### **• Results**

#### **Analysis of root colonization**

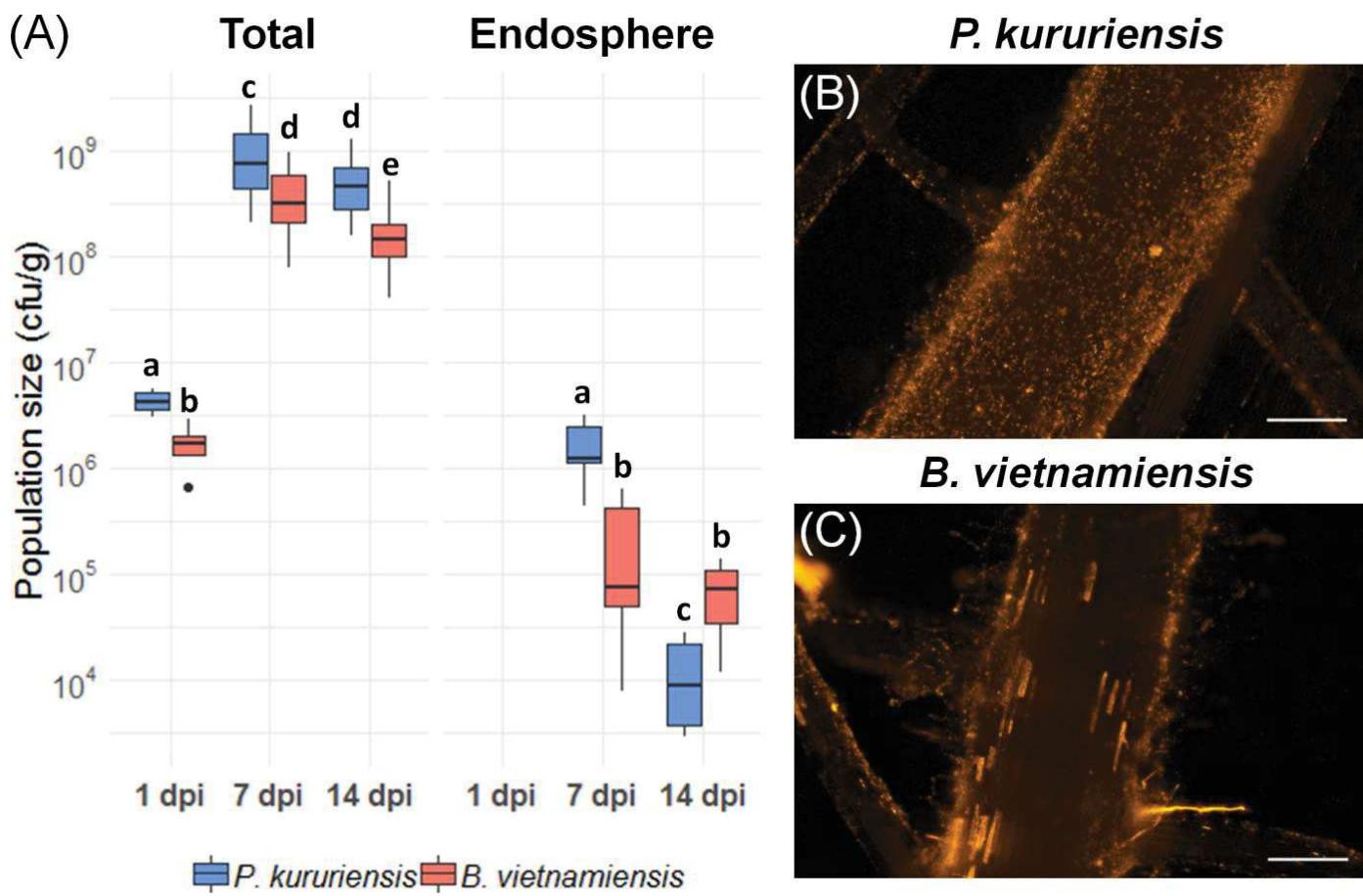
We used hydroponic culture of rice plants, grown in axenic condition and inoculated with DsRed-tagged strains, to monitor the bacterial colonization at 1, 7 and 14 days post inoculation (dpi). First, the root's colonization was measured by counting the bacterial populations on the rhizoplane and endosphere (see Material and Methods). The roots of rice

plants were rapidly colonized by both bacterial strains (Figure 1A). The populations of *Pk* and *Bv* reach a median value of  $4.16 \times 10^6$  and  $1.73 \times 10^6$  cfu.g<sup>-1</sup> respectively at 1 dpi. At 7 dpi, the maximum measured size of the bacterial population is reached for both strains:  $7.49 \times 10^8$  and  $3.18 \times 10^8$  cfu.g<sup>-1</sup> for *Pk* and *Bv* respectively. Then, between 7 and 14 dpi, the size of the total root-associated population decreases for each strain, reaching a median value of  $4.6 \times 10^8$  and  $1.46 \times 10^8$  cfu.g<sup>-1</sup> for *Pk* and *Bv* respectively. Between the same time points the variation of the endophytic population size differs between the two strains. Indeed the endophytic population size of *Pk* decreases from  $1.25 \times 10^6$  to  $1.17 \times 10^4$  cfu.g<sup>-1</sup> between 7 and 14 dpi, while the median number of endophytic *Bv* cells remain stable with  $7.34 \times 10^4$  and  $7.5 \times 10^4$  cfu.g<sup>-1</sup> at 7 and 14 dpi respectively.

Microscopic observations of the primary roots of inoculated rice plants demonstrated that both bacterial strains tagged with the DsRed gene colonized the root surface after inoculation (Figure 1 B, C). Moreover specific zones were more densely colonized by bacteria such as the surface of root hairs and the emergence of lateral roots (Supplementary Figure 1). By comparing the colonization of the two strains, differences can be observed in the way they colonized the surface of the primary root. Several epidermal plant cells seemed colonized intracellularly by the tagged *Bv* cells while the phenomenon was observed less frequently in *Pk*-colonized roots (Figure 1C; Supplementary Figure 1). Observations by confocal microscopy revealed that for *Pk*, the highly colonized epidermal cells appear to be only colonized on their surface as the whole outline of epidermal cells as well as in intercellular spaces (Figure 2A,C) while for *Bv* most of the observations show that bacterial cells were able to cross the cell wall and were observed in the cytoplasm of the cell (Figure 2B,D). Thus, both strains colonized the roots of the Nipponbare cultivar both externally and endophytically but through apparent different intensities and entry roads. We then wondered if the host plant induces contrasted transcriptional regulations associated to the differential colonization pattern of each strain.

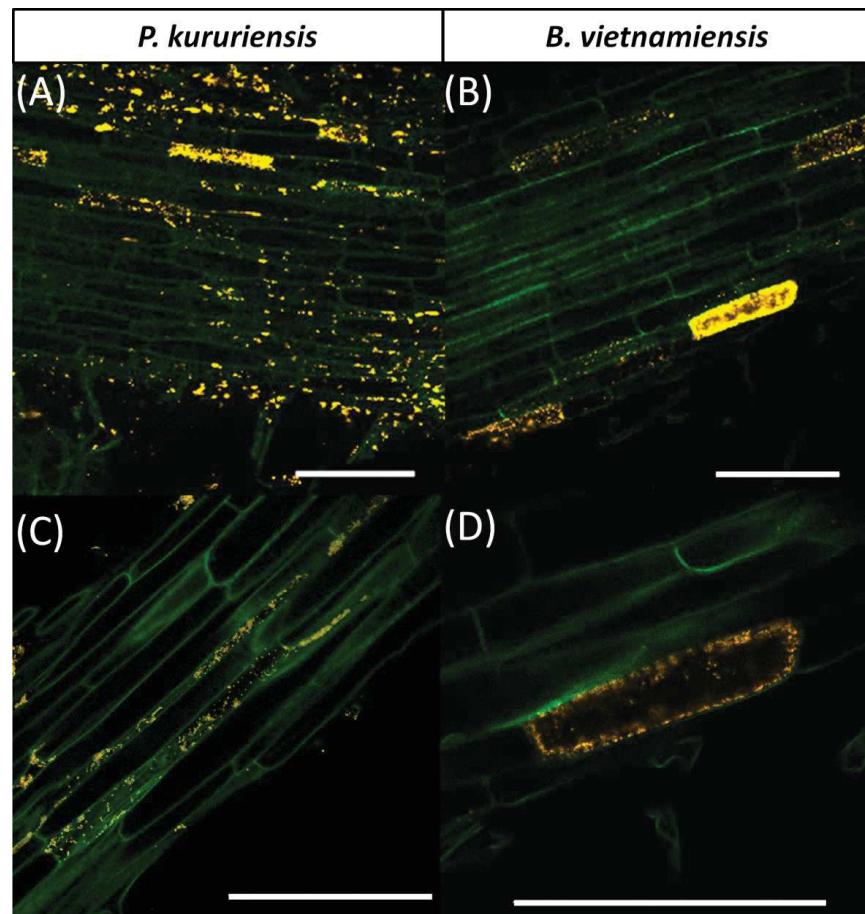
### **Transcriptional response of rice to bacterial inoculation**

In order to identify changes in *O. sativa* transcriptome in response to both strains, we performed RNA-Seq on leaves and roots of non-inoculated controls, *Pk*-inoculated and *Bv*-inoculated plants at 7 dpi. We chose this time point to avoid the initial plant defense burst due to a bacterial inoculation in hydroponic system (hours to 1 dpi) and allow an advanced colonization stage of the roots but without any visible developmental effect such as increased growth. To confirm that the inoculated and non-inoculated control plants were at the same developmental stage, we measured the dry weight of the plants and couldn't detect any



**Figure 1: Colonization of the roots of hydroponically grown rice plants by *Bv* and *Pk***

(A) Population dynamics of DsRed-tagged *Bv* and *Pk* associated to rice roots and inside the plant roots. The data reported are the median of bacterial population size from 18 plants and two independent experiments for rhizosphere compartment and five plants for the endophytic compartment. The letters indicate the significance groups in each compartment according to post-hoc tests on a generalized linear model. Epifluorescence microscopy pictures of the colonization of rice primary roots at seven days post inoculation by *Pk* (B) and *Bv* (C) DsRed cells. White bars represent 200 $\mu$ m.



**Figure 2: Endophytic colonization of the roots of hydroponically grown rice plants by *Bv* and *Pk***

Confocal microscopy observations of *Pk* and *Bv* DsRed-tagged cells colonizing the inner tissues of rice roots at 14 days post inoculation. (A) *Pk* cells colonizing the surface and the intercellular spaces of epidermic cells (B) Multiple epidermic cells colonized by *Bv* cells (C) Apoplastic colonization by *Pk* cells (D) Rice root epidermic intracellular colonization by *Bv* cells. White bars represent 100 $\mu$ m.

significant impact of the inoculation on biomass production (Supplementary Figure 2) nor on plant height. Therefore, we assume that the differences between the transcriptomes of inoculated plants and non-inoculated controls should be related to bacterial colonization of the roots rather than a developmental impact of inoculation. A total of 843 million reads were sequenced with an average of 47 million reads per sample (Table 1). An average of 68% of reads was uniquely mapped to the *O. sativa* genome per sample. A principal component analysis also discriminates the transcriptome of non-inoculated plants compared to the inoculated ones as well as the plant responses to each bacterial strain (Figure 3A,B). Differential expression analysis yields a total of 4951 and 5275 significantly ( $p < 0,01$ ) differentially expressed genes (DEGs) in response to *Bv* and *Pk* respectively. Comparing leaves and roots transcriptomes reveals that there are 5 and 8 times more DEGs detected in leaves in response to *Bv* and *Pk* respectively compared to roots (Figure 3C,D). When comparing the response to *Pk* and *Bv*, a large proportion of DEGs are commonly regulated in leaves (around 50% in response to both strains) contrarily to roots in which the transcriptional regulation appears to be more specific to each inoculated strain. Indeed only 33% of the DEGs in response to *Bv* are also differentially expressed in response to *Pk*. The proportions of commonly up-regulated DEGs in roots represent 20% and 27% of DEGs in response to *Bv* and *Pk* respectively.

In order to identify the biological processes transcriptionally regulated by the colonization of both bacterial strains, a GO enrichment analysis was carried out on the commonly regulated DEGs (intersections in Figure 3C,D). Supplementary Figure 3 provides a visualization of the enriched GO terms from the commonly up or down-regulated DEGs in leaves and roots (Complete list of enriched GO terms available in Supplementary Table 4). Three main biological processes are commonly regulated during the interaction with both strains in leaves and roots: response to stimuli, metabolic and also developmental processes. First, stress-related genes are enriched in the commonly DEGs. Indeed, in leaves the “response to abiotic stimulus” as well as the “response to oxidative stress” terms are enriched in the up-regulated DEGs whereas defense-related GO terms are enriched in the down-regulated DEGs in both leaves and roots. Also, several hormone-related GO terms are enriched in the DEGs in both leaves and roots. In leaves, GO terms related to auxins and Abscisic Acid (ABA) response are enriched in the up-regulated genes while in roots, the up-regulated DEGs are enriched in cytokinines (CK), brassinosteroids (BR) and ethylene response terms. Finally, in roots down-

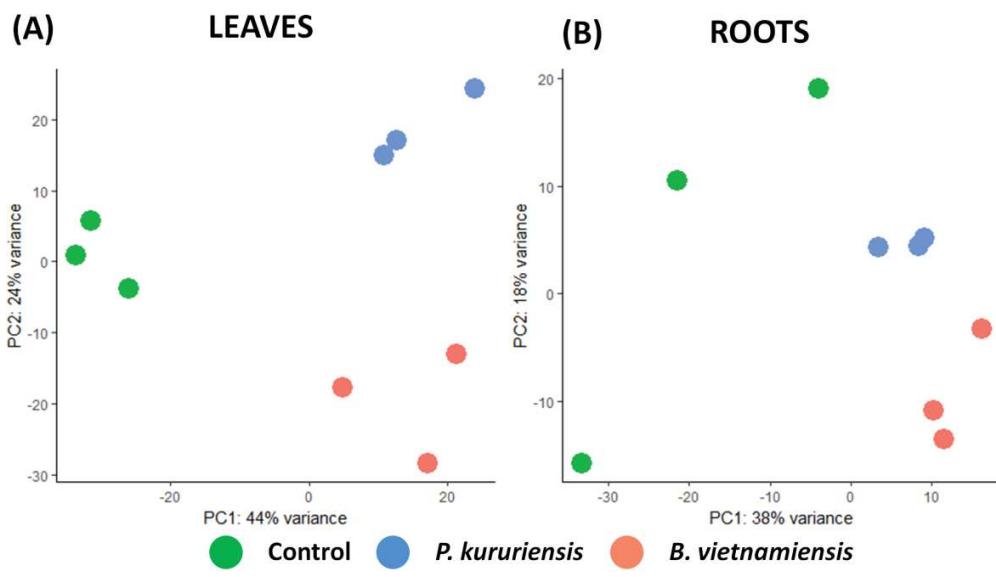
Table 1 : Summary of RNA-Seq data generated for rice transcriptomes

<b>Sample</b>	<b>Organ</b>	<b>Condition</b>	<b>Total of reads</b>	<b>Number of uniquely mapped reads</b>	<b>Proportion of mapped reads</b>
LC1	Leaves	Control	38 533 867	26 161 511	68%
LC2	Leaves	Control	44 792 837	29 952 254	67%
LC3	Leaves	Control	33 331 123	21 989 917	66%
LK1	Leaves	<i>P. kururiensis</i>	46 895 520	32 677 603	70%
LK2	Leaves	<i>P. kururiensis</i>	65 261 206	42 077 779	64%
LK3	Leaves	<i>P. kururiensis</i>	46 707 369	33 553 685	72%
LV1	Leaves	<i>B. vietnamiensis</i>	43 862 789	31 855 527	73%
LV2	Leaves	<i>B. vietnamiensis</i>	41 034 781	27 022 594	66%
LV3	Leaves	<i>B. vietnamiensis</i>	49 608 083	35 455 520	71%
RC1	Roots	Control	38 522 573	27 000 000	70%
RC2	Roots	Control	50 820 144	35 400 000	70%
RC3	Roots	Control	44 458 436	30 800 000	69%
RK1	Roots	<i>P. kururiensis</i>	49 275 052	33 600 000	68%
RK2	Roots	<i>P. kururiensis</i>	52 634 407	34 700 000	66%
RK3	Roots	<i>P. kururiensis</i>	49 754 162	30 900 000	62%
RV1	Roots	<i>B. vietnamiensis</i>	51 314 965	34 700 000	68%
RV2	Roots	<i>B. vietnamiensis</i>	39 023 620	25 800 000	66%
RV3	Roots	<i>B. vietnamiensis</i>	57 406 096	39 300 000	68%

regulated DEGs are enriched in Gibberellic Acid (GA) and Salicylic Acid (SA) response-related terms. The analysis also revealed that metabolic processes are transcriptionally regulated in response to the interaction with both strains: In leaves, the up-regulated DEGs are enriched in the “photosynthesis” and “translation” terms while the down-regulated DEGs are enriched in “starch metabolic process” and “membrane lipid catabolic process” terms.

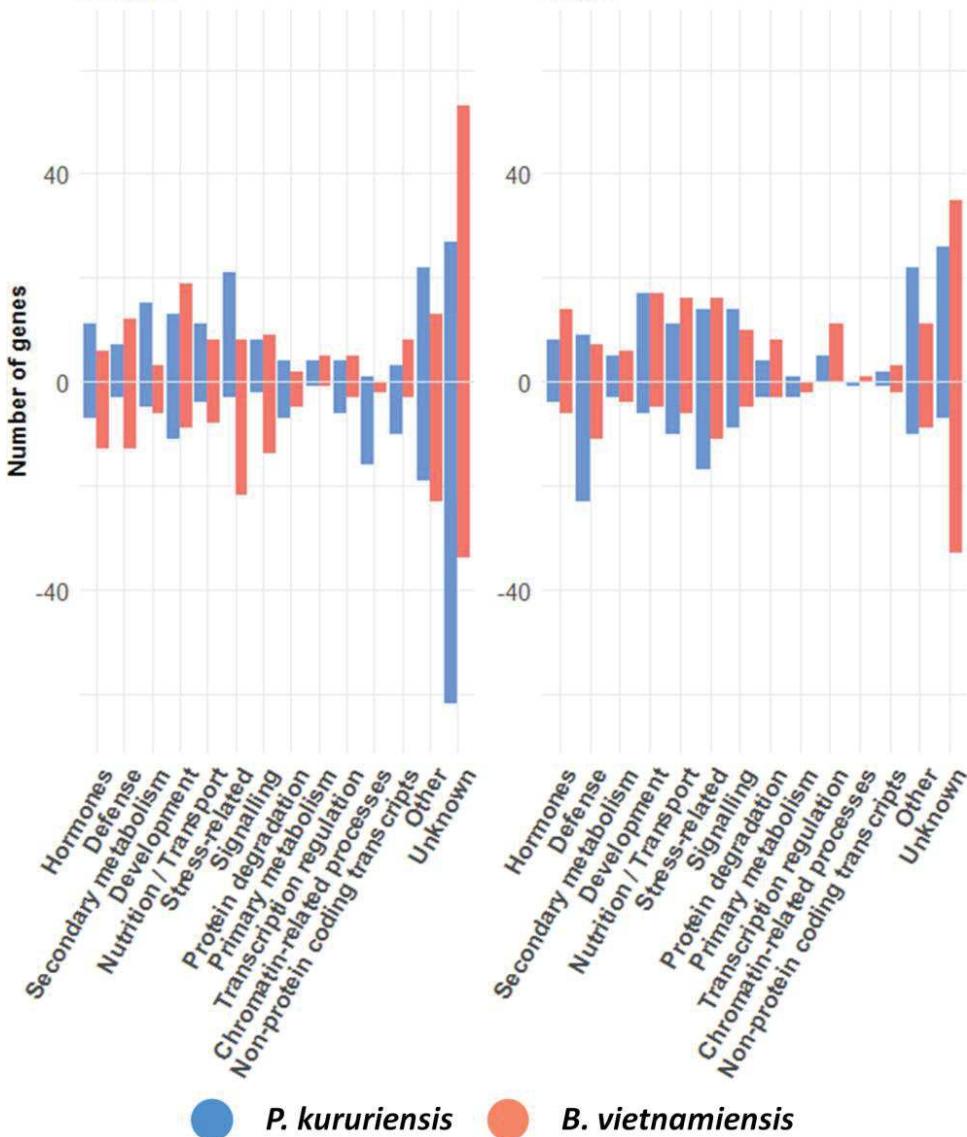
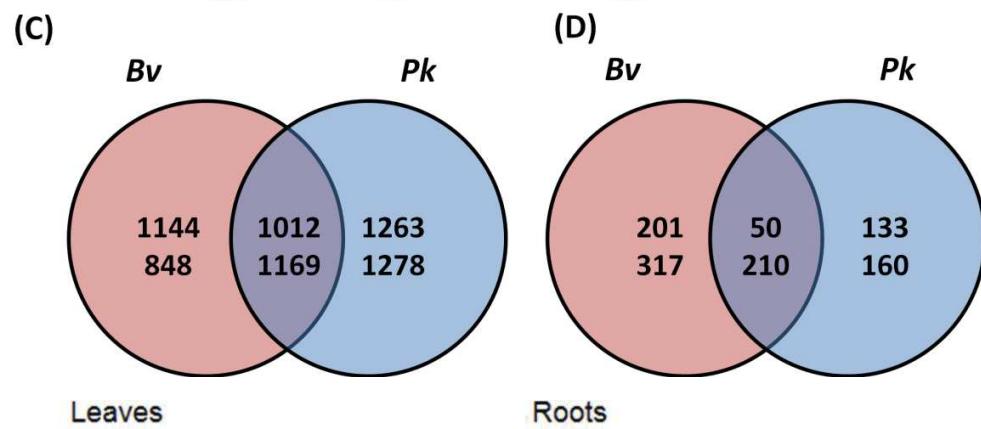
Furthermore, in leaves the “iron ion homeostasis” term is enriched in up-regulated DEGs while the “metal ion transport” term is enriched in the down-regulated DEGs of roots. Although the inoculation of both strains didn’t significantly impact the biomass of rice plants (Supplementary Figure 2), several development-related GO terms are enriched in the DEGs in both leaves and roots. Firstly, in leaves, among others, the “anatomical structure development” term is enriched in the up-regulated DEGs and the “glucan biosynthetic process” term is enriched in the down-regulated DEGs which correspond to genes implicated in cellulose and callose synthesis. Finally, roots transcriptional response is also enriched in development-related GO terms such as the “xylem development” term enriched in the up-regulated DEGs and the “lignin biosynthetic process” term enriched in the down-regulated DEGs.

Additionally, in order to identify the biological processes specifically induced during the interaction with each strain, we performed a GO term enrichment analysis on the DEGs specifically regulated by each strain (Supplementary Figures 4 & 5, Supplementary Tables 5 & 6). This analysis revealed that leaves of plants inoculated with *Pk* up-regulate genes related to biosynthetic process and translation while the interaction with *Bv* induce the up-regulation of genes coding for components of the photosystem II and also the down-regulation of protein folding genes (Supplementary Figure 4, genes listed in Supplementary Table 7). Interestingly, the up-regulated DEGs in response to each strain are enriched in one hormonal signaling pathway. Indeed, the interaction with *Pk* induced the up-regulation of JA-related genes while cytokinin-related genes are enriched in the up-regulated DEGs in the leaves of *Bv*-inoculated plants. The analysis of the specific root transcriptome also revealed processes specifically induced by each strain. Particularly, the response to *Pk* in roots encompasses the down-regulation of oxidative stress response related genes and also chitin catabolic process while the interaction with *Bv* induced the down-regulation of genes involved in defense, JA signaling and response to stimuli (Supplementary Figure 5, corresponding genes in Supplementary Table 8).



**Figure 3: Comparative analysis of leaves and roots transcriptomes in response to *Bv* and *Pk* colonization**

Principal component analysis of the normalized number of reads mapped per gene in leaves (A) and in roots (B). Number of genes regulated in leaves (C) and in roots (D) following bacterial inoculation, FDR < 0.01. The numbers on the upper part correspond to the number of genes up-regulated, inversely for the number of down-regulated genes.



**Figure 4: Functional categories of rice DEGs upon *Bv* and *Pk* colonization**

Number of DEGs related to functional categories among the top 200 up- and down-regulated DEGs in response to each strain. For each graph, positive or negative numbers correspond to the number of up- or down-regulated genes, respectively.

In order to deepen the transcriptome analysis, a functional categorization of the top 200 up and down-regulated DEGs identified in response to each strain was carried out using all available databases for rice annotation (Uniprot, KEGG, RAP-DB and Oryzabase). The proportion of genes related to each category is presented in Figure 4 and exemplify rice specific responses to the two bacterial strains. In leaves, more stress-related genes as well as genes involved in secondary metabolism are up-regulated in response to *Pk* compared to the response to *Bv*. Also, in response to *Pk*, 16 genes related to chromatin remodeling are down-regulated compared to two in response to *Bv*. Inversely, the inoculation of *Bv* induced the down-regulation in leaves of 22 stress-related genes whereas only three are down-regulated in response to *Pk*. In roots, twice as much transcription regulation and protein degradation-related genes are up-regulated in response to *Bv* than in response to *Pk*. Conversely, approximately twice as much “nutrition/transport” and signaling-related genes are down-regulated in response to *Pk* than in response to *Bv*.

In order to identify plant key genes that could be related to the observed patterns of root colonization, we focused our analysis on defense (leaves in Table 2, roots in Table 3) and hormone-related genes (leaves in Table 4, roots in Table 5). Indeed, these processes are enriched among the strain-specific DEGs (Supplementary Figures 4 & 5) and could pinpoint differences in the physiological response of rice following the perception of each strain.

In roots, 13 defense-related genes are commonly regulated while 33 and 18 are specifically *Pk* and *Bv*-regulated (Table 3). Additionally, 12 hormone-related genes (implicated in JA, CK, ethylene, GA and ABA synthesis or signaling) are commonly regulated in response to both strains while 14 and 19 hormone-related genes are *Pk* and *Bv*-regulated respectively (Table 5). The latest encompasses genes implicated in auxins, BR, SA and strigolactones synthesis or signaling. In leaves, 41 defense-related genes are regulated, 6 being commonly regulated while 11 and 24 are specifically induced by *Pk* and *Bv* respectively (Table 2). Also hormone-related DEGs are detected in leaves, 6 genes implicated in GA, JA, auxins, ethylene and ABA synthesis, transport or signaling are commonly regulated. Finally, in response to each strain, 20 hormone-related specific DEGs are detected. Interestingly, only the interaction with *Pk* induced the regulation of BR and SL related genes while only *Pv* induced the regulation of an SA-related gene. Other hormonal pathways, such as CK, ABA, ethylene, JA, auxins and GA are modulated by each strain but induced the regulation of different genes (Table 4).

Table 2: Defense-related DEGs in leaves in response to *P. kururiensis* and *B. vietnamensis*. Presented genes are part of the top 200 up and down-regulated significantly differentially expressed genes (FDR < 0.01), all results for leaves transcriptome can be found in Supplementary Table 6

Gene ID	Log <sub>2</sub> FoldChange		Gene Symbol/Trait Ontology	RAP Annotation
	<i>P. kururiensis</i>	<i>B. vietnamensis</i>		
Os05g0537100	1,70	2,44	<i>WRKY7</i>	WRKY7
Os07g0539900	-2,17	-1,59	<i>PR2</i>	Similar to Beta-1, 3-glucanase-like protein.
Os02g0118875	-1,89	-1,80	NB-ARC	NB-ARC domain containing protein.
Os01g0940800	-1,74	-1,52	<i>Gns6</i>	Similar to Beta-1, 3-glucanase precursor.
Os10g0124400	-1,54	-1,67	NB-ARC	Hypothetical conserved gene.
Os05g0492600	-1,84	-1,59	<i>YR48</i>	Similar to NBS-LRR type resistance protein
Os01g0731100	2,69		<i>PR</i>	Similar to Pathogen-related protein.
Os02g0759400	2,57		<i>RING1</i> / Disease resistance	Zinc finger, RING/FYVE/PHD-type domain containing protein.
Os02g0787300	1,88		<i>MAPKK4</i>	Mitogen-activated protein kinase kinase 4, Defense response
Os02g0626100	1,70		<i>PAL1</i>	Similar to Phenylalanine ammonia-lyase.
Os02g0181300	1,66		<i>WRKY71</i>	WRKY transcription factor, Defense response
Os12g0520200	1,56		<i>PAL3</i>	Similar to phenylalanine ammonia-lyase.
Os01g0855600	1,55		<i>HS1</i>	Similar to Hs1pro-1 protein.
Os11g0227200	-1,57		LRR / Disease resistance	Similar to NBS-LRR disease resistance protein homologue.
Os02g0570400	-1,61		<i>KSL7</i> / Disease resistance	Similar to Ent-kaurene synthase 1A.
Os09g0474000	-1,67		<i>TFX1</i> / Disease resistance	bZIP transcription factor, bZIP-1 domain containing protein.
Os01g0859500	-1,73		<i>LG2</i>	Similar to Basic leucine zipper protein (Ligueless2).
Os05g0375400		2,21	<i>PR2, OsEGL1</i>	Beta-glucanase precursor.
Os03g0320600		1,98	<i>VQ11</i>	VQ domain containing protein.
Os08g0170200		1,88		Disease resistance protein domain containing protein.
Os04g0462500		1,75	<i>GF14b, GF14b, 14-3-3b</i>	Similar to 14-3-3-like protein GF14-6.
Os04g0680800		1,64	<i>MLO4, MLO4</i>	Mlo-related protein family protein.
Os11g0505300		1,63	<i>STV11, SOT1</i>	Sulfotransferase, Resistance to rice stripe virus
Os12g0468300		1,63	NB-ARC	Similar to NB-ARC domain containing protein, expressed.
Os02g0251900		1,61	<i>VQ7</i>	Similar to Tobacco rattle virus-induced protein variant 2.
Os04g0118800		1,54	NB-ARC	NB-ARC domain containing protein.
Os01g0837000		1,54	<i>NPR4</i>	Ankyrin repeat containing protein.
Os11g0604900		1,51	NB-ARC	Similar to NB-ARC domain containing protein.

Os01g0289600	-1,46	<i>WRKY9</i>	Similar to WRKY transcription factor 9
Os01g0382000	-1,47	<i>PR1b</i>	Similar to Pathogenesis-related protein PRB1-2 precursor
Os08g0235800	-1,49	<i>WRKY25</i>	Similar to WRKY transcription factor 25.
Os10g0569400	-1,50	<i>Rir1a</i>	RIR1a protein precursor.
Os08g0386200	-1,55	<i>WRKY69</i>	WRKY transcription factor 69.
Os05g0427400	-1,64	<i>PAL4</i>	Similar to Phenylalanine ammonia-lyase.
Os08g0173600	-1,86	<i>PROPEP4</i> / Anti herbivore response	Conserved hypothetical protein.
Os07g0117900	-1,90	NB-ARC	NB-ARC domain containing protein.
Os06g0244000	-2,09	<i>AAMT</i>	Similar to anthranilic acid methyltransferase 3.
Os04g0205200	-2,09	NB-ARC	NB-ARC domain containing protein.
Os03g0195100	-2,16	<i>ALD1</i>	Putative aminotransferase, Response against blast fungus
Os12g0154800	-2,31	<i>GLP12-2</i>	Germin-like protein 12-2, Disease resistance
Os12g0154700	-2,91	<i>GLP12-1</i>	Germin-like protein 12-1, Disease resistance

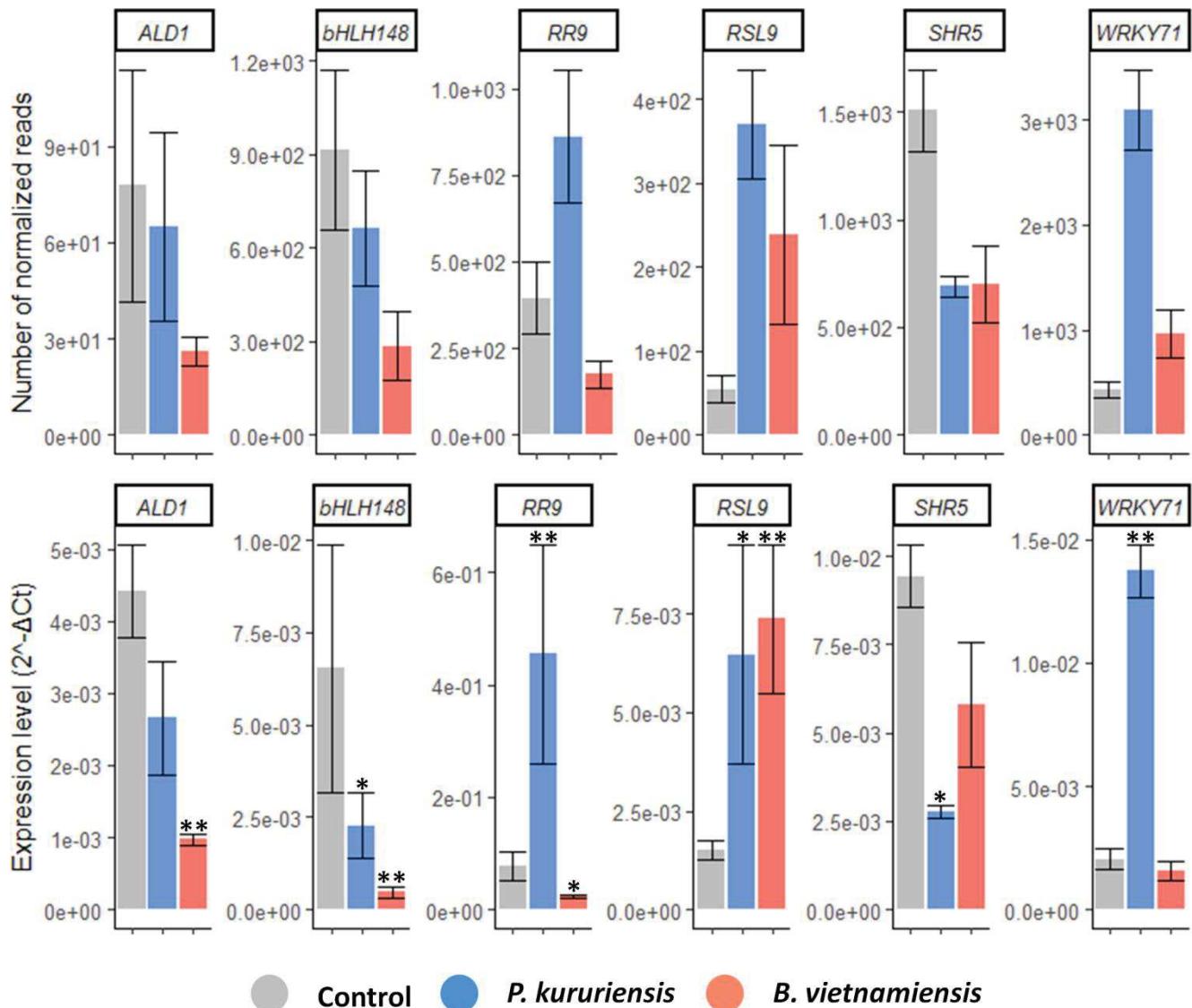
Table 3: Defense-related DEGs in roots in response to *P. kururiensis* and *B. vietnamensis*. Presented genes are part of the top 200 up and down-regulated significantly differentially expressed genes (FDR < 0.01), all results for root transcriptome can be found in Supplementary Table 7

Gene ID	LogFoldChange		Gene Symbol/Trait Ontology	RAP Annotation
	<i>P. kururiensis</i>	<i>B. vietnamensis</i>		
Os08g0332600	1,07	0,74	NB-ARC	Disease resistance protein domain containing protein.
Os08g0189500	-1,15	-1,57	<i>GLP8-6</i>	Germin-like protein 8-6, Disease resistance
Os08g0231400	-1,15	-1,62	<i>GLP8-12</i>	Germin-like protein 8-12, Disease resistance
Os10g0537800	-1,76	-1,80	<i>AP77</i>	Peptidase aspartic, catalytic domain containing protein.
Os12g0500500	-1,72	-1,84	NB-ARC	NB-ARC domain containing protein.
Os01g0508500	-2,07	-1,89	<i>RH2/NRR repressor homolog 2</i>	Conserved hypothetical protein.
Os02g0807900	-1,12	-2,17	<i>WAK21</i>	Conserved hypothetical protein.
Os09g0417600	-1,92	-2,64	<i>WRKY76</i>	WRKY transcription factor, Transcriptional repressor
Os03g0195100	-1,91	-2,65	<i>ALD1</i>	Putative aminotransferase, Response against blast fungus
Os09g0417800	-2,49	-2,66	<i>WRKY62</i>	WRKY transcription factor, Transcriptional repressor
Os01g0508100	-2,07	-2,83	<i>RH3/NRR repressor homolog 3</i>	Ferritin/ribonucleotide reductase-like family protein.
Os06g0105100	-2,75	-3,16	<i>WRKY86</i>	Similar to Legumain.
Os05g0247800	-3,47	-3,74	<i>XIP</i>	Glycoside hydrolase, family 18 protein.
Os08g0332600	1,07		NB-ARC	Disease resistance protein domain containing protein.
Os08g0446200	0,75		<i>PEPRI</i>	Similar to Receptor-like protein kinase precursor
Os12g0448900	0,74		<i>PIOX, RalphaO</i>	Fatty acid alpha-dioxygenase family
Os09g0127300	0,71		<i>CCR17</i>	NAD(P)-binding domain containing protein.
Os12g0199100	0,70		NB-ARC	NB-ARC domain containing protein.
Os07g0273700	0,69		<i>WRKY123</i>	Disease resistance protein domain containing protein.
Os01g0269800	0,68		NB-ARC	NB-ARC domain containing protein.
Os03g0411100	0,64		<i>OshAP2E</i>	Heme activator protein, Biotic and abiotic resistances
Os11g0152700	0,43		<i>OsbZIP79</i>	Similar to Transcription factor HBP-1b(C38) (Fragment).
Os02g0626400	0,41		<i>PAL8</i>	Phenylalanine ammonia-lyase (EC 4.3.1.5).
Os01g0713200	-1,14		<i>Gns10</i>	Similar to Beta-glucanase.
Os02g0629800	-1,15		<i>OsPR12/DEFL7</i>	Similar to Defensin precursor.

Os05g0554000	-1,19	<i>MATE2</i>	Similar to cDNA clone:001-123-D07, full insert sequence.
Os12g0555200	-1,20	<i>PBZ1</i>	Similar to Probenazole-inducible protein PBZ1.
Os05g0247100	-1,24	<i>HI-XIP / Insect resistance</i>	Similar to Glycosyl hydrolases family 18.
Os07g0129200	-1,26	<i>PR1a</i>	Pathogenesis-related 1a protein
Os07g0127500	-1,31	<i>PR1-72</i>	Similar to PR-1a pathogenesis related protein (Hv-1a) precursor.
Os02g0570700	-1,37	<i>Cyp71Z7</i>	Cytochrome P450 family protein.
Os01g0947000	-1,42	Disease resistance	Similar to Beta-1, 3-glucanase precursor.
Os02g0605900	-1,45	<i>CHIT6</i>	Similar to Chitinase (EC 3.2.1.14) A.
Os12g0437800	-1,62	<i>Sci1, PR6</i>	Similar to MPI.
Os11g0700900	-1,67	C10923 / Chitinase	Glycoside hydrolase
Os01g0940700	-1,85	<i>PR2, Glu1</i>	Similar to Glucan endo-1, 3-beta-glucosidase
Os07g0127700	-1,98	Disease resistance	Similar to Pathogenesis-related protein class 1.
Os12g0628600	-1,99	<i>PR5</i>	Similar to Thaumatin-like pathogenesis-related protein 3 precursor.
Os07g0663700	-2,19	<i>SDR110C-MI3/Disease resistance</i>	Similar to Oxidoreductase
Os08g0518900	-2,27	C10122 / Disease resistance	Chitinase (EC 3.2.1.14).
Os03g0130300	-2,54	<i>DEF8</i>	Similar to Cp-thionin.
Os11g0701600	-2,84	Chitinase	Glycoside hydrolase, catalytic domain domain containing protein.
Os04g0316200	-2,92	Gnk2-domain protein	Protein of unknown function DUF26 domain containing protein.
Os11g0701800	-3,16	<i>OsRIXI / Disease resistance</i>	Chitinase (EC 3.2.1.14) III
Os07g0129300	-3,67	<i>PR1a</i>	Similar to PR1a protein.
Os11g0701000	-4,67	Chib3H-c	Class III chitinase homologue (OsChib3H-c).
Os04g0289500	3,58	<i>PR1</i>	Allergen V5/Tpx-1-related domain containing protein.
Os12g0491800	2,15	<i>KSL10</i>	Similar to Ent-kaurene synthase 1A.
Os08g0374000	0,99	<i>OsBetvI</i>	Bet v I allergen family protein.
Os10g0163040	0,96	NB-ARC	Similar to Blast resistance protein.
Os08g0261000	0,91	NB-ARC	NB-ARC domain containing protein.
Os09g0356000	0,91	<i>SIRK1</i>	Similar to OsD305.
Os01g0859500	0,79	<i>LG2</i>	Similar to Basic leucine zipper protein (Liguleless2).
Os09g0517200	-1,65	NB-ARC	Hypothetical conserved gene.
Os05g0478700	-1,67	<i>WRKY84</i>	Hypothetical conserved gene.
Os03g0335200	-1,68	<i>WRKY79</i>	Similar to WRKY1 (WRKY transcription factor 17).

Os11g0117500	-1,75	<i>WRKY40</i>	DNA-binding WRKY domain containing protein.
Os06g0649000	-1,81	<i>WRKY28</i>	PAMP -responsive transrepressor
Os04g0635500	-1,93	Blast/SA induced	Similar to Wound induced protein (Fragment).
Os11g0154500	-2,09	Nac17 / Disease Resistance	Blast disease-responsive transcription factor, Disease resistance
Os11g0462500	-2,66	NB-ARC	Similar to NB-ARC domain containing protein.
Os05g0248200	-4,65	Chitinase	Glycoside hydrolase, family 18 protein.
Os04g0375300	-6,34	rNBS56	Similar to NBS-LRR protein (Fragment).
Os06g0279900	-7,11	NB-ARC	Similar to NB-ARC domain containing protein, expressed.





**Figure 5: Confirmation of genes regulated upon *Bv* and *Pk* colonization**

Gene expression quantified by RNA-Seq (top) and quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR; bottom). For RNA-Seq the data presented are the mean count of normalized reads. For RT-qPCR, transcript levels were normalized to that of the reference gene EF 1 $\alpha$  (Os03g0177400). Stars represent significant differences of mean expression compared to the control condition according to post-hoc test on a generalized linear model. \* corresponds to  $P < 0.05$  and \*\* to  $P < 0.0001$ . Error bars represent the standard deviation ( $n=3$ ).

### **Validation of RNA-Seq data by qPCR**

To validate the RNA-Seq data, we selected genes implicated in defense, hormone signaling or development regulated at 7 dpi by one or both strains (Supplementary Table 9). We measured the level of expression of the selected genes by RT-qPCR in an independently conducted experiment. According to RNA-Seq analysis, in leaves, two defense-related genes are specifically regulated by each strain: *ALD1* is specifically down-regulated in response to *Bv* and *WRKY71* is specifically up-regulated in response to *Pk*. Also, two hormone-related genes were detected as specifically regulated by each strain, namely: *bHLH148*, a JA signaling component, is down-regulated in roots inoculated with *Bv* while *RR9*, a CK signaling component is up-regulated in the leaves of *Pk*-inoculated plants. Finally in roots, *RSL9* is up-regulated and *SHR5* is down-regulated in response to both strains. Gene expression changes obtained through qPCR analysis demonstrated a pattern similar to RNA-Seq (Figure 5) except for the over-expression of *RR9* in response to *Pk* which was not detected as significant by DESeq2.

### **Temporal analysis of strain-specific marker genes**

Following the confirmation of strain-specific transcriptional regulations, we wanted to identify genes which are differentially expressed in both conditions but with opposite regulations. Among all DEGs detected in roots and leaves, only 3 DEGs are potential differential markers: *RERJ1* (Os04g0301500) a JA-responsive gene, *ATL15* (Os01g0597600) a putative amino acid transporter and *DREB1B* (Os09g0522000) a drought-responsive gene. Noteworthy, these three genes are only detected as DEGs in leaves and follow the same pattern of expression as they are up-regulated in response to *Pk* and down-regulated in response to *Bv*.

Among these three genes, two of them, *ATL15* and *RERJ1*, are part of a co-expression network recovered from RiceFRENDB database (Sato *et al.*, 2013) which contains 4 JA-related genes (*JAZ6*, *10*, *12* and *AOS1*) (Figure 6A). As JA is one of the main phytohormones implicated in defense (Pozo *et al.*, 2004) we further wanted to know if the whole co-expression network could act as a differential marker of the response to each bacterial strain. In order to describe the transcriptional regulation of this co-expression network throughout the establishment of the interaction, we produced a transcriptional kinetic of rice leaves tissues at 6 hours post inoculation (hpi), 1 dpi, 7 dpi and 14 dpi and analyzed all 6 genes by RT-qPCR.

Table 4: Hormone-related DEGs in leaves in response to *P. kururiensis* and *B. vietnamiensis*. Presented genes are part of the top 200 up and down-regulated significantly differentially expressed genes (FDR < 0.01), all results for leaves transcriptome can be found in Supplementary Table 6

Gene ID	Log <sub>2</sub> FoldChange		Gene Symbol/Function	RAP Annotation
	<i>P. kururiensis</i>	<i>B. vietnamiensis</i>		
<b>Gibberellic acid</b>				
Os06g0729400	1,85	2,74	<i>GASR8</i>	Similar to Gibberellin-regulated protein 2 precursor.
Os09g0470500	-1,49		<i>Hox4</i>	Homeodomain leucine zipper protein.
Os03g0352450	-1,60		<i>INO80, CHR732</i> GA signaling	Hypothetical conserved gene.
Os08g0560000	-1,66		<i>GA20ox7</i>	Similar to gibberellin 20 oxidase 2.
Os01g0757200		2,11	<i>GA2ox3</i>	GA 2-oxidase3, GA metabolism
Os06g0110000		1,59	<i>KAO</i>	Similar to DWARF3 (Fragment).
Os02g0570400		-1,61	<i>KS7</i>	Similar to Ent-kaurene synthase 1A.
<b>Jasmonate</b>				
Os04g0301500	1,88	-1,65	<i>RERJ1</i>	Helix-loop-helix DNA-binding domain containing protein.
Os03g0767000	2,11		<i>AOS1</i>	Allene oxide synthase (CYP74A1), Biosynthesis of jasmonic acid (JA)
Os01g0705700	1,84		<i>MYL1</i>	Similar to Transcription factor ICE1 (Inducer of CBF expression 1)
Os10g0392400	1,49		<i>JAZ12</i>	Tify domain containing protein.
Os05g0439100		1,46	<i>MYC7E</i>	Similar to Transcription factor MYC7E (Fragment).
Os03g0181100		-1,97	<i>JAZ10</i>	Tify domain containing protein.
<b>Auxin</b>				
Os01g0643300	2,44	2,29	<i>PIN3A</i>	Auxin efflux carrier protein, Auxin transport
Os12g0601300	2,81		<i>IAA30</i>	Similar to Auxin-responsive protein (Aux/IAA) (Fragment).
Os07g0182400		1,93	<i>IAA24</i>	AUX/IAA protein family protein.
Os02g0523800		1,72	<i>IPK2</i>	Inositol polyphosphate kinase, Auxin signaling
Os06g0323100		-1,53	IAA Methylase	Similar to H1005F08.18 protein.
Os06g0671600		-1,69	<i>Small auxin-up RNA 26</i>	Beta tubulin, autoregulation binding site domain containing protein.
Os05g0528600		-2,02	<i>YUCCA2</i>	Flavin monooxygenase-like enzyme, Auxin biosynthesis
<b>Ethylene</b>				
Os09g0522000	2,94	-1,91	<i>DREB1B</i>	Similar to Dehydration-responsive element-binding protein 1B.
Os09g0451000	2,19	1,64	<i>ACO2</i>	ACC oxidase, Ethylene biosynthesis

Os04g0572400	4,19	<i>DREB1E</i>	Similar to CRT/DRE binding factor 1.
Os02g0677300	3,41	<i>DREB1G</i>	Similar to CRT/DRE binding factor 1.
Os10g0562900	2,87	<i>DERF12</i>	Non-protein coding transcript.
Os08g0474000	2,64	<i>DERF3</i>	Similar to AP2 domain containing protein RAP2.6 (Fragment).
Os02g0654700	2,27	<i>BIERF3</i>	AP2/ERF family protein, Abiotic stress response
Os09g0522200	1,83	<i>DREB1A</i>	DRE-binding protein 1A.
Os07g0155600	-1,53	<i>EIN2</i>	Nramp ion-transporter family protein, Ethylene signaling pathway
Os07g0410300	4,78	<i>ERF136</i>	Conserved hypothetical protein.
Os07g0410700	1,93	<i>DERF2</i>	Similar to Ethylene-responsive element binding protein 1.
Os01g0797600	1,66	<i>BIERF2</i>	AP2/ERF family protein, Stress signaling
Os02g0676800	-1,94	<i>ERF20</i>	Similar to Dehydration responsive element binding protein 1E (DREB1E protein).
Os06g0493100	-2,31	<i>bphi008a</i>	Conserved hypothetical protein.
<b>Abscisic acid</b>			
Os02g0636600	-2,27	-1,81	GEM/ABA-related
Os02g0703600	3,07		<i>ABA8OXI</i>
Os07g0569100	1,86		<i>REM4.1</i>
Os07g0281800		-1,57	ABA Synthesis
<b>Cytokinin</b>			
Os02g0182100	-1,80		<i>RR24</i>
Os11g0143300		-1,71	<i>RR9</i>
Os01g0952500		-1,77	<i>RR4</i>
Os08g0460600		-1,89	<i>CKX11</i>
<b>Brassinosteroid</b>			
Os01g0197100	1,72		<i>dwf2, SMG11</i>
Os09g0409950	-1,48		BRI1-interacting protein 120
<b>Strigolactone</b>			
Os01g0935400	1,62	<i>AtLBO</i> ortholog	2OG-Fe(II) oxygenase domain containing protein.
<b>Salicylic acid</b>			
Os04g0581100	-2,01	S3H / SA Conjugation	2OG-Fe(II) oxygenase domain containing protein.

Table 5: Hormone-related DEGs in roots in response to *P. kururiensis* and *B. vietnamensis*. Presented genes are part of the top 200 up and down-regulated significantly differentially expressed genes (FDR < 0.01), all results for root transcriptome can be found in Supplementary Table 7

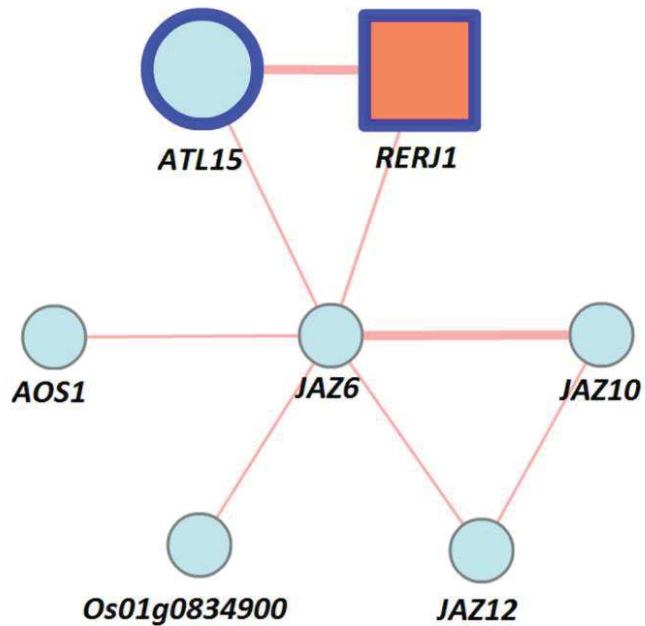
Gene ID	Log2FoldChange		Gene Symbol/Function	RAP Annotation
	<i>P. kururiensis</i>	<i>B. vietnamensis</i>		
<b>Jasmonate</b>				
Os01g0370000	0,87	0,96	<i>OPR9</i>	NADH:flavin oxidoreductase/NADH oxidase
Os05g0362100	0,57		JA synthesis	Similar to Protein MFP-b
Os08g0509100	-2,07		<i>LOX8</i>	Similar to Lipoxygenase, chloroplast precursor (EC 1.13.11.12)
Os03g0181100		-1,62	<i>JAZ10</i>	Tify domain containing protein.
Os03g0741100		-1,93	bHLH148 / JA responsive	Basic helix-loop-helix transcription factor
Os03g0180800		-2,01	<i>JAZ9</i>	TIFY domain-containing transcriptional regulator
<b>Auxin</b>				
Os02g0228900	0,58		<i>IAA7</i>	Similar to Auxin-responsive protein IAA18
Os11g0523800	0,49		<i>ARF1</i>	Similar to Isoform 3 of Auxin response factor 23
Os02g0305950		-2,92	<i>SAUR PROTEIN 7</i>	Similar to calmodulin binding protein.
Os08g0529000		-7,15	<i>PIN5b</i>	Auxin efflux carrier, Auxin homeostasis
<b>Cytokinin</b>				
Os04g0673300	0,66	0,94	<i>RR6</i>	A-type response regulator, Cytokinin signaling
Os04g0556500	-1,35	-1,64	<i>cZOGT1</i>	cis-Zeatin-O-glucosyltransferase
Os07g0449700	1,55		<i>RR7</i>	A-type response regulator, Cytokinin signaling
Os10g0483500	0,66		<i>CKX3</i>	FAD linked oxidase, N-terminal domain containing protein
Os08g0460600		1,29	<i>CKX11</i>	Similar to Cytokinin dehydrogenase 11.
Os11g0143300		1,02	<i>RR9</i>	A-type response regulator, Cytokinin signaling
Os12g0139400		1,00	<i>RR10</i>	A-type response regulator, Cytokinin signaling
Os02g0830200		0,81	<i>RR3</i>	A-type response regulator, Cytokinin signaling
Os05g0591600		-1,76	<i>LOGL8</i> CK synthesis	Similar to Carboxy-lyase.

<b>Ethylene</b>				
Os06g0573900	1,31	2,12	<i>ACO</i>	Similar to 1-aminocyclopropane-1-carboxylic acid oxidase.
Os02g0771600	1,03	1,06	<i>ACO3</i>	Similar to 1-aminocyclopropane-1-carboxylate oxidase
Os04g0610900	0,77	0,93	<i>CTR3</i>	Similar to EDR1.
Os03g0341000	-1,79	-1,99	<i>ERF66</i>	Similar to AP2 domain containing protein RAP2.2
Os07g0674800	-2,11	-3,02	<i>ERF67</i>	Similar to AP2 domain transcription factor EREBP.
Os01g0313300	-1,36	-2,79	<i>ERF68</i>	Similar to EREBP-3 protein (Fragment).
Os07g0575000	1,69		<i>ERF6</i>	ERF, DNA-binding domain containing protein.
Os05g0155200	0,49		<i>ERS2</i>	Similar to Ethylene receptor
Os05g0361700	-1,07		<i>ERF61</i>	Similar to EREBP-2 protein (Fragment).
Os06g0586000	-1,31		ETH responsive	Conserved hypothetical protein
Os03g0183200		1,25	<i>ERF69</i>	Similar to AP2 domain containing protein, expressed.
Os05g0316800		1,03	<i>ERF56</i>	Similar to Ethylene-responsive transcription factor 9
Os10g0390800		0,75	<i>EBL1</i>	Similar to Ethylene-responsive transcription factor 3 (EREBP-3)
Os09g0522000		-2,71	<i>DREB1B</i>	Similar to Dehydration-responsive element-binding protein 1B.
Os02g0677300		-3,39	<i>DREB1G</i>	Similar to CRT/DRE binding factor 1.
<b>Brassinosteroids</b>				
Os11g0289700	1,28		BR synthesis	Cytochrome P450 family protein
Os04g0641700	-1,32		<i>IL11</i>	Similar to H0423H10.4 protein.
Os09g0441400		-1,02	BR synthesis	Similar to Elicitor-inducible cytochrome P450.
<b>Gibberellic acid</b>				
Os05g0560900	-1,15	-1,73	<i>GA2ox8</i>	Similar to gibberellin 2-beta-dioxygenase.
Os06g0729400	0,55		<i>GASR8</i>	Similar to Gibberellin-regulated protein 2 precursor
Os05g0158600		-1,44	<i>GA2ox1</i>	Similar to OsGA2ox1.
<b>Abscisic acid</b>				
Os03g0437200	-1,99	-1,67	Bsr-d1	Abscisic acid-induced antioxidant defence
Os02g0255500	0,42	0,72	<i>PYL3</i>	Similar to Extensin (Fragment).
<b>Salicylic acid</b>				
Os04g0581100	-2,06		S3H/SA conjugation	2OG-Fe(II) oxygenase domain containing protein

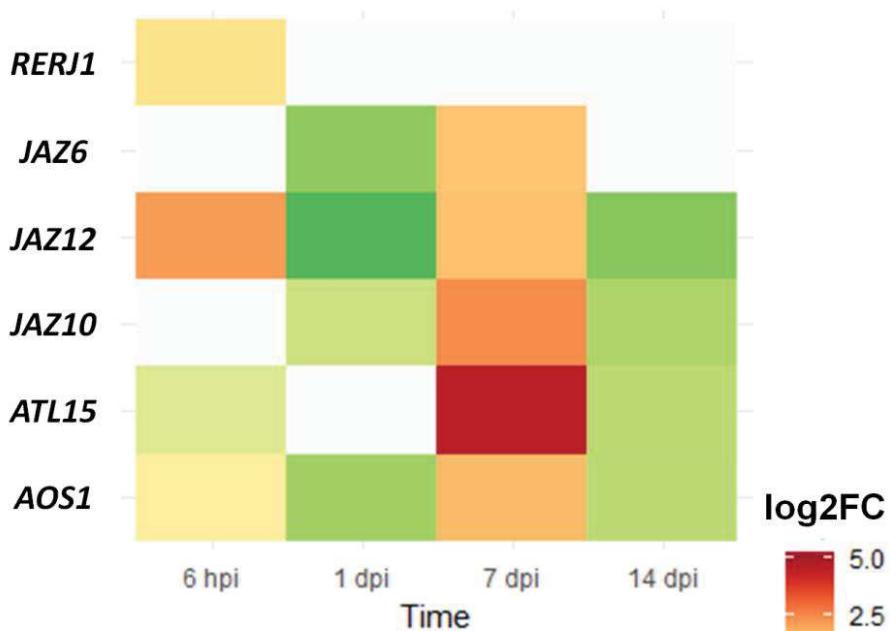
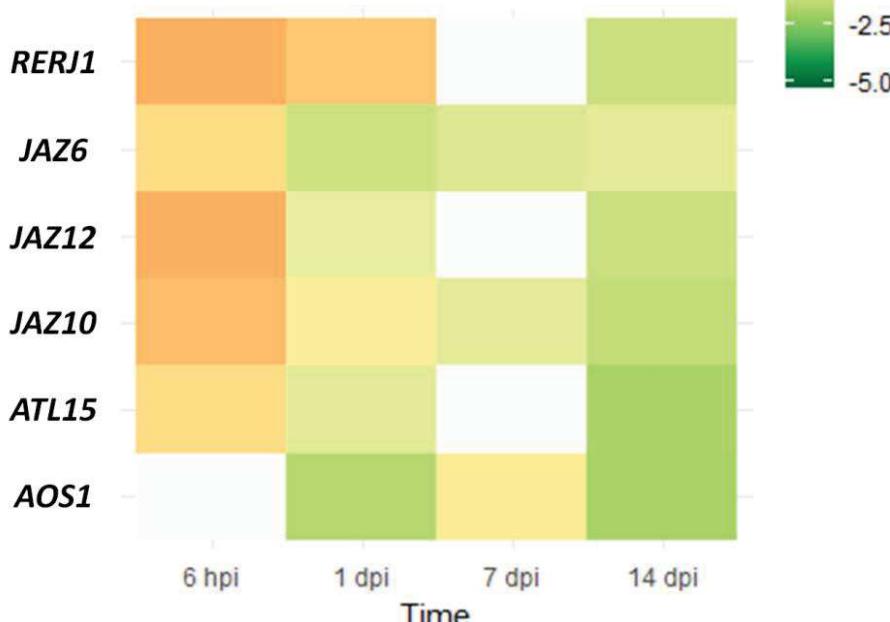
Os11g0259700	2,02	SA Conjugation	Similar to SAM dependent carboxyl methyltransferase
<b>Strigolactones</b>			
Os03g0203200	0,69	<i>dwf14</i>	Strigolactone receptor, Strigolactone perception, Regulation of shoot branching



(A)



(B)

*P. kururiensis**B. vietnamensis*

**Figure 6: JA-related co-expression network transcriptional regulations induced by *Pk* and *Bv* root colonization**

(A) Co-expression network of differential markers (circled in blue), edges width is proportional to the co-expression ratio between each genes according to RiceFRENDB database. (B) Gene expression dynamics of the co-expression network in response to *Pk* and *Bv* quantified by RT-qPCR. Transcript levels were normalized to that of reference gene EF 1 $\alpha$  (Os03g0177400). Values between 0.5 and -0.5 are colored in gray. Data presented are the mean of log2FoldChange (n=3).

Firstly, at 7 dpi we can confirm that depending on the inoculated strains, the regulation of the co-expression network is opposite (Figure 6B, Supplementary Table 10). On one hand *JAZ6*, *JAZ10*, *JAZ12*, *ATL15* and *AOS1* are up-regulated following the inoculation with *Pk* and on the other hand *JAZ6* and *JAZ10* are down-regulated in response to *Bv*. Interestingly, and in sharp contrast to the 7 dpi response, for the short-term response (6 hpi) we observed the up-regulation of 5 out of the 6 genes of the co-expression network in rice leaves after inoculation with *Bv* whereas only *JAZ12* is up-regulated in response to *Pk*. Then, the whole network appears to decline for the rest of the kinetics in response to *Bv*. Eventually, at 14 dpi the expression levels of the 6 genes are quite comparable between the two conditions being all down-regulated compared to non-inoculated controls.

- **Discussion**

The aim of this study was to describe the transcriptional regulations induced by rice following the perception of naturally-associated beneficial bacteria. Additionally, we took advantage of the particular phylogenetic organization of the *Burkholderia* s.l. genus to compare the responses of plants to two closely related beneficial species with different phylogenetic backgrounds in terms of ecology.

***P. kururiensis* M130 and *B. vietnamensis* TVV75 differentially colonize roots of *Oryza sativa* cultivar Nipponbare**

We first observed that both bacteria were able to efficiently colonize the rice Nipponbare roots. The amount of culturable bacterial cells associated with rice roots appears to be coherent with the literature as Compant *et al.* (2010) described that generally a range between  $10^7$ - $10^9$  CFU.g<sup>-1</sup> of root fresh weight are found colonizing roots both externally and internally. Same goes for the endophytic population which generally ranges between  $10^5$  and  $10^7$  CFU.g<sup>-1</sup>. The decrease of the bacterial population size between 7 and 14 dpi may be due to the increase of root biomass by the formation of newly emerged roots which are not importantly colonized at least in the time of our experiments. However, by comparing the colonization of the two strains, two main differences can be observed in the dynamic of root colonization. First, *Pk* forms a significantly bigger population while colonizing rice roots surface than *Bv* at every time post inoculation. Secondly, the dynamic of the endophytic population of both strains is very different as the number of endophytic *Pk* cells declines between 7 and 14 dpi which is not the case for *Bv*. This could be due to the fact that the endophytic colonization by *Pk* is restricted by the plant throughout time contrarily to *Bv* which maintains its population

size. From this observation two hypotheses can be proposed, either the plant is not able to control the colonization by *Bv* or this strain is more efficiently colonizing the newly emerged roots.

Both the maturation zone and the area of lateral root emergence were identified as hotspots for *Pk* and *Bv* colonization. A similar area of colonization was identified for *P. phytofirmans* strain PsJN in grape plants (*Vitis vinifera L.*) (Compant *et al.*, 2005) as well as for *Burkholderia vietnamiensis* MGK3 which also intensively colonizes the same areas of the roots of rice plants (Govindarajan *et al.*, 2008). Root exudates, which contain essential nutrients for microbes, are released in the lateral root emergence zones (Badri & Vivanco, 2009). This may aid colonization and allow the possible entry of bacteria via mechanisms such as “crack entry” into the internal tissues (Hardoim *et al.*, 2008).

Both strains were observed massively colonizing the maturation zone, both on the outside and even the inside of some root hair cells (Supplementary Figure 1). The accumulation of bacterial cells in the root hair zone has already been described for *Pk* (Mattos *et al.*, 2008) and it was proposed to be a common hotspot for rhizobacterial colonization (Compant *et al.*, 2010) as it is correlated to a higher local exudates concentration (Gamalero *et al.*, 2004). Apparent intracellular colonization of epidermal cells was observed for both species but more frequently for *Bv*. In the case of *Bv*, the fact that the vacuole is still intact when observing through the colonized epidermic cell offers evidence that the cell is still living (Figure 2D). Also, as the bacterial cells seem to have passed through the cell wall, the fluorescence signal appears to be cytoplasmic. Intracellular colonization of rice root epidermal cells by bacteria were also observed during the interaction with *Azoarcus* BH72 (Reinhold-Hurek *et al.*, 2006) as well as during the colonization of ryegrass roots by *Paraburkholderia bryophila* Ha185 (Hsu *et al.*, 2018). Furthermore, intracellular colonization of root hair cells was also observed by Prieto *et al.* (Prieto *et al.*, 2011). They showed by confocal microscopy that *Pseudomonas putida* PICP2 and *Pseudomonas fluorescens* PICF7 are able to colonize root hair cells of olive trees (*Olea europaea L.*) and subsequently move into epidermal cells. This observation led them to propose a new route of entry in intern plant tissues for endophytic bacteria which starts with the colonization of single root hairs.

### **Root colonization induces more transcriptional regulations in aerial parts than in roots**

The analysis of root and leaves transcriptomes revealed that colonization of rice roots by both strains induced more transcriptional regulations in leaves than in roots (Figure 3B). This could be due to the fact that the inoculated plants were harvested at seven dpi and therefore at an established state of the interaction between bacteria and rice. In contrast, previous studies

revealed that the inoculation of beneficial rhizobacteria induced the regulation of at least 1000 genes in rice roots at earlier time points after inoculation (Brusamarello-Santos *et al.*, 2018; Drogue *et al.*, 2014; Rekha *et al.*, 2018). Moreover, when comparing to the only study which analyzed the leaves transcriptional response of rice to the inoculation by beneficial rhizobacteria retrieved only 2414 DEGs at early stages of the interaction (Wu *et al.*, 2018) when our data identifies at least 4000 DEGs in leaves in response to both strains.

### **Roots trigger contrasted transcriptional response depending on the inoculated strain**

In response to the colonization by both strains, transcriptional reprogramming of defense-related genes occurs in rice roots (Table 3). Only 13 of them appeared commonly regulated in the same way. Firstly 3 *WRKY* (62, 76 and 86) genes are commonly down-regulated, interestingly those 3 genes are negative regulator of defense (Peng *et al.*, 2008; Yokotani *et al.*, 2013) - putatively for *WRKY86* (Choi *et al.*, 2017). Also two negative regulator of *NHI*, the rice *NPRI* homolog of the major SA response regulator (Chern *et al.*, 2012; Yuan *et al.*, 2007), namely *RH2* and *RH3* are also down-regulated (Chern *et al.*, 2014). Taken together as these genes are described as negative regulators of defense, their down-regulation may reflect a common defense response towards root colonizing bacteria.

The remaining 51 genes were differentially regulated in response to each strain (33 responding to *Pk* and 18 to *Bv*, respectively). Indeed roots colonized with *Pk* induce the down-regulation of 11 PRs genes as well as 5 chitinases and 2 xylanase inhibitors. Namely one of the down-regulated PR genes is *PBZ1* (Os12g0555200), it is described as a defense marker in leaves but it is also up-regulated in response to root invasion by *Magnaporthe oryzae* (Marcel *et al.*, 2010). Moreover, *bZIP79* which is described as a phytoalexin synthesis suppressor (Miyamoto *et al.*, 2015) is up-regulated. On the other hand, roots colonization by *Bv* induce the down-regulation of only one chitinase whereas two PRs genes are up-regulated, of which one gene, *BetvI* is targeted by parasitic nematode to suppress root defense (Chen *et al.*, 2018). Furthermore, 4 *WRKY* genes of which two are thought to encode negative regulators: *WRKY28* (Chujo *et al.*, 2013), *WRKY79* (Choi *et al.*, 2017) and *WRKY40* which is up-regulated in striga-resistant rice roots (Swarbrick *et al.* 2008.) are here down-regulated. Taken together these results show that the colonization of rice roots by *Pk* is associated with a down-regulation of *PRs* genes while the colonization by *Bv* is associated with the down-regulation of defense-suppressing *WRKY* genes. This transcriptional regulation of defense-related genes could be the consequence of the more invasive colonization pattern of *Bv* cells described above.

As previously described, (Drogue *et al.*, 2014; Rekha *et al.*, 2018; Vacheron *et al.*, 2013) the inoculation of beneficial rhizobacteria induces important hormone-related transcriptional regulation in rice roots (Table 5). Similarly, the inoculation of *Pk* and *Bv* induced the regulation of genes encoding for signaling components of CK and ethylene. Particularly, both bacterial strains induced the up-regulation of two genes encoding for ACO which are enzymes implicated in the synthesis of ethylene (Ravanbakhsh *et al.*, 2018). Interestingly, in response to the inoculation of other beneficial bacteria, genes coding for ACOs were down-regulated in rice roots (Brusamarello-Santos *et al.*, 2018; Drogue *et al.*, 2014). Also important transcriptional regulations of Ethylene Responsive Factors (ERFs) occur in response to both strains. In the same way, cytokinine signaling is impacted as both conditions induce the up-regulation of at least 2 *RR* genes and 1 cytokinine oxidase encoding gene (Tsai *et al.*, 2012) however it's not the same genes that are regulated by each bacteria.

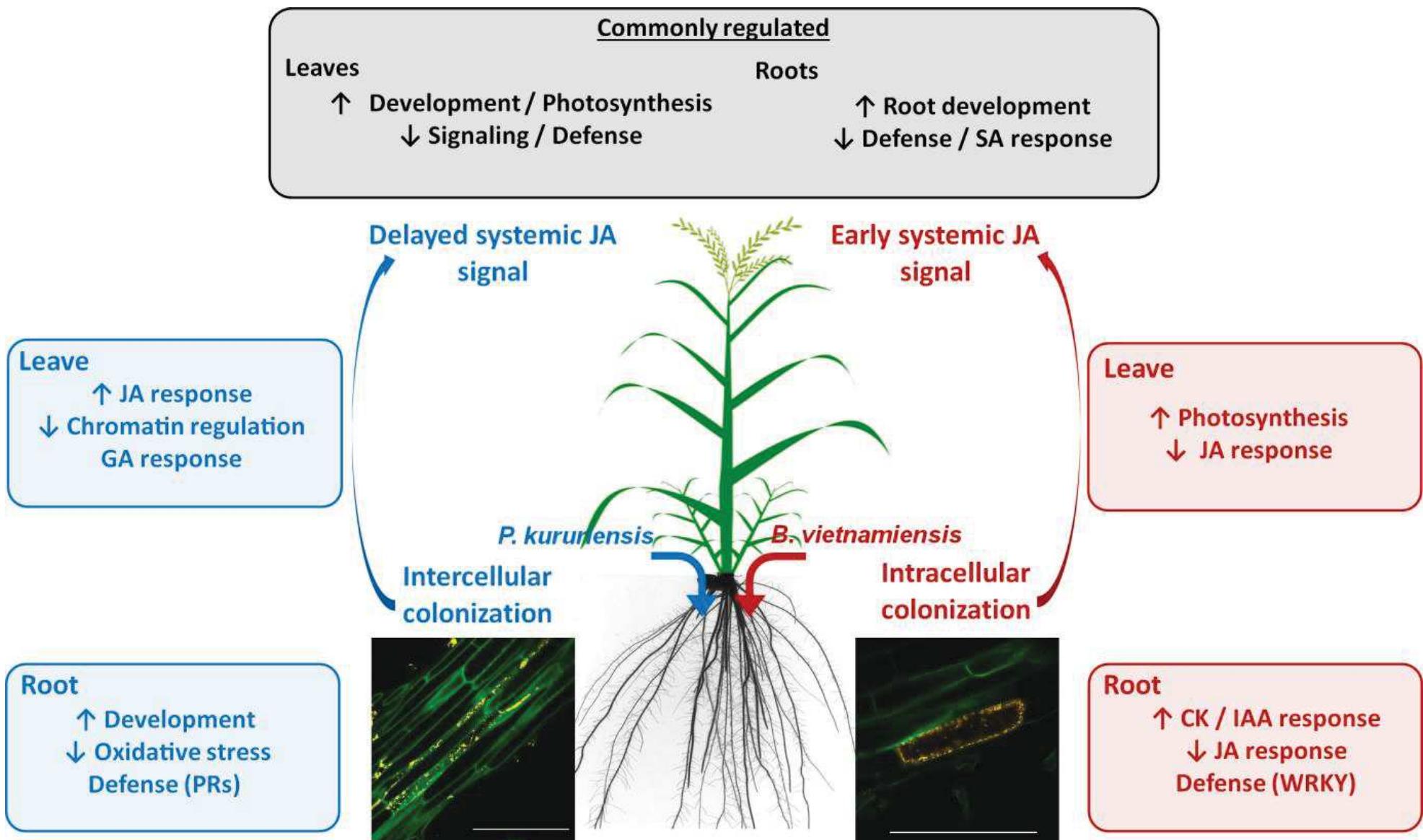
### **Systemic responses associated with root inoculation suggests a time shift in defense response between the two species**

Interestingly the RNA-Seq analysis revealed that the inoculation of rhizobacteria induced major transcriptional regulations in leaves (Table 2, Table 4, and Supplementary Table 5). As previously described (Campos-Soriano *et al.* 2012; Verhagen *et al.* 2004; Persello-Cartieaux *et al.* 2003), the colonization of beneficial microbes induce transcriptional regulations of defense-related genes in the leaves of the inoculated plants. Both strains induced the up-regulation of *WRKY7* as well as the down-regulation of 3 putative R genes and 2 PRs genes. *Pk* induced the up-regulation of *WRKY71* which is known to confer enhanced disease resistance to *Xoo* (Liu *et al.*, 2007) and the down-regulation of *TFX1* which is known as a susceptibility gene for *Xoo* (Sugio *et al.*, 2007). On the other hand *Bv* induce the up-regulation of 5 R genes: 3 *NB-ARC*, *MLO4* and *STV11* (Wang *et al.*, 2014), *NPR4* as well as the down-regulation of three *WRKY* genes (9, 25 and 69) of which two are SA-responsive (Choi *et al.*, 2017; Liu *et al.*, 2007) and also the down regulation of *ALD1*, a basal immunity regulator needed for the accumulation of SA (Jung *et al.*, 2016).

It is also in this organ only that differential markers have been detected. Some of which designated the JA signaling pathway as a putative marker of the interaction with the two bacterial species. Also, interestingly, in opposition with the regulation of JA-related genes, several GA-related genes seem to be down-regulated in response to *Pk* and up-regulated in response to *Bv*. This antagonism between JA and GA has been described (Yang *et al.*, 2012; Yimer *et al.*, 2018) and proposed as one of the ways for plants to fine tune the balance between growth and defense (Huot *et al.*, 2014). Nonetheless, this apparent opposition in

terms of JA-related genes transcriptional regulation at 7 dpi may only be a consequence of a delayed JA systemic signaling in response to *Pk* compared to the response to *Bv* which induced the up-regulation of the co-expression network as soon as 6 hpi. As JA-induced plant defenses have been proposed to contribute to the restriction of endophytic colonization in grasses (Miché *et al.*, 2006) this temporal shift in the induction of JA-related genes between the responses to the two bacterial strains could be associated with a delayed JA-induced defense signal. We propose the following interpretation: the perception of *Bv* induce very early (6 hpi) the up-regulation of JA-related genes in leaves to restrict the colonization whereas in response to *Pk* this JA signal happens at 7 dpi and result in the decrease of the bacterial population (Figure 1A).

The comparison of the interactions between rice and *Pk* and between rice and *Bv* revealed important differences in the process of root colonization and rice transcriptional regulations induced by each strain, that we summarized in Figure 7. First, the numerous intracellular colonization of root epidermic cells by *Bv* resemble a pathogen infection compared to the apoplastic colonization observed for *Pk* and other beneficial endophytes (McCully, 2001; Reinhold-Hurek *et al.*, 2007). However, such intracellular colonization by beneficial endophytes has already been observed (Reinhold-Hurek and Hurek, 1998). Also the specific root response to *Pk* is characterized by the down-regulation of genes coding for chitinase and PRs proteins while *Bv* colonization specifically induced the down-regulation of several *WRKY* genes (Table 3). Moreover, *Bv* specifically induced the down-regulation of JA-signaling genes (Supplementary Figure 5 and Supplementary Table 6) in addition to the common SA-related down-regulation in roots. Therefore, the strategies to circumvent the immune system of roots appear to be different between the two strains. Finally, the fact that the root inoculation of *Bv* induced the up-regulation of JA-related genes in leaves only 6 hpi compared to the delayed similar signal in response to *Pk* colonization at 7 dpi also supports the fact that *Bv* induces a response similar to pathogens. Indeed, the rice root colonization by *Magnaporthe oryzae* induced the up-regulation of JA-related genes in leaves at 3 and 4 dpi (Marcel *et al.*, 2010). All these elements support the fact that *Bv* appears to have a much more invasive colonization strategy both in terms of patterns and modulation of the plant immune system. This statement is in accordance with the opportunistic and pathogenic background of the *Burkholderia* s.s. genus (Eberl and Vandamme, 2016). Consequently, it would be of interest to analyze the response of the cultivar *Nipponbare* to a larger diversity of plant-associated *Burkholderia* and *Paraburkholderia* species to investigate if the differences observed between the two strains can be extrapolated to other species of the respective clades.



**Figure 7: Local and systemic transcriptional regulations of rice in response to *Pk* and *Bv* root colonization**

Common response includes the up-regulation of development-related genes and the down-regulation of defense-related genes in both leaves and roots. Strain-specific regulations in roots encompass the down-regulation of different defense-related genes: while *Pk* induced the down-regulation of Pathogenesis-Related (PRs) genes, *Bv* induced the down-regulation of WRKY transcription factors. Root colonization also induced a systemic up-regulation of Jasmonic Acid-related (JA) genes in leaves however not at the same time for each strain: while *Bv* induced this signal at early stages of the interaction, *Pk* induced a transient delayed systemic up-regulation of JA-related genes.

Another interesting conclusion from this study is the importance of the JA signaling in the interaction with beneficial rhizobacteria. As previously discussed, this major component of plant defense classically associated to the resistance to herbivores and necrotrophic pathogens appears to be involved in a larger diversity of biotic interactions (Thaler *et al.* 2004). We have demonstrated that there is a temporal delay in the induction of JA-related genes in leaves following the colonization by *Pk* comparatively to the response to *Bv*. It would be of interest to investigate the role of JA in the establishment of these interactions in terms of colonization level and plant defense status using JA-deficient mutants.

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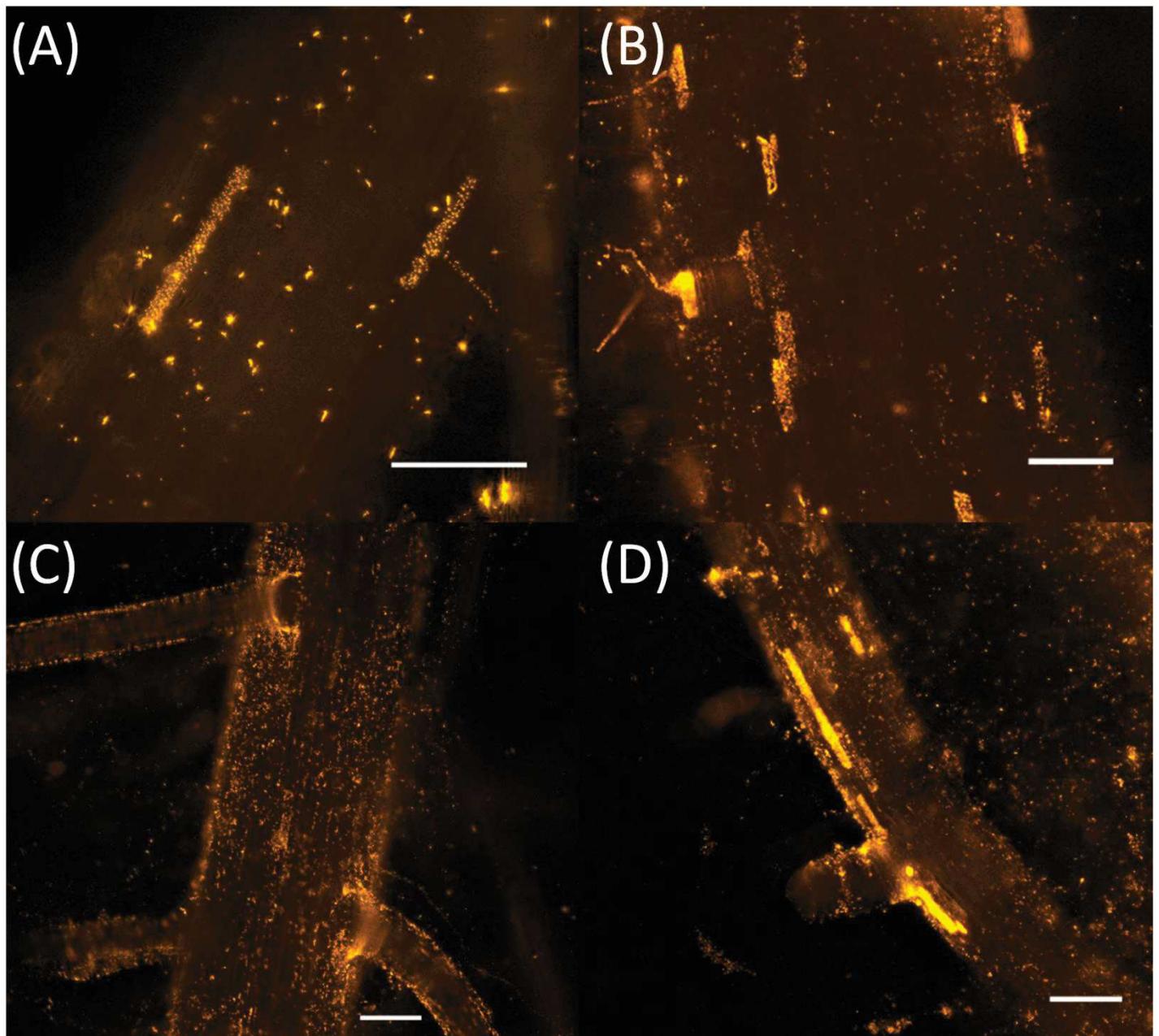
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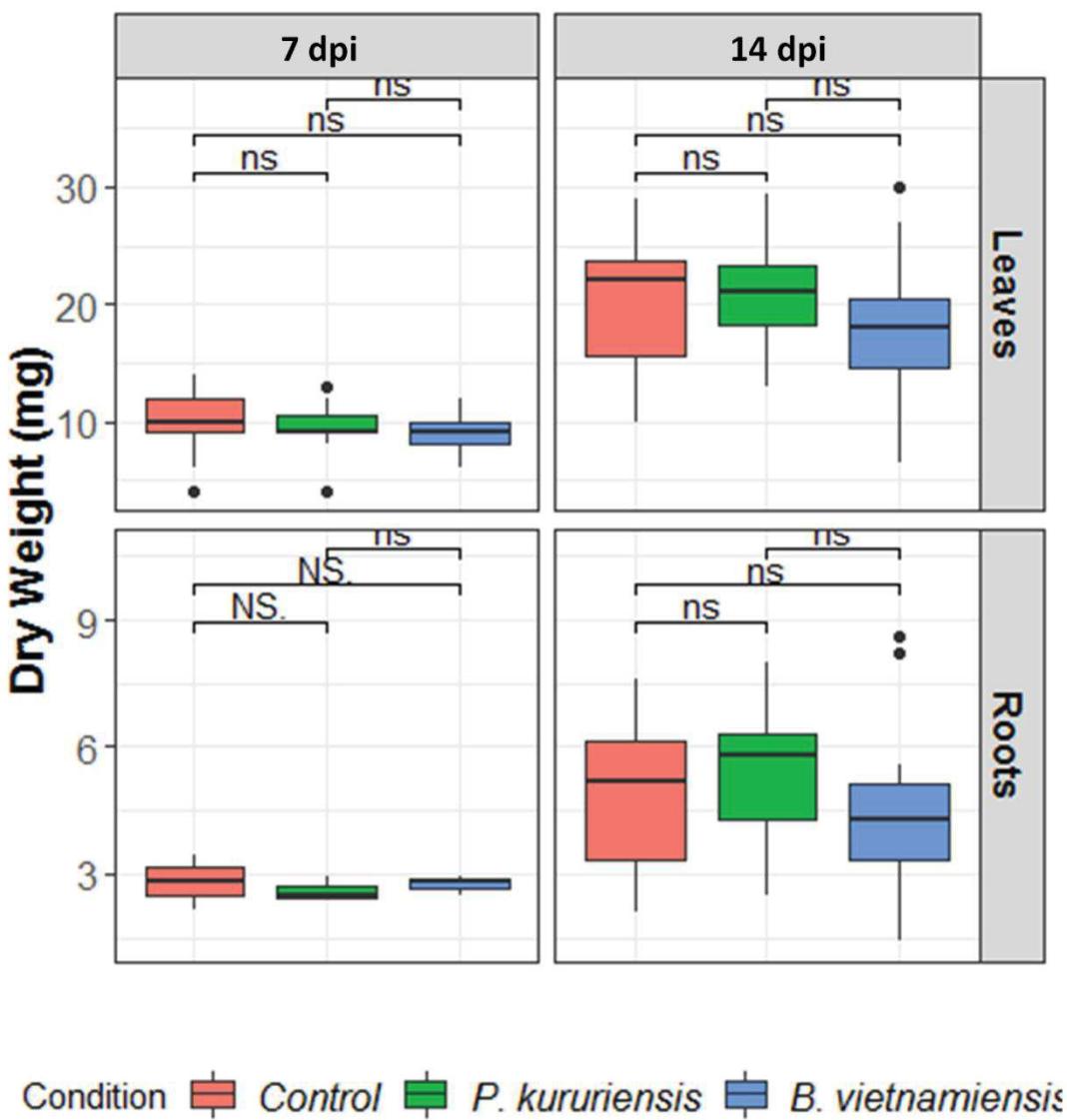
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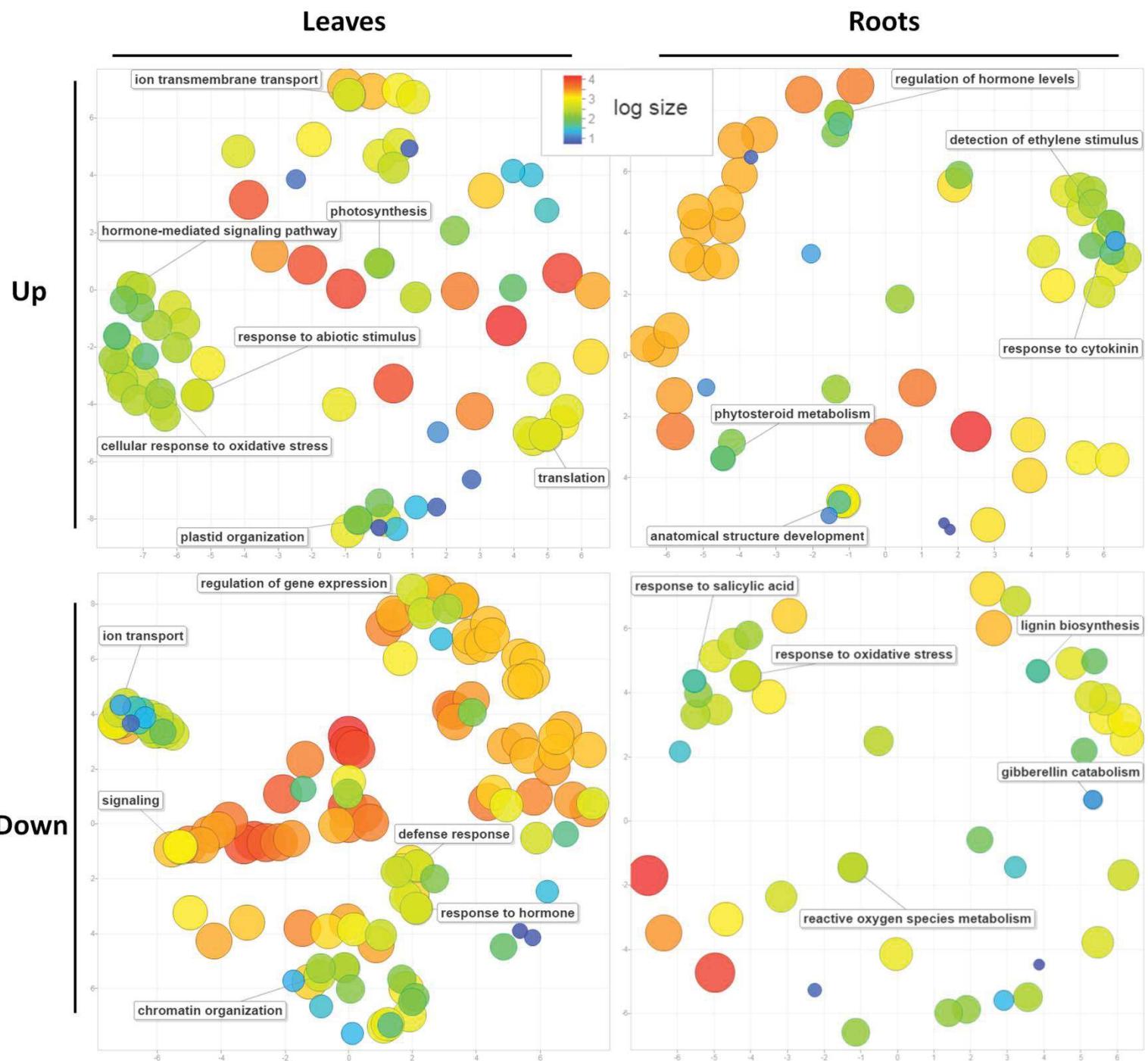
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**Supplementary Figure 1.** Rice roots colonization hotspots of *P. kururiensis* and *B. vietnamiensis*. Epifluorescence microscopy pictures of the colonization of axenically hydroponically grown rice primary roots at 7 days post inoculation by *P. kururiensis* (A, C) and *B. vietnamiensis* (B, D) DsRed cells. Panels A and B presents root hair colonization by bacterial cells and panels C and D presents the colonisation at the emergence of lateral roots. White bars represent 100 $\mu$ m

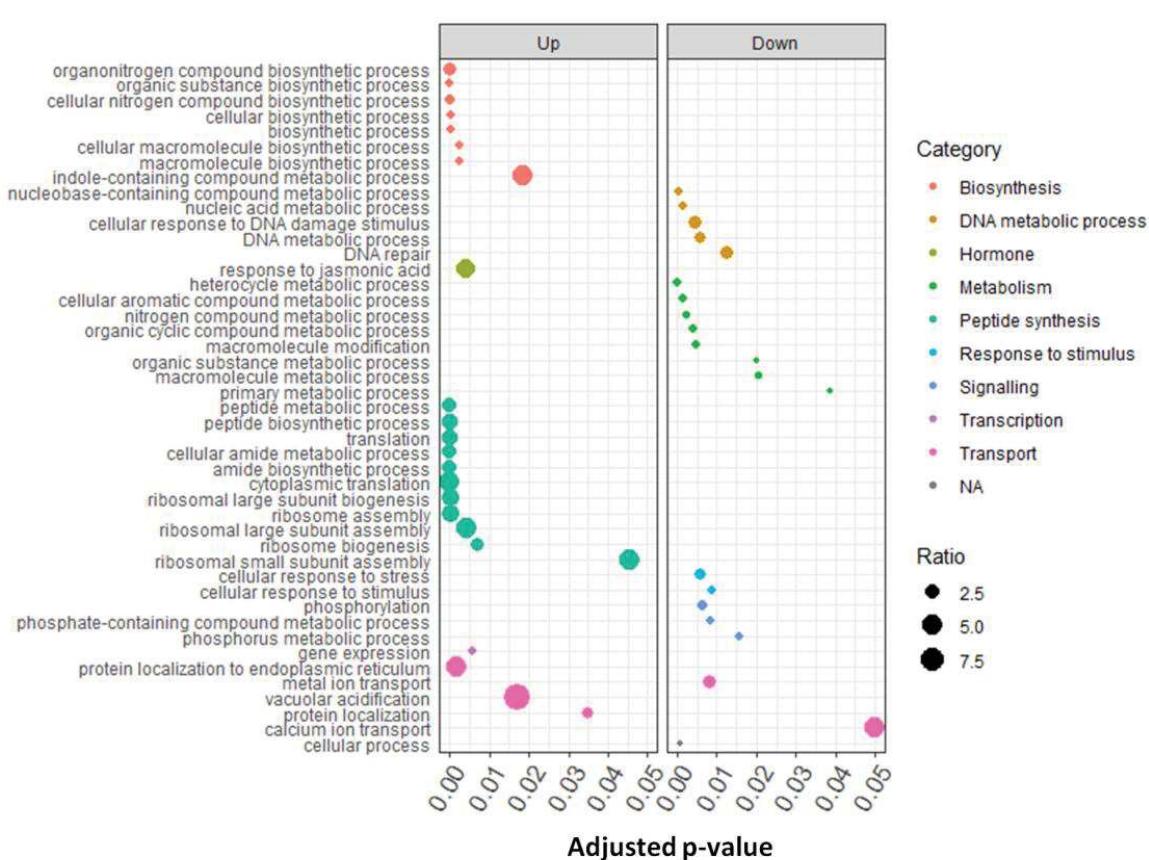


**Supplementary Figure 2.** Impact of the bacterial inoculation on rice biomass. Hydroponically grown rice plants were harvested at 7 and 14 days post inoculation (dpi) or mock-inoculation for controls. Rice tissues were harvested and oven-dry for 6 days. Statistical analysis depicts the result of a Wilcoxon rank test (n=15)

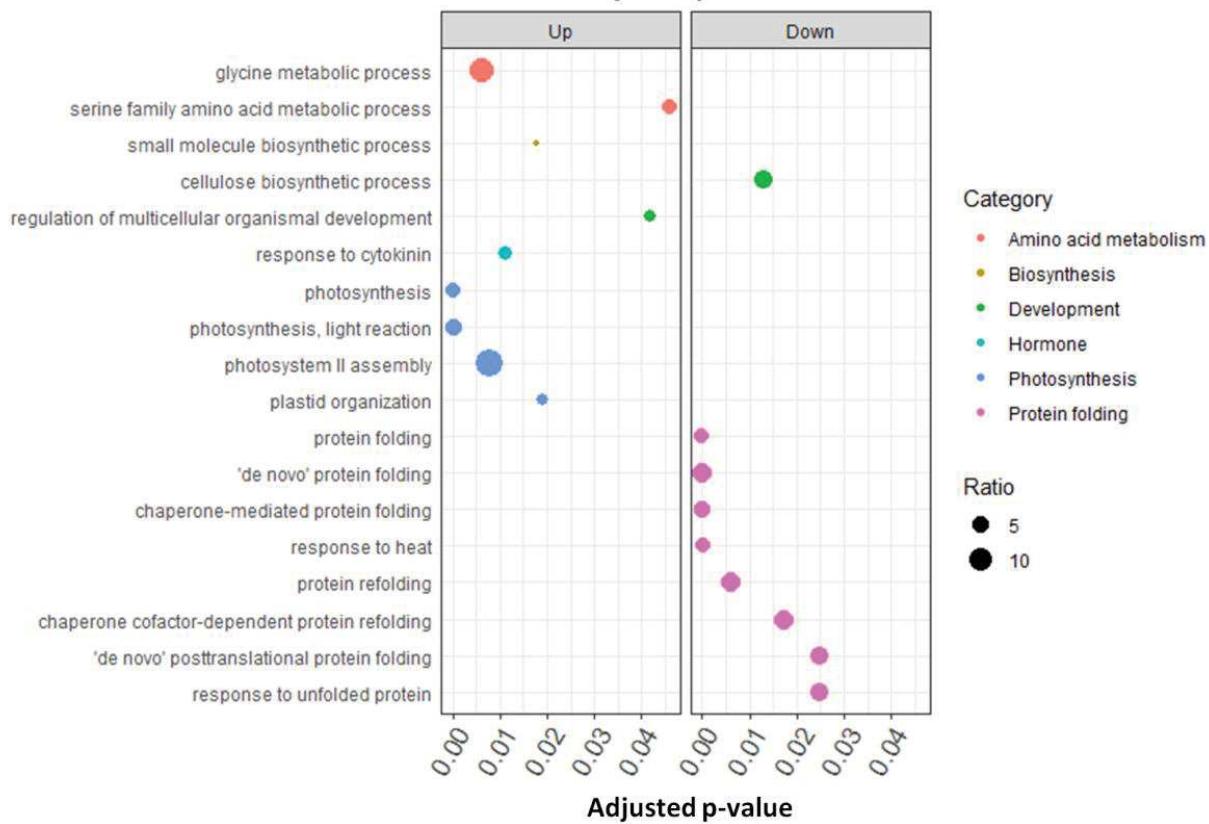


**Supplementary Figure 3.** Enrichment analysis of Biological Process GO terms of common leaves and roots transcriptomes in response to *B. vietnamensis* and *P. kururiensis* colonization. ReviGO (Supek *et al.*, 2011) display of Biological Process GO terms significantly enriched (FDR < 0.05) in the commonly regulated DEGs. The GO terms with higher frequency are displayed in shades of red while the ones with lower frequency are displayed with shades of blue. The GO terms with higher semantic similarity are closer on the plot.

(A)

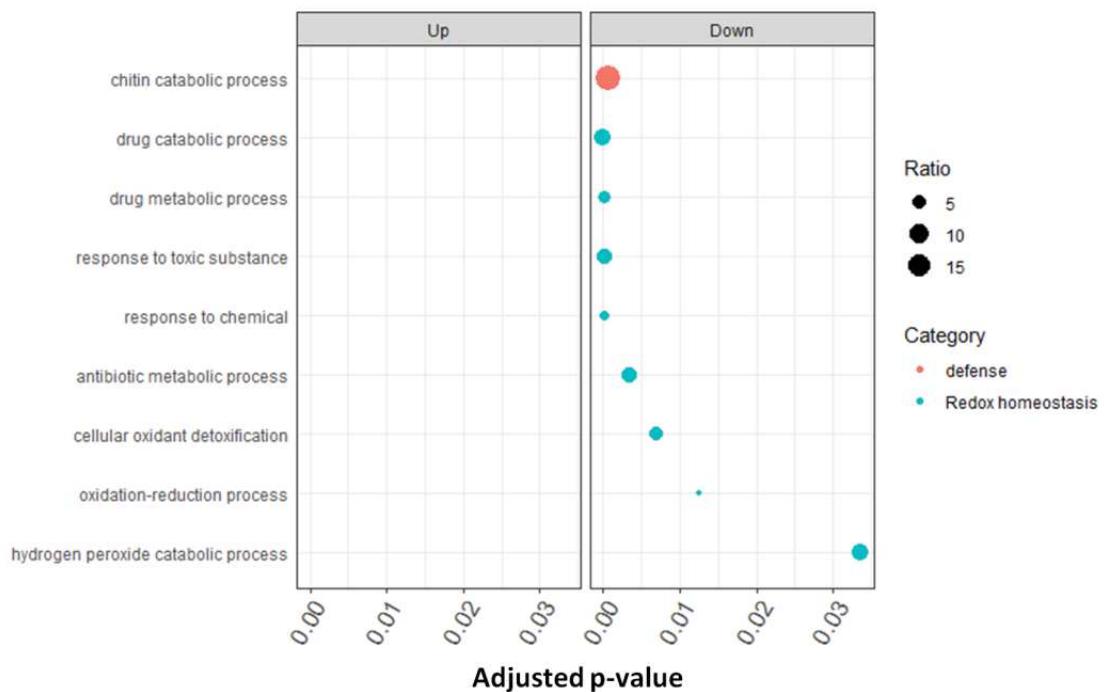


(B)

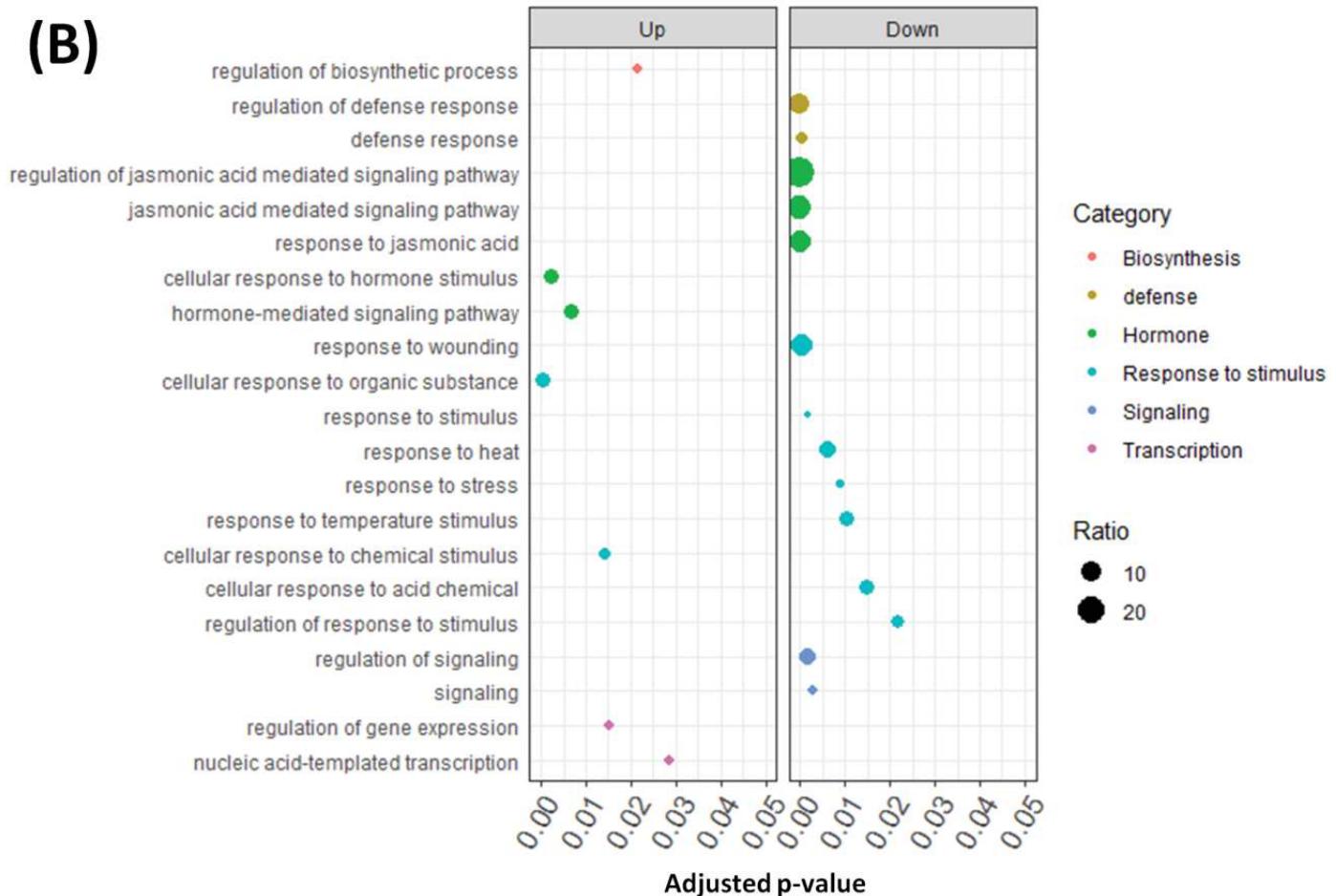


**Supplementary Figure 4.** GO terms enrichment analysis of strain-specific leaves transcriptomes. Enrichment analysis of Biological Process GO terms of specific leaves transcriptomes in response to (A) *P. kururiensis* and (B) *B. vietnamensis* colonization. Y-axis corresponds to the significantly enriched GO terms ( $FDR < 0.05$ ), X-axis corresponds to the adjusted p-value, the size of dots corresponds to the ratio between the proportion of genes related to the given GO term in the transcriptome and the proportion of genes related to the given GO term in the rice genome.

(A)



(B)



**Supplementary Figure 5.** GO terms enrichment analysis of strain-specific roots transcriptomes. Enrichment analysis of Biological Process GO terms of specific roots transcriptomes in response to (A) *P. kururiensis* and (B) *B. vietnamiensis* colonization. Y-axis corresponds to the significantly enriched GO terms (FDR < 0.05). X-axis corresponds to the adjusted p-value, the size of dots corresponds to the ratio between the proportion of genes related to the given GO term in the transcriptome and the proportion of genes related to the given GO term in the rice genome.

Supplementary Table 1 : Hydroponic medium

<b>Solutions</b>	<b>Salts</b>	<b>Final concentration</b>
NH <sub>4</sub> + Mg	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0,5 mM
	MgSO <sub>4</sub> .7H <sub>2</sub> O	1,6 mM
Ca + K	Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	1,2 mM
	KNO <sub>3</sub>	0,7 mM
Oligo-éléments	MnSO <sub>4</sub> .H <sub>2</sub> O	10 µM
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	0,16 µM
	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0,7 µM
	CuSO <sub>4</sub> .5H <sub>2</sub> O	0,8 µM
	H <sub>3</sub> BO <sub>3</sub>	22,6 µM
FeEDTA	FeSO <sub>4</sub> .7H <sub>2</sub> O	90 µM
	EDTA	90 µM
KH <sub>2</sub> PO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>	0,4 mM
Si	CaSiO <sub>3</sub> .9H <sub>2</sub> O	1,70 mM

Supplementary Table 2: Bacterial strains used in this study

Species name	Culture medium	Strain	Transformation	Reference
<i>Escherichia coli</i>	LB Cm 30 µg/mL	DH5 $\alpha$	plasmid pIN29 : <i>ori</i> <sub>pBBR</sub> $\Delta$ <i>mob</i> , Cm <sup>r</sup> , DSRed	Vergunst <i>et al.</i> , 2010
<i>Paraburkholderia kururiensis</i>	LB low salt	M130	Wild strain	Baldani <i>et al.</i> , 1997
<i>Burkholderia vietnamiensis</i>	LB low salt	TVV75 (= LMG 10929 <sup>T</sup> )	Wild strain	Tran Van <i>et al.</i> , 1996
<i>Paraburkholderia kururiensis</i>	LB low salt Cm 200 µg/mL	M130 + pIN29	pIN29 by electroporation	This study
<i>Burkholderia vietnamiensis</i>	LB low salt Cm 200 µg/mL	TVV75 (= LMG 10929 <sup>T</sup> ) + pIN29	pIN29 by electroporation	This study

Supplementary Table 3: Primers used in this study

RAP ID	Gene name	Primers sequence	Reference
Os03g0195100	<i>ALDI</i>	AGGCCACAAGAACATCTGAGGAAG TCTGAAGACGAGCAATGTCACA	This study
Os03g0741100	<i>bHLH148</i>	ACGATTGCGGCTTGTGAAGT TGTCCAGCCTTGCTTCCA	Caldana <i>et al.</i> , 2007
Os11g0143300	<i>RR9</i>	TCATGAGGACAGCCAAATTCTA TGCAGTAGTCTGTGATGATCAGGTT	Jain <i>et al.</i> , 2006
Os12g0589000	<i>RSL9</i>	GGGCCACGAGGATCTTGATT CCACAGTTGCTTGGCCTTC	This study
Os08g0203400	<i>SHR5</i>	ATCTCTTGAGTGGGCTTGGAG CCCTGCTGAATT CCTCAAGTCT	This study
Os02g0181300	<i>WRKY71</i>	AGCCTGGTGGTGAAGATGGGTA CATCTGAAGTAGGCTTGGGCAG	Caldana <i>et al.</i> , 2007
Os04g0301500	<i>RERJ1</i>	ATGGAGTCATGCGTTTGGC TGGGGTGTGCGAAAAATGAC	This study
Os03g0402800	<i>JAZ6</i>	TTGATGACTTCCCAGCTGAGAA GCGCTGTGGAGGAACCTCTG	Lu <i>et al.</i> , 2016
Os03g0181100	<i>JAZ10</i>	TCTTCCCACCCCGTCAAAT CCTCGCTGGTGCTTGCT	Zong <i>et al.</i> , 2016
Os10g0392400	<i>JAZ12</i>	TGCCGATCGCGAGGAA GGTCGCTCGTTGTCGTGAT	Hou <i>et al.</i> , 2009
Os03g0767000	<i>AOS1</i>	TTCCTCCGATACGACTCCTTC AGGTGACGGTGACAGATGAG	This study

Supplementary Table 4a : Gene Ontology term enrichment analysis of commonly up-regulated DEGs in leaves

<b>Term</b>	<b>padj</b>
biological_process	2,24E-08
oxidation-reduction process	1,04E-07
response to chemical	1,04E-07
metabolic process	2,95E-07
cellular response to chemical stimulus	1,17E-06
photosynthesis	1,78E-05
response to salt stress	3,06E-05
chloroplast rRNA processing	3,06E-05
response to osmotic stress	4,04E-05
NADH dehydrogenase complex (plastoquinone) assembly	8,69E-05
cellular process	1,32E-04
cellular metabolic process	1,32E-04
response to abiotic stimulus	3,23E-04
cellular response to toxic substance	4,13E-04
cellular oxidant detoxification	4,13E-04
cellular detoxification	4,13E-04
translation	4,30E-04
amide biosynthetic process	4,72E-04
cellular amide metabolic process	5,37E-04
peptide metabolic process	5,89E-04
peptide biosynthetic process	5,89E-04
response to hormone	6,08E-04
response to oxidative stress	6,72E-04
response to acid chemical	6,93E-04
response to endogenous stimulus	7,31E-04
photosynthesis, light reaction	8,50E-04
organic substance metabolic process	1,01E-03
response to water deprivation	1,75E-03
response to inorganic substance	1,75E-03
iron-sulfur cluster assembly	1,75E-03
cell redox homeostasis	1,75E-03
detoxification	2,01E-03
response to toxic substance	2,01E-03
cellular response to oxidative stress	2,67E-03
cellular component biogenesis	2,67E-03
response to organic substance	2,70E-03
response to light stimulus	2,78E-03
NADH dehydrogenase complex assembly	2,78E-03
hormone-mediated signaling pathway	2,88E-03
protein repair	3,42E-03
gene expression	3,42E-03
photosynthetic electron transport chain	4,29E-03
response to oxygen-containing compound	4,29E-03
ion transport	4,42E-03
electron transport chain	5,83E-03
response to radiation	5,85E-03
multicellular organism development	5,85E-03
cellular response to hormone stimulus	6,34E-03

multicellular organismal process	7,57E-03
ion transmembrane transport	8,40E-03
cellular response to endogenous stimulus	8,40E-03
generation of precursor metabolites and energy	8,57E-03
response to red or far red light	8,61E-03
organonitrogen compound metabolic process	9,02E-03
cellular response to organic substance	1,00E-02
photomorphogenesis	1,03E-02
primary root development	1,03E-02
post-embryonic development	1,03E-02
transmembrane transport	1,07E-02
response to stimulus	1,17E-02
anatomical structure development	1,22E-02
cellular response to acid chemical	1,40E-02
auxin-activated signaling pathway	1,40E-02
regulation of response to osmotic stress	1,42E-02
cellular response to auxin stimulus	1,58E-02
primary metabolic process	1,69E-02
nucleoid organization	1,78E-02
glycerol ether metabolic process	1,88E-02
transport	1,95E-02
cellular component assembly	1,95E-02
small molecule metabolic process	2,16E-02
biosynthetic process	2,18E-02
response to lipid	2,19E-02
plastid organization	2,72E-02
establishment of localization	2,78E-02
plastid translation	3,06E-02
response to abscisic acid	3,20E-02
cellular homeostasis	3,25E-02
organonitrogen compound biosynthetic process	3,41E-02
positive regulation of response to salt stress	3,57E-02
cellular biosynthetic process	3,71E-02
iron ion homeostasis	3,87E-02
protein stabilization	3,87E-02
regulation of biological quality	3,87E-02
cellular response to UV-B	4,08E-02
cellular response to oxygen-containing compound	4,46E-02
sucrose metabolic process	4,75E-02
photosynthetic electron transport in photosystem I	4,98E-02

Supplementary Table 4b : Gene Ontology term enrichment analysis of commonly down-regulated DEGs in leaves

<b>Term</b>	<b>padj</b>
cellular process	2,30E-23
biological_process	2,34E-20
organic substance metabolic process	2,70E-10
cellular metabolic process	3,89E-10
primary metabolic process	3,89E-10
biological regulation	8,20E-10
cellular nitrogen compound metabolic process	1,85E-09
RNA metabolic process	2,36E-09
regulation of biological process	1,14E-08
macromolecule metabolic process	1,30E-08
heterocycle metabolic process	1,83E-08
nucleobase-containing compound metabolic process	2,16E-08
nitrogen compound metabolic process	5,45E-08
gene expression	1,09E-07
nucleic acid metabolic process	1,49E-07
cellular aromatic compound metabolic process	1,67E-07
organic cyclic compound metabolic process	2,33E-07
metabolic process	8,98E-07
signal transduction	1,36E-06
regulation of cellular process	1,58E-06
signaling	1,58E-06
cell communication	2,53E-06
cellular response to stimulus	4,52E-06
localization	5,26E-06
transport	1,24E-05
establishment of localization	1,44E-05
glycolipid catabolic process	6,10E-05
glycosphingolipid catabolic process	6,10E-05
glucosylceramide catabolic process	6,10E-05
glycosylceramide catabolic process	6,10E-05
response to stimulus	6,18E-05
protein folding	9,69E-05
macromolecule localization	1,08E-04
cellular macromolecule metabolic process	1,08E-04
regulation of gene expression	1,25E-04
cellular macromolecule biosynthetic process	1,54E-04
cellular protein localization	1,55E-04
cellular biosynthetic process	1,75E-04
cellular macromolecule localization	2,02E-04
ceramide catabolic process	2,08E-04
glycosphingolipid metabolic process	2,08E-04
glucosylceramide metabolic process	2,08E-04
glycosylceramide metabolic process	2,08E-04
regulation of metabolic process	2,53E-04
macromolecule modification	3,06E-04
macromolecule biosynthetic process	3,37E-04
endocytosis	3,51E-04

cellular localization	3,51E-04
organic substance biosynthetic process	4,47E-04
sphingolipid catabolic process	5,12E-04
membrane lipid catabolic process	5,12E-04
protein localization	5,62E-04
cellular nitrogen compound biosynthetic process	5,62E-04
negative regulation of gene expression	6,22E-04
regulation of macromolecule metabolic process	8,45E-04
cellular component organization or biogenesis	9,45E-04
gene silencing	1,09E-03
starch biosynthetic process	1,27E-03
protein transport	1,37E-03
vesicle-mediated transport	1,37E-03
establishment of protein localization	1,54E-03
gene silencing by RNA	1,62E-03
import into cell	1,65E-03
organic substance transport	1,78E-03
amide transport	1,96E-03
transcription, DNA-templated	2,00E-03
intracellular protein transport	2,00E-03
phosphorus metabolic process	2,02E-03
nucleic acid-templated transcription	2,26E-03
biosynthetic process	2,29E-03
RNA biosynthetic process	2,29E-03
peptide transport	2,29E-03
nucleocytoplasmic transport	2,29E-03
nuclear transport	2,29E-03
heterocycle biosynthetic process	2,40E-03
response to stress	2,40E-03
energy reserve metabolic process	2,40E-03
glycogen metabolic process	2,40E-03
phosphate-containing compound metabolic process	2,48E-03
starch metabolic process	2,59E-03
nucleobase-containing compound biosynthetic process	3,38E-03
nucleic acid phosphodiester bond hydrolysis	3,41E-03
intracellular transport	3,41E-03
chromatin organization	3,41E-03
regulation of nucleobase-containing compound metabolic process	3,70E-03
glucan biosynthetic process	3,82E-03
establishment of localization in cell	4,24E-03
maturity of SSU-rRNA	4,38E-03
regulation of cellular macromolecule biosynthetic process	4,61E-03
organelle organization	4,61E-03
phosphorylation	5,35E-03
regulation of transcription, DNA-templated	5,35E-03
nitrogen compound transport	5,35E-03
regulation of macromolecule biosynthetic process	5,73E-03
glycoside catabolic process	5,89E-03
glycoside metabolic process	5,89E-03
regulation of RNA biosynthetic process	6,05E-03

regulation of nucleic acid-templated transcription	6,05E-03
cellular glucan metabolic process	6,05E-03
histone modification	6,05E-03
RNA export from nucleus	6,05E-03
regulation of cellular biosynthetic process	7,01E-03
covalent chromatin modification	7,02E-03
regulation of RNA metabolic process	7,22E-03
regulation of biosynthetic process	7,33E-03
glucan metabolic process	7,86E-03
glucose metabolic process	8,43E-03
aromatic compound biosynthetic process	9,08E-03
organonitrogen compound metabolic process	9,30E-03
nuclear export	9,30E-03
valyl-tRNA aminoacylation	9,43E-03
cellular component organization	1,08E-02
sulfur compound biosynthetic process	1,19E-02
cellular protein metabolic process	1,29E-02
negative regulation of transcription by RNA polymerase II	1,33E-02
cellular carbohydrate biosynthetic process	1,48E-02
organic cyclic compound biosynthetic process	1,55E-02
DNA demethylation	1,60E-02
ribosome-associated ubiquitin-dependent protein catabolic process	1,61E-02
regulation of cellular metabolic process	1,63E-02
ribonucleoprotein complex biogenesis	1,65E-02
defense response	1,71E-02
RNA transport	1,71E-02
establishment of RNA localization	1,71E-02
nucleic acid transport	1,71E-02
Golgi vesicle transport	1,84E-02
phospholipid transport	1,87E-02
cellular carbohydrate metabolic process	1,90E-02
protein phosphorylation	1,91E-02
ATP-dependent chromatin remodeling	1,91E-02
cellular protein modification process	1,94E-02
protein modification process	1,94E-02
response to hormone	2,21E-02
peptidyl-threonine phosphorylation	2,29E-02
peptidyl-threonine modification	2,29E-02
regulation of nitrogen compound metabolic process	2,35E-02
RNA modification	2,35E-02
protein-containing complex localization	2,46E-02
tRNA aminoacylation for protein translation	2,46E-02
chaperone-mediated protein folding	2,51E-02
protein metabolic process	2,57E-02
small molecule metabolic process	2,58E-02
response to endogenous stimulus	2,59E-02
chromosome organization	2,59E-02
cellular response to auxin stimulus	2,69E-02
receptor-mediated endocytosis	2,69E-02
aminoacyl-tRNA metabolism involved in translational fidelity	2,69E-02

regulation of primary metabolic process	2,71E-02
carboxylic acid metabolic process	2,71E-02
RNA localization	2,74E-02
cellular polysaccharide biosynthetic process	2,75E-02
ceramide metabolic process	2,78E-02
negative regulation of biological process	2,82E-02
amino acid activation	2,87E-02
tRNA aminoacylation	2,87E-02
response to organic substance	2,95E-02
oxoacid metabolic process	3,00E-02
negative regulation of metabolic process	3,00E-02
organic acid metabolic process	3,10E-02
protein export from nucleus	3,10E-02
energy derivation by oxidation of organic compounds	3,15E-02
negative regulation of macromolecule metabolic process	3,20E-02
cellular amide metabolic process	3,37E-02
nuclear pore distribution	3,37E-02
glucose catabolic process	3,37E-02
nuclear pore localization	3,37E-02
Golgi vesicle docking	3,37E-02
ribosome biogenesis	3,44E-02
ion transport	3,60E-02
cellular lipid catabolic process	3,63E-02
metal ion transport	3,68E-02
protein targeting to vacuole	3,79E-02
cellular response to hormone stimulus	3,83E-02
carbohydrate metabolic process	4,11E-02
ribosomal small subunit biogenesis	4,17E-02
demethylation	4,23E-02
thioester biosynthetic process	4,53E-02
DNA dealkylation	4,53E-02
acyl-CoA biosynthetic process	4,53E-02
monosaccharide metabolic process	4,71E-02
ionotropic glutamate receptor signaling pathway	4,71E-02
glutamate receptor signaling pathway	4,71E-02
cellular response to endogenous stimulus	4,75E-02

Supplementary Table 4c : Gene Ontology term enrichment analysis of commonly up-regulated DEGs in roots

<b>Term</b>	<b>padj</b>
developmental process	3,20E-02
brassinosteroid metabolic process	3,20E-02
brassinosteroid biosynthetic process	3,20E-02
brassinosteroid homeostasis	3,20E-02
cellular response to hormone stimulus	3,20E-02
hormone metabolic process	3,20E-02
hormone biosynthetic process	3,20E-02
seed germination	3,20E-02
hormone-mediated signaling pathway	3,20E-02
cytokinin-activated signaling pathway	3,20E-02
response to cytokinin	3,20E-02
anatomical structure development	3,20E-02
lipid homeostasis	3,20E-02
phytosteroid biosynthetic process	3,20E-02
response to hormone	3,20E-02
biological regulation	3,20E-02
response to endogenous stimulus	3,20E-02
cellular response to organic substance	3,20E-02
cellular response to cytokinin stimulus	3,20E-02
cell communication	3,20E-02
cellular response to lipid	3,20E-02
cellular response to endogenous stimulus	3,20E-02
seedling development	3,20E-02
response to ethylene	3,20E-02
phytosteroid metabolic process	3,20E-02
biological_process	3,41E-02
cellular response to acid chemical	3,74E-02
response to fatty acid	4,14E-02
cellular response to chemical stimulus	4,14E-02
multicellular organismal process	4,14E-02
cellular response to fatty acid	4,14E-02
phosphorelay signal transduction system	4,14E-02
regulation of hormone levels	4,14E-02
detection of ethylene stimulus	4,14E-02
response to organic substance	4,14E-02
regulation of biological process	4,73E-02
oxygen transport	4,91E-02
detection of chemical stimulus	4,91E-02
biosynthetic process	4,91E-02
steroid metabolic process	4,91E-02
cell recognition	4,91E-02
signal transduction	4,91E-02
steroid biosynthetic process	4,91E-02
S-adenosylmethionine biosynthetic process	4,91E-02
regulation of transcription, DNA-templated	4,91E-02
cellular response to nitric oxide	4,91E-02
detection of hormone stimulus	4,91E-02

regulation of primary metabolic process	4,91E-02
transcription, DNA-templated	4,91E-02
response to bronchodilator	4,91E-02
nucleic acid-templated transcription	4,91E-02
organic cyclic compound biosynthetic process	4,91E-02
organic substance biosynthetic process	4,91E-02
cellular response to oxygen-containing compound	4,91E-02
regulation of nucleic acid-templated transcription	4,91E-02
negative regulation of protein serine/threonine phosphatase activity	4,91E-02
response to nitric oxide	4,91E-02
detection of endogenous stimulus	4,91E-02
regulation of monopolar cell growth	4,91E-02
sterol metabolic process	4,91E-02
gas transport	4,91E-02
regulation of nucleobase-containing compound metabolic process	4,91E-02
signaling	4,91E-02
regulation of cellular metabolic process	4,91E-02
xylem development	4,91E-02
regulation of cellular macromolecule biosynthetic process	4,91E-02
RNA biosynthetic process	4,91E-02
response to lipid	4,91E-02
transmembrane transport	4,91E-02
response to chemical	4,91E-02
monopolar cell growth	4,91E-02
recognition of pollen	4,91E-02
chemical homeostasis	4,91E-02
regulation of cellular process	4,91E-02
regulation of nitrogen compound metabolic process	4,91E-02
regulation of RNA metabolic process	4,91E-02
regulation of unidimensional cell growth	4,91E-02
positive regulation of seed germination	4,91E-02
regulation of RNA biosynthetic process	4,91E-02
pollen-pistil interaction	4,93E-02
regulation of macromolecule biosynthetic process	4,99E-02

Supplementary Table 4d : Gene Ontology term enrichment analysis of commonly down-regulated DEGs in roots

<b>Term</b>	<b>padj</b>
oxidation-reduction process	2,27E-12
detoxification	7,36E-05
response to toxic substance	2,08E-04
cellular detoxification	3,36E-04
cellular oxidant detoxification	3,36E-04
cellular response to toxic substance	3,36E-04
cofactor metabolic process	5,75E-04
sulfur compound metabolic process	7,57E-04
glutathione metabolic process	3,71E-03
cellular modified amino acid metabolic process	3,73E-03
reactive oxygen species metabolic process	5,25E-03
drug metabolic process	5,25E-03
response to oxidative stress	5,25E-03
carboxylic acid biosynthetic process	5,92E-03
response to chemical	5,92E-03
hydrogen peroxide catabolic process	5,92E-03
cellular homeostasis	5,92E-03
antibiotic catabolic process	5,92E-03
organic acid biosynthetic process	5,92E-03
cell redox homeostasis	5,92E-03
hydrogen peroxide metabolic process	5,92E-03
metabolic process	5,92E-03
drug catabolic process	5,92E-03
response to stress	6,35E-03
response to stimulus	6,88E-03
cofactor catabolic process	9,87E-03
small molecule biosynthetic process	1,19E-02
systemic acquired resistance	1,19E-02
small molecule metabolic process	1,25E-02
lignin biosynthetic process	1,33E-02
oxoacid metabolic process	1,94E-02
regulation of systemic acquired resistance	1,94E-02
response to antibiotic	1,94E-02
response to salicylic acid	1,94E-02
regulation of response to biotic stimulus	1,94E-02
organic acid metabolic process	1,94E-02
amino acid salvage	2,01E-02
regulation of response to external stimulus	2,01E-02
gibberellin catabolic process	2,01E-02
L-methionine salvage from methylthioadenosine	2,01E-02
regulation of biological quality	2,01E-02
diterpenoid catabolic process	2,01E-02
cellular response to chemical stimulus	2,01E-02
L-methionine salvage	2,01E-02
homeostatic process	2,01E-02

regulation of multi-organism process	2,03E-02
carboxylic acid metabolic process	2,88E-02
response to organic cyclic compound	2,90E-02
response to chitin	2,93E-02
L-methionine biosynthetic process	2,93E-02
defense response, incompatible interaction	2,93E-02
aspartate family amino acid biosynthetic process	3,15E-02
terpenoid catabolic process	3,15E-02
hormone catabolic process	3,15E-02
antibiotic metabolic process	3,15E-02
secondary metabolic process	3,44E-02
toxin metabolic process	3,68E-02
cellular response to antibiotic	3,88E-02
biological_process	3,88E-02
phenylpropanoid biosynthetic process	3,88E-02
lignin metabolic process	4,43E-02
glucose metabolic process	4,58E-02
aspartate family amino acid metabolic process	4,72E-02

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Supplementary Table 5 : Gene Ontology term enrichment analysis of differentially expressed genes specifically regulated by each strain in leaves

<b>Condition</b>	<b>Regulation</b>	<b>Category</b>	<b>Term</b>	<b>pval</b>	<b>Ratio</b>
<i>P. kururiensis</i>	Up	Peptide synthesis	peptide metabolic process	1,52E-23	2,92
<i>P. kururiensis</i>	Up	Peptide synthesis	peptide biosynthetic process	6,16E-23	3,09
<i>P. kururiensis</i>	Up	Peptide synthesis	translation	1,75E-22	3,09
<i>P. kururiensis</i>	Up	Peptide synthesis	cellular amide metabolic process	2,57E-21	2,68
<i>P. kururiensis</i>	Up	Peptide synthesis	amide biosynthetic process	3,10E-21	2,90
<i>P. kururiensis</i>	Up	Biosynthesis	organonitrogen compound biosynthetic process	5,26E-17	2,13
<i>P. kururiensis</i>	Up	Peptide synthesis	cytoplasmic translation	3,28E-09	5,59
<i>P. kururiensis</i>	Up	Biosynthesis	organic substance biosynthetic process	1,62E-05	1,33
<i>P. kururiensis</i>	Up	Biosynthesis	cellular nitrogen compound biosynthetic process	2,08E-05	1,40
<i>P. kururiensis</i>	Up	Biosynthesis	cellular biosynthetic process	1,15E-04	1,31
<i>P. kururiensis</i>	Up	Peptide synthesis	ribosomal large subunit biogenesis	1,45E-04	4,06
<i>P. kururiensis</i>	Up	Biosynthesis	biosynthetic process	1,49E-04	1,29
<i>P. kururiensis</i>	Up	Peptide synthesis	ribosome assembly	2,21E-04	3,79
<i>P. kururiensis</i>	Up	Transport	protein localization to endoplasmic reticulum	1,73E-03	5,23
<i>P. kururiensis</i>	Up	Biosynthesis	cellular macromolecule biosynthetic process	2,41E-03	1,33
<i>P. kururiensis</i>	Up	Biosynthesis	macromolecule biosynthetic process	2,57E-03	1,33
<i>P. kururiensis</i>	Up	Hormone	response to jasmonic acid	3,95E-03	4,50
<i>P. kururiensis</i>	Up	Peptide synthesis	ribosomal large subunit assembly	4,15E-03	5,28
<i>P. kururiensis</i>	Up	Transcription	gene expression	5,64E-03	1,30
<i>P. kururiensis</i>	Up	Peptide synthesis	ribosome biogenesis	6,73E-03	2,15
<i>P. kururiensis</i>	Up	Transport	vacuolar acidification	1,71E-02	9,41
<i>P. kururiensis</i>	Up	Biosynthesis	indole-containing compound metabolic process	1,85E-02	5,00
<i>P. kururiensis</i>	Up	Transport	protein localization	3,47E-02	1,74
<i>P. kururiensis</i>	Up	Peptide synthesis	ribosomal small subunit assembly	4,54E-02	5,70
<i>P. kururiensis</i>	Down	Metabolism	heterocycle metabolic process	1,60E-04	1,28
<i>P. kururiensis</i>	Down	DNA metabolic process	nucleobase-containing compound metabolic process	4,05E-04	1,28
<i>P. kururiensis</i>	Down		cellular process	8,44E-04	1,09
<i>P. kururiensis</i>	Down	Metabolism	cellular aromatic compound metabolic process	1,42E-03	1,25
<i>P. kururiensis</i>	Down	DNA metabolic process	nucleic acid metabolic process	1,63E-03	1,29
<i>P. kururiensis</i>	Down	Metabolism	nitrogen compound metabolic process	2,14E-03	1,14

<i>P. kururiensis</i>	Down	Metabolism	organic cyclic compound metabolic process	3,96E-03	1,24
<i>P. kururiensis</i>	Down	DNA metabolic process	cellular response to DNA damage stimulus	4,33E-03	2,18
<i>P. kururiensis</i>	Down	Metabolism	macromolecule modification	4,80E-03	1,28
<i>P. kururiensis</i>	Down	DNA metabolic process	DNA metabolic process	5,88E-03	1,82
<i>P. kururiensis</i>	Down	Response to stimulus	cellular response to stress	5,92E-03	1,79
<i>P. kururiensis</i>	Down	Signalling	phosphorylation	6,09E-03	1,42
<i>P. kururiensis</i>	Down	Transport	metal ion transport	8,10E-03	2,23
<i>P. kururiensis</i>	Down	Signalling	phosphate-containing compound metabolic process	8,25E-03	1,34
<i>P. kururiensis</i>	Down	Response to stimulus	cellular response to stimulus	8,79E-03	1,36
<i>P. kururiensis</i>	Down	DNA metabolic process	DNA repair	1,24E-02	2,16
<i>P. kururiensis</i>	Down	Signalling	phosphorus metabolic process	1,56E-02	1,33
<i>P. kururiensis</i>	Down	Metabolism	organic substance metabolic process	2,00E-02	1,10
<i>P. kururiensis</i>	Down	Metabolism	macromolecule metabolic process	2,04E-02	1,13
<i>P. kururiensis</i>	Down	Metabolism	primary metabolic process	3,87E-02	1,10
<i>P. kururiensis</i>	Down	Transport	calcium ion transport	4,99E-02	5,52
<i>B. vietnamensis</i>	Up	Photosynthesis	photosynthesis	2,33E-08	3,96
<i>B. vietnamensis</i>	Up	Photosynthesis	photosynthesis, light reaction	2,8451E-05	4,77
<i>B. vietnamensis</i>	Up	Amino acid metabolism	glycine metabolic process	0,00616146	11,25
<i>B. vietnamensis</i>	Up	Photosynthesis	photosystem II assembly	0,00782185	14,59
<i>B. vietnamensis</i>	Up	Hormone	response to cytokinin	0,01098367	3,91
<i>B. vietnamensis</i>	Up	Biosynthesis	small molecule biosynthetic process	0,0176506	1,87
<i>B. vietnamensis</i>	Up	Photosynthesis	plastid organization	0,01898361	2,89
<i>B. vietnamensis</i>	Up	Development	regulation of multicellular organismal development	0,04193665	2,82
<i>B. vietnamensis</i>	Up	Amino acid metabolism	serine family amino acid metabolic process	0,04587512	4,61
<i>B. vietnamensis</i>	Down	Protein folding	protein folding	5,60E-07	3,93
<i>B. vietnamensis</i>	Down	Protein folding	'de novo' protein folding	7,7691E-06	6,95
<i>B. vietnamensis</i>	Down	Protein folding	chaperone-mediated protein folding	4,943E-05	5,60
<i>B. vietnamensis</i>	Down	Protein folding	response to heat	0,00013419	4,60
<i>B. vietnamensis</i>	Down	Protein folding	protein refolding	0,00598885	7,60
<i>B. vietnamensis</i>	Down	Development	cellulose biosynthetic process	0,01288375	5,98
<i>B. vietnamensis</i>	Down	Protein folding	chaperone cofactor-dependent protein refolding	0,01714403	6,65
<i>B. vietnamensis</i>	Down	Protein folding	response to unfolded protein	0,0249574	6,33

<i>B. vietnamiensis</i>	Down	Protein folding	'de novo' posttranslational protein folding	0,0249574	6,33
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Supplementary Table 6 : Gene Ontology term enrichment analysis of differentially expressed genes specifically regulated by each strain in roots

Condition	Regulation	Category	Term	pval	Ratio
<i>P. kururiensis</i>	Down	Redox homeostasis	oxidation-reduction process	1,26E-02	2,23
<i>P. kururiensis</i>	Down	Redox homeostasis	response to chemical	1,62E-04	2,97
<i>P. kururiensis</i>	Down	Redox homeostasis	drug metabolic process	1,20E-05	4,27
<i>P. kururiensis</i>	Down	Redox homeostasis	drug catabolic process	3,23E-06	7,63
<i>P. kururiensis</i>	Down	Redox homeostasis	response to toxic substance	6,11E-05	6,04
<i>P. kururiensis</i>	Down	Redox homeostasis	antibiotic metabolic process	3,27E-03	6,31
<i>P. kururiensis</i>	Down	Redox homeostasis	cellular oxidant detoxification	6,95E-03	5,79
<i>P. kururiensis</i>	Down	Redox homeostasis	hydrogen peroxide catabolic process	3,36E-02	7,44
<i>P. kururiensis</i>	Down	defense	chitin catabolic process	5,98E-04	18,63
<i>B. vietnamiensis</i>	Up	Response to stimulus	cellular response to organic substance	4,58E-04	4,77
<i>B. vietnamiensis</i>	Up	Hormone	cellular response to hormone stimulus	2,37E-03	4,83
<i>B. vietnamiensis</i>	Up	Hormone	hormone-mediated signaling pathway	6,77E-03	4,76
<i>B. vietnamiensis</i>	Up	Response to stimulus	cellular response to chemical stimulus	1,43E-02	3,25
<i>B. vietnamiensis</i>	Up	Transcription	regulation of gene expression	1,51E-02	2,04
<i>B. vietnamiensis</i>	Up	Biosynthesis	regulation of biosynthetic process	2,15E-02	2,07
<i>B. vietnamiensis</i>	Up	Transcription	nucleic acid-templated transcription	2,87E-02	2,07
<i>B. vietnamiensis</i>	Down	Hormone	regulation of jasmonic acid mediated signaling pathway	2,52E-06	27,58
<i>B. vietnamiensis</i>	Down	defense	regulation of defense response	5,18E-06	10,49
<i>B. vietnamiensis</i>	Down	Hormone	jasmonic acid mediated signaling pathway	2,31E-05	15,76
<i>B. vietnamiensis</i>	Down	Hormone	response to jasmonic acid	3,78E-05	12,00
<i>B. vietnamiensis</i>	Down	Response to stimulus	response to wounding	1,32E-04	12,72
<i>B. vietnamiensis</i>	Down	defense	defense response	2,38E-04	3,07
<i>B. vietnamiensis</i>	Down	Signaling	regulation of signaling	1,58E-03	6,01
<i>B. vietnamiensis</i>	Down	Response to stimulus	response to stimulus	1,62E-03	1,66
<i>B. vietnamiensis</i>	Down	Signaling	signaling	2,78E-03	2,08
<i>B. vietnamiensis</i>	Down	Response to stimulus	response to heat	6,00E-03	6,63
<i>B. vietnamiensis</i>	Down	Response to stimulus	response to stress	8,85E-03	1,92
<i>B. vietnamiensis</i>	Down	Response to stimulus	response to temperature stimulus	1,04E-02	4,51

<i>B. vietnamensis</i>	Down	Response to stimulus	cellular response to acid chemical	1,48E-02	4,75
<i>B. vietnamensis</i>	Down	Response to stimulus	regulation of response to stimulus	2,17E-02	3,66

Supplementary Table 9 : Genes used to confirm RNA-Seq and their function

RAP-ID	Gene name	Organ	Regulation according to RNA-Seq	Function	Reference
Os03g0195100	<i>ALD1</i>	Leaves	Down-regulated by <i>B. vietnamiensis</i>	Systemic resistance	Jung <i>et al.</i> , 2016
Os03g0741100	<i>bHLH148</i>	Roots	Down-regulated by <i>B. vietnamiensis</i>	JA responsive	Seo <i>et al.</i> , 2011
Os11g0143300	<i>RR9</i>	Leaves	Down-regulated by <i>B. vietnamiensis</i>	CK signaling	Ito and Kurata, 2006
Os12g0589000	<i>RSL9</i>	Roots	Commonly up-regulated by both strains	Root hair formation Homolog of sugarcane LRR-RLK	Min Kim <i>et al.</i> , 2017
Os08g0203400	<i>SHR5</i>	Roots	Down-regulated by <i>P. kururiensis</i>	involved in plant-N2 fixing endophytic association	Vinagre <i>et al.</i> , 2006
Os02g0181300	<i>WRKY71</i>	Leaves	Up-regulated by <i>P. kururiensis</i>	Disease resistance	Liu <i>et al.</i> , 2007

Supplementary Table 10: Expression profiles of the JA network in leaves. Values are log<sub>2</sub>FoldChange of expression compared to non-inoculated plants measured by RT-qPCR

	6 hpi		1 dpi		7 dpi		14 dpi	
	<i>Pk</i>	<i>Bv</i>	<i>Pk</i>	<i>Bv</i>	<i>Pk</i>	<i>Bv</i>	<i>Pk</i>	<i>Bv</i>
<i>ATL15</i>	-0,80	1,05	-0,28	-0,69	4,60	-0,04	-1,52	-1,86
<i>RERJ1</i>	0,89	1,93	-0,22	1,49	0,40	0,49	-0,36	-1,24
<i>AOS1</i>	0,56	0,36	-2,05	-1,66	1,71	0,67	-1,54	-1,92
<i>JAZ6</i>	-0,16	1,04	-2,33	-1,19	1,53	-0,82	-0,28	-0,66
<i>JAZ10</i>	0,12	1,68	-1,20	0,60	2,52	-0,64	-1,79	-1,40
<i>JAZ12</i>	2,26	1,92	-3,19	-0,54	1,61	-0,42	-2,46	-1,24



# **CHAPITRE 3**

Le chapitre 3 traite de la réponse transcriptomique et physiologique du riz à l'interaction avec une souche endophyte modèle, *Paraburkholderia phytofirmans* PsJN.

Tout d'abord une analyse de la colonisation racinaire a pu mettre en évidence pour la première fois la capacité de la souche *Paraburkholderia phytofirmans* PsJN à coloniser massivement la surface des racines de riz. L'analyse de la réponse transcriptomique du riz à l'interaction avec cette souche a mis en évidence d'importantes régulations dans les racines, en particulier la diminution de l'expression de gènes liés à l'immunité ainsi que la modulation de la signalisation hormonale des cytokinines, de l'éthylène et de l'acide jasmonique. La réponse transcriptomique des feuilles a quand à elle montré une augmentation de l'expression de gènes impliqués dans la chaîne de transport d'électrons de la photosynthèse ainsi que le cycle de Calvin. L'hypothèse que l'inoculation de *P. phytofirmans* induisait une augmentation de l'efficacité de la photosynthèse a été testée par des approches de mesure de la fluorescence chlorophyllienne. Les résultats montrent qu'à long terme, la colonisation des racines du riz en condition non stérile induit une augmentation significative de l'indice de performance photosynthétique, PI<sub>ABS</sub>, suggérant un meilleur rendement énergétique de la photosynthèse.

Pour des raisons de place, les tableaux supplémentaires 2, 3 et 6 ne sont pas présents dans ce chapitre.

# Transcriptomic response of rice to root colonization by the model endophyte *Paraburkholderia phytofirmans* PsJN

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## • Introduction

In order to reduce the negative impacts caused by the use of chemical pesticides and fertilizers in agriculture, the potential of plant-associated microbes is nowadays emerging as a promising alternative solution. Indeed, the application of beneficial microbes can enhance plant production by improving nutrient uptake or alleviating the impact of environmental stresses through various mechanisms (Backer *et al.*, 2018). Of particular interest is the use of endophytes which are microbes able to colonize the inner tissues of plants and provide beneficial effects to the plants throughout its life cycle. Among the diversity of microbial endophytes, one particular bacterial strain, *Paraburkholderia phytofirmans* PsJN, hereafter termed PsJN, has the ability to colonize the endosphere of numerous plants species such as *Arabidopsis*, potato, tomato, grapevine, maize and barley (Mitter *et al.*, 2013). PsJN also exhibit multiple beneficial effects to plants, in particular by increasing the tolerance of inoculated plants to biotic and abiotic stresses (Esmaeel *et al.*, 2018).

In order to identify the molecular mechanisms implicated in the beneficial effects of PsJN, several studies analyzed the transcriptional regulations of PsJN-treated *Arabidopsis* plants. For direct beneficial effects, PsJN-triggered root growth promotion by involving hormone signaling, particularly auxin and ethylene (Poupin *et al.*, 2016). Also, systemic transcriptional regulations lead to life cycle shortening through the early up-regulation of flowering control genes (Poupin *et al.*, 2013). Transcriptional analyses were also carried out in the context of PsJN-induced resistance to pathogens (Su *et al.*, 2017; Timmermann *et al.*, 2019), salt (Pinedo *et al.*, 2015) and cold tolerance (Su *et al.*, 2015). However, the interaction between the model endophyte PsJN and rice (*Oryza sativa*) which is both a first-rank scientific model and a major crop for human consumption submitted to important environmental stresses such as drought and diseases, has never been studied.

In order to study the response of rice to PsJN, we first analyzed the colonization of the roots of *Oryza sativa* cultivar Nipponbare by PsJN. Then, the transcriptomes of leaves and roots of control and PsJN-colonized plants were produced to characterize the molecular response of

rice to PsJN. In particular, the analysis of leaves transcriptomes revealed a transcriptional up-regulation of genes implicated in photosynthesis, which was confirmed by measuring the photosynthetic capacity of plants.

- **Material & Methods**

### **Plants & bacterial cultivation**

*Oryza sativa L. ssp. japonica* cv. Nipponbare was used in this study. For all experiments, Plants were grown in sterile conditions using a hydroponic system following the same protocol described in King *et al.* (2019). Plants were grown in a growth chamber (16 h light; 8 h dark; 28°C; 70% humidity). *Paraburkholderia phytofirmans* PsJN<sup>T</sup> was cultured and the bacterial inoculum was prepared as described in King *et al.* (2019). Each plantlet was inoculated with 10<sup>7</sup> bacterial cells 4 days after sowing in hydroponic system.

For greenhouse experiments (28°C, 60% humidity), homogeneously germinated seeds were inoculated with 10<sup>7</sup> bacterial cells (1 ml at 10<sup>7</sup> per seed sown in soil) when transferred in round small pots ( $\varnothing = 5\text{cm}$ ,  $h = 7\text{cm}$ ). At 15 days post-inoculation (dpi), homogeneously grown plantlets were transferred to 12 x 12 x 18.7 cm pots (1 per pot). The substrate used was the GO M2 growing media (Jiffy).

### **Bacterial transformation**

*Paraburkholderia phytofirmans* PsJN<sup>T</sup> cells were transformed by electroporation with the pIN29 plasmid (Vergunst *et al.*, 2010) and its derivative pINGUS. The latest was obtained by replacing the gene encoding for the DsRed fluorescent protein by the *gusA* gene from the GusSH vector (Pasquali *et al.*, 1994) which encodes for a β-glucuronidase. Both plasmids comprise a chloramphenicol resistance gene as well as the DsRed or *gusA* genes under the control of a constitutive TAC promoter. After 24 hours of incubation on selective medium LBm Cm (200 µg.mL<sup>-1</sup>) at 28°C, the most fluorescent colonies were selected (for DsRed constructs).

### **Rice root colonization assays**

The roots of PsJN::pIN29-inoculated plants grown in hydroponics were harvested at 1, 7 and 14 dpi, weighted and grinded in sterile water with a sterile ceramic bead using a FastPrep-24<sup>TM</sup> 5G at 6 m.s<sup>-1</sup> for 40 seconds. The same protocol was applied for plants grown in soil, harvested at 20 dpi. The solution was then diluted and inoculated on low salt LB selective

medium containing 200 µg.mL<sup>-1</sup> of chloramphenicol and incubated at 28°C for 24h. Colony forming unit were then enumerated.

### **Visualization of root-colonizing bacteria**

All microscopic observations of the bacterial colonization were restricted to the primary root in order to compare the colonization patterns on roots which have been in contact with the bacterial population for the same amount of time. Primary roots were harvested at 7 dpi and mounted between slide and slips cover and directly examined with an Epifluorescence Nikon Eclipse Ni-E microscope.

Histochemical localization of β-glucuronidase activity was performed by vacuum-infiltrating a GUS staining solution containing 50 µg.mL<sup>-1</sup> X-Gluc (Duchefa Biochemie) for 20 min in mock and PsJN::pINGUS-treated root systems in separate containers. The containers were then incubated overnight at 37°C, then upon blue coloration visualization, roots systems were rinsed three times using a sterile phosphate buffer solution (AMRESCO). Roots were then visualized using a Nikon SMZ1500 stereomicroscope.

### **RNA extraction**

For the analysis of roots and leaves transcriptional profiles, plants' tissues were harvested in triplicate at 7 dpi. Roots and leaves of untreated plants were collected at the same time. Each biological replicate consisted of five pooled root system or 5 pooled last mature leaves harvested from a single hydroponic system. After harvest, samples were snap-frozen in liquid nitrogen and stored at -80°C. Rice roots were homogenized in liquid nitrogen using cooled mortar and pestles. Rice leaves were grinded using a TissueLyser II (Retsch) set to 30 Hz for 30 sec. Total RNA extraction using TRI-reagent (Sigma) was performed according to manufacturer's instructions. All samples were treated with DNase I (Ambion) and purified using the RNA Clean & Concentrator kit (Zymo) according to manufacturer's instructions. The integrity and quality of the total RNA was confirmed using a Nanodrop™ 1000 spectrophotometer (ThermoFisher) and a 2100 BioAnalyzer (Agilent).

### **RNA sequencing & mapping of reads**

Quality of RNA was checked by determining the RNA Integrity Number (RIN) with a Fragment Analyzer (Agilent). For the library preparation, samples with a RIN value > 6 were used. 12 RNA libraries were prepared using an Illumina TruSeq stranded mRNA sample preparation kit by MGX-Montpellier GenomiX core facility (MGX) France (<https://www.mgx.cnrs.fr/>). Library construction, sequencing and quality assessment was

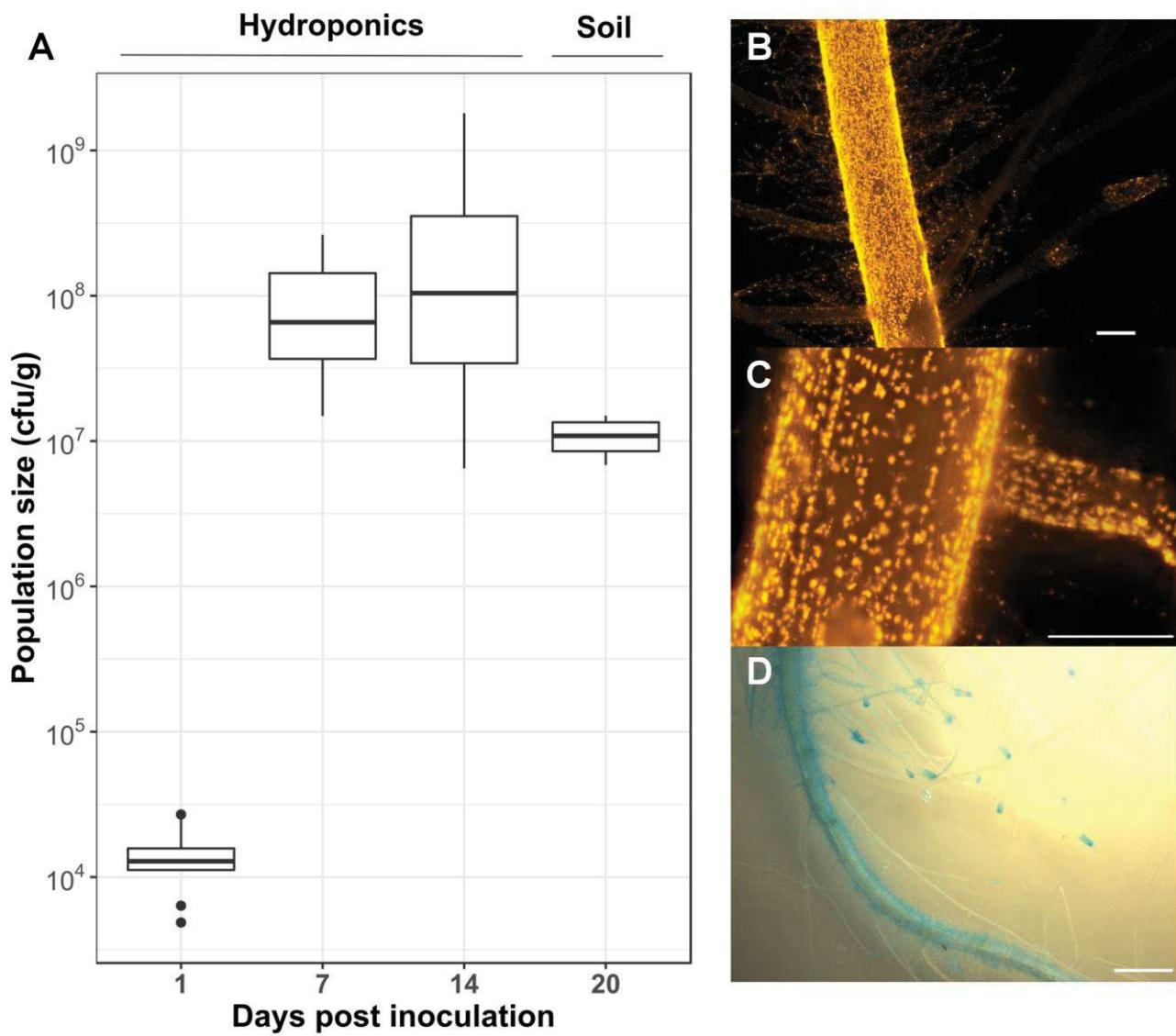
performed as described in King *et al.* (2019) on an Illumina HiSeq 2500. RNA-Seq reads were aligned to the IRGSP 1.0 version of the rice genome using HISAT2 v2.0.5.1 (Kim *et al.*, 2015). The number of reads mapped to each gene locus was counted using HTSeq-count v0.6.0 (Anders *et al.*, 2015).

### **Differential gene expression & Gene Ontology (GO) term enrichment analysis**

DESeq2 v3.7 (Love *et al.*, 2014) was used to calculate differential gene expression between non-inoculated and inoculated conditions. All genes having an adjusted p-value inferior to 0.01 were considered as significantly differentially expressed. All functional enrichment analysis was performed using g:Profiler (version e95\_eg42\_p13\_f6e58b9) with g:SCS multiple testing correction method applying significance threshold of 0.01 (Raudvere *et al.*, 2019; Reimand *et al.*, 2007). To avoid redundancy between GO terms, we used ReviGO (Supek *et al.*, 2011) to reduce the number of enriched GO terms.

### **Chlorophyll-a fluorescence measurements**

OJIP transients were measured for both non-inoculated controls and PsJN-treated plants using a pocket PEA chlorophyll fluorimeter (Hansatech Instruments, UK). The last fully expanded leaf of each plant was used for measurements following 15 min of dark acclimation. Six plants of each condition (Control and PsJN-treated) were measured at the same time. The Fv/Fm and PI<sub>ABS</sub> parameters were calculated as described in Strasser *et al.* (2000).



**Figure 1: Colonization of rice roots by PsJN**

(A) Population dynamics of DsRed-tagged PsJN associated with rice roots. The data reported are the median of bacterial population size from 18 plants and two independent experiments for hydroponic condition and 5 plants for soil condition (B, C) Epifluorescence microscopy pictures of the colonization of rice primary roots at 7 days post-inoculation by PsJN::pIN29 cells. White bars represent 200  $\mu$ m (D) Colonization of rice primary root picture by PsJN::pINGUS. White bar represents 1 mm.

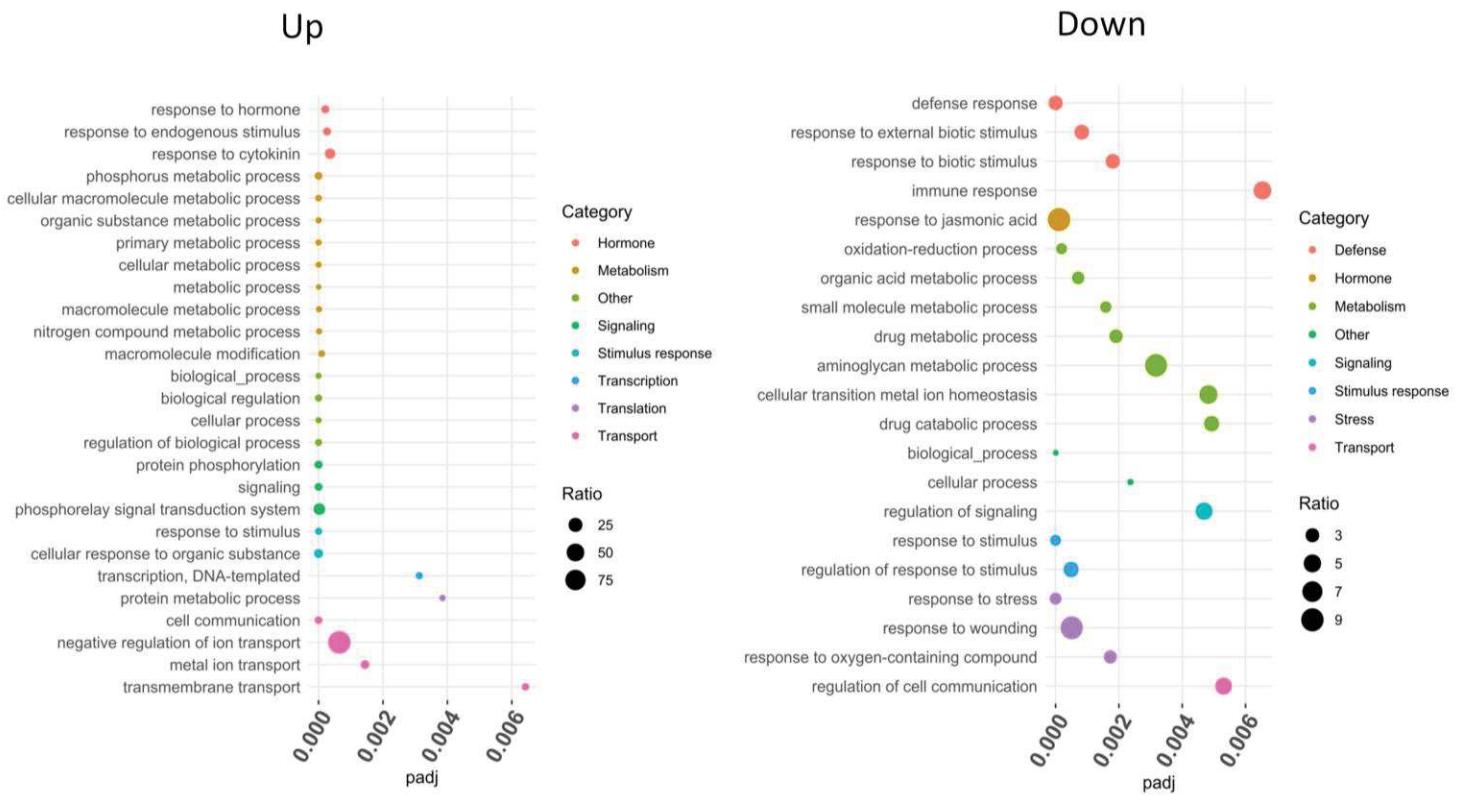
- **Results**

### **Analysis of PsJN colonization of rice roots**

We first wanted to check if PsJN was able to colonize Nipponbare rice roots, to do so we inoculated a DsRed-tagged strain on rice roots of plants grown in hydroponics condition. PsJN colonization was first evaluated by estimating the size of the bacterial population associated to roots (Fig. 1A). PsJN rapidly colonize rice roots by reaching a median value of population size of  $1.28 \times 10^4$  cfu.g<sup>-1</sup>. Afterwards from 1 to 7 dpi, the population size of PsJN massively increases to reach a median value of  $6.57 \times 10^7$  cfu.g<sup>-1</sup>. Finally from 7 to 14 dpi, the bacterial population size doubled to reach a median value of  $1.26 \times 10^8$  cfu.g<sup>-1</sup>. We also evaluated the ability of PsJN to colonize rice roots in non-sterile conditions. PsJN was able to colonize rice roots in soil although at a lower level regarding the median value of  $1.09 \times 10^7$  cfu.g<sup>-1</sup>. We visualized DsRed-tagged PsJN cells colonizing rice roots by microscopy at 7 dpi (Fig 1). This analysis revealed that PsJN cells are able to colonize the surface of root hairs, the root cap of lateral roots and the surface of the primary root (Fig 1B). However, the surface of lateral roots appears to be less colonized by bacterial cells. This observation is confirmed by GUS coloration of PsJN strains harboring the pIN-GUS plasmid. Indeed, the surface of the primary root is largely colonized as well as the root caps of lateral roots while their surface remains relatively free of bacteria comparatively to the primary root surface (Fig. 1D). When observing the colonization of primary root surface, bacterial aggregates can be observed (Fig 1C).

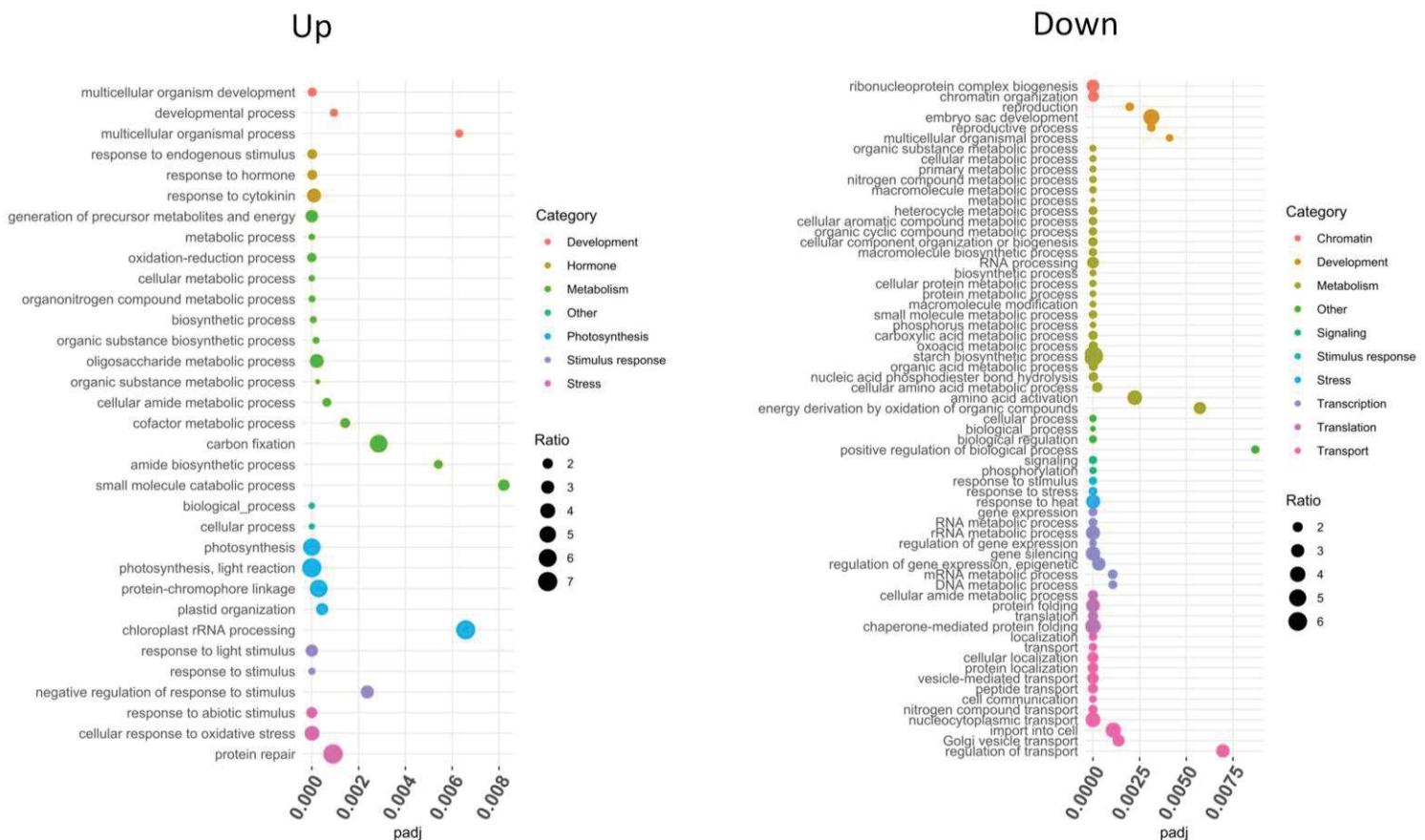
### **Transcriptomic analysis of rice response to PsJN**

In order to identify differentially expressed genes (DEGs) in leaves and roots following PsJN inoculation, we used RNA-sequencing. Total RNA was extracted from three biological replicates per treatment (control or inoculated), each replicates being a pool of 5 plants. On average 42 782 744 reads were sequenced from each sample of which 69% were uniquely mapped to the rice genome (IRGSP, version1) (Supplementary Table 1). Were considered significantly differentially expressed, genes with an FDR adjusted p-value inferior to 0,01. In leaves, 2646 and 2337 DEGs (Supplementary Table 2) were identified as up and down-regulated genes respectively whereas in roots 379 and 609 DEGs, respectively, were identified (Supplementary Table 3).



**Figure 2: GO terms enrichment analysis of DEGs in rice roots in response to PsJN**

Enrichment analysis of Biological Process GO terms of PsJN-treated rice root transcriptome. Y-axis corresponds to the significantly enriched GO terms (FDR < 0.01), X-axis corresponds to the adjusted p-value, the size of dots corresponds to the ratio between the proportion of genes related to the given GO term in the transcriptome and the proportion of genes related to the given GO term in the rice genome



**Figure 3: GO terms enrichment analysis of DEGs in rice leaves in response to PsJN**

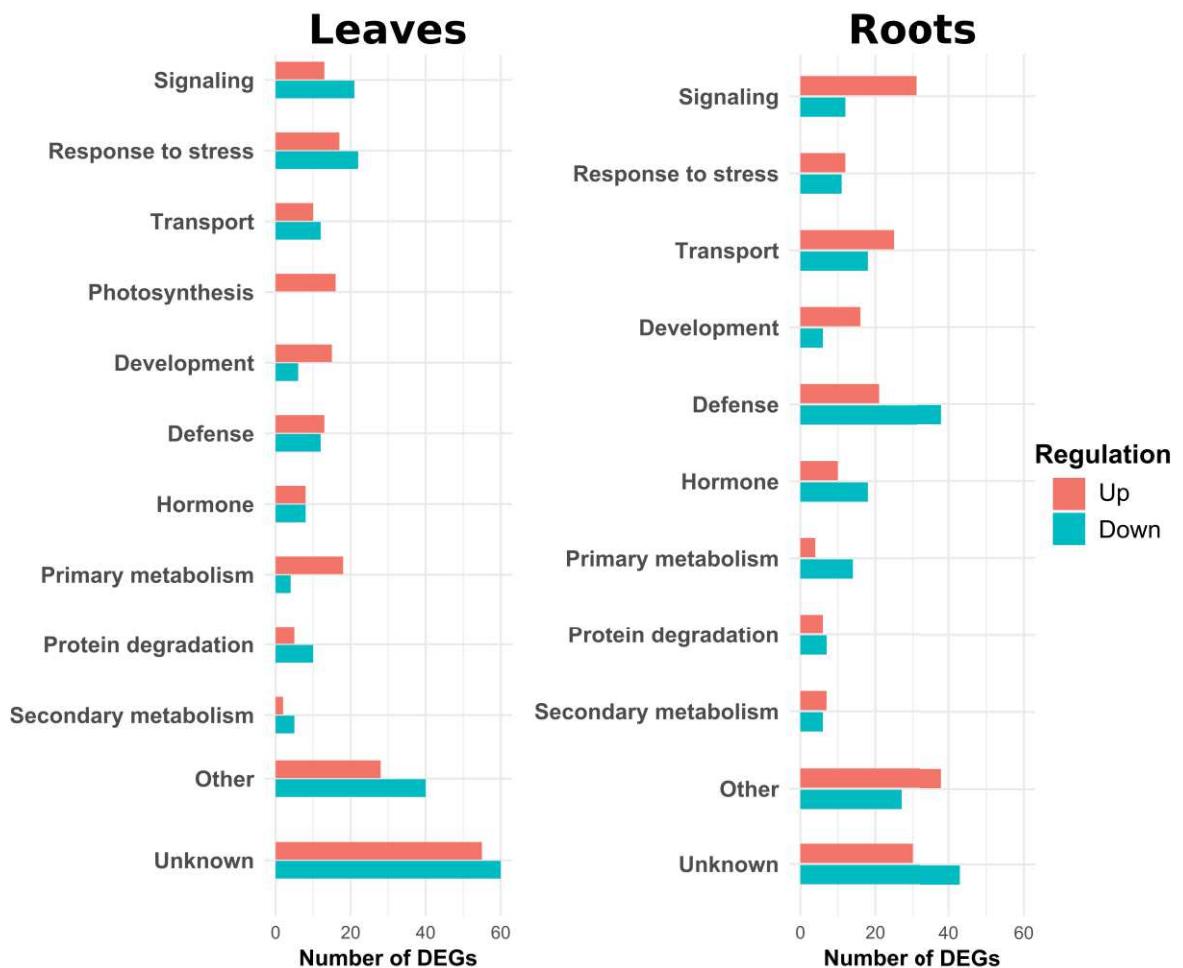
Enrichment analysis of Biological Process GO terms of PsJN-treated rice root transcriptome. Y-axis corresponds to the significantly enriched GO terms (FDR < 0.01), X-axis corresponds to the adjusted p-value, the size of dots corresponds to the ratio between the proportion of genes related to the given GO term in the transcriptome and the proportion of genes related to the given GO term in the rice genome

## **GO term enrichment analysis**

In order to determine the global biological processes transcriptionally regulated in rice during the interaction with PsJN, we performed GO terms and KEGG pathway enrichment analysis (Supplementary Table 4 and 5). Significantly enriched GO terms in root transcriptome encompasses terms related to hormone signaling especially cytokinin (CKs), metal ion transport and also signaling for the up-regulated DEGs and defense response as well as jasmonic acid (JA) response for the down-regulated genes (Figure 2). KEGG pathway enrichment analysis on down-regulated DEGs in roots also pinpoints an enrichment of genes implicated in carbon fixation and secondary metabolism (Supplementary Table 5). In leaves, a large proportion of enriched GO terms are related to primary metabolism: carbon fixation, photosynthesis and amide metabolism are enriched among the up-regulated genes (Figure 3). For the down-regulated genes, namely, carboxylic acid metabolism and starch biosynthesis are enriched. Also GO terms related to developmental process are significantly enriched for both up and down-regulated DEGs.

## **Physiological function mining of transcriptome**

The global analysis of the transcriptome led us to focus on several biological processes. For roots, defense, signaling (both hormonal and receptor kinases) and transport processes appears to be significantly transcriptionally regulated. On the other hand, in leaves, important transcriptional regulations related to primary metabolism and photosynthesis appears to occur. In order to identify highly-regulated genes related to the previously described biological processes, we conducted expert annotation of the top 200 up and down-regulated DEGs in leaves and roots to relate them to a functional category. The functional categorization (Figure 4, Supplementary Table 6) of the top regulated DEGs in roots reveals that 14.75% are related to defense, 10.75% of DEGs are related to transport and signaling and finally 7% of DEGs in roots are hormone-related. In leaves, among the up-regulated DEGs, 9%, 8.5% and 8% of DEGs are related to primary metabolism, response to stress and photosynthesis, respectively. Finally, in leaves, genes related to response to stress and signaling both represent over 10% of the DEGs among the 200 most down-regulated ones.



**Figure 4: Functional categories of rice DEGs upon PsJN colonization**

Number of DEGs related to functional categories among the top 200 up-regulated and down-regulated DEGs in response to PsJN

## **Root response to PsJN**

### **Defense**

In roots, 19% of the down-regulated DEGs are defense-related (Figure 4) among which 9 encode for Pathogenesis-Related proteins (PR) and 11 for WRKY transcription factors (Table 1). Among the up-regulated genes, 7 genes encode for putative disease resistance proteins, 3 genes encode for SA conjugation enzymes and 2 genes encode for germin-like proteins (Os08g0189300, Os08g0189200).

### **Hormones**

Among the root up-regulated genes related to hormone signaling (Table 2), four genes are A-type Response Regulator (RRs) associated with cytokinin (CK) response (Os12g0139400, Os11g0143300, Os04g0673300, Os02g0830200). For the down-regulated DEGs out of the 18 hormone-related genes, seven are related to jasmonic acid (JA) signaling with four *JAZ* genes which are down-regulated (Os03g0180800, Os03g0181100, Os10g0392400, Os07g0153000) as well as *RERJ1* and *bHLH148* which are JA-responsive genes. Another hormonal pathway regulated during the interaction with *P. phytophthora* is the response to ethylene as four Ethylene Response Factor (ERFs) (Os01g0313300, Os07g0410300, Os05g0361700, Os03g0341000) are down-regulated, also two up-regulated genes are encoding for aminocyclopropane-1-carboxylic acid oxidase (ACO), which is an enzyme implicated in ethylene synthesis (Os06g0573900, Os02g0771600).

### **Receptor kinases**

One of the most important functional categories transcriptionally regulated in roots is signaling. Indeed, it represents 15.5% of up-regulated DEGs (Figure 4). Among these, 26 out of 31 DEGs encodes for receptor kinases such as Wall-Associated Kinases (WAKs), Receptor-Like Cytoplasmic Kinase (RLCKs) and putative receptor kinases (Supplementary Table 7). Significantly less signaling-related genes are down-regulated as 7 out of 12 DEGs are annotated as genes encoding for protein kinases.

### **Transport**

In roots, 10.75% of DEGs are implicated in transmembrane transport out of which several sub-categories can be distinguished (Supplementary Table 8): Firstly, transporters implicated in nutrient uptake are up-regulated in roots in particular iron-phytosiderophore transporters such as *YSL12*, *YSL15* and *YSL16* as well as proteins implicated in the transport of zinc

Table 1: List of defense-related DEGs in rice roots in response to PsJN

RAP ID	log <sub>2</sub> FoldChange	Gene name	RAP annotation
Os11g0259700	2,80	SA conjugation	Similar to SAM dependent carboxyl methyltransferase family protein.
Os06g0315000	2,67	SA conjugation	SAM dependent carboxyl methyltransferase domain containing protein.
Os12g0198900	1,81	NB-ARC	Similar to NB-ARC domain containing protein.
Os08g0189300	1,59	<i>OsGLP8-4, GER1</i>	Germin-like protein 8-4, Disease resistance
Os08g0189200	1,42	<i>OsGLP8-3, GER2</i>	Germin-like protein 8-3, Disease resistance
Os08g0374000	1,13	Pathogenesis-Related	Bet v I allergen family protein.
Os11g0179700	1,09	Disease responsive	Similar to Dirigent-like protein.
Os09g0536400	1,06	Pathogenesis-Related	Similar to Thaumatin-like protein 1 precursor.
Os11g0260100	1,06	SA conjugation	SAM dependent carboxyl methyltransferase family protein.
Os06g0693100	0,99	NB-ARC	NB-ARC domain containing protein.
Os06g0158500	0,96	NB-ARC	Conserved hypothetical protein.
Os06g0146250	0,91	<i>OsWRKY73</i>	Similar to WRKY transcription factor 73.
Os08g0170100	0,91	NB-ARC	NB-ARC domain containing protein.
Os01g0781100	0,85	NB-ARC	Similar to NBS-LRR protein (Fragment).
Os02g0286850	0,83	NB-ARC	Similar to Stripe rust resistance protein Yr10.
Os07g0539300	0,81	<i>Gns13</i>	Glycoside hydrolase, family 17 protein.
Os03g0599000	0,78	NB-ARC	NB-ARC domain containing protein.
Os11g0663100	0,77	Putative SA synthesis regulator	Hypothetical conserved gene.
Os01g0859500	0,77	<i>OsLG2, OsbZIP11, BZIP11</i>	Similar to Basic leucine zipper protein (Liguleless2).
Os08g0332600	0,72	NB-ARC	Disease resistance protein domain containing protein.
Os03g0108600	0,65	<i>OsBIRH1</i>	Similar to DEAD-box ATP-dependent RNA helicase 50.
Os01g0826400	-1,34	<i>OsWRKY24</i>	WRKY transcription factor 24 (WRKY24).
Os09g0517200	-1,36	NB-ARC	Hypothetical conserved gene.
Os07g0129200	-1,36	<i>OsPRIa, OsPRI-74, OsSCP</i>	Pathogenesis-related 1a protein, Regulation of abiotic stress responses
Os05g0537100	-1,38	<i>OsWRKY7</i>	WRKY transcription factor 10.
Os04g0412300	-1,42	Beta 1,3-Glucanase	Similar to H0717B12.10 protein.
Os05g0322900	-1,42	<i>OsWRKY45</i>	WRKY transcription factor, Benzothiadiazole (BTH)-inducible blast resistance
Os05g0478700	-1,43	<i>OsWRKY84</i>	Hypothetical conserved gene.
Os02g0654700	-1,44	<i>OsERF91, OsAP59, OsBIERF3</i>	AP2/ERF family protein, Abiotic stress response
Os08g0189500	-1,44	<i>OsGLP8-6</i>	Germin-like protein 8-6, Disease resistance
Os01g0713200	-1,45	<i>Gns10</i>	Similar to Beta-glucanase.
Os12g0116700	-1,51	<i>OsWRKY64</i>	Similar to WRKY transcription factor 64.
Os01g0660200	-1,59	<i>C10728, OsChib3a</i>	Acidic class III chitinase OsChib3a precursor (Chitinase) (EC 3.2.1.14).

Os11g0592000	-1,62	<i>OsPR4c</i>	Similar to Barwin.
Os02g0570700	-1,63	<i>Oscyp71Z7</i>	Cytochrome P450 family protein.
Os02g0181300	-1,64	<i>OsWRKY71, WRKY-71</i>	WRKY transcription factor, Defense response
Os10g0161400	-1,67	NB-ARC	NB-ARC domain containing protein.
Os11g0154500	-1,79	<i>OsNAC111</i>	Blast disease-responsive transcription factor, Disease resistance
Os10g0491000	-1,86	Disease resistance	Plant Basic Secretory Protein family protein.
Os04g0347200	-1,86	Chitinase	Similar to OSIGBa0111I14.7 protein.
Os01g0821600	-1,88	<i>OsWRKY21</i>	WRKY transcription factor 48-like protein (WRKY transcription factor 21).
Os11g0700900	-1,93	<i>C10923</i>	Glycoside hydrolase, subgroup, catalytic core domain containing protein.
Os07g0127500	-2,05	<i>OsPRI#072, OsPRI-72</i>	Similar to PR-1a pathogenesis related protein (Hv-1a) precursor.
Os11g0462500	-2,16	NB-ARC	Similar to NB-ARC domain containing protein.
Os03g0676400	-2,19	<i>OsVQ13</i>	VQ domain containing protein.
Os01g0508500	-2,51	<i>RH2</i>	Conserved hypothetical protein.
Os09g0417800	-2,59	<i>OsWRKY62, XB10</i>	WRKY transcription factor, Transcriptional repressor, Pathogen defense
Os06g0649000	-2,69	<i>OsWRKY28</i>	PAMP (pathogen-associated molecular pattern)-responsive transrepressor
Os03g0335200	-3,02	<i>OsWRKY79, WRKY-79</i>	Similar to WRKY1 (WRKY transcription factor 17).
Os03g0195100	-3,02	<i>OsALD1, ALD1</i>	Putative aminotransferase, Disease resistance response against rice blast
Os09g0417600	-3,22	<i>OsWRKY76</i>	WRKY transcription factor, Transcriptional repressor, Pathogen defense
Os01g0508100	-3,25	<i>RH3</i>	Ferritin/ribonucleotide reductase-like family protein.
Os11g0592200	-3,68	<i>OsPR4, OsPR4a, PR4a, OsPR-4</i>	Similar to Chitin-binding allergen Bra r 2 (Fragments).
Os08g0518900	-4,03	<i>RIXI</i>	Chitinase (EC 3.2.1.14).
Os07g0129300	-4,34	<i>OsPRI, OsPRIa, PR1a, PRI</i>	Similar to PR1a protein.
Os11g0701800	-4,54	<i>C10701</i>	Chitinase III (EC 3.2.1.14) (Class III chitinase homologue (OsChib3H)).
Os02g0808800	-5,84	<i>OsCCR1</i>	Similar to Cinnamoyl-CoA reductase (EC 1.2.1.44).
Os04g0375300	-7,01	<i>rNBS56</i>	Similar to NBS-LRR protein (Fragment).
Os06g0279900	-7,61	NB-ARC	Similar to NB-ARC domain containing protein, expressed.

Table 2: List of hormone-related DEGs in rice roots in response to PsJN

RAP ID	log <sub>2</sub> FoldChange	Gene name	RAP annotation
Os06g0573900	2,16	ACO-encoding	Similar to 1-aminocyclopropane-1-carboxylic acid oxidase.
Os12g0139400	1,42	<i>OsRR10</i>	A-type response regulator, Cytokinin signaling
Os11g0143300	1,36	<i>OsRR9</i>	A-type response regulator, Cytokinin signaling
Os04g0673300	1,25	<i>OsRR6</i>	A-type response regulator, Cytokinin signaling
Os02g0771600	1,23	<i>OsACO3</i>	Similar to 1-aminocyclopropane-1-carboxylate oxidase (Fragment).
Os09g0545300	0,99	<i>OsSAUR39</i>	SAUR family protein, Negative regulator of auxin synthesis and transport
Os03g0352450	0,97	<i>OsINO80</i>	Hypothetical conserved gene.
Os02g0830200	0,91	<i>OsRR3</i>	A-type response regulator, Cytokinin signaling
Os01g0370000	0,79	<i>OsOPR9, OsOPR1-2</i>	NADH:flavin oxidoreductase/NADH oxidase, N-terminal domain containing protein.
Os10g0561800	0,64	<i>Oshox1, OsHox1</i>	Similar to homeodomain leucine zipper protein hox1.
Os07g0153000	-1,36	<i>OsJAZ2</i>	Tify domain containing protein.
Os04g0610400	-1,39	<i>OsAP2-39, OsERF77</i>	APETALA-2-like transcription Factor, Control of the ABA/GA balance
Os05g0158600	-1,45	<i>OsGA2ox1</i>	Similar to OsGA2ox1.
Os05g0361700	-1,48	<i>OsERF61</i>	Similar to EREBP-2 protein (Fragment).
Os10g0392400	-1,48	<i>OsJAZ12</i>	Tify domain containing protein.
Os04g0556500	-1,50	<i>OscZOGT1, cZOGT1</i>	cis-Zeatin-O-glucosyltransferase, Catalyze O-glucosylation of cZ and cZ-riboside
Os07g0410300	-1,59	<i>OsERF136</i>	Conserved hypothetical protein.
Os04g0447100	-1,78	<i>OsLOX6</i>	Similar to Lipoxygenase.
Os05g0591600	-1,79	<i>LOGL8</i>	Similar to Carboxy-lyase.
Os03g0181100	-1,85	<i>OsJAZ10</i>	Tify domain containing protein.
Os05g0560900	-1,96	<i>OsGA2ox8</i>	Similar to gibberellin 2-beta-dioxygenase.
Os03g0741100	-2,14	<i>OsbHLH148</i>	Basic helix-loop-helix transcription factor, Drought tolerance
Os03g0341000	-2,20	<i>OsERF66</i>	Similar to AP2 domain containing protein RAP2.2 (Fragment).
Os04g0581100	-2,35	<i>OsS3H, S3H</i>	2OG-Fe(II) oxygenase domain containing protein.
Os04g0301500	-2,39	<i>OsbHLH006, RERJ1</i>	Helix-loop-helix DNA-binding domain containing protein.
Os06g0592500	-3,11	Ethylene-responsive	Similar to Ethylene-responsive transcriptional coactivator.
Os03g0180800	-3,13	<i>OsJAZ9</i>	TIFY domain-containing transcriptional regulator, Salt and dehydration stress tolerance
Os01g0313300	-3,35	<i>OsERF68</i>	Similar to EREBP-3 protein (Fragment).

(Os05g0472400, Os03g0667500), sulfate (Os03g0196000), potassium (Os08g0206400), phosphate (Os09g0554000) and malate (Os02g0673100). Vacuolar transporters are also regulated in response to *P. phytofirmans* such as *SPX-MFS3*, a vacuolar phosphate efflux transporter being up-regulated, 2 Vacuolar Iron Transporter (VITs) encoding genes, namely *OsVIT2* and *OsVITH2*, are down-regulated. Also, transporters implicated in cell detoxification, such as Heavy Metal ATPase (HMAs) ABC transporters and Multidrug And Toxic compound Extrusion protein (MATE), are detected as transcriptionally regulated both up (Os06g0700700, Os02g0196600, Os10g0195000, Os09g0468000, Os08g0398300) and down (Os02g0584800, Os05g0554000, Os04g0469000, Os02g0584700, Os02g0585200). Moreover, aquaporins such as *NIP3;5*, *NIP3;2* and *PIP2;9* and amino acid transporters such as *GAT1*, *AAP10C* and *ATL7* are also transcriptionally regulated in PsJN-treated roots.

## Leaves response

### Carbon metabolism and photosynthesis

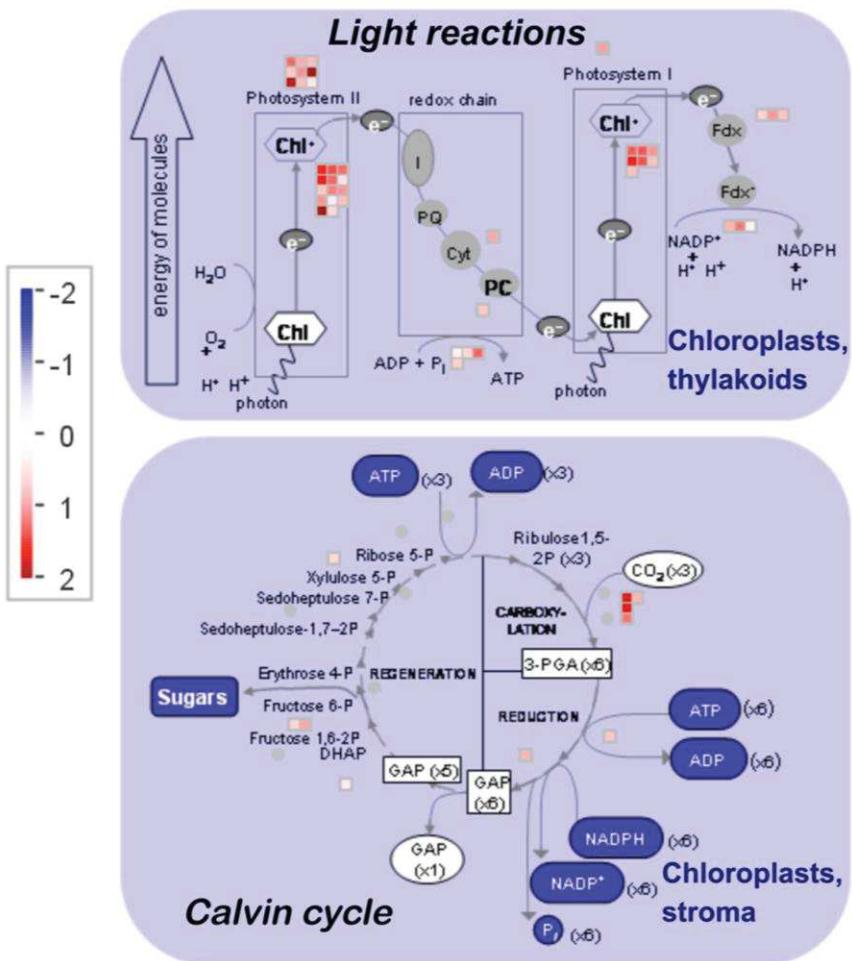
The functional categorization of up-regulated DEGs in leaves revealed that 17% are related to photosynthesis and carbon metabolism (Figure 4). Also, GO term and KEGG pathway enrichment analysis revealed that the most significantly enriched biological process among the up-regulated DEGs is photosynthesis (Supplementary Table 4 and 5). Therefore, we used the Mapman software to visualize the expression of all DEGs in leaves related to photosynthesis and carbon fixation in response to PsJN (Figure 5A, list of DEGs provided in Table 3). Indeed, 43 genes implicated in the light reactions of photosynthesis are differentially expressed in response to PsJN. Respectively, 23 and 8 genes coding for subunits of the photosystem II and I as well as their associated light-harvesting complexes are up-regulated with only one gene being down-regulated. Also, for each redox chain leading to ATP and NADPH synthesis, 6 genes are up-regulated following PsJN inoculation. Furthermore, 10 genes implicated in the Calvin cycle are up-regulated in response to PsJN. Among these, 4 genes encode for RuBisCO subunits, 2 genes are implicated in the reduction phase and 4 in the regeneration phase. Regarding these results, we further wanted to know if PsJN inoculation increased photosynthesis efficiency. Therefore, we measured cholorophyll fluorescence parameters to estimate the impact of PsJN inoculation on photosynthetic capacity of soil-grown rice plants.

Table 3: List of photosynthesis-related DEGs in rice leaves in response to PsJN

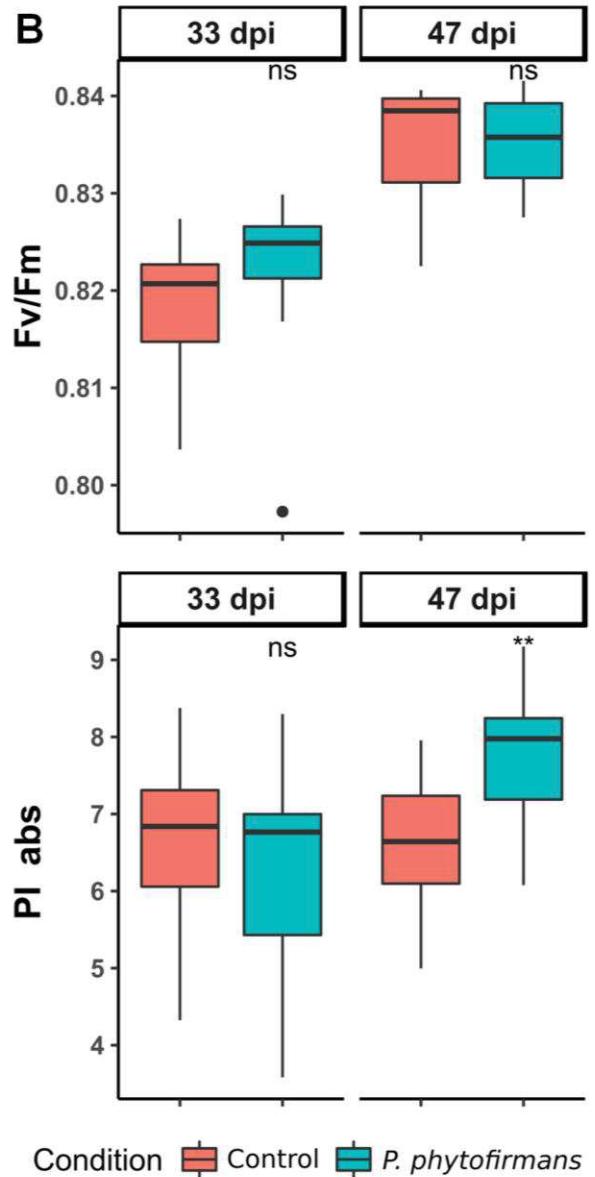
RAP ID	RAP annotation	Gene name	log <sub>2</sub> FoldChange
<b>Photosystem II</b>			
Os08g0504500	Mog1/PsbP/DUF1795, alpha/beta/alpha sandwich domain containing protein.		1,54
Os07g0544800	Similar to Oxygen-evolving enhancer protein 3-2, chloroplast precursor (OEE3)		1,35
Os01g0869800	22-kDa Photosystem II protein, Photoprotection	<i>OsPsbS1, PsbS1</i>	1,22
Os02g0578400	Photosystem II oxygen evolving complex protein PsbQ family protein.		1,62
Os03g0747700	Similar to photosystem II 11 kDa protein-related.		1,24
Os08g0512500	Similar to Thylakoid lumen protein, chloroplast.		0,52
Os01g0805300	Mog1/PsbP, alpha/beta/alpha sandwich domain containing protein.	<i>PsbP</i>	0,75
Os04g0523000	Photosystem II oxygen evolving complex protein PsbQ family protein.		1,12
Os07g0141400	Similar to 23 kDa polypeptide of photosystem II.	<i>PsbP, OsPsbP, psbP</i>	1,02
Os01g0501800	Similar to Photosystem II oxygen-evolving complex protein 1 (Fragment).	<i>PsbO, psbO</i>	1,02
Os12g0190200	Similar to Thylakoid luminal 29.8 kDa protein, chloroplast precursor.		-0,38
Os01g0938100	Photosystem II protein Psb28, class 1 domain containing protein.	<i>Psb28</i>	0,80
Os03g0333400	Similar to photosystem II 11 kD protein.		2,02
Os08g0347500	Similar to PsbP family protein, expressed.		0,62
<b>LHC-II</b>			
Os07g0577600	Similar to Type II chlorophyll a/b binding protein from photosystem I precursor.		1,21
Os11g0242800	Similar to ASCAB9-A (ASCAB9-B) (Fragment).	<i>ASCAB9-A, CP26, Lhcb5</i>	0,91
Os09g0439500	Similar to Type II chlorophyll a/b binding protein from photosystem I precursor.		0,81
Os09g0346500	Similar to Chlorophyll a-b binding protein, chloroplast precursor (LHCII type I CAB) (LHCP).	<i>OsLhcb1, Cab1</i>	0,72
Os03g0592500	Similar to Photosystem II type II chlorophyll a/b binding protein (Fragment).	<i>LHCII, LHCP II, Lhcb2</i>	1,04
Os04g0457000	Similar to Chlorophyll a/b-binding protein CP24, photosystem II (Fragment).	<i>CP24, Lhcb6</i>	2,21
Os01g0600900	Chlorophyll a-b binding protein 2, chloroplast precursor (LHCII type I CAB-2) (LHCP).	<i>OsLhcp, Lhcb1</i>	1,90
Os07g0562700	Similar to Type III chlorophyll a/b-binding protein (Fragment).		0,81
Os09g0296800	Chlorophyll A-B binding protein family protein.	<i>CabE</i>	0,47
<b>Redox chain</b>			
Os07g0556200	Rieske [2Fe-2S] region domain containing protein.	<i>PetC</i>	0,92
Os06g0101600	Plastocyanin, chloroplast precursor.		0,74
<b>ATP synthase</b>			
Os01g0673800	Similar to ATP synthase protein I -related.	<i>OsRopGEF6, RopGEF6</i>	0,36
Os07g0513000	Similar to ATP synthase gamma chain, chloroplast (EC 3.6.1.34) (Fragment).	gamma chain	0,61
Os02g0750200	Non-protein coding transcript.	delta chain	1,26

Os03g0278900	ATPase, F0 complex, subunit B/B', bacterial and chloroplast family protein.	<i>subunit B_ (ATPX)</i>	0,70
<b>Photosystem I</b>			
Os09g0481200	Similar to photosystem I reaction center subunit V.	<i>PsaG</i>	1,27
Os10g0536500	Peptidase S10, serine carboxypeptidase, active site domain containing protein.		1,31
Os07g0435300	Photosystem I reaction center subunit IV, chloroplast precursor (PSI- E)	<i>PsaE</i>	1,02
Os03g0778100	Similar to Photosystem-1 F subunit.	<i>PsaF</i>	1,61
Os04g0414700	Photosystem I PsaO domain containing protein.		1,34
Os12g0189400	Similar to Photosystem I reaction centre subunit N, chloroplast precursor (PSI- N).		0,78
Os08g0560900	Similar to Photosystem I reaction center subunit II, chloroplast precursor	<i>PsaD</i>	0,79
Os01g0589800	Similar to OHP2.	LHC-I	0,97
<b>Ferredoxin redox chain</b>			
Os05g0555300	Similar to electron carrier/ electron transporter/ iron ion binding protein.		0,67
Os07g0489800	Beta-grasp fold, ferredoxin-type domain containing protein.		1,03
Os08g0104600	Ferredoxin I, chloroplast precursor (Anti-disease protein 1).	<i>Fd1, OsFd1, ADII</i>	0,77
Os02g0103800	Leaf-type ferredoxin-NADP+-oxidoreductase, Regulation of electron partitioning in the chloroplast	<i>OsLFNR1, OsLFNR2</i>	0,86
Os06g0107700	Leaf-type ferredoxin-NADP+-oxidoreductase, Regulation of electron partitioning in the chloroplast		1,16
Os02g0277600	Similar to MFDR (NADP adrenodoxin-like ferredoxin reductase).	<i>OsISC21</i>	0,33
<b>Calvin cycle</b>			
<b>Rubisco small subunit</b>			
Os12g0274700	Similar to Petunia ribulose 1, 5-bisphosphate carboxylase small subunit	<i>rbcS, OsRbcS2</i>	1,72
Os12g0291400	Similar to Petunia ribulose 1, 5-bisphosphate carboxylase small subunit.	<i>OsRbcS5</i>	1,72
Os02g0152400	Rubisco small subunit 1, Reguration of Rubisco catalytic activity	<i>OsRbcS1</i>	1,18
Os12g0292400	Similar to Petunia ribulose 1, 5-bisphosphate carboxylase small subunit	<i>OsRbcS4</i>	0,86
<b>Reduction</b>			
Os05g0496200	Similar to 3-phosphoglycerate kinase (Fragment).	Phosphoglycerate kinase	0,74
Os04g0459500	Similar to H0219H12.1 protein.	GAP	0,86
<b>Regeneration</b>			
Os01g0147900	Triosephosphate isomerase, cytosolic (EC 5.3.1.1) (TIM) (Triose- phosphate isomerase).	tpi*, tips, TI	0,42
Os03g0267300	Similar to Fructose-1, 6-bisphosphatase, chloroplast precursor (EC 3.1.3.11) (FBPase).	<i>OsCFR</i>	0,62
Os06g0664200	Inositol monophosphatase/Fructose-1, 6-bisphosphatase domain containing protein.	FBPase	0,94
Os03g0169100	Ribulose-phosphate 3-epimerase, chloroplast precursor (EC 5.1.3.1) (PPE) (RPE) (R5P3E).	RPE	0,61

A



B



**Figure 5: PsJN colonization increases rice photosynthetic efficiency**

(A) Mapman representation of photosynthesis-related DEGs in leaves in response to PsJN (B) Photosynthetic parameters of seed-inoculated PsJN-treated plants grown in the greenhouse. Data reported are the median of 12 plants. ns indicate non-significant difference between PsJN-treated and control plants, \*\* indicate significant difference ( $p < 0.01$ ) between PsJN-treated and control plants according to a non-parametric Wilcoxon test

The first parameter, Fv/Fm, estimates the maximum potential quantum efficiency of photosystem II. At 33 dpi, PsJN-treated plants have a slightly higher, however not significant, Fv/Fm ratio while at 47 dpi there is no difference between control and inoculated plants for this parameter (Figure 5B). The second parameter is the Performance Index ( $PI_{abs}$ ) which corresponds to the potential energy conserved between light harvesting and intersystem electron acceptors. For this parameter, at 33 dpi no significant difference is observed between control and inoculated plants. However, at 47 dpi, a significant increase of  $PI_{abs}$  is measured for PsJN-treated plants compared to control plants (Figure 5B).

- **Discussion**

The work presented here corresponds to the first study of the interaction between rice and *Paraburkholderia phytofirmans* strain PsJN. The analysis of rice root colonization showed that PsJN is able to massively colonize rice roots. However, the different root areas are not equally colonized. Indeed, PsJN cells particularly accumulate in the root hair zone and on lateral root tips. This was also observed during the colonization of grapevine by PsJN (Complant *et al.*, 2008). Interestingly, these root areas correspond to the most nutrient-rich zones. Particularly, the root hair zone is a hotspot in terms of exudates concentration (Gamalero *et al.*, 2004). Also, the root cap cells could be microbial hotspots for several reasons. First, these cells exude large amount of polysaccharide-based mucilage which could be used as nutrients by PsJN cells, as it was previously observed in pea (Knee *et al.*, 2001). Secondly, root cap cells have a shorter life cycle compared to other root cells (Kumpf and Nowack, 2015), which is a consequence of a programmed cell death. This leads to the leakage of the cellular content of which some components could be used as nutrients by bacteria.

In order to reach this level of colonization, PsJN cells need to evade plant immunity. Indeed, the perception of conserved microbe-associated molecular patterns (MAMPs) by plant cells usually leads to the activation of MAMPs-triggered immunity (MTI) (Jones and Dangl, 2006). This basal immune response is mainly characterized by the production of Pathogenesis-Related (PR) proteins during microbial colonization of plant tissues. The transcriptional analysis of defense-related genes in rice roots in response to PsJN shows that MTI is probably suppressed. Indeed, the functional analysis revealed a number of PR genes down-regulated in response to PsJN, mainly from the PR1 and PR3 families.

However, this is not the only MTI-related transcriptional regulations induced by PsJN perception in rice roots. MTI is also characterized by the production of salicylic acid (SA) and

interestingly several DEGs in roots are related to SA metabolism. Indeed, 3 genes encoding for SA methyltransferase are up-regulated in PsJN-treated roots. Interestingly, the overexpression of such enzymes in *A. thaliana* led to an increased susceptibility to pathogens (Koo *et al.*, 2007). Therefore, SA conjugation could also be involved in the suppression of root immunity enabling microbial colonization by beneficial microbes.

Finally, a number of transcription factors of the WRKY family are also regulated in response to PsJN. Particularly, *WRKY45* and *WRKY71* that are down-regulated following PsJN inoculation, where shown to induce enhanced resistance to the leaf pathogen *Xanthomonas oryzae* when over-expressed (Liu *et al.*, 2007; Shimono *et al.*, 2007). Also, the expression of *CCR1*, encoding a Cinnamoyl-CoA reductase which is implicated in lignin synthesis and participates in root immunity (Kawasaki *et al.*, 2006), is here down-regulated. In leaves also, defense-related genes are transcriptionally regulated in response to *P. phytophthora*. Interestingly and in sharp contrast to the root response, important component of plant immunity are systemically transcriptionally up-regulated. First, the chitin elicitor binding protein encoding gene *CEBIP* (Shimizu *et al.*, 2010) is up-regulated which could possibly lead to an enhanced capacity for PsJN-interacting plants to resist fungal colonization. Additionally, the gene *MKK4*, which encodes for a MAPK kinase implicated in rice immunity (Kishi-Kaboshi *et al.*, 2010), is also up-regulated in leaves in response to PsJN.

In roots, among the most highly up-regulated DEGs, the larger category of genes is related to signaling among which the majority of genes encode for receptor-like kinases (RLK). These membrane-bounded receptor proteins are at the basis of environmental stimuli -both biotic and abiotic- sensing by plants (Antolín-Llovera *et al.*, 2012; Osakabe *et al.*, 2013). Among the number of RLK up-regulated in response to PsJN, some have been described as implicated in stress tolerance. For example, 3 genes encoding for abiotic stress-responsive calcium regulated RLK are up-regulated by PsJN, namely *CIPK10*, *CBL8*, *CBL7* (Kanwar *et al.*, 2014). Also, two defense-related RLK are up-regulated in response to PsJN, namely *WAK112* (Delteil *et al.*, 2016) and *SIRK1* (Li *et al.*, 2015). These observations suggest that rice perception of PsJN could lead to an enhanced capacity to respond to abiotic and biotic environment.

As reported in previous studies analyzing plant transcriptional response to beneficial rhizobacteria, the inoculation of PsJN on rice roots induced important transcriptional regulations of hormonal signaling. Particularly, the transcriptome of inoculated roots shows important regulations of genes implicated in SA (discussed above) as well as CK, JA and

ethylene signaling. Firstly, among the up-regulated genes in roots there are 4 genes of the A-type Response Regulators family. These genes act as negative regulators of CK signaling which play a role in the maintenance of rice root meristem (Coudert *et al.*, 2010). Particularly, the overexpression of *RR6* in rice leads to a reduced root development phenotype (Hirose *et al.*, 2007). Also, among the down-regulated genes, two of them are implicated in cytokinin metabolism. The first one is *LOGL8*, the ortholog of *Arabidopsis LOG8* part of a family of genes implicated in CK activation (Kuroha *et al.*, 2009). The second, *cZOGT1* is implicated in the O-glucosylation of cis-zeatin which have inhibitory effect on primary root growth (Kudo *et al.*, 2012). Such important transcriptional regulation related to cytokinins signaling pathway were also reported as part of rice response to the beneficial rhizobacterial strain *Bacillus subtilis* RR4 as well as rice-beneficial *Burkholderia s.l.* strains (King *et al.*, 2019; Rekha *et al.*, 2018).

The second hormonal pathway transcriptionally regulated in roots in response to PsJN is the ethylene one. Indeed two genes encoding for ACC oxidase (ACO) are up-regulated, this enzyme is the last one implicated in the synthesis of ethylene (Wang *et al.*, 2002). This could be related to the fact that PsJN is harboring the gene encoding for ACC deaminase which is a well known plant growth-promoting trait. Indeed, this enzyme is able to cleave ACC, an ethylene synthesis precursor, and therefore lowers plants stress response while maintaining growth (Ravanbakhsh *et al.*, 2018). Although ACC deaminase has a much lower affinity towards ACC than ACO (Glick, 2005), here the microbial population is very high. Therefore, the up-regulation of two ACO-encoding genes could be a way for rice roots to compensate the accumulation of microbial ACC deaminase which possibly led to the down-regulation of the *ERF* genes (Table 2). Finally, PsJN also induced the down-regulation of several JA-related genes. Interestingly, several examples of beneficial microbes suppressing root immunity depending on JA signaling components have been described (Yu *et al.*, 2019). Together with the SA conjugation seen above, this could also be a way for PsJN to accommodate rice immunity to enable root colonization.

The colonization of rice roots by PsJN also led to important transcriptional regulations of transporters. Particularly, three genes encoding for iron-phytosiderophore transporters of the YSL family are up-regulated in response to PsJN. In particular, *OsYSL15* transports Fe3-phytosiderophore complexes from rhizosphere to the roots (Inoue *et al.*, 2009). Such transcriptional regulations were also observed in rice roots inoculated with *Herbaspirillum seropedicae* and rice-beneficial *Burkholderia s.l.* strains (Brusamarello-Santos *et al.*, 2019;

King *et al.*, 2019). Also the fact that iron vacuolar transporters are down-regulated possibly underlines the fact that rice roots are not accumulating iron in their vacuole. This is likely due to the fact that the population of PsJN is in competition for iron with rice roots which could cause this iron deficiency-like transcriptional response (Bashir *et al.*, 2014). This could also be linked to an increase in the photosynthetic capabilities (discussed below), indeed a large part of the iron present in plants is allocated to the photosynthetic process (Briat *et al.*, 2007). Similarly to the fact that PsJN induced the transcriptional regulation of abiotic stress-related RLK, the expression of genes encoding for the transport of water is modulated in PsJN-treated roots. Particularly, two Nodulin 26-like membrane intrinsic proteins (NIPs) encoding genes, namely *OsNIP3;5* and *OsNIP3;2*, are up-regulated while *OsPIP2;9* – encoding for a plasma membrane intrinsic protein (PIP) – is down-regulated in response to PsJN. Apparently, PsJN root colonization induces the transcriptional regulation of genes implicated in water homeostasis in rice. Consequently, it could be interesting to study the impact of PsJN inoculation for rice in a water deficiency context. Indeed, the inoculation of PsJN enhanced drought stress tolerance of wheat (Naveed *et al.*, 2014a) and maize (Naveed *et al.*, 2014b). Finally, root colonization by PsJN induced the up-regulation of *OsALMT7* which encodes for a malate transporter (Heng *et al.*, 2018). Interestingly, root transcriptome and functional experiments of *A. thaliana* interacting with the beneficial *Bacillus subtilis* FB17 showed that *AtALMT1* is implicated in rhizobacteria recruitment (Lakshmanan *et al.*, 2013).

The colonization of rice roots by PsJN not only induced transcriptional regulations at the root level but also in leaves. In fact, more DEGs were detected in the leaves of PsJN-treated plants than in their roots. According to the analysis of the leaf transcriptome of PsJN-treated plants, a significant up-regulation of genes related to photosynthesis and carbon fixation was detected. Particularly, regarding the fact that 23 genes coding for subunits of the photosystem II were up-regulated, we hypothesize that PsJN-treated plants induces an increase in the quantum efficiency of photosystem II. However, the measure of chlorophyll fluorescence didn't reveal any significant change of the Fv/Fm ratio in PsJN-treated plants. This could be due to the fact that plants used for chlorophyll fluorescence measurements were grown in soil while the transcriptomic analysis was performed on hydroponically grown plants. Nonetheless, at 47 dpi, PsJN-treated plants have a significantly higher PI<sub>ABS</sub> value. Therefore, although the inoculation of PsJN didn't induce a significant increase of the photosystem II maximum quantum efficiency, the increase of the vitality index PI<sub>ABS</sub> value suggests that less energy is dissipated during the light reactions of photosynthesis in PsJN-treated plants

(Srivastava *et al.*, 1999). As these parameters are widely used as stress tolerance proxies (Kalaji *et al.*, 2016), it would be interesting to measure the impact of PsJN inoculation on these parameters for rice plants experiencing abiotic stresses. Indeed, the inoculation of PsJN induced an increased photosynthetic efficiency particularly in stressful conditions (Naveed *et al.*, 2014b; Su *et al.*, 2015) which was confirmed by metabolomic analyses (Su *et al.*, 2016).

To conclude, in this study we showed for the first time that PsJN is able to efficiently colonize rice roots and described important transcriptional regulation of various physiological processes triggered by rice following root colonization. Particularly, in roots, the colonization by PsJN is accompanied by a significant down-regulation of defense and an up-regulation of many RLKs. Also, hormonal signaling is modulated in the tissues colonized by PsJN, particularly the CK, ethylene and JA pathways. This latest hormonal pathway is well known to be implicated in defense and is commonly suppressed by beneficial microbes to enable colonization (Yu *et al.*, 2019). Rice roots inoculated with *B. vietnamiensis* TVV75 also induced the down-regulation of JA-related genes, in particular, *JAZ9*, *JAZ10* and *bHLH148* are commonly down-regulated by both strains (King *et al.*, 2019). Consequently, the analysis of the role of JA, in the establishment of these interactions could be very interesting. This could be done by analyzing the colonization of PsJN as well as transcriptional regulations in JA-deficient mutants. We also have shown that important systemic transcriptional regulations occur in rice leaves following root colonization. Particularly, PsJN inoculation induced the up-regulation of genes implicated in photosynthesis and a potential enhancement of photochemistry efficiency. It would be interesting to study the impact of PsJN inoculation on stress tolerance of rice to know if this enhancement of photosynthesis is maintained in stressful conditions.

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Supplementary Table 1: Summary of RNA-Seq data generated for rice transcriptomes

<b>Sample</b>	<b>Organ</b>	<b>Condition</b>	<b>Total of reads</b>	<b>Number of uniquely mapped reads</b>	<b>Proportion of mapped reads</b>
LC1	Leaves	Control	38 533 867	26 161 511	68%
LC2	Leaves	Control	44 792 837	29 952 254	67%
LC3	Leaves	Control	33 331 123	21 989 917	66%
LP1	Leaves	<i>P. phytofirmans</i>	52 174 751	36 881 573	71%
LP2	Leaves	<i>P. phytofirmans</i>	54 686 334	37 061 417	68%
LP3	Leaves	<i>P. phytofirmans</i>	38 487 885	27 734 731	72%
RC1	Roots	Control	38 522 573	27 048 494	70%
RC2	Roots	Control	50 820 144	35 403 810	70%
RC3	Roots	Control	44 458 436	30 782 646	69%
RP1	Roots	<i>P. phytofirmans</i>	40 550 424	27 833 109	69%
RP2	Roots	<i>P. phytofirmans</i>	48 542 587	33 699 410	69%
RP3	Roots	<i>P. phytofirmans</i>	28 491 965	19 362 302	68%

Supplementary Table 4: GO term enrichment analysis

Organ	Regulation	Term	Category	padj	Ratio
Leaves	Up	photosynthesis	Photosynthesis	3,63E-39	5,60
Leaves	Up	photosynthesis, light reaction	Photosynthesis	1,04E-28	6,96
Leaves	Up	biological process	Other	1,06E-22	1,20
Leaves	Up	generation of precursor metabolites and energy	Metabolism	8,40E-18	2,72
Leaves	Up	response to abiotic stimulus	Stress	1,22E-14	2,22
Leaves	Up	metabolic process	Metabolism	1,53E-14	1,21
Leaves	Up	oxidation-reduction process	Metabolism	1,11E-12	1,66
Leaves	Up	cellular process	Other	1,40E-11	1,19
Leaves	Up	cellular metabolic process	Metabolism	1,74E-10	1,21
Leaves	Up	response to light stimulus	Stimulus response	1,98E-10	2,69
Leaves	Up	response to stimulus	Stimulus response	4,53E-06	1,32
Leaves	Up	cellular response to oxidative stress	Stress	9,99E-06	3,88
Leaves	Up	organonitrogen compound metabolic process	Metabolism	1,11E-05	1,24
Leaves	Up	response to endogenous stimulus	Hormone	1,90E-05	1,86
Leaves	Up	response to hormone	Hormone	2,09E-05	1,86
Leaves	Up	multicellular organism development	Development	2,18E-05	1,68
Leaves	Up	biosynthetic process	Metabolism	6,61E-05	1,25
Leaves	Up	response to cytokinin	Hormone	9,54E-05	3,47
Leaves	Up	organic substance biosynthetic process	Metabolism	1,84E-04	1,25
Leaves	Up	oligosaccharide metabolic process	Metabolism	2,13E-04	3,33
Leaves	Up	organic substance metabolic process	Metabolism	2,54E-04	1,14
Leaves	Up	protein-chromophore linkage	Photosynthesis	2,95E-04	5,73
Leaves	Up	plastid organization	Photosynthesis	4,41E-04	2,57
Leaves	Up	cellular amide metabolic process	Metabolism	6,44E-04	1,57
Leaves	Up	protein repair	Stress	9,08E-04	7,17
Leaves	Up	developmental process	Development	9,48E-04	1,49
Leaves	Up	cofactor metabolic process	Metabolism	1,43E-03	1,82
Leaves	Up	negative regulation of response to stimulus	Stimulus response	2,37E-03	3,10
Leaves	Up	carbon fixation	Metabolism	2,86E-03	5,73
Leaves	Up	amide biosynthetic process	Metabolism	5,41E-03	1,58
Leaves	Up	multicellular organismal process	Development	6,30E-03	1,49
Leaves	Up	chloroplast rRNA processing	Photosynthesis	6,58E-03	6,74
Leaves	Up	small molecule catabolic process	Metabolism	8,21E-03	2,33
Leaves	Down	cellular process	Other	2,20E-76	1,50
Leaves	Down	biological process	Other	2,49E-71	1,37
Leaves	Down	organic substance metabolic process	Metabolism	8,07E-51	1,47
Leaves	Down	cellular metabolic process	Metabolism	1,59E-48	1,47
Leaves	Down	primary metabolic process	Metabolism	1,77E-47	1,47
Leaves	Down	nitrogen compound metabolic process	Metabolism	6,05E-47	1,51
Leaves	Down	macromolecule metabolic process	Metabolism	7,43E-42	1,50
Leaves	Down	metabolic process	Metabolism	7,43E-39	1,36

Leaves	Down	heterocycle metabolic process	Metabolism	5,18E-35	1,71
Leaves	Down	gene expression	Transcription	7,28E-33	1,79
Leaves	Down	cellular aromatic compound metabolic process	Metabolism	3,68E-32	1,67
Leaves	Down	organic cyclic compound metabolic process	Metabolism	3,32E-31	1,65
Leaves	Down	RNA metabolic process	Transcription	4,68E-29	1,76
Leaves	Down	biological regulation	Other	1,42E-25	1,54
Leaves	Down	cellular component organization or biogenesis	Metabolism	6,07E-23	1,80
Leaves	Down	macromolecule biosynthetic process	Metabolism	1,71E-20	1,64
Leaves	Down	response to stimulus	Stimulus response	2,63E-20	1,61
Leaves	Down	ribonucleoprotein complex biogenesis	Chromatin	5,76E-19	2,94
Leaves	Down	RNA processing	Metabolism	7,79E-18	2,45
Leaves	Down	localization	Transport	2,07E-16	1,69
Leaves	Down	biosynthetic process	Metabolism	4,91E-16	1,46
Leaves	Down	cellular protein metabolic process	Metabolism	4,83E-15	1,49
Leaves	Down	transport	Transport	5,45E-15	1,68
Leaves	Down	protein metabolic process	Metabolism	2,03E-14	1,44
Leaves	Down	cellular localization	Transport	9,55E-14	2,26
Leaves	Down	rRNA metabolic process	Transcription	4,66E-13	3,48
Leaves	Down	cellular amide metabolic process	Translation	5,29E-12	2,01
Leaves	Down	response to stress	Stress	7,51E-12	1,68
Leaves	Down	protein localization	Transport	8,84E-12	2,21
Leaves	Down	macromolecule modification	Metabolism	1,54E-11	1,46
Leaves	Down	protein folding	Translation	2,42E-10	3,26
Leaves	Down	regulation of gene expression	Transcription	2,70E-10	1,57
Leaves	Down	translation	Translation	1,76E-09	2,07
Leaves	Down	vesicle-mediated transport	Transport	1,01E-08	2,41
Leaves	Down	peptide transport	Transport	2,27E-08	2,02
Leaves	Down	signaling	Signaling	9,19E-08	1,61
Leaves	Down	cell communication	Transport	1,05E-07	1,58
Leaves	Down	gene silencing	Transcription	1,32E-07	3,61
Leaves	Down	small molecule metabolic process	Metabolism	2,20E-07	1,66
Leaves	Down	nitrogen compound transport	Transport	5,36E-07	1,81
Leaves	Down	nucleocytoplasmic transport	Transport	6,30E-07	3,77
Leaves	Down	phosphorus metabolic process	Metabolism	9,89E-07	1,43
Leaves	Down	chaperone-mediated protein folding	Translation	2,18E-06	4,05
Leaves	Down	phosphorylation	Signaling	4,30E-06	1,49
Leaves	Down	carboxylic acid metabolic process	Metabolism	4,90E-06	1,80
Leaves	Down	response to heat	Stress	1,02E-05	3,28
Leaves	Down	oxoacid metabolic process	Metabolism	1,68E-05	1,76
Leaves	Down	starch biosynthetic process	Metabolism	1,87E-05	6,52
Leaves	Down	organic acid metabolic process	Metabolism	1,87E-05	1,75
Leaves	Down	nucleic acid phosphodiester bond hydrolysis	Metabolism	2,07E-05	1,86
Leaves	Down	chromatin organization	Chromatin	3,01E-05	2,32
Leaves	Down	cellular amino acid metabolic process	Metabolism	2,35E-04	2,07

Leaves	Down	regulation of gene expression, epigenetic mRNA metabolic process	Transcription	3,13E-04	3,18
Leaves	Down	DNA metabolic process	Transcription	1,06E-03	1,96
Leaves	Down	import into cell	Transport	1,06E-03	1,79
Leaves	Down	Golgi vesicle transport	Transport	1,09E-03	4,01
Leaves	Down	reproduction	Development	1,37E-03	2,63
Leaves	Down	amino acid activation	Metabolism	1,97E-03	1,70
Leaves	Down	embryo sac development	Development	2,24E-03	3,64
Leaves	Down	reproductive process	Development	3,13E-03	4,44
Leaves	Down	multicellular organismal process	Development	3,13E-03	1,69
Leaves	Down	energy derivation by oxidation of organic compounds	Metabolism	4,11E-03	1,53
Leaves	Down	regulation of transport	Transport	5,73E-03	2,66
Leaves	Down	positive regulation of biological process	Other	6,96E-03	3,11
Leaves	Down			8,70E-03	1,62
Roots	Up	biological process	Other	1,37E-13	1,42
Roots	Up	phosphorus metabolic process	Metabolism	1,04E-12	2,62
Roots	Up	biological regulation	Other	3,69E-11	1,97
Roots	Up	protein phosphorylation	Signaling	4,87E-10	3,02
Roots	Up	cell communication	Transport	2,39E-09	2,79
Roots	Up	cellular process	Other	5,72E-09	1,45
Roots	Up	regulation of biological process	Other	5,75E-09	1,95
Roots	Up	signaling	Signaling	8,81E-09	2,84
Roots	Up	cellular macromolecule metabolic process	Metabolism	2,43E-08	1,71
Roots	Up	organic substance metabolic process	Metabolism	4,13E-08	1,50
Roots	Up	primary metabolic process	Metabolism	4,74E-08	1,52
Roots	Up	response to stimulus	Stimulus response	5,24E-07	1,99
Roots	Up	cellular response to organic substance	Stimulus response	6,75E-07	4,73
Roots	Up	cellular metabolic process	Metabolism	1,48E-06	1,47
Roots	Up	metabolic process	Metabolism	1,88E-06	1,39
Roots	Up	macromolecule metabolic process	Metabolism	1,42E-05	1,51
Roots	Up	nitrogen compound metabolic process	Metabolism	1,79E-05	1,48
Roots	Up	phosphorelay signal transduction system	Signaling	2,47E-05	12,63
Roots	Up	macromolecule modification	Metabolism	9,75E-05	1,82
Roots	Up	response to hormone	Hormone	2,08E-04	3,46
Roots	Up	response to endogenous stimulus	Hormone	2,64E-04	3,41
Roots	Up	response to cytokinin	Hormone	3,58E-04	9,31
Roots	Up	negative regulation of ion transport	Transport	6,46E-04	98,25
Roots	Up	metal ion transport	Transport	1,44E-03	4,37
Roots	Up	transcription, DNA-templated	Transcription	3,13E-03	2,00
Roots	Up	protein metabolic process	Translation	3,85E-03	1,60
Roots	Up	transmembrane transport	Transport	6,43E-03	2,29
Roots	Down	response to stimulus	Stimulus response	3,43E-10	1,92
Roots	Down	response to stress	Stress	1,28E-08	2,26
Roots	Down	defense response	Defense	5,69E-08	3,22

Roots	Down	biological process	Other	7,55E-06	1,23
Roots	Down	response to jasmonic acid	Hormone	1,08E-04	9,06
Roots	Down	oxidation-reduction process	Metabolism	1,88E-04	1,94
Roots	Down	regulation of response to stimulus	Stimulus response	4,91E-04	3,56
Roots	Down	response to wounding	Stress	5,11E-04	8,94
Roots	Down	organic acid metabolic process	Metabolism	7,13E-04	2,42
Roots	Down	response to external biotic stimulus	Defense	8,27E-04	3,45
Roots	Down	small molecule metabolic process	Metabolism	1,59E-03	2,03
Roots	Down	response to oxygen-containing compound	Stress	1,73E-03	2,72
Roots	Down	response to biotic stimulus	Defense	1,81E-03	3,28
Roots	Down	drug metabolic process	Metabolism	1,91E-03	2,76
Roots	Down	cellular process	Other	2,36E-03	1,24
Roots	Down	aminoglycan metabolic process	Metabolism	3,17E-03	8,50
Roots	Down	regulation of signaling	Signaling	4,69E-03	4,58
Roots	Down	cellular transition metal ion homeostasis	Metabolism	4,83E-03	5,42
Roots	Down	drug catabolic process	Metabolism	4,93E-03	3,60
Roots	Down	regulation of cell communication	Transport	5,31E-03	4,53
Roots	Down	immune response	Defense	6,53E-03	5,25

Supplementary Table 5: KEGG pathway enrichment analysis

<b>Organ</b>	<b>Regulation</b>	<b>Term</b>	<b>padj</b>	<b>Ratio</b>
Leaves	Up	Photosynthesis	4,76E-12	6,03
Leaves	Up	Metabolic pathways	5,42E-11	1,71
Leaves	Up	Carbon fixation in photosynthetic organisms	2,08E-06	4,19
Leaves	Up	Photosynthesis - antenna proteins	4,98E-06	8,60
Leaves	Up	Carbon metabolism	8,33E-06	2,50
Leaves	Up	Biosynthesis of secondary metabolites	1,70E-05	1,67
Leaves	Up	MAPK signaling pathway - plant	7,09E-04	2,76
Leaves	Up	Glyoxylate and dicarboxylate metabolism	2,35E-03	3,29
Leaves	Up	Plant hormone signal transduction	5,24E-03	2,15
Leaves	Up	Oxidative phosphorylation	1,13E-02	2,41
Leaves	Up	Ubiquitin mediated proteolysis	1,66E-02	2,65
Leaves	Up	Peroxisome	1,82E-02	2,87
Leaves	Up	Glutathione metabolism	2,68E-02	2,64
Leaves	Up	Pentose phosphate pathway	2,81E-02	3,26
Leaves	Up	Terpenoid backbone biosynthesis	3,04E-02	3,49
Leaves	Down	Ribosome biogenesis in eukaryotes	1,44E-10	5,88
Leaves	Down	Carbon metabolism	1,38E-07	2,88
Leaves	Down	Biosynthesis of amino acids	7,03E-07	2,96
Leaves	Down	Metabolic pathways	3,44E-06	1,56
Leaves	Down	Aminoacyl-tRNA biosynthesis	2,67E-05	5,50
Leaves	Down	Biosynthesis of secondary metabolites	1,06E-04	1,66
Leaves	Down	Glycolysis / Gluconeogenesis	5,17E-04	2,92
Leaves	Down	Purine metabolism	1,70E-03	3,40
Leaves	Down	Alanine, aspartate and glutamate metabolism	1,81E-03	4,46
Leaves	Down	Protein processing in endoplasmic reticulum	8,66E-03	2,29
Leaves	Down	Ribosome	1,34E-02	2,01
Leaves	Down	RNA transport	2,10E-02	2,43
Leaves	Down	Glutathione metabolism	2,90E-02	2,74
Leaves	Down	Phosphatidylinositol signaling system	2,92E-02	3,52
Leaves	Down	Glyoxylate and dicarboxylate metabolism	4,60E-02	2,89
Roots	Up	Plant hormone signal transduction	1,50E-06	7,10
Roots	Down	Metabolic pathways	1,31E-07	2,34
Roots	Down	Biosynthesis of secondary metabolites	8,45E-05	2,45
Roots	Down	Amino sugar and nucleotide sugar metabolism	8,84E-04	5,09
Roots	Down	Carbon fixation in photosynthetic organisms	2,63E-03	6,64
Roots	Down	Phenylpropanoid biosynthesis	7,39E-03	3,95
Roots	Down	Taurine and hypotaurine metabolism	3,21E-02	14,22

Supplementary Table 7: List of signaling-related DEGs in rice roots in response to PsJN

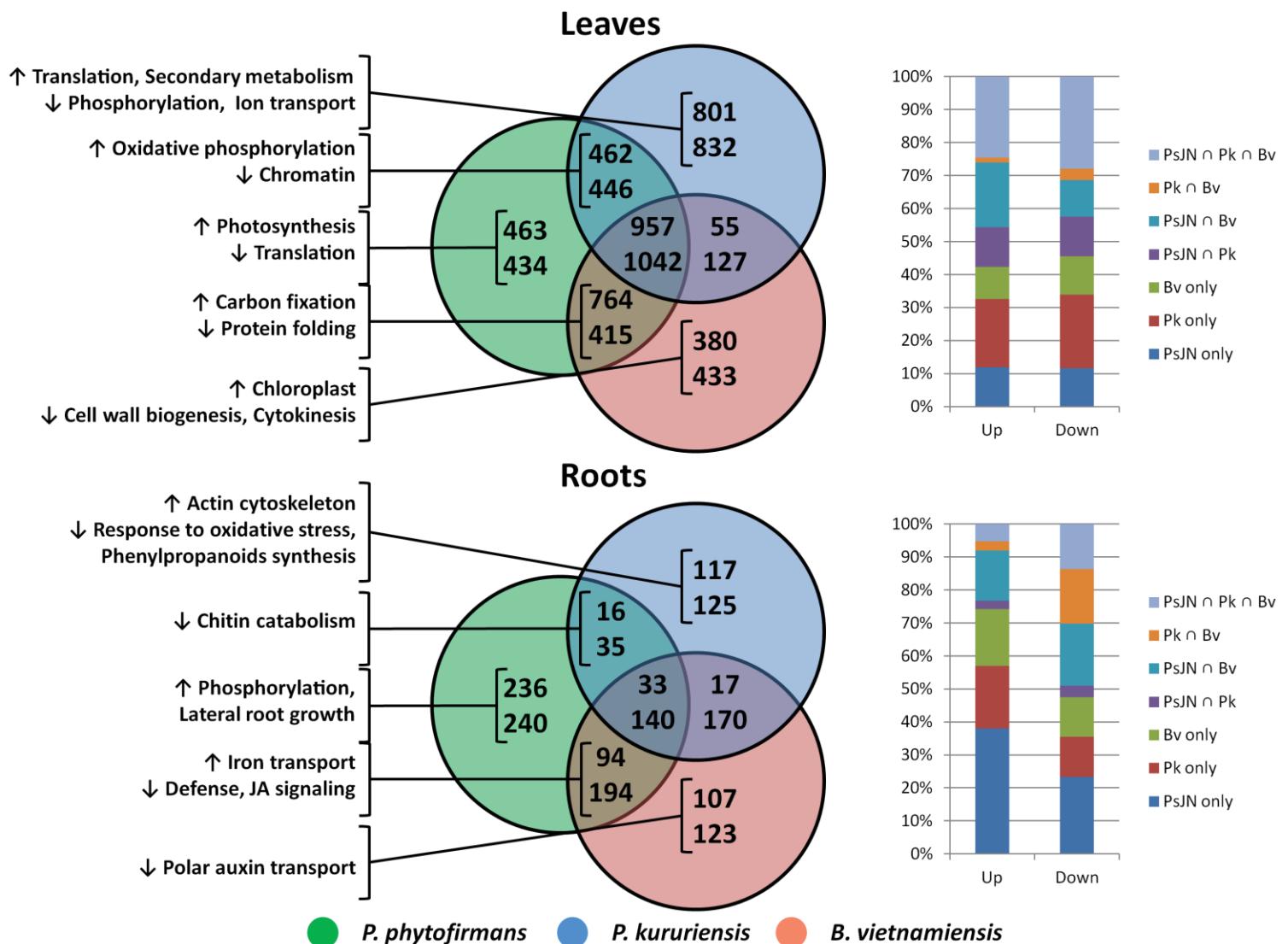
RAP ID	Log <sub>2</sub> FoldChange	Gene name	RAP annotation
Os04g0634400	2,44		Similar to H0315F07.5 protein.
Os06g0534200	1,80		Conserved hypothetical protein.
Os09g0551251	1,79		Similar to H0105C05.10 protein.
Os10g0134700	1,78		Protein kinase-like domain containing protein.
Os04g0633900	1,77		Similar to H0315F07.1 protein.
Os10g0180800	1,49	<i>OsWAK112</i>	Wall-associated kinase, Negative regulation of rice blast resistance
Os05g0231100	1,48		Hypothetical conserved gene.
Os03g0339900	1,45	<i>OsCIPK10</i> ,	Similar to Serine/threonine protein kinase.
Os08g0446400	1,39		Leucine-rich repeat, N-terminal domain containing protein.
Os09g0356000	1,25	<i>OsRLCK266, OsSIRKI</i>	Similar to OsD305.
Os01g0818700	1,24		Leucine-rich repeat, N-terminal domain containing protein.
Os02g0153100	1,13	<i>LRK8</i>	Protein kinase, core domain containing protein.
Os02g0291400	1,07	<i>OsCBL8</i>	Similar to Calcineurin B-like protein 8.
Os09g0471550	1,02		EGF-like calcium-binding domain containing protein.
Os02g0153700	0,99		Serine/threonine protein kinase domain containing protein.
Os02g0291000	0,96	<i>OsCBL7</i>	Calcineurin B (Fragment).
Os05g0495600	0,96	<i>OsACA7, OsPM5ATP4</i>	Similar to autoinhibited calcium ATPase.
Os11g0245200	0,96		Similar to leucine-rich repeat family protein / protein kinase family protein.
Os08g0203700	0,94		Protein kinase, core domain containing protein.
Os01g0816600	0,93		Similar to predicted protein.
Os11g0609500	0,89	<i>OsRLCK339</i>	Similar to Jacalin-like lectin domain containing protein.
Os05g0486100	0,83	<i>OsRPK1</i>	LRR-RLK, Negative regulation of polar auxin transport, Root development
Os01g0852100	0,83	<i>OsRLCK48</i>	Conserved hypothetical protein.
Os11g0609600	0,76	<i>OsGF14h, 14-3-3h</i>	Similar to 14-3-3-like protein.
Os01g0818600	0,73		Similar to Leucine-rich repeat transmembrane protein kinase.
Os07g0542400	0,67		Similar to Receptor protein kinase.

Os05g0258400	0,65		Protein kinase, catalytic domain domain containing protein.
Os12g0210400	0,65		Protein kinase, core domain containing protein.
Os12g0611100	0,65	<i>OsRLCK373</i>	Similar to serine/threonine-protein kinase receptor.
Os01g0782200	0,64		Diacylglycerol kinase, catalytic region domain containing protein.
Os09g0394600	0,63		Endonuclease/exonuclease/phosphatase domain containing protein.
Os01g0783700	-1,43		Similar to EF-hand Ca2+-binding protein CCD1.
Os01g0955100	-1,44	<i>OsCML31, OsMSR2</i>	Similar to Calmodulin-like protein (Fragment).
Os04g0371200	-1,53	<i>OsRLCK148</i>	Similar to OSIGBa0134J07.8 protein.
Os03g0277700	-1,60		Protein of unknown function DUF26 domain containing protein.
Os09g0412300	-1,61		Similar to Calmodulin-like protein.
Os02g0807900	-1,80	<i>OsWAK21</i>	Conserved hypothetical protein.
Os11g0664800	-1,83		Hypothetical conserved gene.
Os04g0586500	-1,88		Malectin-like carbohydrate-binding domain domain containing protein.
Os08g0360300	-1,92		Calmodulin binding protein-like domain containing protein.
Os08g0203400	-2,15	<i>SHR5</i>	Protein kinase, core domain containing protein.
Os03g0643250	-2,53		Similar to Hydrolase-like protein.
Os07g0251900	-3,12		Leucine-rich repeat, N-terminal domain containing protein.

Supplementary Table 8: List of transport-related DEGs in rice roots in response to PsJN

RAP ID	Log <sub>2</sub> FoldChange	Gene name	RAP annotation
Os02g0650300	4,81	<i>OsYSL15</i>	Iron-phytosiderophore transporter, Iron homeostasis
Os12g0204100	2,30	<i>OsNIP3;5</i>	Similar to Major intrinsic protein, expressed.
Os08g0152000	1,95	<i>OsNIP3;2</i>	Nodulin 26-like intrinsic membrane protein, Arsenite (As(III)) uptake by lateral roots
Os03g0196000	1,62	<i>Ossultr1;2, OsSul1;2</i>	Similar to Sulfate transporter.
Os04g0542800	1,62	<i>OsYSL16</i>	Transporter of the Copper-Nicotianamine Complex, Fe(III)-deoxymugineic acid transporter
Os03g0667500	1,38	<i>OsZIP10</i>	Similar to Metal transport protein.
Os03g0197100	1,32		Similar to Sugar transporter protein.
Os04g0491200	1,28		Oligopeptide transporter domain containing protein.
Os05g0472400	1,22		Similar to Zinc transporter 9.
Os07g0257200	1,05	<i>OsNramp5</i>	Manganese and Cadmium transporter, Mn and Cd uptake
Os05g0493800	1,03		Similar to nodulin-like protein.
Os08g0398300	1,03	<i>OsABCA4</i>	ABC transporter-like domain containing protein.
Os09g0429400	1,01	<i>OsGLR2.3</i>	Similar to Glutamate receptor.
Os05g0586500	0,98	<i>OsGAT1</i>	Amino acid transporter, transmembrane family protein.
Os09g0468000	0,93		Multi antimicrobial extrusion protein MatE family protein.
Os10g0195000	0,93	<i>OsSTA240</i>	Multi antimicrobial extrusion protein MatE family protein.
Os06g0700700	0,91	<i>OsHMA2</i>	Similar to P1B-type heavy metal transporting ATPase.
Os02g0673100	0,88	<i>OsALMT7</i>	Similar to ALMT3.
Os09g0429200	0,88	<i>OsGLR2.2</i>	Similar to Glutamate receptor.
Os04g0524600	0,87	<i>OsYSL12</i>	Oligopeptide transporter OPT superfamily protein.
Os06g0129400	0,86	<i>OsSPX-MFS3</i>	Splicing variant of SPX-MFS protein 3
Os08g0206400	0,79	<i>OsHAK12</i>	Similar to Potassium transporter 18.
Os09g0554000	0,69	<i>OsSTA235, OsPT20</i>	Similar to Mitochondrial phosphate transporter.
Os02g0196600	0,66	<i>OsHMA4</i>	Heavy metal P1B-type ATPase, Control of Cu accumulation in rice grain
Os01g0825800	-1,34	<i>OsATL7</i>	Amino acid transporter, transmembrane domain containing protein.
Os07g0448200	-1,38	<i>OsPIP2;9</i>	Similar to cDNA clone:J023132K03, full insert sequence.

Os01g0878400	-1,43	<i>OsAAP10C, OsAAP5</i>	Amino acid transporter, transmembrane domain containing protein.
Os04g0538400	-1,63	<i>OsVITH2</i>	Similar to Nodulin 21 (N-21).
Os02g0585100	-1,65		Similar to ATFP4.
Os01g0905200	-1,70		Exo70 exocyst complex subunit family protein.
Os02g0585200	-1,83		Heavy metal transport/detoxification protein domain containing protein.
Os04g0607600	-1,87	<i>OsHKT7, OsHKT1;4</i>	High-affinity K <sup>+</sup> transportern, Na <sup>+</sup> exclusion from leaf blade upon salt stress
Os10g0419400	-1,89	<i>OsIDI1L/OsARD1</i>	Similar to SIPL.
Os09g0396900	-1,93	<i>OsVIT2, OsVIT1</i>	Protein of unknown function DUF125, transmembrane family protein.
Os02g0584700	-1,95		Heavy metal transport/detoxification protein domain containing protein.
Os04g0469000	-2,21		Heavy metal transport/detoxification protein domain containing protein.
Os05g0554000	-2,22	<i>OsMATE2</i>	Similar to cDNA clone:001-123-D07, full insert sequence.
Os02g0584800	-2,24		Heavy metal transport/detoxification protein domain containing protein.
Os06g0142350	-2,31	<i>UDP-GT, OsENOD93-1</i>	Similar to Early nodulin.
Os01g0905300	-2,41	<i>OsExo70FX14</i>	Similar to Leucine zipper protein-like.
Os02g0802500	-2,50		Similar to H <sup>+</sup> -translocating, inorganic pyrophosphatase beta-1 polypeptide
Os01g0127000	-2,98	<i>OsLPR3</i>	Multicopper oxidase, Maintenance of Pi homeostasis



**Complementary Figure: Comparative analysis of rice transcriptomic response to *P. phytofirmans*, *P. kururiensis* and *B. vietnamiensis***

Venn diagrams and histograms show the number of DEGs in response to each *Burkholderia* s.l. strains as well as the proportion of genes shared between the responses to these three strains. GO terms enrichment analysis on each pool of genes was performed as described in Chapter III and representative significantly enriched terms are related to each pool of shared or specific DEGs

- **Complementary results**

As we previously analyzed the transcriptome of rice in response to *Paraburkholderia kururiensis* M130, hereafter termed *Pk*, and *Burkholderia vietnamiensis* TVV75<sup>T</sup>, hereafter termed *Bv*, which are beneficial endophytes isolated from rice roots and also the response of rice to PsJN, we conducted a comparative analysis of the response of rice to these three strains. Moreover, the root and leave tissues were treated and harvested simultaneously with the material used in our previous study (King *et al.*, 2019). We performed comparative analysis of the transcriptomic responses to PsJN, *Pk* and *Bv* to reveal which physiological processes are commonly and specifically transcriptionally regulated in response to three plant-beneficial *Burkholderia s.l.* (Complementary Figure 1).

Respectively, 24.65% and 27.94% of all up- and down-regulated DEGs in leaves in response to the three *Burkholderia s.l.* are commonly regulated. In sharp contrast to the very low proportion of genes commonly regulated by *Pk* and *Bv*, inferior to 5%, at least 10% of DEGs are regulated commonly by PsJN and *Pk* or PsJN and *Bv*. In particular, almost 20% of up-regulated DEGs in leaves are in common between PsJN and *Bv*-induced transcriptome. Finally, among all DEGs in leaves, approximately 40% are strain-specific with half of them being specifically induced by *Pk*. The comparative analysis of root transcriptomes revealed a very different pattern. Indeed, among all DEGs, 38% and 23% are respectively up and down-regulated in response to PsJN while *Pk* and *Bv*-specific DEGs roughly represent half of this proportion for both up and down-regulated DEGs. Furthermore, in opposition to leaves, a larger proportion of PsJN-induced DEGs in roots is shared with *Bv* than with *Pk*. Indeed, on average between up and down-regulated DEGs, 17% of DEGs are shared in response to PsJN and *Bv* while only 3% are shared in response the two *Paraburkholderia* strains. Finally, a larger proportion of DEGs in common between several conditions are down-regulated. For example, 13.63% of all down-regulated DEGs are commonly responsive to the three strains while the same proportion reaches 5.32% for the up-regulated DEGs. Also, this trend is observed for all non strain-specific DEGs in roots. We then performed GO terms and KEGG pathway enrichment analysis on the different commonly and specifically-induced pool of DEGs. The enrichment analysis of this common pool of DEGs showed an enrichment of stress-related processes such as oxidative stress response. In leaves, as described earlier, more up-regulated DEGs by PsJN are commonly regulated by *Bv* than by *Pk*. Interestingly this pool of 764 DEGs show a significant enrichment in the carbon fixation pathway (Supplementary

Table 5). At the same time, specifically up-regulated DEGs in leaves by PsJN and *Bv* are respectively enriched in light reactions of photosynthesis and chloroplast-related cellular component GO terms. On the other hand, the common pool of up-regulated DEGs by PsJN and *Pk* is enriched in the oxidative phosphorylation pathway while *Pk* specifically induced the up-regulation of secondary metabolism pathways such as flavonoids and phenylpropanoids biosynthesis. Also, an opposite enrichment is observed in leaves between the response to PsJN and *Pk*. Indeed, while *Pk* induced the up-regulation of genes enriched in the process of translation, it is the down-regulated genes which are enriched in this same process in response to PsJN. In roots, the same trend is observed where *Pk* appears to induce specific transcriptional regulations compared to PsJN and *Bv*. Indeed, a larger pool of DEGs is common between the response to PsJN and *Bv*. Enrichment analysis revealed that the common down-regulated DEGs are enriched with defense and JA response processes while *Pk* specifically induced transcriptional down-regulation enriched in the response to oxidative stresses and phenylpropanoids synthesis. However, PsJN and *Pk* both induced the down-regulation of genes encoding for chitinases as demonstrated by the enrichment in chitin catabolism process among the 35 commonly down-regulated DEGs.

In conclusion, the inoculation of the three strains induced important transcriptional regulations in leaves of which more than 25% of DEGs are commonly regulated. This shows that a common response is triggered in systemic tissues regardless of the plant of isolation of the *Burkholderia s.l.* strain. However, while PsJN and *Pk* are both *Paraburkholderia* strains, the transcriptional regulations triggered in systemic tissues by PsJN and *Bv* seem to be more similar. This is the case both in terms of commonly DEGs but also in terms of physiological processes transcriptionally regulated. Indeed, PsJN and *Bv* both induced the up-regulation of genes related to photosynthesis. On the other hand, *Pk*-induced many specific transcriptional regulations particularly related to translation and several secondary metabolism pathways. This trend of rice response to PsJN being more similar to *Bv* than to *Pk* is also observed in roots although PsJN induced a large proportion of specific transcriptional regulations. This could be due to the fact that *Pk* and *Bv* were isolated from rice and not PsJN. However, a major regulation is shared in response to PsJN and *Bv* in roots, it is the down-regulation of JA-related genes. As previously described, this hormonal pathway is targeted by several beneficial microbes to enable immune suppression and tissue colonization. As all three strains are able to colonize rice roots and reach similar population sizes, it suggests that different strategies to avoid defense response are applied by PsJN, *Bv* and *Pk*. Particularly, PsJN and

*Bv*, regarding the down-regulation of JA-related in roots, could use an active process to lower plant defense while *Pk* although it is detected doesn't induce defense response in rice roots. These hypotheses could be verified by comparing the response of rice to living cells, heat-killed cells and MAMPs of each strain. Also, the role of secretion systems in the response of rice to each strain would be very interesting to investigate using bacterial mutants lacking for example their Type 3 secretion system.

# **CHAPITRE 4**

Le chapitre 4 traite d'une analyse comparative des régulations transcriptionnelles liées à l'immunité du riz en réponse à l'inoculation de souches rhizosphériques, endophytes et pathogènes de *Burkholderia* *s.l.* ainsi que des souches PGPR modèles du riz.

Parmi les souches étudiées dans ce chapitre on trouve: quatre souches de *Burkholderia* *s.l.* nouvellement isolées de racines de riz, deux étant identifiées comme des *Paraburkholderia* et les deux autres comme des *Burkholderia* *sensu stricto*; les trois souches endophytes précédemment décrites, *P. kururiensis*, *B. vietnamensis*, *P. phytofirmans*; trois souches de *Burkholderia* pathogènes, *B. glumae*, *B. plantarii* et *B. gladioli*; ainsi que trois souches PGPR modèle du riz appartenant aux espèces *Azospirillum brasiliense*, *Herbaspirillum seropedicae* et *Pseudomonas defensor*. Une analyse comparative des capacités de colonisation a permis de décrire des profils de colonisation contrastés entre les différentes souches à la fois en termes de capacité de colonisation et d'intensité de colonisation. Ensuite, des analyses transcriptionnelles, au cours du temps, de l'expression de gènes de défense, dans les racines, et de gènes liés à l'acide jasmonique, dans les feuilles, en réponse aux 10 souches ont été entreprises. Ces analyses ont permis de mettre en évidence des régulations spécifiques à certains profils de colonisation et types d'interaction en fonction du temps post-inoculation.



# **Comparative analysis of rice defense response to rhizospheric, endophytic and pathogenic *Burkholderia* s.l. strains and model PGPR**

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## **• Introduction**

In natural conditions, plant tissues interact and are colonized by a wide diversity of microorganisms having various effects on plant growth ranging from beneficial, neutral and deleterious (Turner *et al.*, 2013). Whatever the interaction, the perception of microorganisms by plants is based on the recognition of conserved molecular patterns called Microbe-Associated Molecular Patterns (MAMPs) (Jones and Dangl, 2006). The perception of MAMPs by plant receptors triggers an immune response, called MAMP-Triggered Immunity (MTI), characterized by the production of Pathogenesis-Related (PR) proteins and antimicrobial metabolites. However, in the case of beneficial interactions, this basal immune response has to be modulated to enable the establishment of the interaction.

Previous works have described various mechanisms to evade or suppress plant immunity in the context of beneficial plant-microbe interactions (Trdá *et al.*, 2015; Yu *et al.*, 2019). Also, several studies showed that the interaction with so-called Plant-Growth Promoting Microbes (PGPM) induces the down-regulation of defense in roots (Lakshmanan *et al.*, 2012; Millet *et al.*, 2010; Stringlis *et al.*, 2018). However, very few studies have analyzed the immune regulations of plants when interacting with a diversity of naturally-associated, beneficial and pathogenic strains. Indeed, the studies are usually restricted to comparative analysis of the host response to one beneficial and one pathogenic microbe (Adomas *et al.*, 2008; Giovannetti *et al.*, 2015; Guimil *et al.*, 2005; Kelly *et al.*, 2018; Vogel *et al.*, 2016).

To study the transcriptional response of plants to contrasted interactions with microorganisms, we took advantage of the rice-*Burkholderia* *sensu lato* (*s.l.*) model. Indeed, the *Burkholderia* *s.l.* bacterial genus contains both beneficial and pathogenic strains naturally associated with

rice. Interestingly, recent phylogenetic studies defined two genera within the *Burkholderia* clade separating environmental and plant-beneficial species, the *Paraburkholderia* genus, from pathogenic and opportunistic species: the *Burkholderia sensu stricto* (s.s.) genus (Estrada-de los Santos *et al.*, 2018; Sawana *et al.*, 2014). In a previous work, we described the transcriptional regulations in response to two *Burkholderia s.l.* beneficial endophytes from these 2 clades, *P. kururiensis* M130 and *B. vietnamiensis* TVV75 (Govindarajan *et al.*, 2008; Mattos *et al.*, 2008). We especially showed differences in the expression dynamics of a jasmonic acid (JA)-related co-expression network depending on the inoculated strain (King *et al.*, 2019).

In this context, we enlarged our previous study to a diversity of species isolated from rice, with two supplementary *Burkholderia* spp. and two *Paraburkholderia* spp. strains. Additionally, we also included three *Burkholderia* pathogens, *B. glumae* and *B. gladioli*, two causal agents of rice panicle blight (Nandakumar *et al.*, 2009) as well as *B. plantarii* which causes seedling blight in rice (Azegami *et al.*, 1987). To broaden our study, we also have included three beneficial bacterial models in our study, firstly *A. brasiliense* B510 and *H. seropedicae* SmR1, which have been thoroughly studied for their plant-beneficial effects, particularly on cereals (Pérez-Montaña *et al.*, 2014). The third model strain included in the analysis is *P. phytofirmans* PsJN, a model bacterial endophyte with a large host spectrum (Mitter *et al.*, 2013). Finally, we also selected the bacterial strain *Ps. defensor* WCS374 which has been described to trigger Induced Systemic Resistance (ISR) in rice through a JA-dependent signaling pathway (De Vleesschauwer *et al.*, 2008). This rhizobacteria-induced priming state of plant defenses towards pathogens has been largely studied and its molecular bases are well described in *Arabidopsis* (Pieterse *et al.*, 2014).

First, we analyzed the colonization patterns and numbered root-associated populations of the bacterial set on the rice cultivar Nipponbare, then we performed a temporal expression analysis i) on defense-related genes in colonized roots; and ii) on JA-related genes in systemic tissues, to compare rice responses to ecologically and taxonomically diverse bacteria.

## • Material & Methods

### Phylogenetic analysis

Evolutionary history of the studied strains was inferred using the Maximum Likelihood method based on the General Time Reversible mode using MEGA7 (version 7.0.21). Sequences used for phylogenetic analyses were 550 bp fragment sequences of the *recA* gene

which was proven to be a good alternative to 16S DNA sequence (Eisen, 1995), particularly for *Burkholderia* s.l. strains for whom it was demonstrated to provide a greater degree of resolution among closely related species (Payne *et al.*, 2005).

### **Plants & bacterial cultivation**

*Oryza sativa L. ssp. japonica* cv Nipponbare was used in this study. Plants were grown in sterile conditions using a hydroponic system following the same protocol described in King *et al* (2019). Plantlets grown for four days in growth chamber (16 h light; 8 h dark; 28°C; 70% humidity) were inoculated with  $10^7$  bacterial cells. All strains used in this study can be found in Table 1 and Supplementary Table 2, and their phylogenetic proximity is given in Figure 1. Isolation media for ABIP441, ABIP447, ABIP477 and ABIP659 were PCAT (Burbage and Sasser, 1982) and Norris Glucose (Ranganayaki and Mohan, 2007) both free of nitrogen sources.

### **Bacterial transformation**

All bacterial strains were transformed by electroporation with the pIN29 plasmid (Vergunst *et al.*, 2010) which comprise a chloramphenicol resistance gene as well as a DsRed-encoding gene under the control of a constitutive TAC promoter. After 24 hours of incubation on selective medium LB low salt containing chloramphenicol ( $200 \mu\text{g.mL}^{-1}$ ) at 28°C, the most fluorescent colonies were selected.

### **Rice root colonization assays**

The roots of plants grown in hydroponics and inoculated with fluorescent strains were harvested at 7 and 14 days post-inoculation (dpi). For global colonization, the roots were weighted and grinded in sterile water with a sterile ceramic bead using a FastPrep-24™ 5G at  $6 \text{ m.s}^{-1}$  for 40 seconds. The solution was then diluted and inoculated on low salt LB (Sigma-Aldrich) selective medium containing the corresponding antibiotic (Supplementary Table 1) and incubated at 28°C for 24h. Colony forming units (cfu) were then enumerated. In order to measure the size of the endophytic population, we followed the protocol described in King *et al.* (2019). The inoculated rice roots were surface-disinfected for 1 minute using a solution of 1% Chloramine T (Sigma-Aldrich) supplemented with 0.1% Tween 20. Roots were then rinsed 4 times with sterile water. Controls of disinfection were performed by plating rinsing water on TSA medium (Sigma-Aldrich) overnight. Surface-disinfected roots were then treated as described above.

### **Visualization of root-colonizing bacteria**

Fluorescent strains used in this study and their associated growth conditions can be found in Supplementary Table 1. All microscopic observations of the bacterial colonization were restricted to the primary root in order to compare the colonization patterns on roots which have been in contact with the bacterial population for the same amount of time. Primary roots were harvested at 7 and 14 dpi and mounted between slide and slips cover and directly examined under the microscope. Epifluorescence observations were performed using a Zeiss Axio-Imager Z2 microscope.

### **RNA extraction**

For the analysis of roots and leaves transcriptional profiles, both plants' tissues were harvested in triplicate at 6 hours post-inoculation (hpi), 1, 7 and 14 dpi with bacterial cells. Roots and leaves of untreated plants were collected at the same time. Each biological replicate consisted of five pooled root systems or five pooled last mature leaves harvested from a single hydroponic system. After harvest, samples were snap-frozen in liquid nitrogen and stored at -80°C. Rice roots were homogenized in liquid nitrogen using cooled mortar and pestles. Rice leaves were grinded using a TissueLyser II (Qiagen) set to 30 Hz for 30 sec. Total RNA extraction using TRI-reagent (Sigma) was performed according to manufacturer's instructions. All samples were purified using the RNA Clean & Concentrator kit (Zymo) according to manufacturer's instructions. The integrity and quality of the total RNA was confirmed by gel electrophoresis using 1% agarose gel and quantified using a Nanodrop™ One (ThermoFisher). Representative samples of each extraction were also analyzed with an Agilent 2100 Bioanalyzer®.

### **Reverse Transcription and qPCR**

cDNA was synthesized from total RNA using Oligo dT (Invitrogen) and the SuperScript III Reverse Transcriptase (Invitrogen) according to manufacturer instructions. Gene expression measurements were performed using Takyon SYBR 2X qPCR Mastermix Blue (Eurogentec). The gene EF-1 $\alpha$  was used as a reference gene to normalize expression results using the  $\Delta\Delta Ct$  method (Pfaffl, 2001) with non-inoculated samples as experimental controls. Primers used in this study can be found in Supplementary Table 2.

### **Statistical analyses**

All statistical analyses were performed using R software (version 3.5.2). For physiological measurements, Wilcoxon rank tests were performed with non-inoculated samples as reference groups using the “ggpubr” package (version 0.2). For relative abundance of bacterial

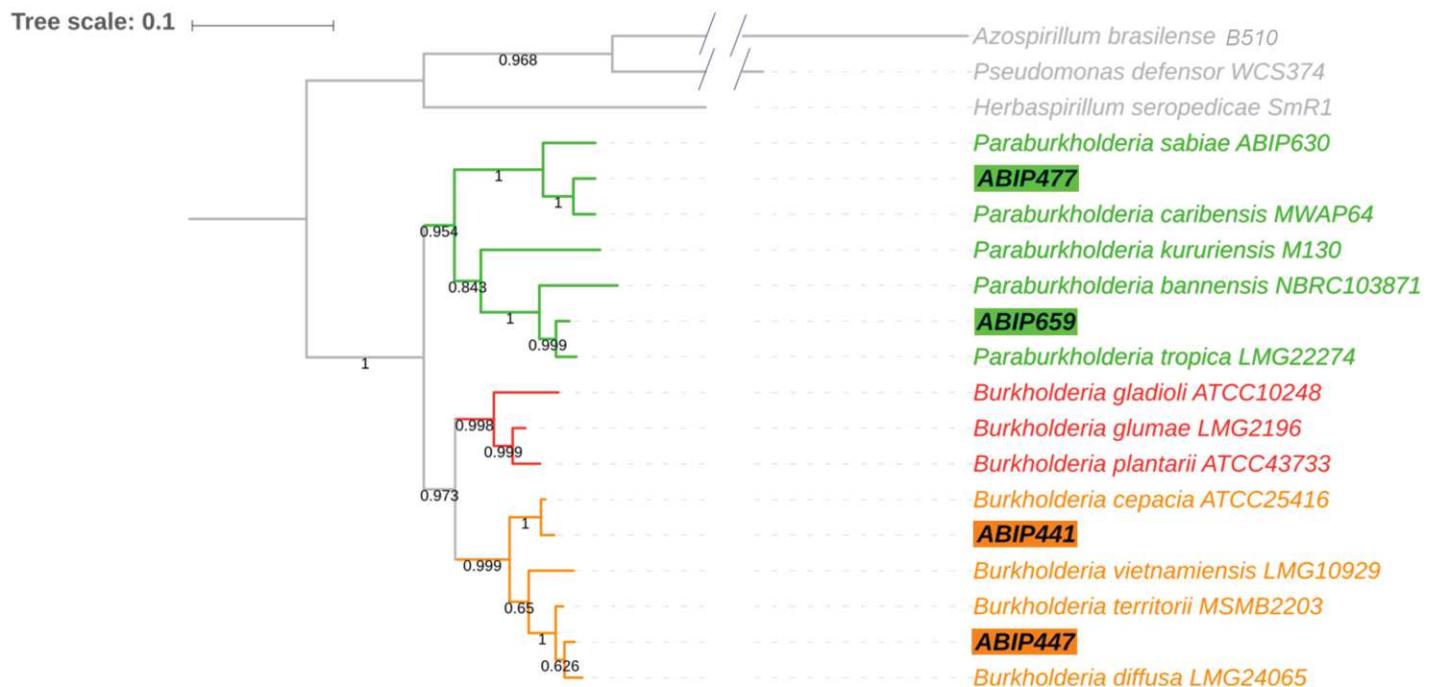
population associated to roots, statistical analysis was performed using a negative binomial zero-inflated model to estimate the differences of colonization between the inoculated strains. Significance groups were defined by Tukey's post-hoc test ( $P < 0.05$ ). Principal component analyses of gene expression data were performed on  $\log_2$  transformed mean fold change values using the "FactoMiner" (version 1.41) and "factoextra" (1.0.5) packages.

- **Results**

### **Rice root colonization analysis of rhizospheric naturally-associated *Burkholderia* s.l. strains, model PGPR and *Burkholderia* s.s. pathogens**

Our collection included four new isolates of *Burkholderia* s.l. isolated from rice in Cameroon and Vietnam. These strains originate from a large sampling of rice-associated *Burkholderia* in Africa and Asia, and were used in this study as representative of new rice-associated species. Their description is part of a separate study. Figure 1 represents a phylogenetic tree based on 550 bp-length *recA* gene fragments and shows the evolutionary relationship between the four new rice isolated strains, other strains used in this study, and type strains of closely related *Burkholderia* s.l. species (Table 1). Strains ABIP477 and ABIP659 were affiliated to the *Paraburkholderia* genus while ABIP441 and ABIP447 belong to the *Burkholderia* s.s. genus. More specifically, ABIP477 and ABIP659 are closely related to *P. caribensis* MWAP64<sup>T</sup> and *P. tropica* LMG22274<sup>T</sup>, respectively, while ABIP441 and ABIP447 are related to *B. cepacia* ATCC25416<sup>T</sup> and *B. diffusa* LMG24065<sup>T</sup>, respectively.

DsRed-tagged derivatives of the strains (Supplementary Table 1) enabled to study their colonization patterns of rice roots in sterile hydroponic conditions. Their respective colonization capacities were studied at 7 and 14 days post-inoculation (dpi) and revealed contrasted patterns between strains (Fig. 2). At 7 dpi, few cells of the ABIP441 and ABIP477 strains were shown to be colonizing rice roots (Fig. 2A, 2C). However, at 14 dpi both of these strains were observed colonizing the surface of the primary root (Fig. 2E, 2G). In contrast, the microscopic colonization analysis of the two other isolates, namely ABIP447 and ABIP659, suggest that they have greater colonization capacities. Indeed, as soon as 7 dpi, large portions of rice roots were heavily colonized by these two strains. Nonetheless, the comparison of their respective colonization patterns also suggests different capacities. Indeed, while ABIP659 is heavily colonizing the surface of root hairs (Fig. 2D, 2H), roots inoculated with DsRed-tagged ABIP447 cells exhibit apparent epidermal and root hair intracellular colonization (Fig. 2B).



**Figure 1: Phylogenetic analysis of the strains used in this study**

The newly isolated strains used in this study are in bold. Maximum Likelihood method was used to construct this tree using a 550 bp fragment sequence of *recA*. Branches are drawn to scale, representing the number of nucleotide substitution per site. Numbers indicate the results of bootstrap tests with 1000 replicates. *Azospirillum brasiliense* B510, *Herbaspirillum seropedicae* SmR1 and *Pseudomonas defensor* WCS374 were used as outgroups

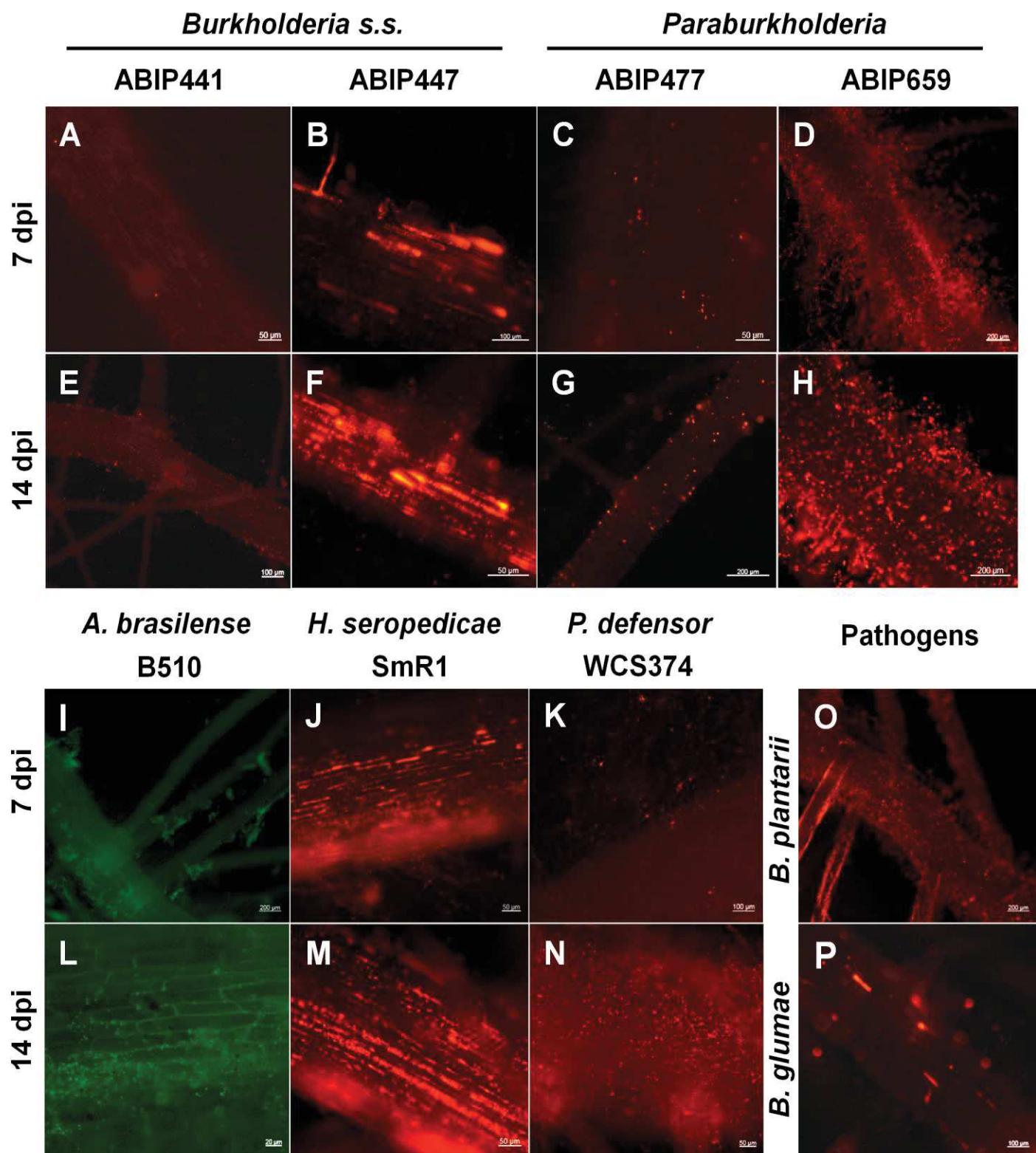
Table 1: List of strains used in this study

Strain	Species	Isolation	Ecology	Reference
ABIP441	<i>Burkholderia</i> sp. (close to <i>B. cepacia</i> )	Rice roots (Cameroun)	Rice-associated	This study
ABIP447	<i>Burkholderia</i> sp. (close to <i>B. diffusa</i> )	Rice roots (Cameroun)	Rice-associated	This study
ABIP477	<i>Paraburkholderia</i> sp. (close to <i>P. caribensis</i> )	Rice roots (Cameroun)	Rice-associated	This study
ABIP659	<i>Paraburkholderia</i> sp. (close to <i>P. tropica</i> )	Rice roots (Vietnam)	Rice-associated	This study
SmR1 <sup>T</sup>	<i>Herbaspirillum seropedicae</i>	Rice roots (Brazil)	Diazotrophic endophyte	Baldani <i>et al.</i> , 1986
B510	<i>Azospirillum brasiliense</i>	Rice roots (Japan)	Diazotrophic endophyte	Elbeltagy <i>et al.</i> , 2001
WCS374 <sup>T</sup>	<i>Pseudomonas defensor</i>	Potato rhizosphere (Netherlands)	ISR-inducer in rice	Geels and Schippers, 1983
LMG9035 <sup>T</sup>	<i>Burkholderia plantarii</i>	Rice seedling (Japan)	Rice pathogen (Seedling blight)	Azegami <i>et al.</i> , 1987
LMG10906	<i>Burkholderia glumae</i>	Rice grain (Japan)	Rice pathogen (Panicle blight)	Kurita and Tabei, 1967
LMG2216 <sup>T</sup>	<i>Burkholderia gladioli</i>	<i>Gladiolus</i> sp. (USA)	Pathogen of onions, Iris, <i>Gladiolus</i> and rice with <i>B. glumae</i>	Severini, 1913

This tendency was also observed at 14 dpi with ABIP447 apparently colonizing intercellular spaces (Fig. 2F) while ABIP659 remains restricted to the surface of root hairs (Fig. 2H).

The colonization of rice roots by the two rice endophyte model strains *Azospirillum brasiliense* B510 and *Herbaspirillum seropedicae* SmR1, was also analyzed in our growth conditions. It revealed important differences in the way they colonize rice roots. Firstly, *A. brasiliense* forms bacterial aggregates on the surface of the principal and lateral roots at 7 dpi (Fig. 2I) and subsequently occupy intercellular spaces at the surface of epidermal cells at 14 dpi (Fig. 2L). On the other hand, as soon as 7 dpi, *H. seropedicae* intensively colonizes the surface of root hairs, intercellular spaces and was also observed colonizing some epidermal cells (Fig. 2J). Finally, at 14 dpi *H. seropedicae* cells appear to accumulate in intercellular spaces as revealed by the straight lines of cells following the cell walls of epidermal cells (Fig. 2M). We also analyzed the capacity of *Pseudomonas (Ps) defensor* WCS374 to colonize rice roots. Similarly to ABIP441 and ABIP477, a relatively light colonization at 7 dpi (Fig. 2K) is followed by an important colonization of the surface of root hairs at 14 dpi (Fig. 2N). Finally, we also analyzed the colonization of rice root by two *Burkholderia* pathogens, *B. plantarii* and *B. glumae*. *B. plantarii* was shown to massively colonize rice roots and to form polysaccharidic structures along most of rice roots (Fig. 2O) while for *B. glumae*, local accumulation of cells and apparent intracellular colonization are observed (Fig. 2P). Clear differences were also observed in the phenotypic response of rice as *B. plantarii*-inoculated roots were strikingly shorter and browner while no visible effects were observed for roots inoculated with *B. glumae* (Data not shown). The global patterns of root colonization by each strain are compiled in Table 2.

We also compared the level of colonization of each studied strain excepting pathogens, by estimating the size of the bacterial population associated to roots by counting colony forming unit (cfu) (Fig. 3). We compared these results to previous ones obtained in parallel for two *Burkholderia* s.l. endophyte strains, namely *Paraburkholderia kururiensis* M130 and *Burkholderia vietnamiensis* LMG10929 (King *et al.*, 2019). For the whole root-associated population, *A. brasiliense* reaches the highest level of colonization with more than  $10^7$  cfu.g<sup>-1</sup>. The size of the populations of ABIP447 and *H. seropedicae* SmR1 both exceeded  $10^6$  cfu.g<sup>-1</sup> with only *H. seropedicae* SmR1 having a significantly lower level of colonization. ABIP659 population size, almost reaching  $10^5$  cfu.g<sup>-1</sup>, was significantly higher than ABIP477 and *Ps. defensor* ones. For ABIP441, only one biological replicate out of five enabled to measure its



**Figure 2: Comparative analysis of rice root colonization**

Epifluorescence microscopy observations of bacterial strains colonizing the roots of rice plants grown hydroponically in sterile conditions. All strains expressed DsRed proteins, excepting *A. brasiliense* B510 which expresses eGFP

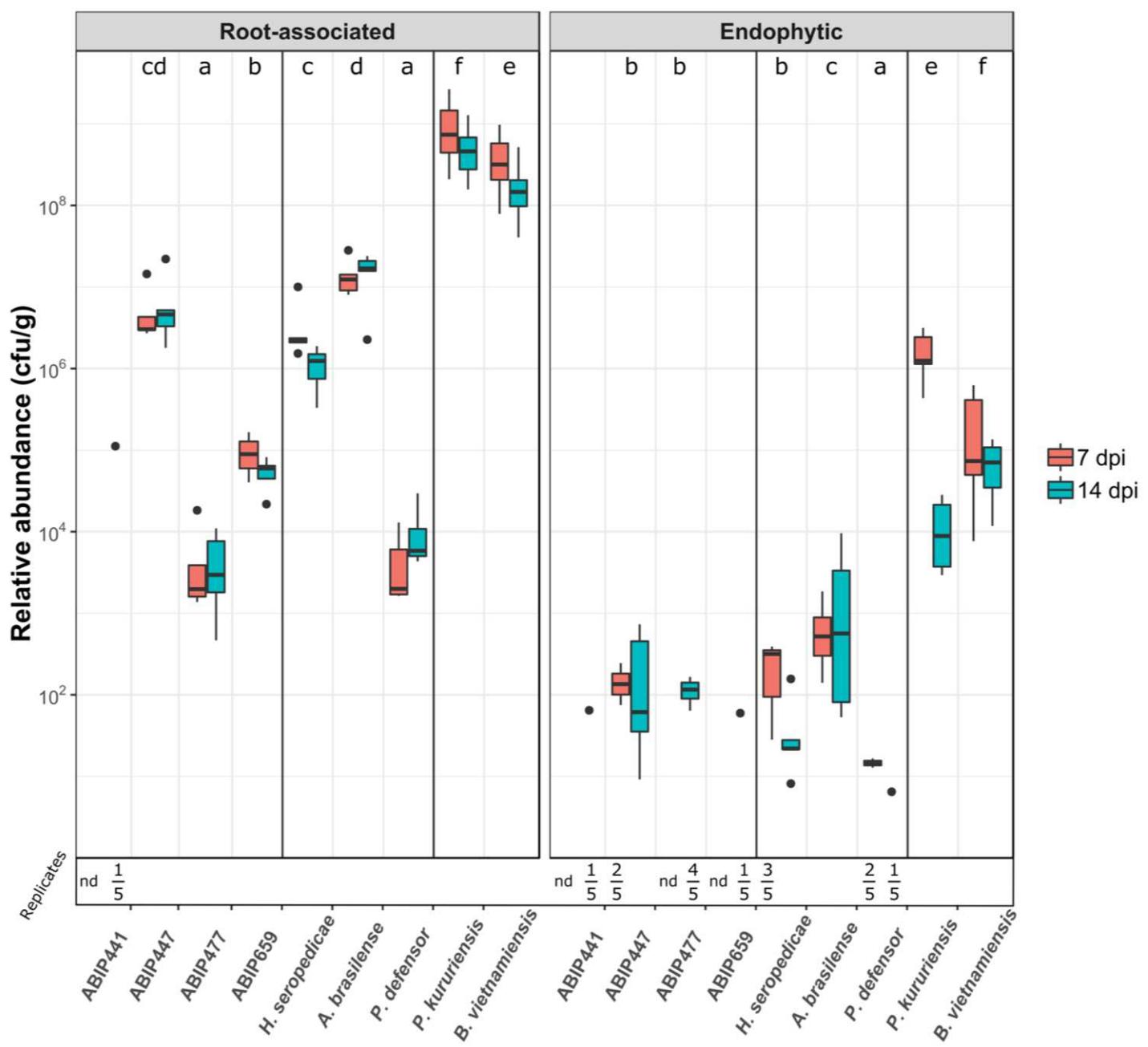
population size at  $10^5$  cfu.g $^{-1}$ . For the endophytic compartment, similarly to whole root-associated population, *A. brasiliense* reaches the highest level of colonization with a median value above  $5 \times 10^2$  cfu.g $^{-1}$  as soon as 7 dpi. At this time point, only a part of the biological replicates enabled to detect endophytic colonization for ABIP447 and *H. seropedicae* and even none for ABIP477. However, they fall in the same significance group, for the level of colonization, at 14 dpi with more than four out of five replicates showing endophytic colonization of approximately  $10^2$  cfu.g $^{-1}$ . Finally, for ABIP441, ABIP659 and *Ps. defensor*, the low number of replicates enabling to detect endophytic colonization suggests that these strains do not consistently colonize rice root endosphere. By comparing with results obtained for *P. kururiensis* and *B. vietnamiensis*, none of the tested strain reached a similar level of colonization either for whole root-associated or endophytic population.

Table 2: Rice root colonization patterns summary

Species	Strain	Root hairs	Lateral root emergence	Intercellular spaces	Epidermal cells
<i>Burkholderia</i> sp. (close to <i>B. cepacia</i> )	ABIP441		✓		
<i>Burkholderia</i> sp. (close to <i>B. diffusa</i> )	ABIP447	Whole cell	✓	✓	✓
<i>Paraburkholderia</i> sp. (close to <i>P. caribensis</i> )	ABIP477	Surface			
<i>Paraburkholderia</i> sp. (close to <i>P. tropica</i> )	ABIP659	Surface	✓		
<i>H. seropedicae</i>	SmR1	Surface	✓	✓	✓
<i>A. brasiliense</i>	B510	Surface			
<i>Ps. defensor</i>	WCS374 <sup>T</sup>	Surface	✓		
<i>P. kururiensis</i>	M130	Surface	✓	✓	
<i>B. vietnamiensis</i>	TVV75 <sup>T</sup>	Whole cell	✓	✓	✓
<i>P. phytofirmans</i>	PsJN <sup>T</sup>	Surface	✓		
<i>B. plantarii</i>	LMG9035 <sup>T</sup>	Surface		✓	
<i>B. glumae</i>	LMG10906	Surface			✓

#### Transcriptional analysis of rice local and systemic response

We analyzed the transcriptional response of rice to the four previously described isolates, three *Burkholderia* pathogens, three rice beneficial model strains and three *Burkholderia* s.l. endophytes. We monitored the expression of defense-related genes in roots and a JA-related co-expression network, described in King and collaborators (2019) as differentially regulated

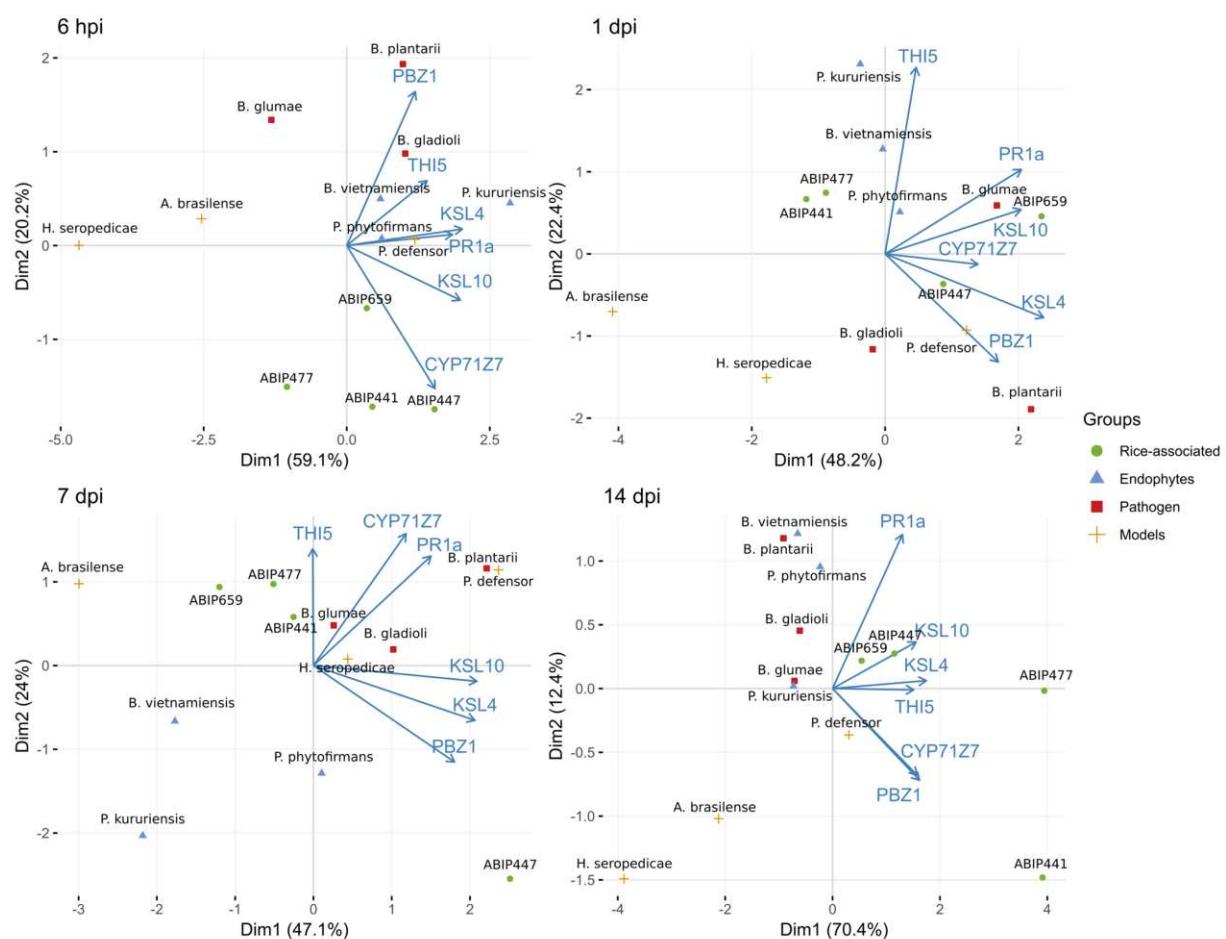
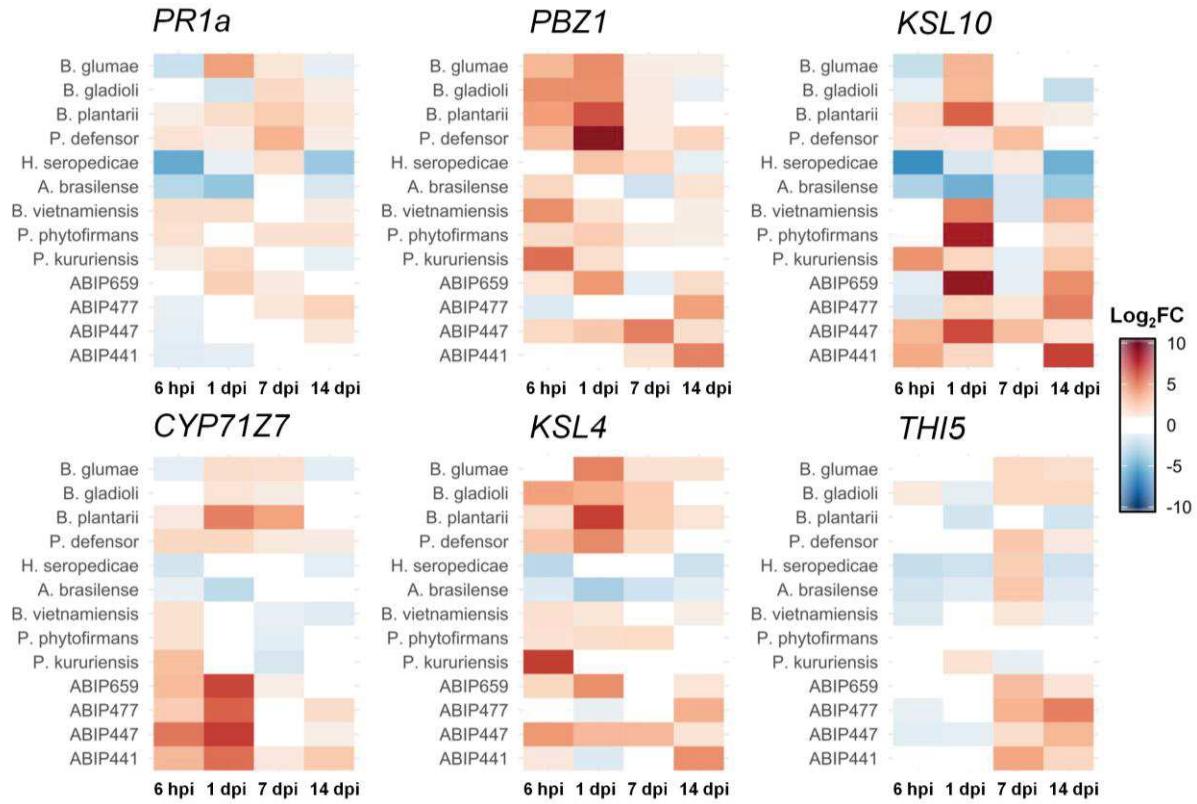


**Figure 3: Relative abundance of root-associated and endophytic inoculated strains**

Plants grown hydroponically in sterile conditions for four days were inoculated with  $10^7$  bacterial cells. Bacterial population sizes were estimated by enumerating colony forming unit (cfu) after root grinding. Endophytic population sizes were estimated by surface disinfection. Letters indicate significantly different groups ( $P < 0.05$ ) according to Tukey's post-hoc test performed on negative binomial zero-inflated model. Fractions below the graph indicate the proportion of biological replicates for which cfu were detected, "nd" indicate that none bacteria were detected. ABIP441 is closely related to *B. cepacia*, ABIP447 is closely related to *B. diffusa*, ABIP477 is closely related to *P. caribensis*, ABIP659 is closely related to *P. tropica*

in leaves of root-inoculated plants depending on the colonization process of two endophytic *Burkholderia* s.l. strains. To describe the defense response of roots towards the different tested strains, we performed expression analysis of six defense-related genes in roots at early time points, 6 hours post-inoculation (hpi) and 1 dpi as well as the same time points chosen for colonization analysis, 7 and 14 dpi. Among the six genes whose expression has been monitored throughout the establishment of the different interactions, two of them encode for Pathogenesis-Related (PR) proteins, namely *PR1a* and *PBZ1*, *THI5* encodes for a thionin, a family of defense peptides and three genes are implicated in phytoalexin synthesis, *KSL4*, *KSL10* and *CYP71Z7*. These genes have been selected as they were shown to be down-regulated in roots colonized by *H. seropedicae* and endophytic *Burkholderia* s.l. strains (Brusamarello-Santos *et al.*, 2019; King *et al.*, 2019). Expression profiles of each genes represented as heatmaps are provided in Figure 4. Among all gene expression profiles, several observations are of particular interest. Firstly, *PBZ1* is up-regulated at early time points, 6 hpi and/or 1 dpi, in response to all tested strains with the exception of ABIP441 and ABIP477. Its highest level of expression measured was measured in response to the three pathogens and *Ps. defensor* at 1 dpi. A similar trend is observed for *KSL4* which is highly up-regulated in response to these last four strains at 1 dpi. For *CYP71Z7*, the inoculation of the three *Burkholderia* s.l. endophytes and the four new rice isolates induced its up-regulation at 6 hpi while only the last four strains induced its up-regulation at 1 dpi. Finally, the expression of *THI5* is mostly up-regulated at late time points, 7 and 14 dpi. It is particularly up-regulated by the four newly isolated strains, *B. glumae* and *B. gladioli* while at 14 dpi, *B. plantarii*, *H. seropedicae*, *A. brasiliense* and *B. vietnamensis* induced its down-regulation. Also, very interestingly, *H. seropedicae* and *A. brasiliense* were the only strains to induce an almost constant down-regulation of *PR1a*, *KSL10*, *CYP71Z7* and *KSL4*. Oppositely, the inoculation of *P. defensor* induced the up-regulated of all tested genes at almost every time points.

We performed a principal component analysis (PCA) at each time point post inoculation to cluster the profiles of expression of defense genes in roots depending on the inoculated strain (Fig. 5). The early response (6 hpi) shows a clear separation of the response towards pathogens and the four isolates ABIP441, ABIP447, ABIP477 and ABIP659. Also, the response to the three *Burkholderia* s.l. endophytes (*P. kururiensis*, *P. phytofirmans* and *B. vietnamensis*) and *Ps. defensor* is in clear opposition with the one to *A. brasiliense* and *H. seropedicae* regarding the horizontal axis of the PCA plot. At 1 dpi, a similar trend is

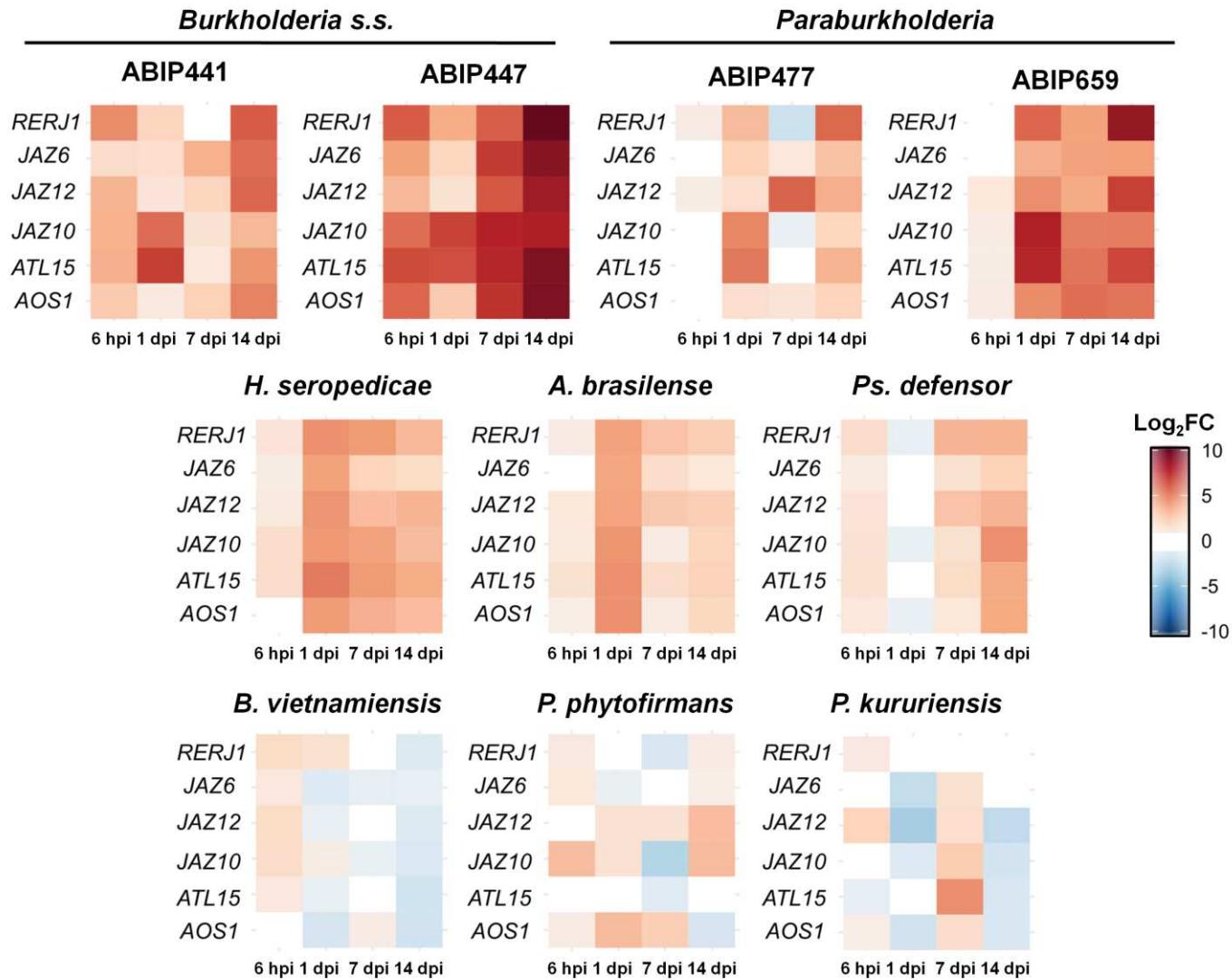


**Figure 5: Principal component analysis of the expression of six defense-related genes expressed in inoculated roots**

PCA analysis was performed on mean  $\log_2$ FoldChange expression of six genes compared to a non-inoculated control at each time point. ABIP441 is closely related to *B. cepacia*, ABIP447 is closely related to *B. diffusa*, ABIP477 is closely related to *P. caribensis*, ABIP659 is closely related to *P. tropica*

observed with the two latest strains inducing a down-regulation of all tested genes excepting *PBZ1* while the response to the other endophytes, ABIP477 and ABIP441 groups together. Also, the response to ABIP659 and *B. glumae* appeared to be similar while *B. plantarii* induced important up-regulation of most tested genes resulting in its separated position on the PCA plot. This is also the case for the roots inoculated with ABIP447 at 7 dpi, which clearly separate from the response to the other tested ABIP isolates. Noteworthy, at this same time point, the responses to the three *Burkholderia s.l.* endophytes groups together. At the latest time point, 14 dpi, the response to pathogens and endophytes groups together. Particularly, the response to *B. plantarii* and *B. vietnamiensis* are very similar, the same observation can be done for *B. glumae* and *P. kururiensis*. Also, the horizontal axis strikingly separates the response to the two model endophytes from the one induced by ABIP477 and ABIP411. Finally, ABIP659 and ABIP447 induced transcriptional regulations close to the “endophyte-pathogen” group.

The analysis of the systemic response at the leaf level excluded pathogen-inoculated plants and focused on the transcriptional regulations of JA-related genes in response to root-associated and beneficial strains. The comparison of the expression dynamics throughout time enabled to compare the regulations induced by each strain and revealed several types of patterns (Fig. 6). Firstly, half of the inoculated strains induced consistent up-regulation of almost all tested genes in leaves, namely ABIP441, ABIP447, ABIP659, *H. seropedicae* and *A. brasiliense*. Among these, strain-specific differences in the level of induction of the genes can be observed. Indeed, ABIP447 and ABIP659 induced the highest level of up-regulation, particularly for ABIP447 which induced constant increase of the expression throughout time reaching a maximum at 14 dpi. For *H. seropedicae* and *A. brasiliense* the highest level of expression of the tested genes is reached at 1 dpi and decreases afterwards especially for *A. brasiliense*. *Ps. defensor* and ABIP477 induced punctual down-regulation of several genes, respectively at 1 and 7 dpi for *RERJ1* and *JAZ10* for both and additionally *AOS1* for *Ps. defensor*. However, at 14 dpi, ABIP477 and *Ps. defensor* induce the highest global level of induction of the genes observed at the tested time points. In the case of the response to *B. vietnamiensis*, *P. phytofirmans* and *P. kururiensis*, the modulation of the expression between up and down-regulation is more variable throughout time. Indeed, the majority of the genes are commonly down-regulated at 1 and 14 dpi for *B. vietnamiensis* and *P. kururiensis* while for *P. phytofirmans* it is at 7 dpi that the highest proportion of down-regulated genes is observed. However, for this latest strain, the global trend shows an up-regulation of JA-related genes in leaves.



**Figure 6: Temporal analysis of JA-related genes expression in leaves in response to root-inoculation**

The values of mean log<sub>2</sub>FoldChange of six genes compared to a non-inoculated control over 2 weeks post-inoculation were represented as heatmaps. Expression level is color-coded with red indicating up-regulation and blue indicating down-regulation. Only values greater than 0.5 or lower than -0.5 were represented. ABIP441 is closely related to *B. cepacia*, ABIP447 is closely related to *B. diffusa*, ABIP477 is closely related to *P. caribensis*, ABIP659 is closely related to *P. tropica*

- **Discussion**

The objectives of this study were to describe the responses of rice to a diversity of bacterial strains having contrasted interaction characteristics with their host, both in terms of root colonization and ecology. To do so, we selected 10 strains of *Burkholderia* s.l.: Three pathogens, *B. glumae* and *B. gladioli*, and *B. plantarii*; three well-described endophytes, *P. kururiensis* M130, *B. vietnamensis* TVV75 and *P. phytofirmans* PsJN; four *Burkholderia* s.l. strains isolated from rice roots, ABIP441, ABIP447, ABIP477 and ABIP659. Finally, we also added three model PGPR strains, namely *Azospirillum brasilense* B510, *Herbaspirillum seropedicae* SmR1 and *Pseudomonas defensor* WCS374.

Among the four rice isolated strains described in this study, ABIP441 and ABIP447 were identified as *Burkholderia* s.s. while ABIP477 and ABIP659 were identified as *Paraburkholderia* species (Figure 1). Two of these strains, ABIP441 and ABIP659, were closely related to species already found to be associated with cereals, respectively *B. cepacia* (Zhang and Xie, 2007) and *P. tropica* (Reis *et al.*, 2004). On the other hand, ABIP447 is closely related to the *B. diffusa* type strain which was isolated from the sputum of cystic fibrosis patients (Vanlaere *et al.*, 2008). ABIP477 is thought to be part of the *P. caribensis* species originally isolated from soil (Achouak *et al.*, 1999) but strains of this species were also isolated from root nodules of tropical legumes (Vandamme *et al.*, 2002). To our knowledge, this is the first time that strains identified as *B. diffusa* and *P. caribensis* are isolated from field-grown rice plants.

Among the four new *Burkholderia* s.l. rice isolates, ABIP441 was scarcely observed on the surface of rice roots at 7 dpi, however at 14 dpi large areas of the primary root and lateral root emergences were colonized by fluorescent bacteria. Even so, no consistent counting of root-associated cells was measurable although clear colonization was observed by microscopy. This could be explained by the small number of detectable cells which are possibly loosely associated to roots and have been lost during the root grinding protocol. In the same way, at 7 dpi, ABIP477 was only found at the surface of roots as isolated cells. However, at 14 dpi, we regularly observed localized aggregates of several bacterial cells showing a higher colonization capacity than ABIP441. Also, at this same time point, we consistently detected endophytic colonization of rice roots by ABIP477. For ABIP659, which is closely related to the *P. tropica* type strain, massive and consistent colonization of root hairs was observed.

Similarly, in tomato, important root hair colonization was also observed for *P. tropica* Mto-293 (Bernabeu *et al.*, 2015). Finally, the most invasive pattern of colonization was observed for ABIP447, indeed patches of epidermic cells and intracellularly colonized root hairs were regularly detected. This typical pattern of colonization was also observed for *B. vietnamiensis* (King *et al.*, 2019) which is also a species found to be a human opportunist (Mahenthiralingam *et al.*, 2008). Rice roots colonization analyses, for the four isolates, suggest two categories of colonization, with ABIP441, close to *B. cepacia*, and ABIP477, close to *P. caribensis*, being light colonizers in comparison to ABIP447, close to *B. diffusa* and ABIP659, close to *P. tropica*, which can be considered as heavy colonizers. Therefore, the phylogenetic background of the strains does not seem to drive the intensity of the colonization. However, for ABIP447-inoculated roots, the frequent observation of epidermic cells apparently colonized from the inside and the consistent counting of colonies from disinfected roots suggest an endophytic colonization which is not the case for ABIP659. Also, similarly to what was observed for *P. kururiensis* M130 and *B. vietnamiensis* LMG10929 (King *et al.*, 2019), the *Burkholderia* s.s. strain, here ABIP447, show a more invasive colonization pattern of rice roots.

We also confirmed a high level of root colonization for *A. brasiliense* B510 and *H. seropedicae* SmR1 as well as consistent endophytic colonization for both strains at 14 dpi. However, by comparing with previous results obtained for *P. kururiensis* M130 and *B. vietnamiensis* LMG10929 (King *et al.*, 2019), they appear to reach a significantly lower population size for both whole-root associated and endophytic populations. For *A. brasiliense* B510, the reduced number of bacterial aggregates between 14 and 7 dpi is coherent with the fact that the colonization of this bacteria is non-persistent (F. Wisniewski-Dyé, personal communication). Additionally, the analysis of *Ps. defensor* root colonization by microscopy revealed that this strain accumulates particularly in the root hair zone. However, at the whole root level the relative abundance of the bacterial cells is apparently low, similarly to ABIP477. Also, the low proportion of replicates detecting potential endophytic colonization suggests that its colonization is restricted to the first cell layer of roots. Finally, root colonization analysis of two *Burkholderia* rice pathogens revealed that *B. plantarii* colonizes much more efficiently rice roots than *B. glumae*. This is consistent with the fact that *B. glumae* is inducing symptoms in the mature aboveground parts of rice plants contrarily to *B. plantarii* which causes severe damages on rice seedlings (Solis *et al.*, 2006). However, the observation of apparent intracellular colonization of epidermal root cells by *B. glumae* could

be a way for this bacterium to maintain itself and possibly systemically colonize the plants before exerting its pathogenic effects in the floral parts.

Having described strain-specific root colonization patterns, we further wanted to analyze the root immune response induced in inoculated plants. However, as plant immunity has been mostly studied in leaves, few immune markers adapted to roots response are widely used. We monitored the expression of two *PR* genes, including *PBZ1* which has been described as an adapted gene to monitor both leaves and roots defense response to fungal colonization (Marcel *et al.*, 2010). Also, phytoalexins are antimicrobial compounds produced by plants during pathogen infection (Ahuja *et al.*, 2012) which are also released in the rhizosphere and could alter surrounding soil microbiota (Kato-Noguchi *et al.*, 2008). Finally, we also selected a gene encoding for a thionin, an antimicrobial peptide (García-Olmedo *et al.*, 1998) which was down-regulated in rice roots during the interaction with *H. seropedicae* (Brusamarello-Santos *et al.*, 2012).

The use of principal component analysis on the expression pattern of these 6 genes enabled to compare the root immune response of the different tested strains throughout time. Interestingly, the early regulations regroup together all four newly described rice isolates, the three pathogens and the non-*Burkholderia* endophytes. This suggests that monitoring the expression of the selected genes during the initial perception of bacterial cells is sufficient to separate the response to contrasted ecological interactions. However, following regulations tends to separate these initial pools of immune responses and seems to be driven by the colonization characteristics of the different strains. For example, at 1 dpi, the responses to the *Burkholderia s.l.* endophytes and the low-colonizer isolates, ABIP477 and ABIP441, are grouped together. We could hypothesize that the response to endophytes is similar to a response induced by limited microbial colonization of root surface. This is in clear opposition to the high level of induction of *PBZ1* at this time point caused by pathogens and *Ps. defensor*. However, the response to *Burkholderia s.l.* endophytes is more clearly separated from the other tested strains, particularly for *P. kururiensis* and *B. vietnamensis*. Indeed, these two strains induced the down-regulation of two genes implicated in phytoalexin synthesis, *CYP71Z7* and *KSL10*. Finally, at 14 dpi, surprisingly the PCA axis projection regroups the response induced by the *Burkholderia s.l.* endophytes and pathogens revealing that an invasive colonization induces a comparable regulation of the selected genes. This trend is also observed for the responses to ABIP447 and ABIP659 while the low colonizers,

ABIP477 and ABIP441, induce the highest induction at 14 dpi of *PBZ1*, *CYP71Z7*, *KSL10* and *KSL4* among all tested strains.

At 6 hpi, 1 and 14 dpi, the interaction with *A. brasiliense* and *H. seropedicae* induced the down-regulation of most of the genes selected, *PR1a*, *KSL4* and *KSL10* most particularly (Figure 4). This is in opposition with previous studies analyzing the response to *A. brasiliense* which usually showed an increase in *PR* genes expression in roots (Drogue *et al.*, 2014; Fukami *et al.*, 2017). Oppositely, *H. seropedicae* was shown to down-regulate the expression of *PR10* in roots (Brusamarello-Santos *et al.*, 2012, 2019). On the other hand, *P. kururiensis* and *B. vietnamiensis* inoculations mostly induced the up-regulation of these same genes at the previously given time points. However, as we monitored their expression at several time points, we show expression modulations throughout time while previous analyses were performed at single time points. This provides evidence that endophytic colonization is not always accompanied by a constant down-regulation of defense-related genes in roots and that it is most likely dynamic oscillating from up to down-regulation of defenses following inoculation. Besides this, the expression of *PBZ1* is up-regulated by all inoculated strains at least for one of the time points tested. Therefore, this gene can be considered as a local sensor of root colonization whatever the type of interaction although the inoculation of pathogens and *Ps. defensor* caused the higher level of induction at early stages of the interactions.

Similarly, to roots, the transcriptional regulations of JA-related genes in leaves can be associated to particular colonization capacities. This was previously described for the two *Burkholderia s.l.* rice endophytes, *P. kururiensis* and *B. vietnamiensis* (King *et al.*, 2019). Interestingly, the response of rice to the studied rice isolates and non-*Burkholderia* endophytes exhibit different patterns. Indeed, the highest level of induction of the monitored JA-related genes is measured in response to the four rice isolates, particularly ABIP447 and ABIP659. Regarding the fact that these strains were both described as the most invasive root colonizers, the level of induction of these genes could be proposed as a proxy for the intensity of root colonization. It is also in accordance with the relative low level of induction observed for ABIP441 and ABIP477. This proposition is consistent with the involvement of JA in the systemic regulation to biotic and mechanical stresses such as wounding (Reymond and Farmer, 1998; Schilmiller and Howe, 2005). Similarly to what was observed for root colonization, the genus of the *Burkholderia s.l.* strain does not seem to drive rice transcriptional response but rather the colonization intensity. Indeed, we described, for both *Burkholderia s.l.* genera, a low colonizing and a heavy colonizing strain, respectively

ABIP441 and ABIP447 for *Burkholderia* s.s.; also respectively, ABIP477 and ABIP659 for *Paraburkholderia*. Additionally, when comparing the response triggered by endophytes, inner tissues colonization appears to cause lower level of induction and apparent modulation of this systemic JA response over time. Furthermore, by comparing the response to the different endophytes, similarly to what is observed in roots, the response to *Burkholderia* s.l. endophytes suggests a specific capacity to modulate this JA systemic response.

In conclusion, the combination of quantitative and qualitative colonization analysis enabled to describe contrasted types of interactions between rice and naturally-associated *Burkholderia* s.l. strains. Furthermore, we compared the local and systemic transcriptional regulations of rice in response to a diversity of root-inoculated bacterial strains. This led us to describe transcriptional states associated to particular colonization processes and ecological interactions in the rice-*Burkholderia* s.l. model and their specificities towards the model rice PGPRs *Azospirillum brasiliense* and *Herbaspirillum seropedicae*.

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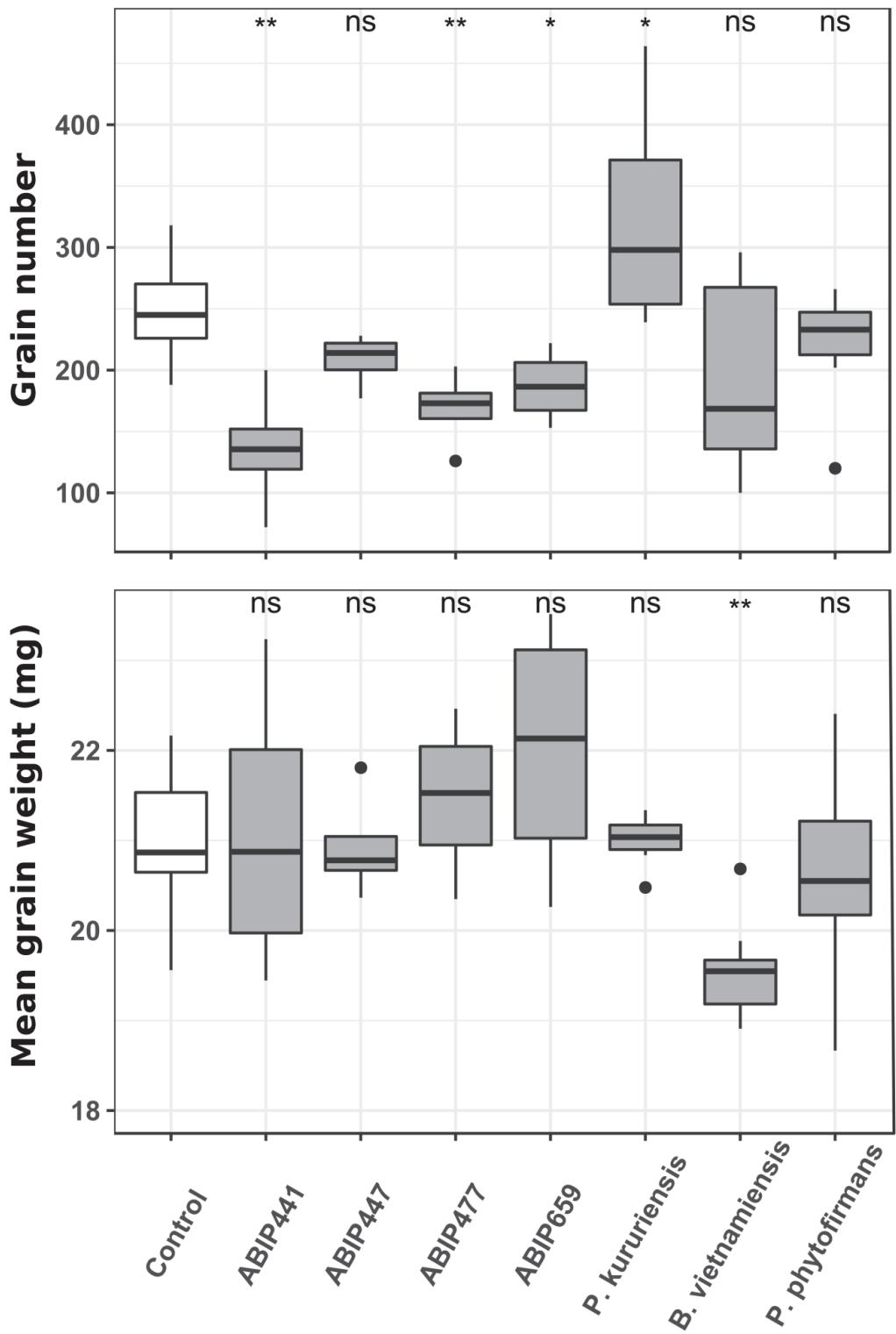
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Supplementary Table 1: List and growth conditions of fluorescent strains

Species	Wild-type strain	Transformation	Antibiotic	Fluorescence	Reference
<i>Burkholderia</i> sp.	ABIP441	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Burkholderia</i> sp.	ABIP447	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Paraburkholderia</i> sp.	ABIP477	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Paraburkholderia</i> sp.	ABIP659	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Burkholderia plantarii</i>	LMG9035 <sup>T</sup>	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Burkholderia glumae</i>	LMG10906	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Burkholderia gladioli</i>	LMG2216 <sup>T</sup>	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study
<i>Azospirillum brasiliense</i>	B510	pMP2444	Gentamycin (10 µg/mL)	eGFP	Valette <i>et al.</i> , 2019
<i>Herbaspirillum seropedicae</i>	SmR1 <sup>T</sup>	Tn5-mediated	Streptomycin (20 µg/mL) Kanamycin (500 µg/mL)	DsRed	Monteiro <i>et al.</i> , 2008
<i>Pseudomonas defensor</i>	WCS374 <sup>T</sup>	pIN29	Chloramphenicol (200 µg/mL)	DsRed	This study

Supplementary Table 2: Primers used in this study

RAP ID	Name	Function	Sequences		Tm	Reference
Os06g0513862	<i>OsTHIS</i>	Thionin family protein	Fwd	GCTGAGGAATCGGAGGTAGTTG	60°C	Brusamarello-Santos <i>et al.</i> , 2012
			Rev	TTCTCATGGTGCTGCACACA		
Os07g0129200	<i>OsPRIa</i>	Pathogenesis related	Fwd	TCGTATGCTATGCTACGTGTT	60°C	Spence <i>et al.</i> , 2014
			Rev	CACTAAGCAAATACGGCTGACA		
Os12g0555500	<i>OsPBZ1</i>	Probenazole-inducible protein	Fwd	GCGTTGAGTCCGTGAGAGT	58°C	Vergne <i>et al.</i> , 2010
			Rev	TCACCCATTGATGAAGCAAA		
Os04g0179700	<i>OsKSL4</i>	Momilactone A synthesis	Fwd	TCCATCTTAATTGGCTGAGAAA	58°C	Miyamoto <i>et al.</i> , 2015
			Rev	GGCTATAAGACCAGCAGCTA		
Os12g0491800	<i>OsKSL10</i>	Oryzalexin D-E synthesis	Fwd	CTGACAGCGGCAATACC	58°C	Toyomasu <i>et al.</i> , 2008
			Rev	CCTTAGGTGTGCGGTAGG		
Os02g0570700	<i>OsCyp71Z7</i>	Phytocassane synthesis	Fwd	CCCTATGTATTCTCAAGTTGCTTG	58°C	Miyamoto <i>et al.</i> , 2015
			Rev	TTGTTTTATGGAAGTGGTAACAA		
Os04g0301500	<i>OsRERJ1</i>	Responsive to JA	Fwd	ATGGAGTCATGCCTTTGGC	60°C	King <i>et al.</i> , 2019
			Rev	TGGGGTGTGCAAAAATGAC		
Os03g0402800	<i>OsJAZ6</i>	Jasmonate-ZIM-domain protein 6	Fwd	TTGATGACTTCCCAGCTGAGAA	60°C	Lu <i>et al.</i> , 2016
			Rev	GCGCTGTGGAGGAACCTTG		
Os03g0181100	<i>OsJAZ10</i>	Jasmonate-ZIM-domain protein 10	Fwd	TCTTCCCACCCGTCAAAT	60°C	Zong <i>et al.</i> , 2016
			Rev	CCTCGCTGGTGCTTGCT		
Os10g0392400	<i>OsJAZ12</i>	Jasmonate-ZIM-domain protein 12	Fwd	TGCCGATCGCGAGGAA	60°C	Hou <i>et al.</i> , 2009
			Rev	GGTCGCTCGTTGCGTGAT		
Os01g0597600	<i>OsATL15</i>	Amino acid Transporter-Like 15	Fwd	CATGTCATTATCGGCTCGTT	60°C	King <i>et al.</i> , 2019
			Rev	CCATCGGCCTTGTAGATCTGA		
Os03g0767000	<i>OsaOS1</i>	Allene Oxide Synthase (JA biosynthetic pathway)	Fwd	TTCCTCCGATACGACTCCTTC	60°C	King <i>et al.</i> , 2019
			Rev	AGGTGACGGTGACAGATGAG		
<i>Burkholderia spp.</i>	<i>recA</i>	Bacterial Recombinase A	Fwd	AGGACGATTATGGAAGAWAGC	56°C	Spilker <i>et al.</i> , 2009
			Rev	GACGCACYGAYGMRTAGAACTT		



**Complementary Figure: Impact of the root inoculation of *Burkholderia* s.l. strains on rice grain yield and mean grain weight**

Rice plantlets of the Nipponbare cultivar grown in the greenhouse were inoculated one week after planting with  $10^8$  bacterial cells and harvested four months after inoculation. Results corresponds to the number of grains produced by 8 pots containing 4 plants each for the conditions inoculated with *P. kururiensis*, *B. vietnamiensis* and *P. phytofirmans* and mock-inoculated controls. Results corresponds to the number of grains produced by 4 pots containing 4 plants each for the conditions inoculated with ABIP441, ABIP447, ABIP477 and ABIP659. ABIP441 is closely related to *B. cepacia*, ABIP447 is closely related to *B. diffusa*, ABIP477 is closely related to *P. caribensis*, ABIP659 is closely related to *P. tropica*. Asterisks represents significant differences compared to mock-inoculated controls according to a non-parametric Wilcoxon test (\*,  $P < 0.05$ , \*\*,  $P < 0.01$ )

- **Complementary Results**

We further wanted to describe long-term impact of the *Burkholderia s.l.* strains studied in the chapter 4 on rice yield. The complementary figure shows the number of grain produced depending on the strain inoculated as well as the mean grain weight of each inoculation condition. Among all tested strains, only *P. kururiensis* M130 induced a significant yield increase. Furthermore, ABIP477, *B. vietnamiensis* and *P. phytofirmans* didn't induce significant differences compared to control plants according to statistical tests. However, slight decrease of rice yield is observable regarding the median values. Finally, ABIP441, ABIP447 and ABIP659 induced a significant diminution of rice yield. Regarding mean grain weight, *B. vietnamiensis* induced a significant diminution of the median value. Although, no significant differences were observed compared to non-inoculated plants, ABIP477 and ABIP659 appear to induce a slight increase of the median grain weight.

# **CHAPITRE 5**

## **DISCUSSION GENERALE**

L'objectif de ces travaux de thèse était de décrire les réponses physiologiques et transcriptionnelles du riz au cours de l'interaction avec des bactéries appartenant aux genres *Burkholderia* et *Paraburkholderia* entretenant avec le riz des types d'interactions variées, allant du mutualisme à la pathogénie. L'hypothèse initiale de ce projet était que la séparation phylogénétique entre les genres *Burkholderia s.s.* et *Paraburkholderia*, marquée par des lignées aux écologies contrastées, serait associée à des stratégies de colonisation différentes et induirait par conséquent des réponses différentielles d'une plante hôte commune. Ces travaux ont ainsi consisté à analyser les régulations transcriptionnelles du riz au cours de l'interaction avec différentes souches de *Burkholderia s.l.*. Une première approche a consisté dans la production et l'analyse du transcriptome du riz au cours de l'interaction avec *Paraburkholderia kururiensis* M130 et *Burkholderia vietnamiensis* TVV75, deux souches endophytes bénéfiques du riz. Un second volet du projet a consisté à analyser les réponses transcriptionnelle et physiologique du riz à une souche endophage à large spectre, *Paraburkholderia phytofirmans* PsJN, dont l'interaction avec le riz n'avait jamais été décrite. Enfin, en s'appuyant sur les résultats précédemment obtenus, une analyse comparative des réponses immunitaires du riz face à une plus large diversité de souches, bénéfiques à pathogènes, ne se limitant pas aux genres *Paraburkholderia* et *Burkholderia s.s.* a été entreprise, dans le but d'identifier des profils de régulation communs ou spécifiques à différents modes de colonisation et/ou types d'interactions.

#### Importance et diversité des interactions riz-*Burkholderia s.l.*

Parmi la diversité des microorganismes associés au riz, le choix et l'intérêt du genre *Burkholderia s.l.* se justifie par plusieurs facteurs. Premièrement, d'un point de vue fondamental, comme précédemment décrit, cela tient compte de la diversité des types d'interactions avec le riz que l'on retrouve au sein du genre ainsi que son histoire évolutive particulière. Ensuite c'est un genre de bétaprotéobactéries, or l'analyse métagénomique de référence du riz a démontré un enrichissement de taxons de protéobactéries au niveau du rhizoplan et de l'endosphère (Edwards *et al.*, 2015). De plus, la classe des betaprotéobactéries est la plus représentée parmi les colonisateurs précoces et tardifs au cours du cycle de vie du riz (Edwards *et al.*, 2018). Enfin, une méta-analyse des microbiotes de plusieurs plantes modèles -notamment le riz, *Arabidopsis*, le maïs et la vigne- a pu mettre en évidence un enrichissement particulier au riz de certaines lignées de Burkholderiales (Hacquard *et al.*, 2015). Ainsi, le genre *Burkholderia* fait partie de ces classes très abondantes au sein du microbiote du riz. Ajouté au potentiel agronomique de nombreuses espèces au sein de ce

genre, son étude est d'un intérêt tout particulier. Ces arguments sont également appuyés par des travaux menés au sein de l'équipe de recherche ABIP en parallèle de ces travaux. Par des analyses d'amplicons 16S obtenus à partir d'ADN extrait de la rhizosphère ou de racines du riz, la forte abondance relative et la récurrence de séquences affiliées au genre *Burkholderia s.l.* dans plusieurs pays et conditions de culture ont pu être démontrées (Données non publiées).

#### Des modes de colonisation du riz contrastés chez les burkholderias

Les travaux de description de l'interaction entre le riz et différentes souches de *Burkholderia s.l.* ont pu mettre en évidence des capacités de colonisation contrastées. En effet, malgré le fait que la colonisation racinaire par des bactéries soit un phénomène complexe influencé par de nombreux facteurs (Benizri *et al.*, 2001), un certain nombre d'observations récurrentes ont pu mettre en évidence des profils de colonisation particuliers aux différentes souches. Parmi ceux-ci, on retrouve la formation de microcolonies à la surface des racines, la colonisation de la zone des poils racinaires, l'accumulation dans les espaces intercellulaires de l'épiderme racinaire ainsi que l'accumulation au niveau des émergences de racines latérales (Chapitre 2, 3 et 4). Egalement, un certain nombre des événements de colonisation qui ont pu être observés ont été proposés comme des voies d'entrées et/ou des caractéristiques d'endophytes (Complant *et al.*, 2010; Gaiero *et al.*, 2013). Parmi celles-ci on trouve la colonisation intracellulaire de cellules épidermiques, de cellules bordant les émergences de racines latérales ainsi que la colonisation des poils racinaires (Chapitre 2 et 4). Néanmoins, la majorité de ces analyses se sont limitées à la racine principale du riz, or d'importantes variations de la colonisation par des microorganismes bénéfiques en fonction du type racinaire ont pu être précédemment mises en évidence (Gutjahr *et al.*, 2009). Par ailleurs, par des approches de désinfection de surface, une estimation de la quantité de cellules colonisant les tissus internes a pu être effectuée et suggère la qualité d'endophage de certaines souches de *Burkholderia s.l.* isolées au sein du laboratoire (Chapitre 4). Egalement, les résultats de cette analyse ont démontré une capacité accrue -dans nos conditions expérimentales- des deux souches étudiées au cours du chapitre 2, *P. kururiensis* et *B. vietnamiensis*, à coloniser l'endosphère du riz par rapport à d'autres souches modèles des symbioses associatives telles que *Azospirillum brasilense* et *Herbaspirillum seropedicae*. (Chapitre 4). Ces résultats sont néanmoins à tempérer au vu du système expérimental utilisé. En effet, des analyses de colonisation effectuées en condition stérile ne sont pas représentatives de la colonisation par ces mêmes souches dans un sol. En effet, les résultats obtenus pour *P. phytofirmans* (Chapitre 3) démontrent bien cette tendance à avoir un niveau de colonisation dans un sol qui soit plus faible que dans des conditions

stériles. Cette même tendance a été observée lors d'analyses microscopiques de colonisation racinaire de plantes cultivées en sol (Données non présentées).

Les perspectives de ces travaux sont multiples. Tout d'abord, les analyses microscopiques de colonisation racinaire pourraient être approfondies pour les souches nouvellement décrites dans le chapitre 4. Particulièrement, en élargissant les observations à d'autres types racinaires que la racine principale. Egalement, en procédant à des analyses en microscopie confocale afin, entre autre, d'être capable de démontrer le caractère endophyte dans nos conditions des souches étudiées dans le chapitre 4. Ensuite, la description des capacités de colonisation pourrait être étendue à une plus large diversité de souches. Les mesures quantitatives par comptage de colonies pourraient être remplacées par des approches indépendantes de la culture *in vitro* de microorganismes. Des mesures plus robustes pourraient être obtenues par qPCR voir digital droplet PCR (ddPCR), cette dernière approche permettant une quantification absolue du nombre de cellules colonisant les tissus de plantes inoculées. De plus cette approche permettrait de mesurer la capacité de colonisation de tissus où la densité de la population endophyte sera faible, par exemple les parties aériennes. Une autre perspective de ces travaux serait de décrire de manière plus poussée les évènements de colonisation intracellulaire observée pour *B. vietnamensis* TVV75 et la souche ABIP447 (Chapitre 2 et 4). Tout d'abord, des analyses par microscopie électronique permettraient de démontrer une réelle colonisation intracellulaire. Dans le même objectif, des approches de microscopie confocale couplées à des marquages des membranes ou des compartiments cellulaires pourraient aussi le mettre en évidence. Par ailleurs, l'un des questionnements découlant de ces observations concerne la viabilité de la cellule colonisée ainsi que le lien potentiel entre ces évènements de colonisation cellulaire et celle des tissus internes.

L'analyse des réponses phénotypiques a montré des variations qui rendent difficile la démonstration d'effets bénéfiques des bactéries étudiées en termes de croissance ou de rendement du riz. Des facteurs cruciaux pour mettre en évidence de tels phénotypes en condition contrôlée sont le substrat utilisé, la concentration de l'inoculum ainsi que le génotype de la plante hôte. Néanmoins, pour le génotype Nipponbare, l'inoculation par *P. kururiensis* a induit une augmentation du rendement tandis que l'inoculation par *B. vietnamensis* a significativement diminué le poids moyen des grains (Chapitre 4, Figure complémentaire). Par ailleurs, les souches montrant des capacités de colonisation réduites en condition hydroponique ont globalement eu des effets négatifs sur le rendement. Egalement une tendance à l'augmentation de la masse moyenne des grains induite par les deux souches

de *Paraburkholderia*, ABIP477 et ABIP659, a été mesurée. Ces résultats exposent les limites de la définition de microorganismes bénéfiques pour les plantes. En effet, les phénotypes de promotion de croissance sont extrêmement dépendants des conditions environnementales et de la combinaison hôte-souche. Pour approfondir cette partie des travaux, une optimisation des conditions de culture et d'inoculation est nécessaire afin d'être capable de mettre en évidence des effets clairs et répliquables de promotion de croissance. Egalement un élargissement de la diversité de génotypes de plantes étudiées pourrait amener des résultats intéressants en termes de variations de réponses.

#### L'analyse transcriptomique de la réponse du riz à des endophytes révèle les régulations physiologiques induites au cours de ce type d'interaction

Afin de décrire les régulations physiologiques induites par le riz au cours de l'interaction avec différentes souches endophytes de *Burkholderia s.l.*, nous avons procédé à une analyse transcriptomique par RNA-Seq. Dans cet objectif et comme explicité dans le chapitre 2, les plants de riz ont été cultivés en condition hydroponique stérile et récoltés à des stades où l'inoculation n'avait pas induit de changements phénotypiques observables. Le choix de la méthode RNA-Seq présente des avantages certains, il permet de décrire la régulation de gènes aux fonctions inconnues pouvant ainsi alimenter des bases de données ou des méta-analyses qui pourront orienter les investigations du rôle d'un gène vers des processus physiologiques voire des fonctions. Egalement, elle permet potentiellement d'identifier des nouveaux transcrits ainsi que des régulations post-transcriptionnelles potentiellement impliquées dans la réponse à la condition étudiée. C'est cependant une méthode sensible qui implique la mise en place de conditions contrôlées afin de limiter la variabilité biologique en réponse à un traitement. Cette approche nécessite également une certaine quantité de matériel biologique initial relativement importante pour des protocoles de séquençage classiques. Ce dernier facteur a impliqué pour l'étude du transcriptome racinaire un prélèvement et une extraction du système racinaire entier. Or, comme décrit précédemment la colonisation des tissus par des rhizobactéries n'est pas homogène, ainsi on peut s'attendre à des réponses contrastées en fonction des types racinaires, des zones racinaires voire même des types cellulaires. Néanmoins, l'analyse de la réponse du système racinaire entier permet de décrire les régulations les plus importantes à l'échelle de l'organe. D'ailleurs, les régulations transcriptionnelles mesurées par cette approche ont pu être confirmées par RT-qPCR au cours d'une expérience indépendante (Chapitre 2). Enfin, c'est également une approche exploratoire

qui n'est qu'un point de départ dans l'analyse de systèmes biologiques complexes comme l'interaction entre plantes et microorganismes.

L'analyse globale des transcriptomes produits a permis de mettre en évidence une tendance contre-intuitive. En effet, l'inoculation de rhizobactéries a induit la régulation transcriptionnelle à 7 jours post-inoculation d'un plus grand nombre de gènes dans les parties aériennes que dans les racines. Comme discuté dans le chapitre 2, ceci peut-être dû au temps auquel la réponse est étudiée, cependant l'unique étude publiée dans laquelle le transcriptome des racines et des feuilles de riz au cours d'une symbiose associative sont étudiés montrent cette même tendance (Xie *et al.*, 2017). Ceci semble être particulier à l'interaction avec des microorganismes bénéfiques, en effet la colonisation des racines par un pathogène a mis en évidence plus de 2000 gènes différentiellement exprimé dans les racines (Marcel *et al.*, 2010). Cette tendance est encore plus marquée en réponse aux endophytes isolées du riz par rapport à la réponse à *P. phytofirmans* (Chapitre 3, Figure complémentaire). Ceci pourrait s'expliquer par un effet d'adaptation à la plante hôte, les deux souches naturellement associées au riz induisant une réponse moins marquée dans les racines de leur plante d'isolement. On pourrait émettre l'hypothèse qu'au cours d'un processus de co-évolution, les bactéries pourraient être sélectionnées pour une capacité à atténuer la réponse de leur hôte ou encore à mieux s'en camoufler. Cette proposition est appuyée par la large proportion de gènes spécifiquement induit ou réprimés en réponse à l'interaction avec *P. phytofirmans*, endophage modèle mais non isolé du riz. A l'inverse, la réponse transcriptomique des feuilles à l'inoculation aux trois souches étudiées montre que plus de 50% des gènes différentiellement exprimés le sont communément à au moins 2 souches. Ces observations permettent de supposer que la majorité des régulations physiologiques induites par l'interaction avec les trois souches étudiées semblent avoir lieu dans les feuilles. Ou encore que l'impact au niveau racinaire, qu'il soit positif ou non, se répercute aussi au niveau foliaire ne serait ce que pour adapter développement, métabolisme et photosynthèse par exemple.

L'analyse fonctionnelle des transcriptomes produits a mis en évidence la régulation transcriptionnelle de plusieurs processus physiologiques précédemment décrits comme étant impactés et impliqués dans la réponse aux rhizobactéries (Chapitre 1, Tableau 4). Parmi eux, on trouve des voies de signalisation hormonale liées à l'auxine, aux cytokinines, à l'éthylène, au jasmonate, à l'acide abscissique et à l'acide salicylique. Globalement, les régulations de ces voies hormonales n'ont pas montré de différences majeures en réponse aux trois souches étudiées, en particulier certains gènes impliqués dans la synthèse et la signalisation des

cytokinines et de l'éthylène sont communément régulés. On trouve cependant une exception, la régulation de l'acide jasmonique (JA). En effet, en plus du signal systémique décrit dans le chapitre 2 (King et al, 2019), une diminution de l'expression de gènes impliqués dans la signalisation et la synthèse du JA est observée dans les racines en réponse à *B. vietnamensis* et *P. phytofirmans*. Ainsi, l'une des perspectives majeures de ces travaux serait de décrire le rôle de cette phytohormone dans l'établissement des interactions riz-*Burkholderia s.l.*, voire de l'étendre à la perception des endophytes. Des approches fonctionnelles qui décriraient la colonisation, la réponse immunitaire dans des lignées de riz déficientes en JA ou mutées dans la voie de signalisation, en particulier les gènes *JAZ*, sont d'ores et déjà envisagées. Egalement, des approches fonctionnelles du côté bactérien, pourraient explorer le rôle de certains mécanismes bactériens, comme le système de sécrétion de type 3 et les effecteurs associés ou le quorum sensing, sur la régulation des défenses de la plante. De plus, un certains nombre de processus physiologiques pourraient être plus particulièrement investigués (i) l'impact développemental des interactions étudiées, le rôle d'un certain nombre de gènes impliqués dans la formation des racines latérales ou encore la synthèse des parois cellulaires des plantes comme décrit dans la réponse du riz à *Bacillus subtilis* RR4 et la réponse de la canne à sucre à *Burkholderia* Q208 (Paungfoo-Lonhienne et al., 2016; Rekha et al., 2018)(ii) le rôle et la régulation de la synthèse de l'éthylène et sa signalisation comme discuté dans le chapitre 3 au vu du nombre d'études mettant en évidence des régulations transcriptionnelles liées à cette phytohormone (Brusamarello-Santos et al., 2019; Camilios-Neto et al., 2014; Paungfoo-Lonhienne et al., 2016; Rekha et al., 2018; Thomas et al., 2019; Xie et al., 2017) (iii) l'homéostasie du fer dans l'interaction riz-*Burkholderia*, des gènes impliqués dans la synthèse ou l'homéostasie de phytosidérophores et de transport du fer. En effet, les gènes les plus fortement régulés dans les racines sont liés à ces processus de la même manière qu'en réponse à l'inoculation par *Herbaspirillum seropedicae* SmR1(Brusamarello-Santos et al., 2019).

#### Vers la définition d'états transcriptionnels associés à des types d'interaction

Les résultats obtenus à la suite des analyses transcriptomiques par RNA-Seq ont permis d'identifier des gènes d'intérêt dont l'expression a été mesurée, par RT-qPCR, de façon cinétique au cours de l'établissement de l'interaction entre le riz et une diversité de souches (chapitre 4). A notre connaissance, c'est la première étude décrivant les régulations transcriptionnelles, au cours du temps, en réponse à une diversité de souches naturellement associées à l'hôte étudié, en incluant des pathogènes ainsi que des souches PGPR modèles.

L'analyse des régulations systémiques du réseau JA identifié dans le chapitre 2 devait nous permettre de tester l'hypothèse proposée précédemment de « signature » dans la réponse du riz à des souches de *Burkholderia* s.s. et *Paraburkholderia*. Les résultats obtenus invalident cette hypothèse et suggèrent plutôt que le facteur principal est l'intensité et le type de colonisation mis en place par les bactéries inoculées. Cette tendance était déjà suggérée par le fait que les transcriptomes de riz inoculés avec *P. phytofirmans* étaient plus proche de la réponse à *B. vietnamensis* qu'à *P. kururiensis* (Chapitre 3, Résultats complémentaires). Néanmoins, comme décrit dans le chapitre 4, les profils d'expression de ce réseau de gènes permettent d'établir des groupes de réponse en fonction de l'intensité des profils de colonisation - modéré versus intensif - et du type de colonisation - colonisation du rhizoplan versus endosphère -. Une perspective directe de ces travaux serait de décrire les régulations de ce réseau au cours de l'interaction avec des pathogènes ainsi qu'à une plus large diversité de souches, afin de valider ces gènes comme marqueurs de type de colonisation (et potentiellement de l'interaction). D'autre part, les analyses transcriptomiques couplées à des recherches bibliographiques nous ont permis de sélectionner des gènes régulés dans les racines au cours de l'interaction avec des rhizobactéries bénéfiques. En effet, il existe peu de marqueurs de défense racinaire, en particulier chez le riz. De même que pour les régulations dans les parties aériennes, les analyses temporelles d'expression des gènes sélectionnés couplées aux descriptions des capacités de colonisation ont permis de regrouper des profils de réponse en fonction de l'intensité de colonisation ainsi que du type d'interaction.

L'un des objectifs annoncés de ces travaux était d'identifier des gènes marqueurs de certains types d'interaction ou encore de la réponse à des phylums bactériens en particulier. Cependant, les analyses transcriptomiques ainsi que les cinétiques d'expression qui en ont découlées remettent en cause cette vision. En effet, peu de différences marquées ou opposées ont pu être mises en évidence, ainsi au lieu de tenter d'identifier des gènes marqueurs, il semble désormais plus judicieux de tenter d'identifier des « états transcriptionnels ». C'est en effet la mesure de l'expression de plusieurs gènes voire du transcriptome, et ce au cours du temps, qui pourrait permettre de décrire plus clairement les régulations transcriptionnelles et physiologiques induites par les plantes en réponse à des conditions environnementales changeantes. Avec l'émergence des approches de biologie des systèmes permettant de définir des réseaux de co-expression voir de régulations de gènes, la définition, à l'échelle du génome, d'états transcriptionnels associés à des conditions environnementales est de plus en plus envisageable. Ainsi, une des perspectives de ces travaux pourrait viser à décrire les

réseaux de gènes impliqués dans les interactions riz-*Burkholderia* par des approches d'inférence basées sur des cinétiques de réponse transcriptomique. Ce type d'approche a déjà été appliqué chez les plantes pour décrire les régulations impliquées dans la nutrition, le développement, l'immunité ou encore la signalisation hormonale (Dong *et al.*, 2015; Gaudinier *et al.*, 2018; Hickman *et al.*, 2017; Lavarenne *et al.*, 2019; Varala *et al.*, 2018) et permettrait de décrire les régulations transcriptionnelles globales au cours de l'interaction avec des bactéries bénéfiques.

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Dans les conditions naturelles, les plantes interagissent avec une grande diversité de microorganismes, entretenant avec elles des types d’interactions variées allant du mutualisme à la pathogénie. Quelque soit le type d’interaction mis en place, les plantes sont capables de reconnaître des motifs moléculaires microbiens conservés qui induisent l’activation d’une réponse immunitaire dite « non-hôte ». Cette réponse immunitaire basale a largement été étudiée dans le cas des interactions avec des microorganismes mutualistes et pathogènes. Cependant, dans le cas des « symbioses associatives » avec des rhizobactéries ou des bactéries endophytes bénéfiques, regroupées sous le terme de Plant Growth-Promoting Rhizobacteria (PGPR), les réponses immunitaires et physiologiques des plantes ont très peu été décrites. Dans ce contexte, ce projet de thèse a eu pour objectif de décrire les régulations transcriptionnelles de la monocotylédone modèle, le riz, en réponse à l’interaction avec des bactéries bénéfiques -rhizosphériques ou endophytes- et pathogènes du genre *Burkholderia* *sensu lato* (*s.l.*). Ce genre ubiquiste de bêta-protéobactéries a la particularité d’avoir été subdivisé en deux genres aux écologies distinctes : le genre *Paraburkholderia* regroupant des espèces environnementales et bénéfiques des plantes, et le genre *Burkholderia* *sensu stricto* (*s.s.*) qui regroupe des espèces opportunistes et pathogènes de l’homme ainsi que des pathogènes de plantes, mais aussi des espèces phytobénéfiques (comme *B. vietnamiensis*). L’analyse par RNA-Seq de la réponse transcriptomique du riz à trois souches endophytes, *Paraburkholderia kururiensis* M130, *Burkholderia vietnamiensis* TVV75 et *Paraburkholderia phytofirmans* PsJN, ont permis de mettre en évidence des régulations physiologiques contrastées en fonction de la souche inoculée. De plus, des analyses comparatives de la colonisation des tissus racinaires par ces souches ont pu associer certaines de ces régulations à différents modes de colonisation. Enfin, l’expression de gènes impliqués dans la réponse immunitaire des plantes, identifiés par l’analyse fonctionnelle des transcriptomes, a été mesurée au cours de cinétiques d’interaction avec une plus large diversité de souches. Pour cela, dix souches de *Burkholderia* *s.l.*, comprenant trois souches pathogènes, ainsi que trois souches PGPR modèles du riz des genres *Azospirillum*, *Herbaspirillum* et *Pseudomonas* ont été choisies. Cette dernière approche a permis de mettre en évidence des régulations transcriptionnelles associées à des types de colonisation, rhizosphérique et endophytique, ou d’interactions, bénéfiques et délétères. Ces travaux s’intègrent dans la caractérisation des bases moléculaires de la réponse des plantes aux microorganismes bénéfiques qui représentent un potentiel important pour le développement de solutions agronomiques durables favorisant la nutrition et la résistance des plantes aux maladies.

Mot-clés : Riz, *Burkholderia*, Endophage, Rhizobactéries, Transcriptomique, PGPR

In natural conditions, plants interact with a large diversity of microorganisms maintaining with them various types of interaction ranging from mutualism to pathogenesis. Whatever the type of interaction established, the plants are able to recognize conserved microbial molecular motifs which trigger a so-called “non-host” immune response when perceived. This basal immune response has been extensively studied in the case of interactions with mutualistic and pathogenic microorganisms. However, in the case of “associative symbiosis” with beneficial rhizobacteria or bacterial endophytes, grouped under the term Plant Growth-Promoting Rhizobacteria (PGPR), the immune and physiological responses of plants have been scarcely described. In this context, this thesis project aimed at describing the transcriptional regulations of the model monocotyledonous rice, in response to the interaction with beneficial -rhizospheric or endophytic- and pathogenic bacteria of the genus *Burkholderia* *sensu lato* (*s.l.*). This ubiquitous genus of beta-proteobacteria has the particularity of having been subdivided into two genera with distinct ecologies: the genus *Paraburkholderia*, which groups together environmental and plant-associated species, and the genus *Burkholderia* *sensu stricto* (*s.s.*), which groups together human opportunistic and pathogenic species but also phytobeneficial species such as *B. vietnamiensis*. RNA-Seq analysis of the transcriptomic response of rice to three endophytic strains, *Paraburkholderia kururiensis* M130, *Burkholderia vietnamiensis* TVV75 and *Paraburkholderia phytofirmans* PsJN, revealed contrasting physiological regulations depending on the inoculated strain; in addition, comparative analyses of root tissue colonization by these strains enabled to associate some of these regulations with different colonization patterns. Finally, the expression of genes involved in the immune response of plants, identified by the functional analysis of transcriptomes, was measured during interaction kinetics with a wider diversity of strains. For this, ten strains of *Burkholderia* *s.l.*, comprising three pathogenic strains, as well as three model rice PGPR strains of the genera *Azospirillum*, *Herbaspirillum* and *Pseudomonas* were selected. This last approach highlighted transcriptional regulations associated with the types of colonization, rhizospheric and endophytic, or interaction, beneficial and deleterious. This work is part of the characterization of the molecular bases of plants’ response to beneficial microorganisms which represent an important potential for the development of sustainable agronomic solutions favoring nutrition and plant resistance to diseases.

Keywords : Rice, *Burkholderia*, Endophage, Rhizobactéries, Transcriptomics, PGPR