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Metabolic and hormonal characterisation of plant growth-promoting effect of *Paraburkholderia phytofirmans* PsJN in *Arabidopsis thaliana*

Tesis entregada a la Pontificia Universidad Católica de Chile en cumplimiento parcial de los requisitos para optar al Grado de Doctor en Ciencias Biológicas con mención en Genética Molecular y Microbiología

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There's magic everywhere
Look at me standing
Here on my own again
Up straight in the sunshine

No need to run and hide
It's a wonderful, wonderful life
No need to laugh and cry
It's a wonderful, wonderful life

Wonderful life
Colin Vearncombe

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GENERAL INDEX

RESUMEN

Paraburkholderia phytofirmans PsJN es una rizobacteria promotora del crecimiento de plantas (*Plant Growth-Promoting Rhizobacterium*; PGPR), capaz de colonizar la rizósfera, rizoplano y endósfera de diferentes plantas, estableciendo una relación mutualista con éstas. Se ha descrito que la cepa PsJN mejora el crecimiento de varias plantas, generando en éstas efectos como: mayor número de raíces laterales y pelos radiculares, aumento en el peso fresco, y aceleramiento del ciclo de vida.

Se han propuesto una serie de mecanismos para explicar la forma en la cual esta PGPR es capaz de mejorar el crecimiento de las plantas, los cuales han sido atribuidos a ciertos genes cuyas funciones putativas corresponden a enzimas de detoxificación, sistemas de secreción y modulación hormonal, entre otras. En este sentido, el rol de PsJN en la regulación hormonal es unos de los mecanismos más estudiados. Lo anterior, debido a su capacidad de sintetizar y degradar auxina, y expresar la enzima ACC desaminasa, la cual cataliza la degradación de 1-aminociclopropano-carboxilato, el precursor inmediato de etileno.

Además, se ha propuesto que PsJN puede proteger a las plantas de condiciones de estrés biótico y abiótico, a través de la activación de resistencia sistémica inducida (ISR) y tolerancia sistémica inducida (IST). Estas respuestas han sido asociadas con cambios transcripcionales en genes de rutas hormonales de la planta tales como, jasmonato, etileno, ácido salicílico y ácido abscisíco, permitiendo que ésta responda rápidamente mediante adaptaciones metabólicas y fisiológicas a condiciones de estrés.

Debido a su complejidad, el crecimiento y desarrollo de la planta son el resultado de un sistema fino de regulación que incorpora la interacción de señales internas y ambientales tales

como luz, temperatura, nutrientes, agua, fitohormonas, metabolitos, e interacciones bióticas, entre otras. Sin embargo, poco se sabe sobre cómo esta PGPR es percibida por las plantas, y cómo esta información es procesada e integrada en la regulación del crecimiento y desarrollo de estas.

En este contexto, esta tesis tuvo como objetivo realizar la caracterización metabólica y hormonal de la planta tras los efectos fenotípicos que ejerce PsJN en la promoción del crecimiento de tejido aéreo vegetativo, utilizando como modelo a *Arabidopsis thaliana*. Para este propósito, se estudió si los efectos producidos por PsJN en la planta están relacionados con la disponibilidad de carbono, para determinar su posible rol en la regulación del crecimiento a través del metabolismo primario. Además, se analizó el metabolismo secundario y rutas hormonales en la planta, para determinar la contribución de PsJN en la promoción de crecimiento vegetativo de tejido aéreo en Arabidopsis.

Mediante el análisis fenotípico del desarrollo vegetativo de tejido aéreo, se logró establecer que PsJN tiene un mayor efecto en crecimiento de la roseta cuando las plantas son sometidas a un régimen de baja disponibilidad de carbono, utilizando fotoperiodo de día corto. También, se observó que PsJN acelera el proceso de emergencia de hojas verdaderas, sin afectar la tasa de germinación de las semillas. Por otra parte, se determinó que el aumento del tamaño de la roseta en respuesta a PsJN está dado principalmente por una mayor expansión celular y elongación de las hojas. Asimismo, a través del análisis molecular de rosetas de Arabidopsis en el mismo estadío de desarrollo vegetativo, de plantas crecidas en presencia y ausencia de PsJN, se observó una acumulación diferenciada de metabolitos secundarios y transcritos relacionados a rutas hormonales de la planta, los que están asociados al crecimiento celular, desarrollo de las hojas y el *priming*.

Este estudio corresponde al primer reporte que describe algunos de los mecanismos moleculares involucrados en la promoción del crecimiento aéreo vegetativo de Arabidopsis gatillado por PsJN. Además, los hallazgos de este estudio muestran que PsJN induce rutas hormonales asociadas a *priming*, las que estarían relacionadas con la capacidad de activar ISR e IST frente a condiciones de estrés biótico y abiótico en Arabidopsis.

ABSTRACT

Paraburkholderia phytofirmans PsJN is a plant growth-promoting rhizobacterium (PGPR) able to colonise rhizosphere, rhizoplane and endosphere of different plants, establishing mutualistic relations with them. This rhizobacterium enhances plant growth, where inoculated plants exhibit changes such as, higher number of lateral roots and root hairs, increase of fresh weight, and the acceleration of their whole lifecycle.

Several mechanisms have been proposed to explain how this PGPR can improve plant's growth, which has been attributed to some of its genetic traits with putative functions such as, detoxification enzymes, secretion and delivery systems, phytohormonal modulation, among others. The role of PsJN in phytohormonal modulation is one of the most studied mechanisms. Due to its capacity to synthesise and degrade auxin, and to express the enzyme ACC deaminase, which catalyses the degradation of 1-aminocyclopropane-carboxylate, the immediate precursor of ethylene.

Besides, it has been reported that PsJN can protect plants from biotic and abiotic stress conditions, by activating induced systemic resistance (ISR) and induced systemic tolerance (IST). These responses have been associated with changes in gene expression of hormones such as jasmonate, ethylene, salicylic acid and abscisic acid, allowing the plant to respond faster to stress conditions by conducting metabolic and physiological adaptations.

Due to its complexity, plant growth and development are the outcomes of a fine-tunning regulatory system that incorporates the interaction of internal and environmental cues such as, light, temperature, nutrients, water, hormones, metabolites, biotic interactions, among others.

Little is known about how the plant senses this PGPR and processes it as a signal to be integrated into the whole orchestration of plant growth and development.

Therefore, this study aimed to characterise the metabolic and hormonal changes underlying the phenotypic effects of vegetative growth-promotion of aerial tissue driven by PsJN using *Arabidopsis thaliana* as a plant model. For this purpose, it was determined if PsJN effects were dependent on carbon availability, to dissect its possible role in the regulation of the primary metabolism to promote growth. Additionally, secondary metabolism and phytohormones were also analysed for deciphering PsJN's contribution in the vegetative aerial growth-promotion phenotype exhibited in Arabidopsis.

Through the phenotypical analysis of vegetative development of aerial tissues, it was determined that PsJN aerial growth-promotion is stronger when plants are submitted to low carbon availability in short-day photoperiod. Likewise, it was observed that PsJN accelerates the emergence of true leaves, without affecting the germination rate. Cell expansion and leaf elongation were found to be the main factor related to rosette growth. Furthermore, by analysing Arabidopsis rosettes in the same vegetative developmental stage, from plants grown with and without PsJN's inoculation it was found that aerial tissues exhibited changes in the accumulation of secondary metabolites, as well as in transcripts accumulation related to hormonal pathways, and cell division, expansion and differentiation. These changes can be associated with cell growth, leaf development, and priming.

Overall, this study is the first report which describes some of the Arabidopsis' molecular mechanisms involved in the growth promotion of the aerial vegetative tissues of Arabidopsis triggered by PsJN. Likewise, it shows how PsJN activates hormonal pathways as a priming

response, which might be associated with the display of ISR and IST when plants face biotic and abiotic stressors, respectively.

INTRODUCTION

During millions of years, prokaryotes and eukaryotes have co-existed on Earth (Hughes & Sperandio, 2008). The interaction between kingdoms has evolved into chemical signalling that allows organisms to communicate and discern between beneficial and detrimental relationships (Badri et al., 2009). Plants, among other eukaryotes, are colonised by microorganisms that conform the plant microbiome. Bacteria, as a part of this microbiome, have a spectrum of different effects on plants (Hughes & Sperandio, 2008; Quiza et al., 2015). In the past decades, a selected group of bacteria has been described to have positive outcomes in plant growth (Compant et al., 2010; Dimkpa et al., 2009; Glick, 2012) and are classified as plant growth-promoting rhizobacteria (PGPR). Nonetheless, the mechanisms by which these effects are mediated are not fully understood.

Paraburkholderia phytofirmans PsJN: lifestyle and colonization pathway.

PGPR are a group of bacteria that colonise the rhizosphere as well as roots surfaces and establish mutualistic relations with plants (Bresson et al., 2013; Vacheron et al., 2013). The proposed mechanisms explaining how PGPR can enhance the plant's growth, are classified as direct or indirect (Glick, 2012). Direct mechanisms are those involved in hormone level modulation, and those facilitating nutrient uptake such as nitrogen fixation, phosphate solubilisation, and iron sequestering by siderophore biosynthesis. Indirect mechanisms are those related to bacterial functions as biocontrol, to protect plants of pathogen-attack, and abiotic stress (Mitter et al., 2013a) (FIGURE 1A). For instance, niche competition with pathogens through the synthesis of antimicrobial molecules, production of chemical compounds that may

influence plant growth (e.g., volatile organic compounds), and the activation of induced systemic resistance (ISR) and tolerance (IST), which are enhanced defensive responses to a wide range of biotic and abiotic stressors, respectively (Glick, 2012; Hardoim et al., 2015; Poupin et al., 2013; Santoyo et al., 2016; Vacheron et al., 2013; Pieterse et al., 2014). It has been proposed that these last responses are mainly triggered by priming, which is defined as "the sensibilisation of the whole plant for enhanced defence, which is characterised by faster and stronger activation of cellular defences upon pathogen invasion" (Pieterse et al., 2014). The strain *P. phytofirmans* PsJN (PsJN hereinafter) is an aerobic, rod-shaped, non-sporulating, and motile Gram-negative β-proteobacterium used as a PGPR model. This bacterium was first isolated from surface-sterilised onion roots as an endophyte (Frommel et al., 1991), meaning that lives inside plant tissues (endosphere) without causing harm to its host (Hardoim et al., 2015). The endophytic colonisation of PsJN follows different stages. First, the bacterium moves towards the root system in response to chemical signals from roots exudates (Kost et al., 2013). Then, it attaches to root surfaces, and rhizoplane populations grow, being bacteria-to-bacteria communication (quorum sensing; QS) a crucial factor for this step (Zúñiga et al., 2013). Once PsJN has achieved rhizoplane colonisation, it can get access into the plant tissues by employing different routes, such as tissue wounds, root cracks, germinating radicles, among others (Weilharter et al., 2011). Using a PsJN GFP-labelled strain, Poupin et al. (2013) showed that one probable colonisation pathway in *A. thaliana* plants (Arabidopsis hereinafter) is through cracks at the lateral root emergence zone. In *Vitis vinifera* L. PsJN was shown to colonise sequentially rhizoplane and endosphere where it can get to the xylem vessels, the pathway used for migrating to distal parts of the plant as pedicels, inflorescences and young berries (Compant et al., 2008; Compant et al., 2005) (FIGURE 1B).

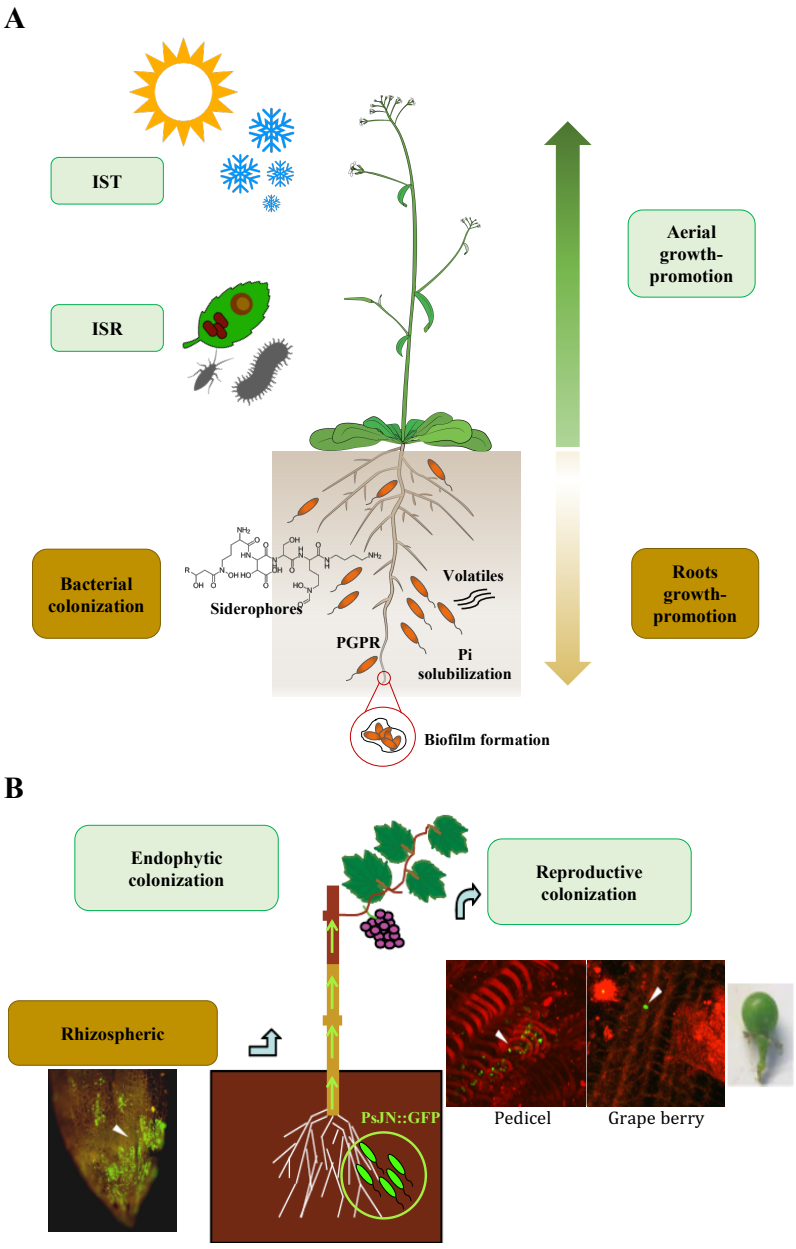


FIGURE 1. Plant growth-promoting rhizobacteria (PGPR) effects and colonization in plants. (A) A general view of PGPR effects to promote shoot and root growth, as well as distal defence responses. Through the colonisation of the root system, PGPR can contribute to plant's growth by producing biomolecules such as phosphate-solubilization enzymes, siderophores (e.g., ornibactin) and through defence responses to biotic and abiotic stresses (e.g., low and high temperatures, drought and bacterial molecules) by activating induced systemic resistance and tolerance (ISR and IST, respectively). **(B)** PsJN proposed colonisation pathway of rhizosphere, endosphere and aerial vegetative and reproductive organs in *Vitis vinifera* model. The colonisation pathway was proposed by tracking PsJN::GFP, a PsJN strain which constitutively expresses the green fluorescent protein (Modified from Mitter et al. (2013a)).

Vegetative plant growth-promoting effects triggered by PsJN colonisation has been described in Arabidopsis (Poupin et al., 2013), potato (Frommel et al., 1991), tomato (Pillay & Nowak, 1997), grapevine (Compant et al., 2005), switchgrass (Kim et al., 2012) and maize (Naveed et al., 2014). Some of the beneficial effects are enhanced development of the root system, higher number of lateral roots and root hairs, increase in lignin deposits around the vascular system, better functioning stomata, increased photosynthetic rate and augmented fresh weight of plants. Besides, PsJN have been proved to drive an acceleration of the whole life cycle of Arabidopsis, where PsJN-treated plants exhibit a higher rosette growth rate during the vegetative development, leading to early flowering and senescence (Poupin et al., 2013). Therefore, PsJN seems to have a pivotal role promoting rosette growth during the vegetative development. This last makes relevant to consider when to analyse PsJN effects in inoculated plants, if based on the chronological age or the morphological age (developmental stage). To make this choice, further characterisation of the changes in the vegetative aerial tissue caused by PsJN need to be addressed.

PsJN displays broad plasticity and adaptive capability to different environmental conditions, as well as hosts species (Ali et al., 2014; Mitter et al., 2013b). This phenotypic plasticity has been attributed to its large genome (8.2 Mbp) in comparison with other sequenced endophytes. Its genome bears a high number of genetic traits with putative functions related to adaptive features as detoxification enzymes, secretion and delivery system, quorum sensing, motility and phytohormones modulation (Mitter et al., 2013b; Weilharter et al., 2011). Thus, several molecular mechanisms could potentially be involved in the strain's function as PGPR.

PsJN role in phytohormonal modulation.

Phytohormones correspond to a specific group of compounds which govern several regulatory aspects of plant growth and development, as well as their interaction with environmental factors (Munné-Bosch & Müller, 2013). Phytohormonal modulation is one of the proposed mechanisms to explain how PGPR are able to enhance plant growth. Particularly, the indole-3-acetic acid (IAA), the most abundant naturally occurring auxin in plants (Soeno et al., 2010), has been described as a major hormone regulator of plant growth and plant responses to environmental cues through the integration of other hormone's functions (Jaillais & Chory, 2010). It has been proposed a potential role for IAA signalling in the establishment of plant-bacteria interactions, since auxin synthesising bacteria can produce spatiotemporal modulation of IAA levels in the plant (Spaepen et al., 2007). Evidence has arisen regarding the role of auxin signalling in PsJN's plant growth-promoting effect. PsJN genome encodes at least three putative IAA biosynthetic pathways (Mitter et al., 2013b; Zúñiga et al., 2013), plus an IAA degradation pathway encoded by the *iac* operon (Donoso et al., 2017; Zúñiga et al., 2013). Also, the *acdS* gene, that encodes the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which catalyses the degradation of ACC, the direct precursor of ethylene (ET), another phytohormone implicated in the control of plant development and stress responses (Thain et al. 2004).

Several reports have shown that Arabidopsis plantlets inoculated with PsJN exhibit transcriptional induction of genes involved in synthesis, perception and signalling of auxin (Poupin et al., 2016; Poupin et al., 2013; Zúñiga et al., 2013). Besides, PsJN is unable to develop endophytic populations and promote plant growth in Arabidopsis ox-miR393 transgenic line (Zúñiga et al., 2013), this line overexpresses the miRNA miR393 targeting auxin receptors of TIR1 family (Chen et al., 2012).

In the same work, Zúñiga et al. (2013) evaluated the role of ACC deaminase and IAA degradation of PsJN in Arabidopsis growth-promotion. The authors reported that plantlets inoculated with PsJN Δ*iacC* and Δ*acdS* mutant strains failed to promote plant growth. They proposed that IAA degradation would be essential for primary root elongation and fresh weight augmentation, and ACC deaminase for root development. Additionally, Poupin et al. (2016) showed that PsJN produces transcriptional changes in genes of auxin and ethylene pathways in Arabidopsis shoots and roots. It should be mentioned that these findings corresponded to a rapid response of plantlets from 12 to 96 h after the inoculation with PsJN wild-type strain. Furthermore, Arabidopsis mutant lines defective for auxin synthesis and transport did not exhibit growth-promotion by PsJN; similar to mutant lines of genes related to ethylene perception and synthesis (Poupin et al., 2016). Overall, these observations demonstrate that both auxin and ethylene hormones are indispensable for the Arabidopsis's response to PsJN.

Recent evidence suggests that PsJN also has the ability to modulate plant's hormonal pathways in response to stress, specifically by ISR and IST activation. For instance, protection upon pathogen attack has been demonstrated in PsJN-inoculated Arabidopsis plants (Bordiec et al., 2011; Su et al., 2017; Timmermann et al., 2017). PsJN-inoculated Arabidopsis plants challenged with the virulent strain *P. syringae* pv. *tomato* DC3000, exhibit a significantly less diseased severity percentage than non-inoculated plants until 28 days post infection, as well as lower pathogen proliferation (disease progression) in rosettes (Timmermann et al., 2017). Gene expression analysis of ISR markers in plants inoculated with PsJN showed that protective effect of PsJN is given by a faster specific transcriptional response of jasmonate (JA), salicylic acid (SA) and ET hormonal pathways, all associated with biotic stress responses (Timmermann et al. 2017; Timmermann, et al. 2019).

Additionally, PsJN activation of IST to cold stress has been reported in *V. vinifera* (Theocharis et al., 2012) and Arabidopsis (Su et al., 2015). Pinedo et al. (2015) studied short and long-term effects of PsJN in Arabidopsis under salt stress conditions. Their findings demonstrated that PsJN induces Arabidopsis short-term salt-tolerance by upregulating expression of genes involved in abscisic acid (ABA) responses, JA synthesis, and in reactive oxygen species (ROS) and methylglyoxal detoxification. Proline levels were also significantly higher in inoculated plants, which acts as an osmoprotectant compound to face possible damages in plant tissues caused by abiotic stresses (Singh et al., 2015). Noteworthy, PsJN conducted plant growth-promotion in salt-stressed plants along their whole life cycle (Pinedo et al., 2015). These evidences indicate that PsJN drives to hormonal and metabolic changes in plants.

FIGURE 2 summarises some of the PsJN's proposed mechanisms to promote plant-growth based on its genetic and phenotypic traits. Although, plant-induced hormonal responses to PsJN have been well documented, there is still a gap on how these transcriptional changes in hormonal pathways modulate downstream phenotypic effects induced by PsJN in Arabidopsis.

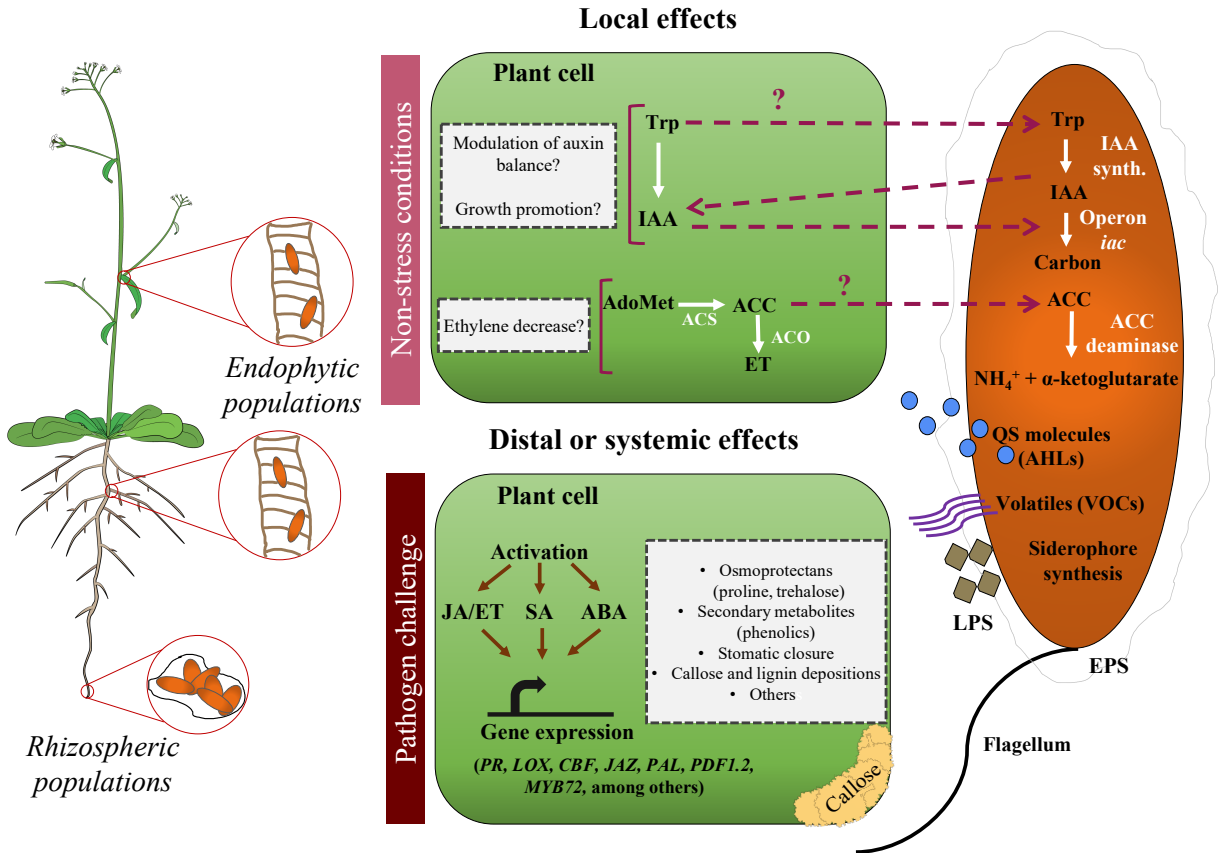


FIGURE 2. Molecular signalling proposed for plant-PsJN interactions. Rhizospheric and endophytic colonisation induces local and systemic effects in the plant. Under non-stress conditions, PsJN could be promoting plant growth by modulating auxin (IAA) balance, due to its ability to produce and degrade this phytohormone, as well as decreasing ethylene levels by producing ACC deaminase. The upstream precursor of ethylene (ET) synthesis is S-adenosyl methionine (AdoMet), which is sequentially transformed to 1-aminocyclopropane-1-carboxylate (ACC) and then to ET by ACC synthase (ACS) and ACC oxidase (ACO), respectively. Besides, other molecules such as, volatile organic compounds (VOCs) and siderophores can also contribute to plant growth. Plant perception of PsJN quorum sensing (QS) signals (N-acyl-homoserine-lactones; AHLs), lipopolysaccharide (LPS), exopolysaccharide (EPS) and flagellin (structural protein of flagellum) can lead to plant immunity responses activating stress phytohormones such as, jasmonate (JA), ET, salicylic acid (SA) and abscisic acid (ABA). Therefore, primmed plants can face biotic or abiotic stressors (e.g., pathogen challenge) by triggering early defence responses through induced systemic resistance and tolerance (ISR and IST, respectively).

Role of primary metabolism in plant growth.

Plant growth and development are regulated by the interaction of external and internal cues such as light, nutrients and water availability, phytohormones, biotic interactions, and therefore through the metabolic pathways (Eveland & Jackson, 2012; Hansen et al., 2002).

Plant central or primary metabolism comprises anabolic and catabolic processes as photosynthesis, respiration, the synthesis of nucleic acids, lipids and proteins (Sulpice & McKeown, 2015). Plants as photoautotrophic organisms grow proportionally to their photosynthetic and metabolic performances, and thus, to their carbon content (Hansen et al., 2002; Meyer et al., 2007). Consequently, plant growth is highly modulated by light. They have to adapt continuously to short-term variations as daily light/darkness cycles, as well as to long-term changes such as daylength and irradiance (given by the seasonal time), which have a high impact in carbon availability (Fernandez et al., 2017). Hence, the biomass yielded during the plant vegetative growth phase, has been proposed as the final expression of the plant metabolic capacity (Meyer et al., 2007). Therefore, primary metabolism participates in the regulation of plant growth and development in two ways: i) by providing the fuel (energy) and the building blocks (biomolecules) to support growth; and ii) through its own adaptation to balance growth with given environmental conditions (Meyer et al., 2007; Sulpice & McKeown, 2015).

Metabolic and hormonal crosstalk in plant growth regulation.

Plant primary metabolism itself actively contributes to plant growth and development, however, it has been described that metabolism and hormones work tightly interconnected in regulating plant growth and development (Eveland & Jackson, 2012; Li & Sheen, 2016; Ruan, 2014; Sakr et al., 2018; Sulpice et al., 2010; Wang & Ruan, 2013). The fundamental processes involved in plant growth and development are cell growth, division, expansion and

differentiation, as well as morphogenesis (Doerner, 2008; Lu Wang & Ruan, 2013). Signalling pathways of sugars as sucrose and the intermediate for trehalose synthesis, trehalose-6-phosphate (Tre6P) have been involved directly in the regulation of plant growth (Figueroa & Lunn, 2016; Ruan, 2014). Sucrose is the primary end product of photosynthesis acting as the sugar for systemic source-to-sink translocation for itself, as well as an element/companion cell complex in the phloem. In this way, sucrose serves as a pathway for transporting water, nutrients and other signal molecules into sink organs including roots, shoots, flowers, developing fruits, and meristematic tissues (Ruan, 2014; Sakr et al., 2018; Wang & Ruan, 2013).

Sucrose, on its role as signalling molecule, interplays with auxin to control cell proliferation and expansion (Wang & Ruan, 2013). This has been also proposed for glucose, one of the sucrose breakdown products (Sairanen et al., 2013). Glucose can stimulate auxin synthesis (Sairanen et al., 2013), and both molecules can cooperate to activate cell cycle components such as, cyclins and cyclin-dependent kinases (Skylar et al., 2011). On the other hand, Tre6P, has been proposed as a carbon status signal in plants. Tre6P modulates photosynthate partitioning during the day and regulates transitory starch degradation in leaves during the night. Therefore, having a main function in the negative feedback regulation of sucrose levels (Figueroa & Lunn, 2016; Lunn et al., 2006). Likewise, evidence suggests that Tre6P promotes plant growth through inhibiting SnRK1, a conserved protein kinase activated under energy deficit, driving to the inhibition of biosynthetic pathways to limit energy consumption (Caldana et al., 2019; Figueroa & Lunn, 2016).

Trehalose metabolism also responds to biotic and abiotic stresses. This disaccharide has been proposed to provide tolerance to extreme temperatures, drought, salinity, and protection against ROS synthesised after pathogen attack (Lunn et al., 2014). Fernandez et al. (2012) have

proposed that PsJN could induce systemic tolerance to cold stress in grapevine leaves by activating trehalose synthesis. This molecule is a soluble non-reducing sugar used as osmoprotectant and carbon storage for organisms such as bacteria, fungi, invertebrates and plants (Lunn et al., 2014).

In response to environmental signals, plants also synthesise secondary metabolites, which have been described as regulators of plant defence (Burow & Halkier, 2017). For instance, callose formation – one of the phenotypic effects of PGPR observed in Arabidopsis and other species – has been proved to be regulated by glucosinolates, a group of defence secondary metabolites, in a highly specific manner (Li et al., 2018). Likewise, in the recent years, new evidence has arisen, which implicate secondary metabolites in the regulation of plant growth and development. Katz et al. (2015) determined that indole-3-carbinol, a breakdown product of indole glucosinolate, binds to the auxin receptor TIR1 and represses auxin signal transduction. Flavonoids, other types of secondary metabolites, have been also proposed to modulate auxin transport and ROS accumulation (Erb & Kliebenstein, 2020). Moreover, it has been proposed that secondary metabolites could be recycled back to primary metabolism serving as precursors, as a mechanism that could be reducing cell energetic costs (Erb & Kliebenstein, 2020). These evidences strongly suggest a potential role of primary and secondary metabolites in PGPR growth-promoting effects.

The characterisation of PsJN effects have centred primarily in elucidate mechanistic responses of the plant root system. However, more studies are needed to have a better understanding of the aerial vegetative responses to this PGPR, given its effects observed in rosettes' growth-promotion in Arabidopsis.

What are the molecular mechanisms behind Arabidopsis aerial vegetative tissues growth-promotion driven by PsJN?

In past years, several studies have centred primarily in elucidate mechanistic responses of the root system to PsJN (Frommel et al., 1991; Poupin et al., 2016; Sun et al., 2009; Zúñiga et al., 2013) or using the entire plant (Da et al., 2012; Poupin et al., 2013; Timmermann et al., 2017; Timmermann, 2019; Wang et al., 2015). However, pivotal aspects of plant growth and development regulation, such as, primary metabolism occur in aerial photosynthetic tissues during the vegetative development. Besides, given the phenotypic changes that exhibit aerial tissues along vegetative development driven by PsJN, it is supported that more in-depth aerial plant molecular responses need to be studied. As was stated above, plant growth is tightly regulated by carbon availability and primary metabolism, as well as secondary metabolism, phytohormones and stress responses. In this regard, some open questions about how PsJN could be modulating aspects related to metabolism and phytohormones, and how this potential modulation is involved in the enhancement of plant growth need to be addressed. Moreover, there are still several gaps in our understanding of the link between PsJN's genetic traits and Arabidopsis plant growth-promotion phenotype.

Therefore, this thesis work focused on unveiling PsJN-driven molecular responses in Arabidopsis aerial vegetative tissues. For this purpose, phenotypical characterisation of aerial vegetative responses of Arabidopsis to PsJN were conducted. Plants were seed-inoculated with PsJN under in vitro conditions, and then transplanted to soil. Non-inoculated plants were used as control. Following, metabolic and hormonal analyses were conducted in rosettes from PsJN and non-inoculated conditions harvested at two different vegetative developmental stages, at the end of the day and the end of the night. Through the analysis of vegetative development of aerial

tissues, it was determined that PsJN accelerates the emergence of true leaves, without affecting the germination rate. Hence, the characterization of metabolic and hormonal changes in Arabidopsis shoots of PsJN- treated and -untreated plants were conducted at the same phenological developmental stage. As the main results of this thesis, it was found that PsJN produces molecular responses in Arabidopsis rosettes conducting to changes in the accumulation of secondary metabolites and induction of genes related to hormonal pathways. These changes can be associated with cell growth, leaf development, and induced systemic resistance and tolerance.

HYPOTHESIS

P. phytofirmans PsJN promotes the growth of *A. thaliana* aerial vegetative tissue through metabolic and hormonal modulation.

GENERAL OBJECTIVE

To determine the effects of *P. phytofirmans* PsJN upon the metabolism and hormonal pathways in *A. thaliana* aerial vegetative tissues' development.

SPECIFIC OBJECTIVES

To study the impact of growth conditions in *P. phytofirmans* PsJN effects in *A. thaliana* rosette growth.

To characterise changes in primary and secondary metabolism in *A. thaliana* rosettes in response to the growth-promotion driven by *P. phytofirmans* PsJN.

To analyse changes in hormonal pathways in *A. thaliana* rosettes in response to the growth-promotion driven by *P. phytofirmans* PsJN.

MATERIALS AND METHODS

Bacterial culture and inoculant preparation.

P. phytofirmans PsJN (DSM 17436, DSMZ GmbH, Germany) was grown on Dorn mineral salts medium (Dorn, Hellwig, Reineke, & Knackmuss, 1974) supplemented with 10 mM fructose as carbon source, at 28-30ºC in an orbital shaker (180 rpm) for 24 h. Bacterial cell suspensions were adjusted to 10^8 colony forming units per millilitre (CFU/mL) with phosphate buffer pH 7.2, as determined by plate counting. Subsequently, the adjusted cell suspension was used to prepare 0.8% agar plates containing half-strength Murashige & Skoog (MS) basal salts medium (Murashige & Skoog, 1962) (Sigma-Aldrich, St. Louis) at different final levels of PsJN (10^2, 10^4 and 10^6 [CFU/mL]).

Plant growth conditions and treatments.

A. thaliana Columbia (Col-0) accession was used for all experiments. Seeds were surfaced sterilised with 50% [v/v] commercial bleach containing 0.1% Tween-20 for 10 min and washed three times in sterile distilled water. Then, seeds were kept at 4ºC in darkness for two or five days to synchronise germination. Seeds were sown in 0.8% agar half-strength MS plates previously inoculated with PsJN at different dilutions (10^2, 10^4 and 10^6 [CFU/mL]). As a control a 0.8% agar half-strength MS plates without inoculation was used. Plates were placed horizontally in a growth chamber at 21ºC, under 12:12h light-darkness cycle and 130 [mol m^{-2} s^{-1}] light intensity.

For experiments in soil, seeds were sown in 0.8% agar half-strength MS plates with or without PsJN (10^6 [CFU/mL]), and then grown at 21ºC under 16:8, 12:12, 8:16 or 6:18h light-

darkness cycles with a light intensity of 130 [mol m^{-2} s^{-1}]. At 7 days after sowing (DAS), plantlets were transplanted to pots with peat/vermiculite at a 1:1 ratio. Transferred plants were maintained under the same environmental conditions and were watered with sterile tap water three times a week.

Determination of plant growth parameters.

Fresh and dry weight of rosettes were determined with a Shimadzu analytical balance (Shimadzu Corporation, Japan). For dry weight, Arabidopsis rosettes of inoculated and non-inoculated conditions were cut, individually packed in aluminium paper and dried at 70ºC for 48 h. For measuring rosette area, 30 plants were photographed every 1 to 2 days, starting two days after transplantation (9 DAS). For leaf area measuring, the first two true leaves were analysed from photographs taken of rosettes at 21 DAS from randomly selected plants (n=5) from PsJN and non-inoculated conditions. The images were analysed using ImageJ version 2.0.0 software (http://imagej.nih.gov). The number of emerged leaves per day was determined by counting the number of leaves per plant every day.

Microscopy analysis.

To determine possible changes in the cell size of true leaves, cell area was measured using light microscopy. The first two true leaves were cut from rosettes at 21 DAS from plants of both, inoculated and non-inoculated conditions. Five plants were analysed per condition. Every pair of leaves of each plant were placed on an individual well in a 12-wells microplate containing 12.5% solution of glacial acetic acid in ethanol and incubated for 1 h in a rotary shaker until leaves were completely cleared of chlorophyll. Subsequently, leaves were washed

with 50% ethanol for 20 min, followed by a wash with milli-Q water for 5 min. Leaves were re-suspended in 1.0 M KOH solution and stored at 4ºC until observation. To determine cell size and stomatal index, each pair of leaves from different plants were placed under a coverslip and observed on a widefield light microscope fitted with a digital camera. Images were taken in the middle portion of each leaf at both sides of the midrib of abaxial and adaxial epidermal layers, and palisade mesophyll layer. Stomata were counted in the abaxial epidermal layer. Cells of the three layers were measured using ImageJ 2.0.0 software (http://imagej.nih.gov).

Plant sample harvest and preparation for metabolic, hormonal and transcriptional analyses.

Plants grown in soil using 8:16 h photoperiod were harvested at the vegetative growth stages 1.04 (4 L) and 1.06 (6 L), which correspond to 4 and 6 leaves, respectively (Boyes, 2001). Whole rosettes were cut at ground level, placed in plastic scintillation vials and flash-frozen in liquid nitrogen. Samples were collected at the end of the night (EN) during the last 20 min of the darkness period under low-intensity green light, and at the end of the day (ED) during the last 20 min of the light period, avoiding shading the plants. Five biological replicates were harvested for all developmental stages, time points, and conditions. Approximately, 200 - 300 mg of fresh tissue was collected for each replicate, obtained from 60 to 70 rosettes and 20 to 24 rosettes from samples at developmental stage 4 L and 6 L, respectively. Frozen rosettes were homogenised to a fine powder by vigorous shaking with steel beads on liquid nitrogen in the same plastic vials used for harvesting using a ball mill (Retsch Technology, Haan, Germany). The frozen tissue powder was then stored at -80ºC until use.

Primary metabolite analyses.

Sucrose, glucose, fructose, starch, chlorophylls, total proteins, total amino acids and nitrate were extracted from aliquots of 15-20 mg frozen tissue powder with a solution of ethanol 80% / 10 mM HEPES-KOH pH 7.0 (TABLE 1). The measurement of soluble sugars and chlorophylls was performed as is described in Stitt et al. (1989). Amino acids and nitrate were assayed as is described in Bantan-Polak et al. (2001) and Cross et al. (2006), respectively. Finally, total proteins and starch content were determined following the protocol described in Hendriks et al. (2003). The extraction of trehalose 6-phosphate (Tre6P) and other compounds (TABLE 1) as sucrose 6-phosphate, glucose 6-phosphate, glucose 1-phosphate, glucose 1,6-bisphosphate, fructose 6-phosphate, fructose 1-phosphate, fructose 1,6-bisphosphate, galactose 1-phosphate, mannose 1-phosphate, ADP-glucose, UDP-glucose, UDP-galactose, glycerol 3-phosphate, glycerate, 3-phosphoglycerate, pyruvate, phosphoenolpyruvate, 2-oxoglutarate, aconitate, shikimate, iso-citrate, citrate, succinate, malate and fumarate, were carried out from aliquots of 15-20 mg frozen tissue powder with a solution of $CHCl_3$ / CH_3OH (3:7 v/v). Metabolites were assayed by using Liquid Chromatography coupled to a triple quadrupole mass spectrometer (LC/MS-Q3). The quantification of the metabolites was performed by the comparison of the integrated MS-Q3 signal peak area with a calibration curve obtained using authentic standards as is described by Lunn et al. (2006).

TABLE 1. Primary metabolites measured in Arabidopsis rosettes. Four types of primary metabolites, belonging to nitrogen and carbon central metabolism, were measured. The class and detail of metabolites measured, plus the analytical platform used to conduct their quantification, are indicated.

Metabolite class	Compounds	Analytic platform
Nitrogen-related	Total proteins, total amino acids, nitrate	Enzymatic assay
Non-phosphate sugars	Starch, sucrose, glucose, fructose	Enzymatic assay
Phosphate intermediaries	Sucrose 6-phosphate, trehalose 6-phosphate, galactose 1-phosphate, glucose 1-phosphate, fructose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, mannose 1-phosphate, glucose 1,6-bisphosphate, fructose 1,6-bisphosphate, glycerol 3-phosphate, phosphoenolpyruvate and 3-phosphoglycerate, ADP-glucose, UDP-galactose, UDP-glucose.	LC/MS-Q3
Organic acids	Succinate, fumarate, malate, citrate, iso-citrate, aconitate 2-oxoglutarate, shikimate, pyruvate and glycerate	LC/MS-Q3

Maximum enzymatic activity.

Maximum enzymatic activities were deteremined by using stopped assays, in which aliquots of extracts were incubated with their substrates for a fixed time before stopping the reaction and the product then determined in a second assay (Ap Rees & Hill, 1994; Gibon et al., 2002; Gibon et al., 2004). Briefly, aliquots of 15 – 20 mg of frozen tissue powder were used to conduct enzymes measurements (TABLE 2). NAD-GAPDH (GAPGH; Glyceraldehyde 3-Phosphate Dehydrogenase), NADP-GAPDH, SPS (sucrose phosphate synthase), G6PDH (glucose 6-phosphate dehydrogenase), HK (hexokinases), Ac-INV, N-INV (acid and neutral invertases), Fd-GOGAT (ferredoxin-dependent glutamate synthase), NR (nitrate reductase) and GS (glutamine synthetase) were assayed as described in Gibon et al. (2004); TPI (triose phosphate isomerase) Burrell et al. (1994); cPGI (cytoplasmic phosphoglucoisomerase) and total-PGI (total phosphoglucoisomerase) Weeden and Gottlieb (1982); PGM (phosphoglucomutase) Manjunath et al. (1998), and RubisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) Lilley and Walker (1975).

TABLE 2. Enzymatic activities of carbon-nitrogen metabolism measured in Arabidopsis rosettes. Enzymes are organized by the metabolic pathways in which they are involved in.

Metabolic pathway	Enzymes	Abbreviation
Nitrogen assimilation	Nitrate reductase	NR
	Glutamine synthetase	GS
	Glutamate synthase (ferredoxin)	Fd-GOGAT
Glycolisis	Phosphoglucose isomerase (cytosolic)*	cPGI
	Triose phosphate isomerase*	TPI
	Glyceraldehyde 3-phosphate dehydrogenase (NAD)	NAD-GAPDH
Sucrose synthesis	Phosphoglucose isomerase (cytosolic)*	cPGI
	Phosphoglucomutase	PGM
	Sucrose phosphate synthase	SPS
Starch synthesis	Phosphoglucose isomerase (total)	t-PGI
	Phosphoglucomutase	PGM
Hexoses metabolism	Acid invertases	Ac-INV
	Neutral invertases	N-INV
	Hexokinases	HK
	Glucose 6-phosphate dehydrogenase	G6PDH
Calvin cycle	Ribulose 1,5-bisphosphate carboxylase/oxigenase (initial)	In-RubisCO
	Ribulose 1,5-bisphosphate carboxylase/oxigenase (total)	t-RubisCO
	Glyceraldehyde 3-phosphate dehydrogenase (NADP)	NADP-GAPDH
	Triose phosphate isomerase*	TPI

*Enzymes participating in more than one pathway.

Secondary metabolites analysis.

The protocol described by Tohge and Fernie (2010) was followed for secondary metabolites extraction. Briefly, aliquots of 30 – 40 mg of frozen tissue powder were transferred to pre-cooled 2 mL safe-lock microcentrifugue tubes. Five μL of CH_3OH 80% ice-cold were used as extraction buffer per mg of frozen powder material. The Waters Acquity Ultra Performance Liquid Chromatography (UPLC) System coupled to Extractive Orbitrap mass detector was used to obtain a secondary metabolites profiling of targeted- and untargeted compounds (41 and 671, respectively) according to the protocol described by Giavalisco et al. (2009). The UPLC was connected to an Exactive Orbitrap (Thermo Scientific, Carlsbad, United States) via a heated electrospray source (Thermo Scientific, Carlsbad, United States). RefinerMS (version 5.3; GeneData), Metalign (Lommen & Kools, 2012), and Xcalibur (Thermo Scientific, Carlsbad, United States) softwares were used to extract molecular masses, retention times, and associated peak intensities from raw data. Metabolite identification and annotation were performed using standard compounds, literature and Arabidopsis metabolomics databases (Rohrmann et al., 2011; Tohge & Fernie, 2009, 2010) (TABLE 3).

TABLE 3. Targeted secondary metabolites measured in Arabidopsis rosettes. A total of 41 secondary metabolites were measured by Ultra Performance Liquid Chromatography coupled to Extractive Orbitrap mass detector (UPLC-MS/MS).

Metabolite class	Compounds
Phenylpropanoids	2,3-, 2,5- dihydroxybenzoic acid 5-O-β-D-glucoside; 2,3-, 2,5- dihydroxybenzoic acid 5-O-β-D-xyloside; 5-hydroxyferulic acid glucoside; coumaric acid glucoside; quercetin; 1,2-disinapoylglucose
Flavonoids	kaempferol-3-O-(-O-hexosyl) glucoside-7-O-rhamnoside; kaempferol-3-O-(2"-O-rhamnosyl) glucoside-7-O-rhamnoside; kaempferol 3-O-glucosyl-glucoside 7-O-rhamnoside; kaempferol-3-O-glucoside-7-O-rhamnoside: keampferol-3-O-rhamnoside-7-O-rhamnoside;quercetin-3-O-(2"-O-rhamnosyl) glucoside-7-O-rhamnoside; quercetin-(2"-O-hexo)glucoside-7-O-rhamnoside; quercetin-hexo-rhamnosyl-hexo; Rutin
Glucosinolates	3-methylsulfinylpropyl-glucosinolate; 4-methylsulfinylbutyl glucosinolate; 5-methylsulfinylpentyl glucosinolate; 3-butenylglucosinolate; 4-hydroxy-3-indolylmethyl-glucosinolate; 6-methylsulfinylhexyl glucosinolate; 4-pentenylglucosinolate; 7-methylsulfinylheptyl glucosinolate; 4-methylthiobutyl glucosinolate; 3-indolymethyl glucosinolate; 8-Methylsulfinyloctyl glucosinolate; 5-methylthiopentyl glucosinolate; 4-methoxy-3-indolylmethyl-glucosinolate; 6-methylthiohexyl glucosinolate; 1-methoxy-3-indolylmethyl-glucosinolate; 7-methylthioheptyl glucosinolate; 8-methylthiooctyl glucosinolate
Anthocyanins	Anthocyanin_A12 A11/cyanidin 3-O-[2"-O-(6"'-O-(sinapoyl) xylosyl) 6"-O-(p-O-(glucosyl)-p-coumaroyl) glucoside] 5-O-(6""-O-malonyl) glucoside Cyanidin 3-O-[2"-O-(2"'-O-(sinapoyl) xylosyl) 6"-O-(p-O-coumaroyl) glucoside] 5-O-[6""-O-(malonyl) glucoside] Anthocyanin (m/z=1181.29; RT=7.07)* Anthocyanin (m/z=1181.29, RT=7.84)*
Antioxidant	Glutathione

*m/z: mass-to-charge ratio; RT: retention time.

RNA extraction, cDNA synthesis and gene expression analysis.

The total RNA extraction was carried out using aliquots of 10 – 15 mg of frozen tissue powder from each sample. The tissue was thawed by adding CTAB 2X buffer, and incubating for 30 min at 56ºC. RNA was isolated using TRIzol™ Reagent (Invitrogen™, Carlsbad, United States) according the manufacturer's recommendations. Lithium chloride (2M) was used for RNA precipitation, and the obtained pellets were washed using cold-freeze 80% ethanol. RNA quantity and quality were assayed via NanoDrop™ (Thermo Scientific, Carlsbad, United States) and 2100 BioAnalyzer system (Agilent, Santa Clara, United States). Four μg of RNA were used for cDNA synthesis with SuperScript™ II Reverse Transcriptase (Invitrogen™, Carlsbad, United States) followed by DNase treatment with Ambion® TURBO™ DNA-Free (Invitrogen™, Carlsbad, United States) according manufacturer's recommendations. Quantitative q-PCR reactions were conducted using optical 384-well plates with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster city, United States) using SYBR green PCR Master Mix (Applied Biosystems, Foster city, United States) for dsDNA synthesis detection in 5 μL of reaction volume. The following standard program was used for all q-PCR reactions: 50 ºC for 2 min, 95 ºC for 10 min, 40 cycles of 95 ºC for 15 s, and 60 ºC for 1 min. Amplicon melting curves, were recorded after cycle 40 by heating from 60 ºC to 95ºC to assess primers specificity.

cDNAs first strand was analysed for integrity using 5'/3' amplification ratio of GAPDH $[(1+E_{GAPDH5'})^{C_t\ GAPDH5'}/(1+E_{GAPDH3'})^{C_t\ GAPDH3'}$, ($E$; PCR efficiency) which also works as an indicator for processivity of the reverse transcriptase (Czechowski et al., 2005). Forty-five different genes involved in phytohormonal pathways and cell growth were analysed, and 4 genes were selected as reference genes using primers previously reported and validated (Czechowski

et al., 2005). All primers used in this study are detailed in TABLE 4. The expression level of each gene of interest (GOI) was determined using $(2)^{-\Delta Ct}$; with $\Delta Ct = Ct_{GOI} - Ct_{RG}$ (RG: reference gene) (Czechowski et al., 2005). The expression ratios PsJN/non-inoculated control were obtained from the equation $(2)^{-\Delta Ct\ PsJN} / (2)^{-\Delta Ct\ Control}$. Log_2 of fold change analysis values were used to plot heat maps. To select the reference gene for normalization, expression stability using the Ct values obtained for all the samples was assessed using geNORM (Vandesompele et al., 2002), NormFinder (Andersen et al., 2004) and Bestkeeper (Pfaffl et al., 2004) softwares.

TABLE 4. Genes selected for relative transcriptional analysis in Arabidopsis rosettes in response to PsJN. Enzymes are classified by the metabolic pathways in which they are involved in.

GI (locus tag)	Gene	Primer name	Primer sequence (5' - 3')	Cell process	Reference
AT1G25220	*ASB1*	AtASB1 F	ACCACTCGCCGCCTAAACC	Auxin biosynthesis	Poupin et al. (2016)
		AtASB1 R	ATTGGACCATGCTGCTTAGAGG		
AT1G70560	*TAA1*	AtTAA1 F	CAAGTGGGAAGGAGACGCAT		
		AtTAA1 R	GGTTCACCACCGTCTCTCTG		
AT5G20730	*ARF7*	AtARF7 F	TTTCTACAACCCGAGGGCTGCT	Auxin signalling	Czechowski et al. (2004)
		AtARF7 R	ACCGCATACCGAGGGAAACTTGA		
AT4G14560	*IAA1*	AtIAA1 F	ATCTGCTCCTCCTCCTGCAAAAAC		
		AtIAA1 R	CGGTTAGATCTCACTGGAGGCCAT		
AT3G23630	*IPT7*	AtIPT7 F	CCTCACCACTTGCTTGGAGT	Cytokinin biosynthesis	This study*
		AtIPT7 R	GGGAAGCTTGTTGTTCGCTG		
AT3G63110	*IPT3*	AtIPT3 F	CGGTTTCTGCTGGACATTGC		
		AtIPT3 R	CACTAGACACCGCGACAACT		
AT2G01830	*AHK4*	AtAHK4 F	CCTCTCACAACTCATTACAGCTCA	Cytokinin perception	This study
		AtAHK4 R	CCACCACCACCCAGTTGATAA		
AT5G35750	*AHK2*	AtAHK2 F	ACTTTGATCCCGCACCGATT		
		AtAHK2 R	GATGCCCTTGCCCGTAAGAT		
AT3G62670	*CGA1*	AtCGA1 F	GCTCCGATTGTAACACAACCAA	Cytokinin signaling	Czechowski et al. (2004)
		AtCGA2 R	CCACAAGCGTTACAAAGAGACTTG		
AT5G56860	*GNC*	AtGNC F	GGCCAAGATGTTTGTGGCTAAC		
		AtGNC R	GTCTCCTTCTTTGGCACCATGT		
AT4G26150	*ARR2*	AtARR2 F	ACGCAACAGTTGTGGGTGAG		
		AtARR2 R	TGATACAGATTCCGGCTCGG		
AT3G30180	*BR6ox2*	AtBR6ox2 F	GGGCTGGCCAATATTTGGTG	Brassinosteroids biosynthesis	This study
		AtBR6ox2 R	GGTAACCGGCAACAAGTCCT		
AT3G50660	*DWF4*	AtDWF4 F	TCTGTAGCCATTGCTCTCGC		
		AtDWF4 R	TAGTTCCTTCTTGGCCCTCG		
AT1G73830	*BEE3*	AtBEE3 F	TGTGGAATCCATGCAGAAGGCAAA	Brassinosteroids signalling	Czechowski et al. (2004)
		AtBEE3 R	ACAGAACTCCCATCCCTCCCTTGA		
AT1G78440	*GA2ox1*	AtGA2ox1 F	GGATCTCTGTCCCTCCCGAT	Gibberellin biosynthesis	This study
		AtGA2ox1 R	AAACCCTATGCCTCACGCTC		
AT1G15550	*GA3ox1*	AtGA3ox1 F	AGCAAATGTGGTCCGAAGGT		
		AtGA3ox1 R	GTCTTCTTCGCTGACCCCAA		

GI (locus tag)	Gene	Primer name	Primer sequence (5' - 3')	Cell process	Reference
AT3G05120	*GID1A*	AtGID1A F	TCTAAGTGTGTGGCGGTCTG	Gibberellin perception	This study
		AtGID1A R	CGCCTCACTGTTCTTCCACA		
AT2G01570	*RGA1*	AtRGA1 F	TCCACTCATTACCACCTCCGCTTG	Gibberellin signalling	Czechowski et al. (2004)
		AtRGA1 R	TTCACTCGACTCGACTCCACCA		
AT3G49950	*GRASS*	AtGRASS F	TCGGGTGGGGAATGAAGAAGGAAG		
		AtGRASS R	AAATGGGAACCCAAACGGTAGCAA		
AT2G19590	*ACO1*	AtACO1 F	TGGGTTCCTATACCGCCATC	Ethylene biosynthesis	Poupin et al. (2016)
		AtACO1 R	ACCAGCCGGATTGTAAAACG		
AT1G62380	*ACO2*	AtACO2 F	TGCAGGAGGCATCATCTTGT		
		AtACO2 R	TGCAACCGACATCCTGTTTC		
AT1G66340	*ETR1*	AtETR1 F	AACTCGTTGAAGTCGTCGCT	Ethylene perception	This study
		AtERF1 R	GAAGGTCCCTAGCTCGCATC		
AT3G23150	*ETR2*	AtETR2 F	GTGCAGGTGAGTTTCCTTACA		
		AtETR2 R	AACCGAAGGAGCAACGACAT		
AT3G23240	*ERF1*	AtERF1 F	GGTGTACGGACGAAACCCTAGCTT	Ethylene signalling	Czechowski et al. (2004)
		AtERF1 R	AAATCTCCCCAGCTCTCGGTGAAG		
AT2G31230	*ERF15*	AtERF15 F	TTTCCCGGTGGAAGTGGTTAGAGA		
		AtERF15 R	TCAAGGCCATAACCGGAGATCCT		
AT5G42650	*AOS*	AtAOS F	CACGATGGGAGCGATTGAGA	Jasmonate biosynthesis	This study
		AtAOS R	TCGTCGCCAACGGTTGATAA		
AT3G45140	*LOX2*	AtLOX2 F	AGACTGACCAGCGGATTACG		
		AtLOX2 R	TCAGGCATCTCAAACTCGCA		
AT1G70700	*JAZ9*	AtJAZ9 F	CAGGAGAAGACGTTAGGGCG	Jasmonate perception	This study
		AtJAZ9 R	TCCTACAATAAACAGACCAAAGCA		
AT5G44420	*PDF1.2*	AtPDF1.2 F	TCTTTGGTGCTAAATCGTGTGT	Jasmonate signalling	This study
		AtPDF1.2 R	TTCAGTGGTCCTGTTGTAGAC		
AT5G46760	*MYC3*	AtMYC3 F	TGGCAGATCTCACACGACTTCGAT		Czechowski et al. (2004)
		AtMYC3 R	CCCAGCCGAGGATCACTGTGTTAT		
AT2G14610	*PR1*	AtPR1 F	ACGGGGAAAACTTAGCCTGG	Salicilic acid signalling	This study
		AtPR1 R	TTGGCACATCCGAGTCTCAC		
AT3G56400	*WRKY70*	AtWRKY70 F	GTTTGAAGATTCCGGCGATAGTC		Czechowski et al. (2004)
		AtWRKY70 R	ACACGTCTCCGATCTCTTTTTTCT		
AT1G49720	*ABF1*	AtABF1 F	GGCCTGGAGAAGGTTGTTGAGAGA	Abscisic acid signalling	Czechowski et al. (2004)
		AtABF1 R	GCCTGTTTTCGAGCCCTTGATCTA		

GI (locus tag)	Gene	Primer name	Primer sequence (5' - 3')	Cell process	Reference
AT1G01720	*ATAF1*	AtATAF1 F	TCAGGCTGGATGATTGGGTTCTCT	Abscisic acid response	Czechowski et al. (2004)
		AtATAF1 R	GCCTCTCGGTAGCTCCTTTTTTGT		
AT2G46270	*GFB3*	AtGBF3 F	TGGGATCACTGCCTCAAGGTCAAA		
		AtGBF3 R	TCAAAAGCGTCCCCGGAGTTGTTA		
AT2G01850	*EXGTA3*	AtEXGTA3 F	GGGACGGTTCTAAATGGGCA	Cell expansion	This study
		AtEXGTA3 R	CGAGAACCGCGCAATGTAAG		
AT3G29030	*EXPA5*	AtEXPA5 F	AAAGGCCGATCCATTGTGGT		
		AtEXPA5 R	TCCCACCACTTCTCTTGCAC		
AT3G02150	*PTF1*	AtPTF1 F	TCCGAGGCAAATGGTAAGCC		
		AtPTF1 R	GTCGAAATGTTTTGGGAAGACGA		
AT3G61890	*HB12*	AtHBT12 F	TGTGGTGATCAAGGACTGGC		
		AtHBT12 R	AACCTCCCTTCTGGCTCACT		
AT4G37490	*CYCB1*	AtCYCB1 F	GGCGTATCAGCAATGGAAGC	Cell division	This study
		AtCYCB1 R	CTTTGGCCGGAGGGATCAAA		
AT4G00150	*SCL6-IV*	AtSCL6 F	ATGTTGGCAAAGGACAGAACTCGT	Cell differenciation	Czechowski et al. (2004)
		AtSCL6 R	AACTCCGGTGGAAATCAGGAGGA		
AT1G06180	*MYB13*	AtMYB13 F	CCTAAACTAGCCGGGCTACTTC		
		AtMYB13 R	GACCATCTGTTGCCTAAGAGTTGA		
AT5G11260	*HY5*	AtHY5 F	GGCGACTGTCGGAGAAAGTCAAAG	Photomorpho_ genesis	Czechowski et al. (2004)
		AtHY5 R	TCAACAACCTCTTCAGCCGCTTG		
AT2G25000	*WRKY60*	AtWRKY60 F	TTGGTGCAACAAATGGCTTC	Biotic stress	Czechowski et al. (2004)
		AtWRKY60 R	TCGCAAGAGCTGCAGTGAAC		
AT4G25490	*CBF1*	AtCBF1 F	CCGCCGTCTGTTCAATGGAATCAT	Abiotic stress	Czechowski et al. (2004)
		AtCBF1 R	TCCAAAGCGACACGTCACCATCTC		
AT4G25480	*CBF3*	AtCBF3 F	GGATCATGGCTTCGACATGG		
		AtCBF3 R	GCTCTGTTCCGCCGTGTAAA		
Reference genes					
AT5G62690	*TUB*	AtTUB F	GAGCCTTACAACGCTACTCTGTCTGTC	HK**	Czechowski et al. (2004)
		AtTUB R	ACACCAGACATAGTAGCAGAAATCAAG		
AT4G05320	*UBI*	AtUBI F	CACACTCCACTTGGTCTTGCGT	HK**	
		AtUBI R	TGGTCTTTCCGGTGAGAGTCTTCA		
AT1G13320	*PDF2*	AtPDF2 F	TAACGTGGCCAAAATGATGC	HK**	
		AtPDF2 R	GTTCTCCACAACCGCTTGGT		
AT2G28390	*SAND*	AtSAND F	AACTCTATGCAGCATTTGATCCACT	HK**	
		AtSAND R	TGATTGCATATCTTTATCGCCATC		

cDNA integrity control					
AT1G13440	*GAPDH5'*	GAPDH5 F	TCTCGATCTCAATTTCGCAAAA	HK**	Czechowski et al. (2004)
		GAPDH5 R	CGAAACCGTTGATTCCGATTC		
	GAPDH3'	GAPDH3 F	TTGGTGACAACAGGTCAAGCA	HK**	
		GAPDH3 R	AAACTTGTCGCTCAATGCAATC		

*Primers designed in this study using PRIMER-BLAST.
** HK: housekeeping.

Statistical data analysis.

The statistical analysis of plant growth parameters was carried out using Graphpad Prism version 6.0 software (La Jolla, California, United States, URL http://www.graphpad.com). Rosette absolute and relative growth rates were analysed using RStudio version 1.2.5033 (RStudio, Inc., Boston, United States, URL http://www.rstudio.com/). The explorative analyses of metabolomic data were done in MetaboAnalyst software (http://www.metaboanalyst.ca) following developer's instructions (Chong, Wishart, & Xia, 2019). Statistical analyses of metabolomic and gene expression data were done in RStudio version 1.2.5033 (RStudio, Inc., Boston, United States, URL http://www.rstudio.com/) using the packages Tidyverse version 1.3.0 and rstatix version 0.5.0. Heat map plot of gene expression data was done in RStudio version 1.2.5033 (RStudio, Inc., Boston, United States, URL http://www.rstudio.com/) using the package ggplot2 version 3.3.1.

Dr. Saleh Alseekh (MPIMP, Central Metabolism Lab) kindly performed an analysis of correlation using the raw data (mass and retention times) to classify the untargeted metabolites of interest in this study.

RESULTS

OBJECTIVE 1: To study the impact of growth conditions in *P. phytofirmans* PsJN effects in *A. thaliana* rosette growth.

Setting of Arabidopsis-PsJN interaction system culture conditions for plant growth-promoting effect.

To conduct the characterisation of the plant growth-promoting effects of PsJN in Arabidopsis vegetative aerial tissue, growth parameters related to rosette, leaf and cell growth were analysed. Also, to study the possible impact of carbon availability on this phenomenon, four different photoperiods were used to determine potential differences in Arabidopsis responses to PsJN.

To set the appropriate culture conditions for evaluation of phenotypic and molecular responses in Arabidopsis inoculated with PsJN, exploratory assays and protocol standardisation were initially conducted. Thus, different variables of culture conditions of the Arabidopsis-PsJN interaction system were evaluated such as, the time for seeds stratification (2 and 5 days) and different PsJN dilutions to inoculate seeds (10^2, 10^4, 10^6 [CFU/mL]). This last, due to there is no one unique set of standard conditions established in the literature for this purpose. The germination percentage was determined at 3 DAS in all conditions. It was found that neither the time of seed stratification nor PsJN inoculation level affected seed germination (FIGURE 3A). Subsequently, plant growth parameters as fresh weight and area of rosettes were analysed from PsJN-treated and control plantlets germinated from seeds treated with short or and long stratification.

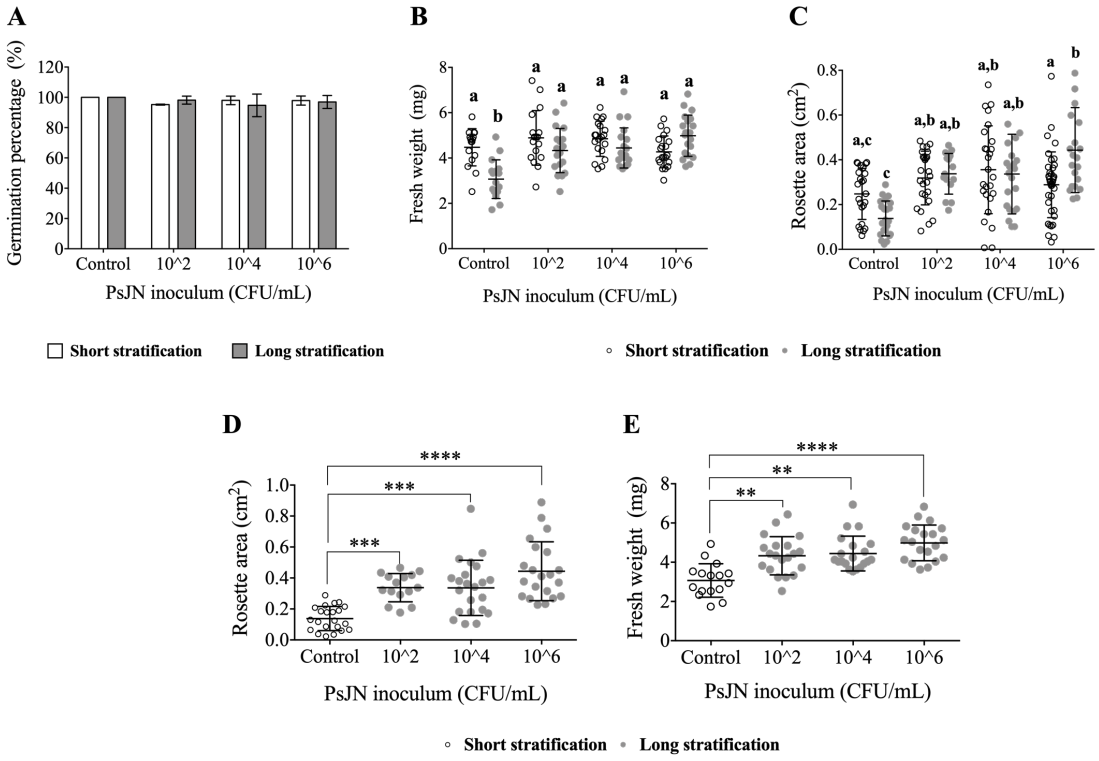


FIGURE 3. Effect different factors of Arabidopsis-PsJN culture conditions on the growth-promotion phenotype. Arabidopsis Col-0 seeds were treated for stratification for two or five days, sown on MS culture medium inoculated with different dilutions of PsJN at 10^2, 10^4 or 10^6 colony forming units per millilitre [CFU/mL], and non-inoculated as control condition. **(A)** Germination percentage of seeds was evaluated 3 DAS in all conditions. Bar plots represent mean and standard deviation (SD). **(B)** Fresh weight and **(C)** rosette area, were measured at 14 DAS in all conditions. Scatter dot plots represent mean and standard deviation (SD). Same letters indicate non-significant differences among treatments (two-way ANOVA, Tukey's multiple comparisons test (p-value>0.05); n= 20-47).

Rosette growth parameters of Arabidopsis Col-0 plants germinated from the seeds stratified for five days **(D)** Fresh weight, and **(E)** Rosette area. Scatter plots represent mean and standard deviation (SD). Asterisks indicate statistical significance (Left: ANOVA, Kruskal-Wallis multiple comparisons test (p-value>0.05); n=20-47). Right: ANOVA, Tukey's multiple comparisons test p-value>0.05); n=20-47).

The results obtained showed that long seed stratification for five days (FIGURES 3B and C) and a PsJN concentration of 10^6 CFU/mL as an inoculant (FIGURES 3D and E) were the conditions where PsJN showed an enhanced effect in aerial vegetative growth in Arabidopsis. Based on these observations, these conditions were used for further analysis of Arabidopsis-PsJN interaction system.

PsJN plant-growth promotion effect in aerial vegetative tissue is given by an enhancement of leaf and cell enlargement.

To test how PsJN enhances aerial tissue growth parameters, the rosette area growth curve, leaf and cell sizes were analysed. Rosette and leaf area, leaf length and width, as well as cell area were measured using culture conditions previously set under neutral days (12:12 h photoperiod) for 21 days. FIGURE 4 shows representative photographs of rosette phenotype of plants from PsJN-inoculated and non-inoculated conditions at 21 DAS.

The rosette growth curves in inoculated and non-inoculated conditions, were established by analysing digital images taken from the aerial zone of the plants obtained from 2 to 14 days after transplantation to soil (9 to 21 DAS, respectively). Rosette area was significantly higher in plants from PsJN-treated condition, at 9, 15, 16, 17, 19 and 21 DAS (FIGURE 5A).

At 21 DAS, the first-two true leaves were harvested in plants of both conditions, to proceed with measurements of leaf area, length and width. Leaf area was bigger in PsJN-inoculated plants than in non-inoculated condition, with 0.28±0.01 and 0.23±0.01 [cm^2], respectively (t-Test; p-value= 0.0128) (FIGURE 5B). Likewise, leaf length was also higher in inoculated plants with 5.44±0.22 [mm] in comparison with non-inoculated plants, where leaf length was 4.90±0.22 [mm].

FIGURE 4. Aerial growth phenotype in Arabidopsis plants inoculated with PsJN. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation for control condition. Plants were grown under neutral days 12:12 h light/ darkness cycle. Photographs are representative of rosettes from plants grown for 14 days under PsJN and non-inoculated conditions (white bar represent 2 cm).

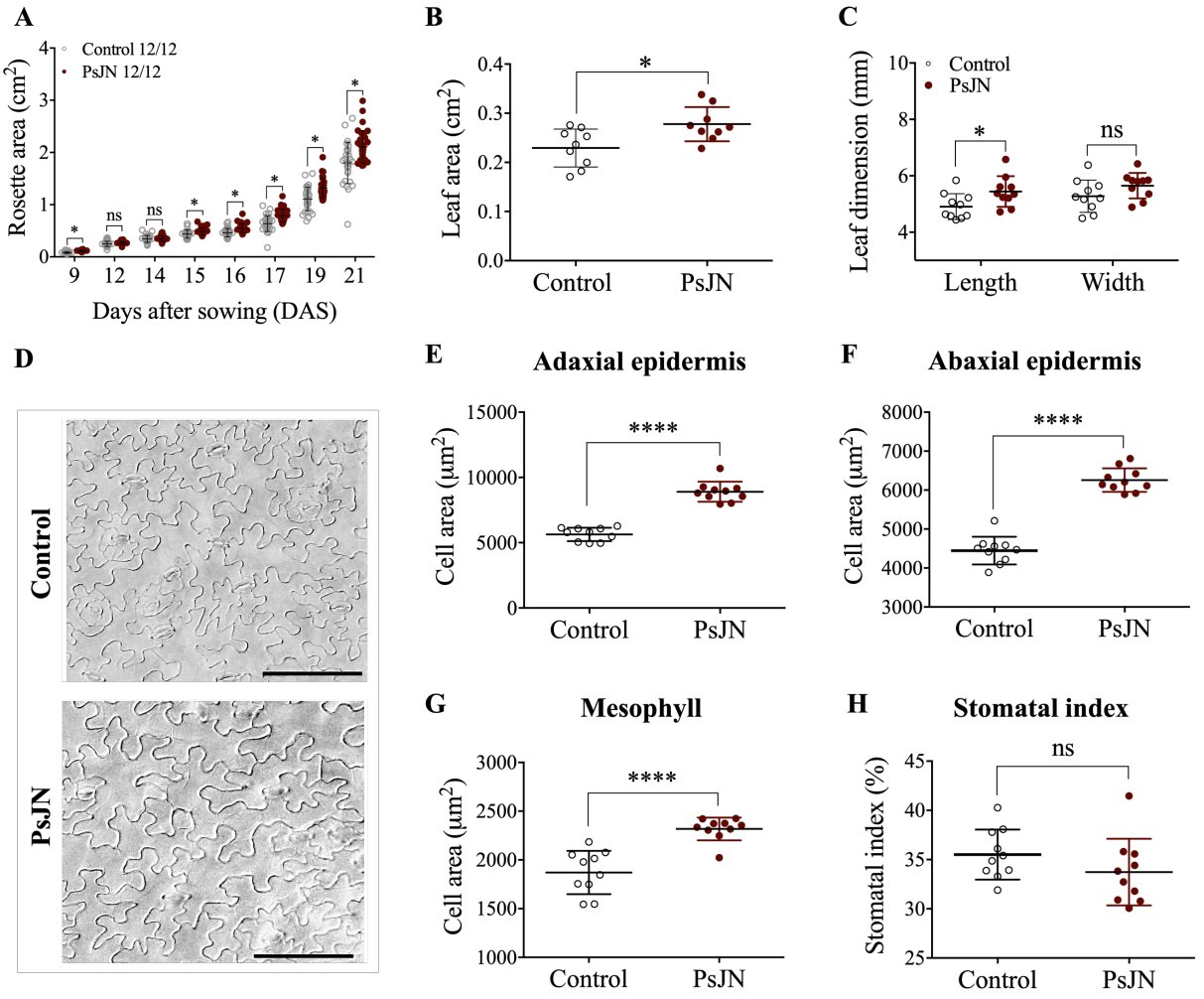


FIGURE 5. Characterisation of the effect of PsJN in rosette, leaves and their cells in Arabidopsis. Arabidopsis plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated were grown in 12:12 h photoperiod for 21 days. **(A)** Rosette area growth from the first 12 days after transplanting to soil. Scatter dot plots represent mean values and standard deviation (SD). Asterisk indicates statistical significance (Multiple t-tests ($p<0.05$); Holm-Sidak multiple comparisons method $n_{12/12}= 30$ $n_{8/16}=28$-30 $n_{6/18}=31$). **(B)** Leaf area of the two-first true leaves (unpaired t-Test, (p-value=0.0127); n=9). **(C)** Leaf length and width of the two-first true leaves (two-way ANOVA, Tukey's multiple comparisons test (p-value=0.0052); n=10). **(D)** Representative micrographs of abaxial epidermal cells form non-inoculated plants (above) and PsJN-inoculated plants (bottom). Black bar: 100 µm. **(E)** Cell area from adaxial epidermis; **(F)** Cell area from abaxial epidermis**; (G)** Cell area from mesophyll layer; and, **(H)** Stomatal index in abaxial epidermis. (t-Test ($p<0.0001$); n=10). Scatter dot plots represent mean values and standard deviation (SD). Asterisks indicate statistical significance. Ns: not significant.

In contrast, leaf width did not show significant differences between PsJN-treated and untreated plants (FIGURE 5C).

Cell area was determined in three different cell layers: adaxial and abaxial epidermis, and mesophyll. Representative images clearly show larger epidermal cells in inoculated than in non-inoculated plants FIGURE 5D. Epidermal cells area from adaxial layer was higher in PsJN treated plants than in non-inoculated plants: 8902±243.4 and 5631±162.9 [μm^2], respectively (FIGURE 5E), whereas for the abaxial layer were 6257±95.8 and 4445±112.6 [μm^2], respectively (FIGURE 5F). Cells from mesophyll layer also exhibited a higher area in PsJN-inoculated plants than non-inoculated plants (2318±36.8 and 18.71±70.4 [μm^2]), respectively (FIGURE 5G). Finally, stomatal index was determined as control for stable environmental plant growth-conditions (Casson & Gray, 2008), which did not show differences between both conditions (33.73±1.07 and 35.51±0.80 [μm^2]) (FIGURE 5H). These results show that Arabidopsis responded to PsJN, by developing a more significant rosette area, mainly due to longer cells and leaves.

PsJN produces a stronger plant growth-promotion effect on Arabidopsis aerial tissue in short days regime.

Light is a pivotal environmental factor for the adjustment of photosynthetic traits in plants. Hence, day length affects the plant's photosynthetic performance, growth and development (Lepistö & Rintamäki, 2011). Therefore, rosette growth curves in the presence and absence of PsJN inoculation, under long and neutral days (16:8 h and 12:12 h light/ darkness cycles, respectively) were evaluated. In long and neutral days, PsJN-inoculated plants exhibited the same pattern of rosette area augmentation, however, only under neutral days it was possible

to observe differences in aerial growth between PsJN-treated and untreated plants (FIGURE 6A). These results indicate that PsJN enhances plant growth when plants are grown in neutral-day regime, but not under a long-day light regime. Due to shorter light periods produce a decreased carbon supply for plants (Gibon et al., 2009), it was explored if short light regimes could affect PsJN's growth-promotion. Specifically, the PsJN's effect in two short-day photoperiods of 8 and 6 hours of light and neutral-day as control were tested. In the three photoperiods assayed, PsJN-inoculated plants developed a big rosette size in comparison with non-inoculated plants.

The analysis of the rosette growth curves in neutral and short-day photoperiods showed similar trends during the first two weeks after transplantation where rosette size was significantly increased in PsJN plants compared to non-inoculated condition at most time points measured (FIGURES 6B-D). Although only the 8:16 h regime showed significant increase in rosette size in all time points tested (FIGURE 6C). In the 6:18h regime, rosette growth was slower in comparison to the other photoperiods tested and, after 16 DAS, differences in rosette area between PsJN-treated and untreated plants were not observed (FIGURE 6D). SUPPLEMENTARY TABLE 1, APPENDIX, shows the detail of the results obtained in the statistical analysis.

For further understanding how PsJN enhances rosette size, the absolute growth rate (AGR), was determined. The ARG corresponds to the plant area that is formed in a unit of time (or growth velocity) (Vanhaeren et al., 2015). PsJN-treated plants exhibited a higher rosettes' ARG than inoculated plants, independently of the photoperiod used for cultivation (SUPPLEMENTARY FIGURE 1A, APPENDIX).

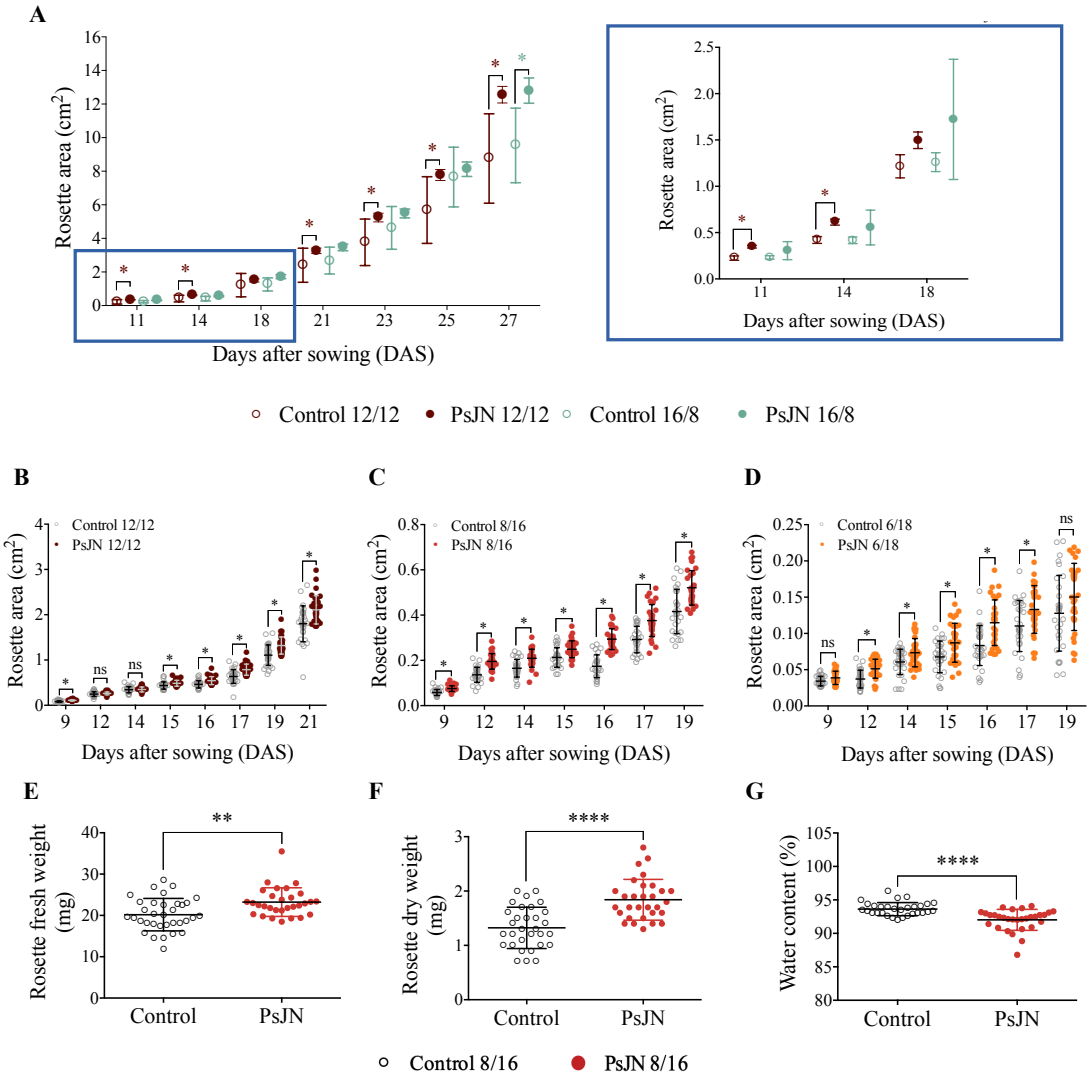


FIGURE 6. Photoperiod effects on vegetative aerial growth of Arabidopsis interacting with PsJN. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation for control condition. Plants were grown under long, neutral and short days (16:8 h, 12:12 h, 8:16 h and 6:18 h of light/ darkness cycle, respectively). **(A)** Left: rosette area growth curve under long and neutral days. Right: Zoom in of rosette area growth curve under long and neutral days at 11, 14 and 18 DAS. Symbols represent mean and standard deviation (SD) (Multiple t-tests ($p<0.05$); Holm-Sidak multiple comparisons method; $n_{16/8}$= 15 $n_{12/12}$=15). **(B, C, D)** Rosette area growth curve under neutral and short days (12:12 h, 8:16 h and 6:18 h of light/ darkness cycle, respectively). Scatter dot plots represent mean values and standard deviation (SD) (Multiple t-tests ($p<0.05$); Holm-Sidak multiple comparisons method $n_{12/12}$= 30 $n_{8/16}$=28-30 $n_{6/18}$=31). **(E, F, G)** Rosette dry weight fresh weight, and water content of 21 days-old plants grown under 8:16 h light/ darkness cycle. Scatter dot plots represent mean values and standard deviation (SD) (Mann-Whitney test; n = 30 **(E)** p-value=0.0023; **(F)** p-value<0.0001; and **(G)** p-value<0.0001). Asterisks indicate statistical significance. Ns: not significant.

Over the first 9 days after transplantation to the soil in neutral-day (at 16 DAS), AGR were 0.062±0.002 and 0.056±0.002 [cm^2/day] for PsJN-treated and untreated plants, respectively (SUPPLEMENTARY TABLE 2, APPENDIX). In 8:16 h photoperiod differences in AGR started from the day 9 after transplantation (at 16 DAS), 0.096±0.005 and 0.079±0.005 [cm^2/day] for PsJN-treated and untreated plants, respectively. Relative growth rates (RGR), which is the ratio of rosette to its size on a previous timepoint (Vanhaeren et al., 2015) did not show differences between treated and control plants in the photoperiods tested (SUPPLEMENTARY FIGURE 1B, APPENDIX).

As the 8:16 h photoperiod showed the most significant differences between PsJN- treated and untreated plants, all further experiments were conducted using this condition. To support the observations of rosette growth, fresh and dry weight of rosettes were measured as complementary parameters indicative of plant biomass augmentation, at 21 DAS in PsJN-inoculated and non-inoculated plants grown under 8 h of light. Rosettes from PsJN condition showed higher biomass in terms of fresh and dry weight than those of the non-inoculated condition. The fresh weight obtained was 23.22±0.63 and 20.18±0.67 [mg], in PsJN and control, respectively (FIGURE 6E). Rosettes dry weight was 1.840±0.068 and 1.023±0.068 [mg] in PsJN-treated and non-inoculated plants, respectively (FIGURE 6F). Water content of rosettes from PsJN condition was decreased compared to non-inoculated condition (92.02±1.57 93.63±0.98 [%]) (FIGURE 6G). These observations corroborate that the growth-promotion of aerial tissue in PsJN-interacting plants is explained not only by a bigger rosette size but also by biomass augmentation.

PsJN induces faster growth and early vegetative development of Arabidopsis aerial tissue.

To establish if the enhancement of plant growth driven by PsJN proceeds through a faster development in Arabidopsis plants, leaf emergence times over the first 14 days after the transplantation to soil were studied. It was observed that the emergence and development of new true leaves occurred at different times between PsJN-inoculated and non-inoculated plants. FIGURE 7A shows photographs of representative rosettes of plants from both conditions where it is possible to observe that the development of the 3rd, 4th and 6th leaf at three different time points is out of phase in PsJN-treated plants. The development of true leaves started at 12 DAS in treated plants, whereas this process happened two days later (at 14 DAS) in non-inoculated plants. In addition, it was determined that since day 16 PsJN-inoculated plants exhibited an acceleration in the emergence of the next true leaves (FIGURE 7B), reaching vegetative developmental stages before untreated plants (TABLE 5).

To corroborate if this phenomenon also occurred in other photoperiods, the same experiment was carried out using neutral-day. In this case, the same result was observed, with the difference that under neutral-day the first-two true leaves emerged the same day (12 DAS) with or without inoculation with PsJN. The emergence and development of the next leaves were out of phase as was observed under short-day. In addition, it was also determined the total number of true leaves when plants were at the reproductive developmental stage of the first flower aperture. The results showed no differences between plants inoculated or not with PsJN (FIGURE 7C). Therefore, PsJN is accelerating leaf emergence and development times, without affecting the number of total true leaves at the initiation of the reproductive stage.

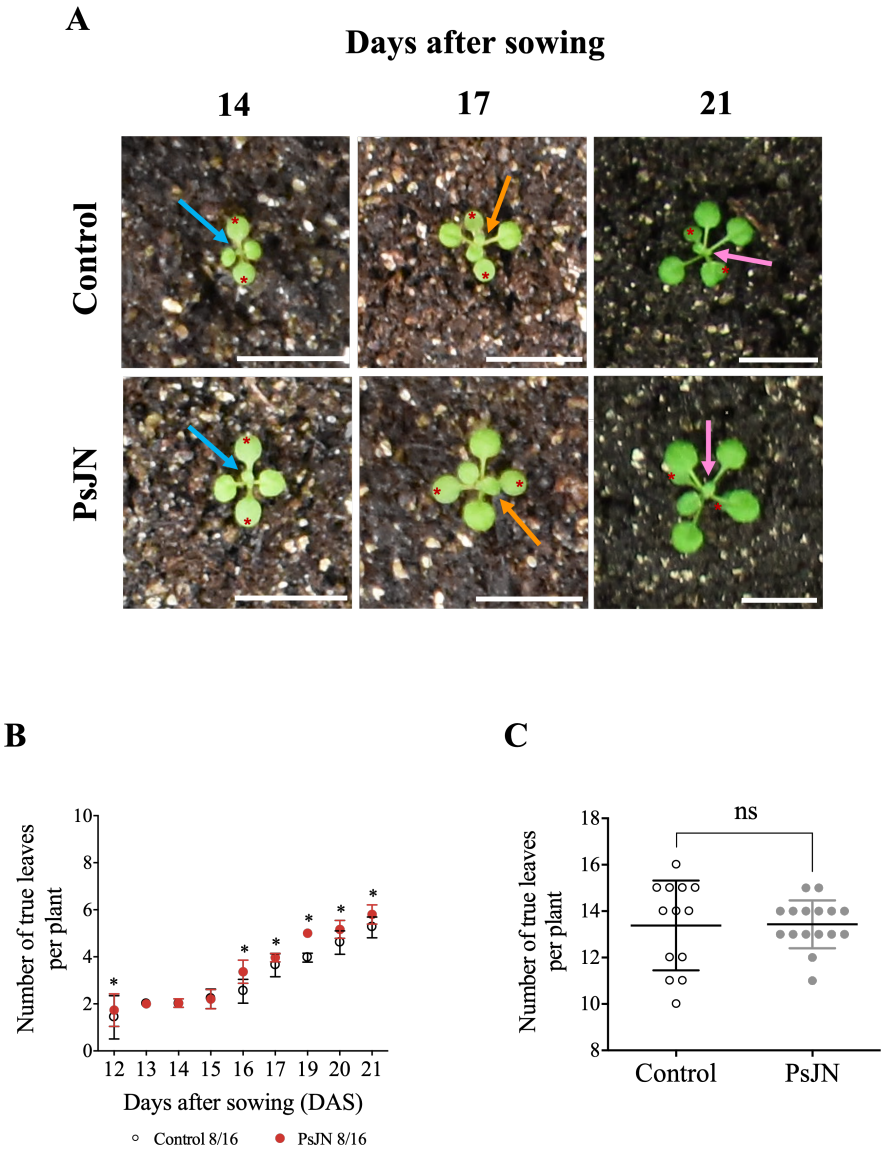


FIGURE 7. Arabidopsis-PsJN interaction accelerates leaf emergence. Arabidopsis plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated as control condition were grown under 8:16 h light/ darkness cycle for 21 days. The number of true leaves were counted every day since the emergence of the first pair of true leaves. **(A)** Representative rosettes of PsJN and non-inoculated conditions at 14, 17 and 21 DAS. Light blue arrows indicate the 3rd leaf at 14 DAS; orange arrows indicate the 4th leaf at 17 DAS; and pink arrows indicate the 6th leaf at 21 DAS. Red asterisks indicate cotyledons. **(B)** Number of true leaves per day in plants grown under 8:16 h photoperiod. Multiple t-tests ($p<0.05$); $n_{12/12}= 30$ $n_{8/16}=28$-30. Symbols represent mean values and standard deviation (SD). Asterisks indicate statistical significance. **(C)** Number of true leaves per plant grown under neutral days until the aperture of the first flower. t-Test ($p=0.9256$); n= 13-16. Scatter plots represent mean values and standard deviation (SD).

TABLE 5. Arabidopsis-PsJN interaction accelerates vegetative development. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) or without inoculation as control condition. Plants were grown under two photoperiods 8:16 h and 12:12 h light/ darkness cycles. Vegetative developmental stages were established by analysing images taken every 1 - 2 days until 6 L stage. The day for leaf development was determined when percentile 95 of plants was reached.

Vegetative developmental stage		**Age of the plant according to growth condition**			
Developmental stage [a]	Parameter [a]	PsJN 8:16 h	Control 8:16 h	PsJN 12:12 h	Control 12:12 h
1.02 (2 L)	2 rosette leaves > 1mm in length	12 [b]	14 [b]	12 [b]	12 [b]
1.04 (4 L)	4th rosette leaf > 1mm in length	17 [b]	18 [b]	14 [b]	15 [b]
1.05 (5 L)	5th rosette leaf > 1mm in length	19 [b]	21 [b]	16 [b]	17 [b]
1.06 (6 L)	6th rosette leaf > 1mm in length	22 [b]	24 [b]	17 [b]	19 [b]

[a] Developmental stages defined by Boyes et al. (2001).

[b] Days after sowing

The results obtained in this objective were crucial to establish the experimental conditions to carry out the molecular characterisation of the plant growth-promoting effect of PsJN in Arabidopsis aerial tissue. The fact that PsJN enhances aerial vegetative growth in Arabidopsis when plants are under short-day photoperiod, suggest an effect upon carbon use efficiency. Therefore, the following conditions were established to conduct the corresponding metabolic and hormonal responses of Arabidopsis vegetative aerial tissue to PsJN:

i. To synchronise seed germination by stratification for five days;

ii. To use PsJN inoculum at 10^6 CFU/mL;

iii. To grow plants under short-day photoperiod (8:16 h light/darkness cycle).

Furthermore, it was established for the first time that PsJN promotes rosettes growth by accelerating the emergence of true leaves after the seed germination, which leads treated plants to achieve vegetative developmental stages earlier than in control non-inoculated plants. In this regard, the molecular analyses to characterise the plant growth-promotion of Arabidopsis rosettes must be carried out by comparing plants at the same developmental stage. Thus, it was established:

iv. To analyse samples at the same vegetative developmental stage instead of the same plant chronological age; in order to compare plants at the same phenological state.

OBJECTIVE 2: To characterise changes in primary and secondary metabolism in *A. thaliana* rosettes in response to the growth-promotion driven by *P. phytofirmans* PsJN.

PsJN triggers global changes in Arabidopsis metabolism at 4 L and 6 L developmental stages.

To determine if accelerating vegetative aerial growth in Arabidopsis young plants was associated with changes in plant metabolism, several primary and secondary metabolites were measured in 4 L and 6 L rosettes of plants grown under 8:16 h photoperiod. A total of 751 compounds from primary and secondary metabolism were measured at ED and EN. To conduct these analyses, rosettes were harvested in two early vegetative developmental stages: four and six true leaves (4 L and 6 L, respectively), of plants grown in presence and absence of PsJN.

As an explorative analysis of the metabolic datasets obtained, Partial Least Squares – Discriminant Analysis (PLS-DA) and hierarchical clustering were conducted, separately, for ED and EN metabolites. PLS-DA score plots grouped by treatment (with or without PsJN) and developmental stages (4 L and 6 L), differentiating four groups (FIGURES 8A-B). At ED component 1 explains 7.3% of the total variability observed in the dataset (FIGURE 8A). The dataset of EN metabolism displayed the same tendency, where component 1 explained a 10.9% of the variability (FIGURE 8B).

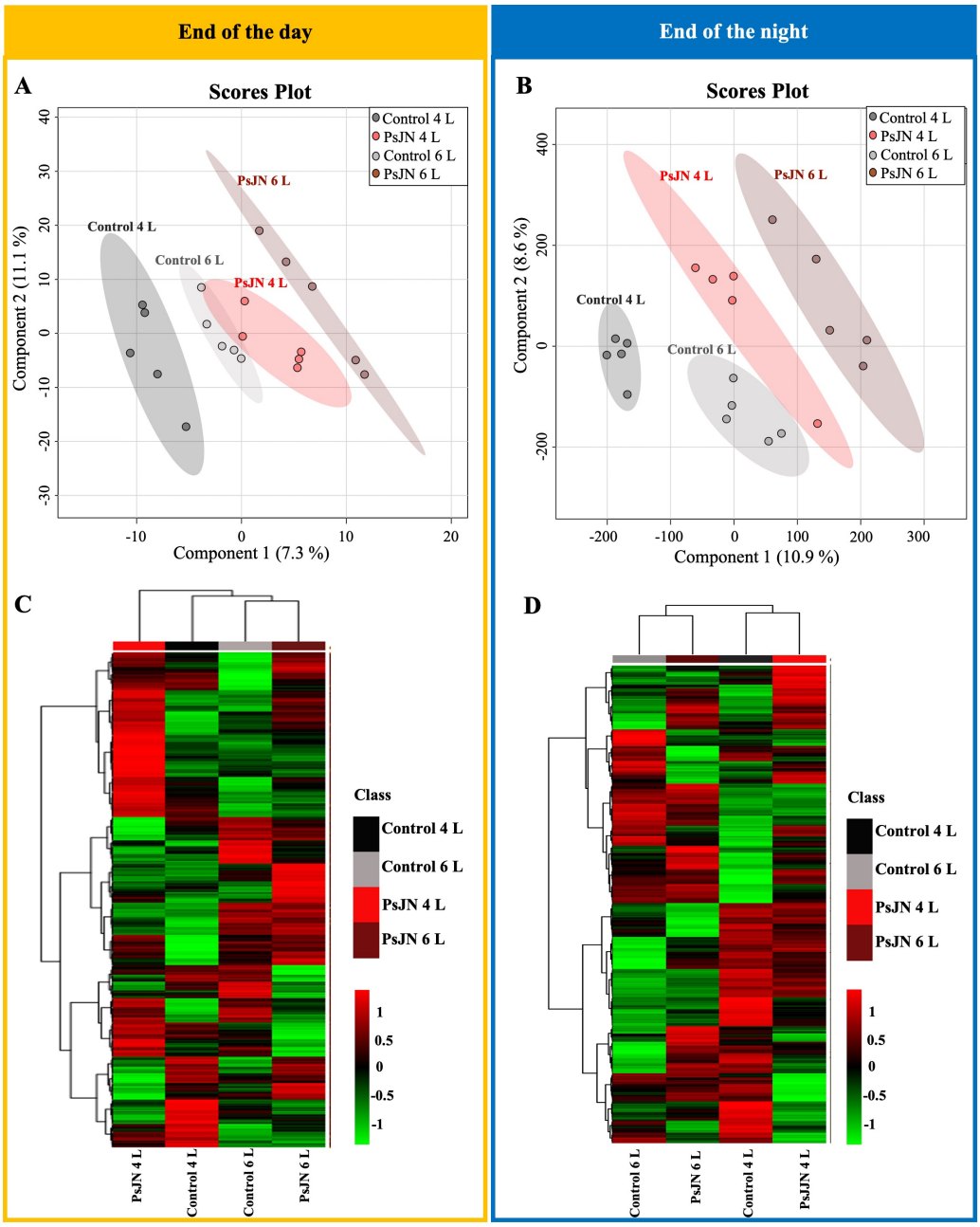


FIGURE 8. Exploratory analysis for the whole metabolic data set partitioned as day and night metabolism. Arabidopsis plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated as control condition were grown under 8:16 h light/ darkness cycle. Rosettes for both conditions were harvested at 4 L and 6 L developmental stages at the end of the day (ED), and at the end of the night (EN). The exploratory analysis was carried out using the whole metabolic data set obtained from primary and secondary metabolites. Plot scores obtained by Partial Least Squares – Discriminant Analysis (PLS-DA) of samples harvested at **ED (A)**, and at **EN (B).** Shaded area corresponds to 95% trust of each sample group. Heatmap obtained by Hierarchical clustering cluster analysis of data at **ED (C)** and at **EN (D)** (Similarity measure: Euclidean distance; clustering algorithm: Ward's linkage).

Moreover, the metabolic profiles at the ED and EN obtained by hierarchical clustering analysis exhibited changes in abundance of primary and secondary metabolites in PsJN and non-inoculated conditions at both developmental stages (FIGURES 8C-D). At ED 4 L rosettes from PsJN-treated condition exhibited the most divergent metabolite accumulation pattern (FIGURE 8C). On the other hand, the heatmap clustering of samples harvested at EN was due to the effect of the developmental stage, suggesting that PsJN is not the main contributing factor to the changes observed (FIGURE 8D). However, there are evident differences between PsJN-treated and control metabolic profiles.

Global metabolic analysis suggests that PsJN treatment has subtle effects on the metabolic profile of Arabidopsis aerial tissue. Further data analysis was carried out separately for primary and secondary metabolites, to discriminate the molecules that could explain these metabolic changes between conditions.

Day and night primary metabolism of Arabidopsis rosettes were not affected by PsJN.

To characterise possible changes in primary metabolites led by PsJN, a total of 33 compounds of carbon and nitrogen central metabolism pathways were analysed in rosettes at 4 L and 6 L developmental stages. Statistical analysis was carried out for ED and EN datasets separately in each developmental stage between PsJN and non-inoculated conditions, to determine if there were differences in diurnal and nocturnal metabolic output in response to PsJN. Through two-way ANOVA analysis it was not found any significant differences between PsJN and non-inoculated conditions in the primary metabolites measured, neither at ED nor at EN. FIGURES 9 and 10 shows the results obtained for some representative primary metabolites measured at ED and EN, respectively

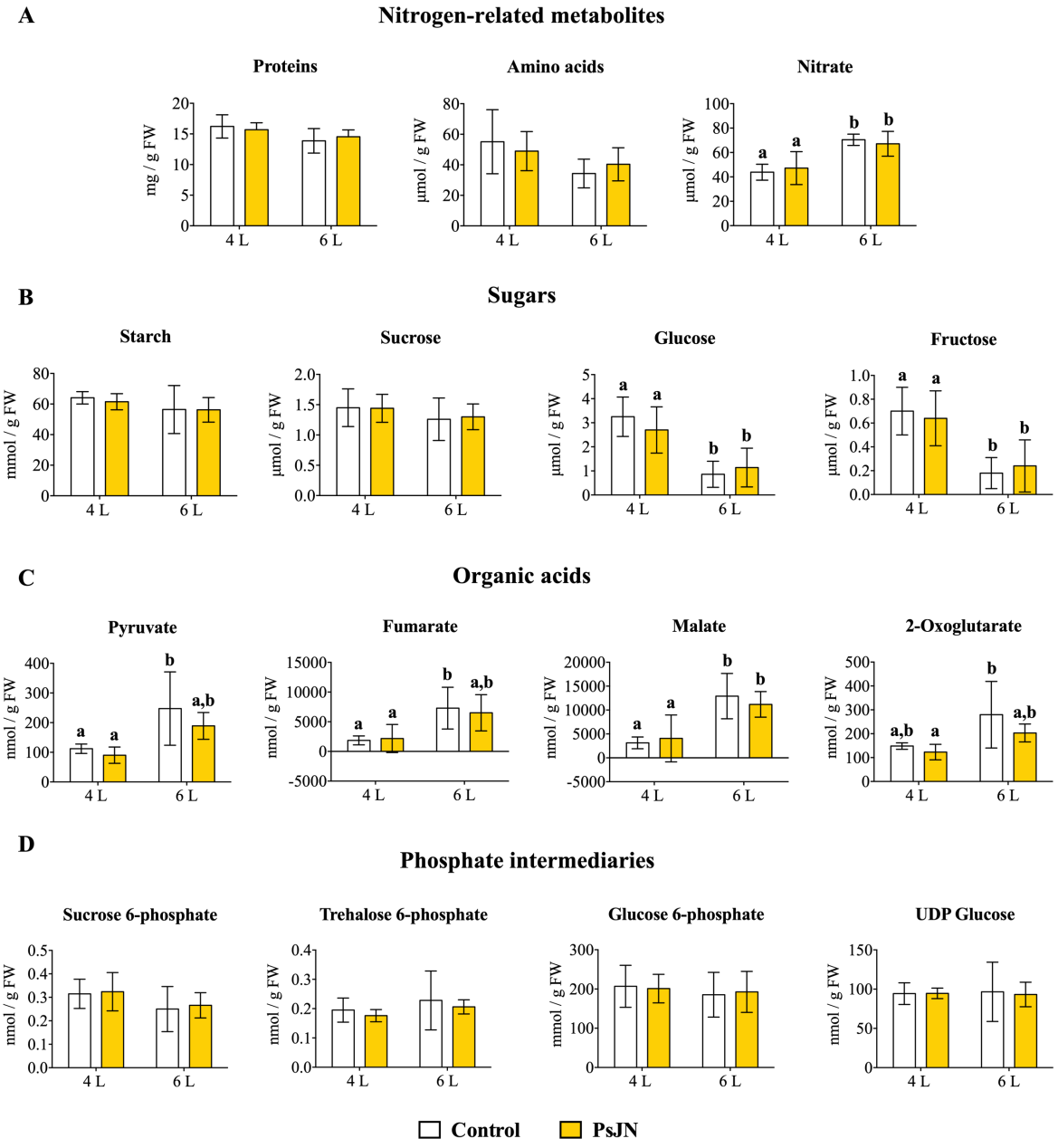


FIGURE 9. Effect of PsJN in primary metabolites of Arabidopsis shoots at the end of the day (ED). Representative primary metabolites measured at ED in Arabidopsis rosettes at 4 L and 6 L developmental stages. Plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated control were grown in 8:16 h photoperiod. Five biological replicates were analysed per developmental stage. The concentration of all metabolites measured was normalised to fresh weight (FW) of each sample. **(A)** Nitrogen-related metabolites. **(B)** Sugars. **(C)** Organic acids. **(D)** Phosphate intermediaries. Bar plots represent mean and SD values. Letters indicate statistical significance (Two-way ANOVA; Tukey's multiple comparisons test (p-value >0.005) n= 5).

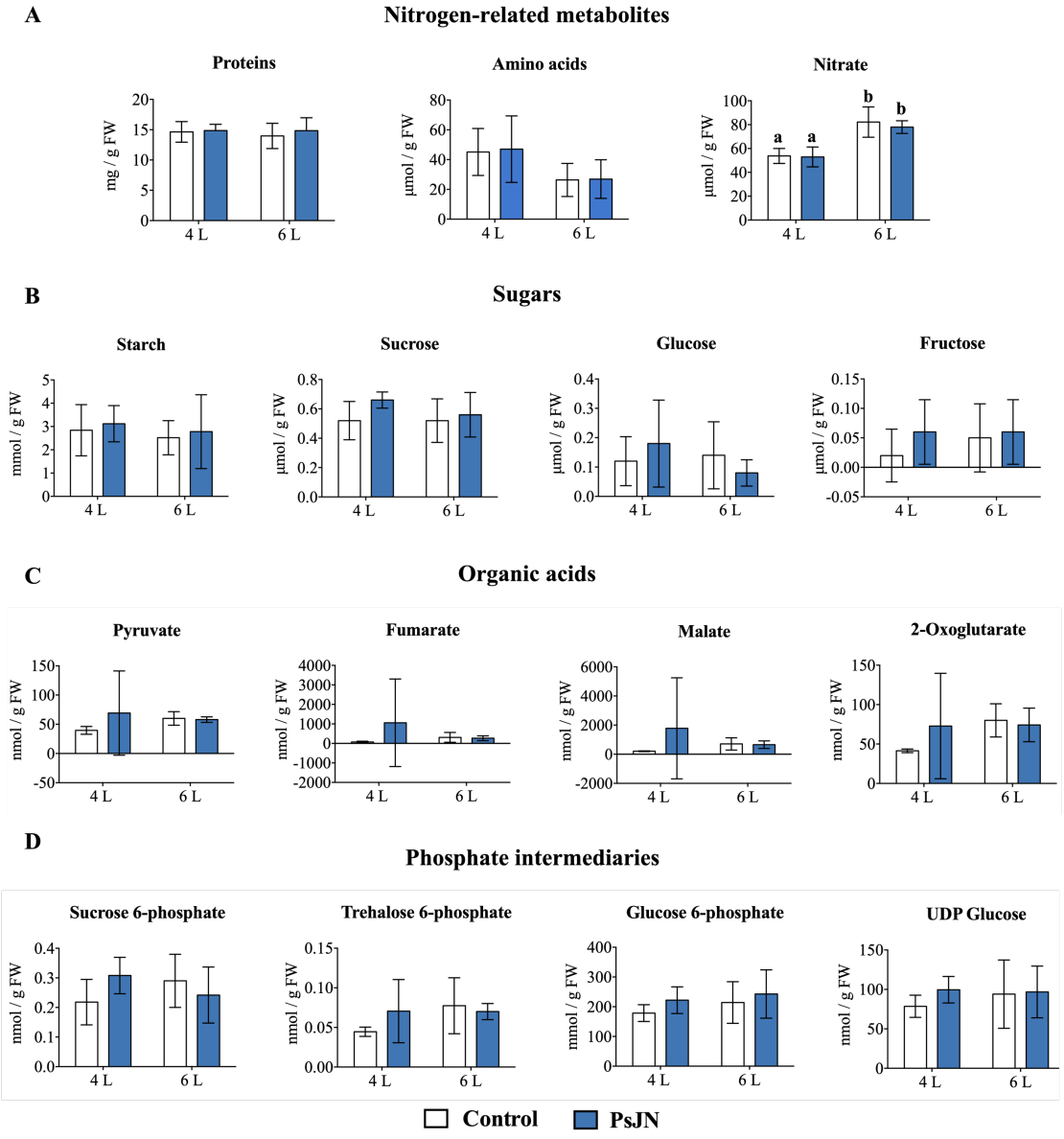


FIGURE 10. Effect of PsJN in primary metabolites of Arabidopsis shoots at the end of the night (EN). Representative primary metabolites measured at EN in Arabidopsis rosettes at 4 L and 6 L developmental stages. Plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated controls were grown under 8:16 h photoperiod. Five biological replicates were analysed per developmental stage. The concentration of all metabolites measured was normalised to fresh weight (FW) of each sample. **(A)** Nitrogen-related metabolites. **(B)** Sugars. **(C)** Organic acids. **(D)** Phosphate intermediaries. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

What did show differences in these metabolites was the transition of developmental stage, where it was found that a few compounds from the whole set of primary metabolites showed statistical differences. Among nitrogen-related metabolites, nitrate was the only compound that showed an increase (over a 51%) on their levels in both, PsJN (47.22±6.01 to 67.14±6.01 [µmol/gFW], respectively) and non-inoculated (43.85±6.37 to 70.43±6.37 [µmol/gFW], respectively) conditions, when plants switched stages from 4 L to 6 L at ED (FIGURE 9A). The same tendency was observed at EN, where the nitrate increment was up to 50% from stage 4 L to 6 L in PsJN (52.95±5.47 to 77.95±5.47 [µmol/gFW], respectively) and non-inoculated (53.77±5.47 to 82.19±5.47 [µmol/gFW], respectively) conditions (FIGURE 10A). Among carbon-related metabolites, starch and sucrose did not exhibit variations in response to developmental stage or PsJN treatment, although the quantities of them changed in response to the day/night metabolism (FIGURES 9B and 10B). In contrast, glucose and fructose levels dropped in the transition from 4 L to 6 L stage at ED in PsJN (glucose: 2.70±0.5 to 1.14±0.5 [µmol/gFW]; fructose: 0.64±0.13 to 0.24±0.13 [µmol/gFW]) and non-inoculated conditions (glucose: 3.25±0.53 to 0.86±0.55 [µmol/gFW]; fructose: 0.7±0.13 to 0.18±0.13 [µmol/gFW]) at ED (FIGURE 8B). However, at EN, glucose and fructose levels were too close to their detection limit; therefore, no comparison could be established (FIGURE 10B). Only four of the ten organic acids measured (TABLE 1) exhibited changes on their levels responding mainly due to the switch between developmental stages (FIGURES 9C and 10C). Malate presented a significative increase from stage 4 L to 6 L in PsJN (4068±2418 to 11182±2418 [nmol/gFW]) and non-inoculated (3134±2565 to 12912±2565 [nmol/gFW]) conditions at ED,

but not at EN, where its total amount was very low and close to their limit of detection, in all conditions.

Furthermore, pyruvate and fumarate exhibited a significant increase in non-inoculated conditions when going from 4L to 6L at ED, although this change was not observed in rosettes inoculated with PsJN (FIGURE 9C). At EN, low levels of these two organic acids were detected in comparison with ED, and there were no changes at different developmental stages, or PsJN treatment (FIGURE 10C). The quantity of all phosphate intermediaries measured was similar among all conditions and did not respond to the developmental stage or PsJN treatment (FIGURES 9D and 10D).

Overall, there were no differences in the primary metabolites analysed between PsJN-treated and non-inoculated samples within each developmental stage, neither at ED nor EN. Hence, PsJN did not induce significant changes in the accumulation pattern of primary metabolites analysed in both time points used in this study.

Based on these observations, it was then determined if the lack of response to PsJN in metabolite quantification was due to a regulation of metabolite levels triggered by another factor. It has been described that primary metabolism is highly regulated by changes in the activity of key enzymes at a post-translational level (Stitt & Gibon, 2014). Therefore, the next step was to measure the maximum enzymatic activity of a group of enzymes involved in the carbon-nitrogen metabolism.

Maximum enzymatic activity was measured for 16 different enzymes involved in distinct metabolic pathways in 4 L and 6 L rosettes from PsJN and non-inoculated conditions in both at ED and at EN (TABLE 2). Statistical analysis did not show significant differences between treated and untreated rosettes in both developmental stages, nor at ED or EN. However,

significant differences were detected in the activity of four of these 16 enzymes at the ED in response to the switch of the developmental stage of rosettes (FIGURE 11), although at EN, the enzymatic activities remained unchanged in all conditions (SUPPLEMENTARY FIGURE 2, APPENDIX).

At ED, NAD-GAPDH, an enzyme involved in glycolysis, showed an increase on its activity only in non-inoculated condition from 4L to 6 L developmental stage (789.7±128 to 1182±128 [mmol*min^{-1}*g^{-1}]) (FIGURE 11). Another enzyme, part of carbon metabolism, that showed changes on its initial activity was RubisCo, which had a significative activity rise in non-inoculated condition rosettes at 6 L compared with rosettes of PsJN condition at 4 L (211.5±24.33 and 282.5±24.33 mmol*min^{-1}*g^{-1}; respectively). Two enzymes involved in nitrogen metabolism NR and Fd-GOGAT also exhibited changes in their maximum activities. The enzyme NR, which produces the reduction of nitrate to nitrite, showed an increase in its activity only in non-inoculated condition rosettes from 4 L to 6 L stage (7.347±2.31 to 19.24±2.31 [mmol*min^{-1}*g^{-1}]. Whereas rosettes of PsJN plants did not show such increase. In the case of Fd-GOGAT, the only significant change on its activity was between 4 L rosettes from non-inoculated plants and 6 L rosettes from PsJN-inoculated plants (34.81±5.25 and 51.99±5.25 mmol*min^{-1}*g^{-1}, respectively). The results observed in NAD-GAPDH and NR activities, suggest that PsJN could have an effect by maintaining unchanged their maximum activities in the transitioning from 4 L to 6 L developmental stages. Contrary, to the significant increase on their activities in this transition as observed in rosettes of non-inoculated condition. Even though there were some significant changes in other enzymatic activities measured such as, the initial activity of RubisCo and Fd-GOGAT, these results lack of biological meaning.

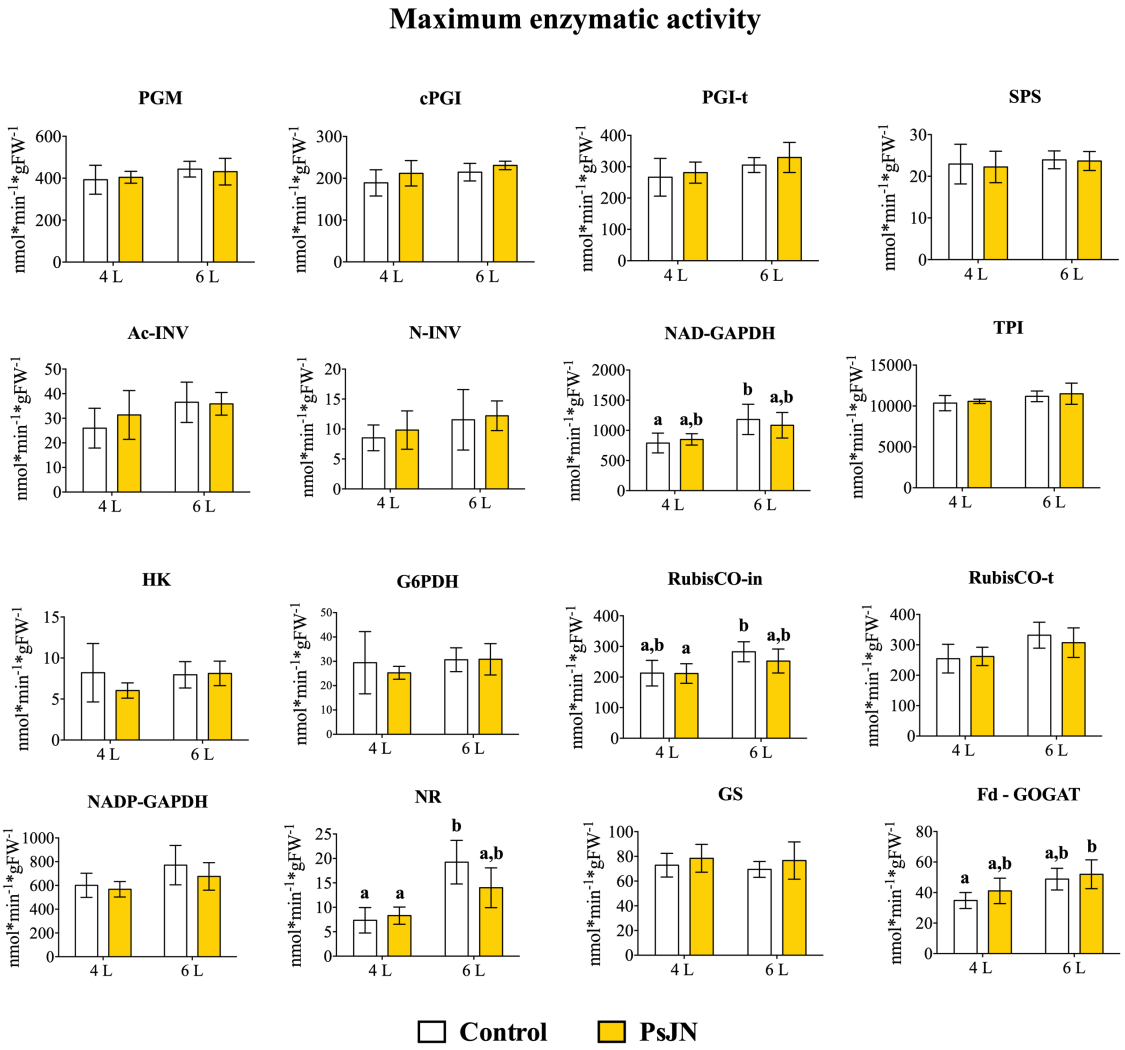


FIGURE 11. Effect of PsJN in enzymatic activity of enzymes form carbon – nitrogen metabolism in Arabidopsis at the end of the day (ED). Sixteen enzymatic activities were determined for enzymes involved in primary metabolism of Arabidopsis. The measurements were carried out in rosettes form plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated for control condition grown under 8:16 h photoperiod and harvested at ED. Five biological replicates were analysed per developmental stage. Maximum enzymatic activities were normalised to fresh weight (FW) and total protein content of each sample. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

Targeted secondary metabolites of Arabidopsis rosettes change mainly in response to developmental stage transitioning.

A total of 712 secondary metabolites were detected by UPLC-MS/MS, corresponding to 41 of the targeted and 671 untargeted compounds. Similarly, to what was observed in the analysis of the primary metabolism, the statistical analysis of targeted metabolites data did not show significant differences between PsJN and non-inoculated conditions within 4 L and 6 L developmental stages. However, a set of 12 (out of 41) compounds showed differences on its levels at ED (FIGURE 12) and EN (FIGURE 13) in response to the transition of developmental stages. From those 12 compounds, eight exhibited changes at ED. Four of them corresponded to flavonoids: quercetin-hexo-rhamnosyl-hexo, quercetin-(2’’-O-hexo) glucoside-7-O-rhamnoside, quercetin-3-O-(2’’-O-hexo) glucoside-7-O-rhamnoside and rutin, which levels exhibited a significant decrease from 4 L to 6 L only in PsJN-treated samples (FIGURE 12A-D). Three dihydroxybenzoic acid glucosides showed changes in their levels. 2,3-dihydroxybenzoic acid 5-O-β-D-glucoside, and 2,5-dihydroxybenzoic acid 5-O-β-D-glucoside increased their levels from 4 L to 6 L only in non-inoculated rosettes (FIGURES 12E-F). 2,3-dihydroxybenzoic acid 5-O-β-D-xyloside, as well as 4-methoxy-3-indolymethyl-glucosinolate (4-methoxyglucobrassicin) showed the same trend observed in flavonoids, where their levels exhibited a significant increase from 4 L to 6 L in PsJN condition (FIGURES 12G and H, respectively). These results suggest that at ED, PsJN has an effect in the accumulation levels of quercetin glucosides, rutin, 2,3-dihydroxybenzoic acid 5-O-β-D-xyloside and 4-methoxy-3-indolymethyl-glucosinolate, driving to a significant increase form 4 L to 6 L stages. This change does not occur in non-inoculated plants.

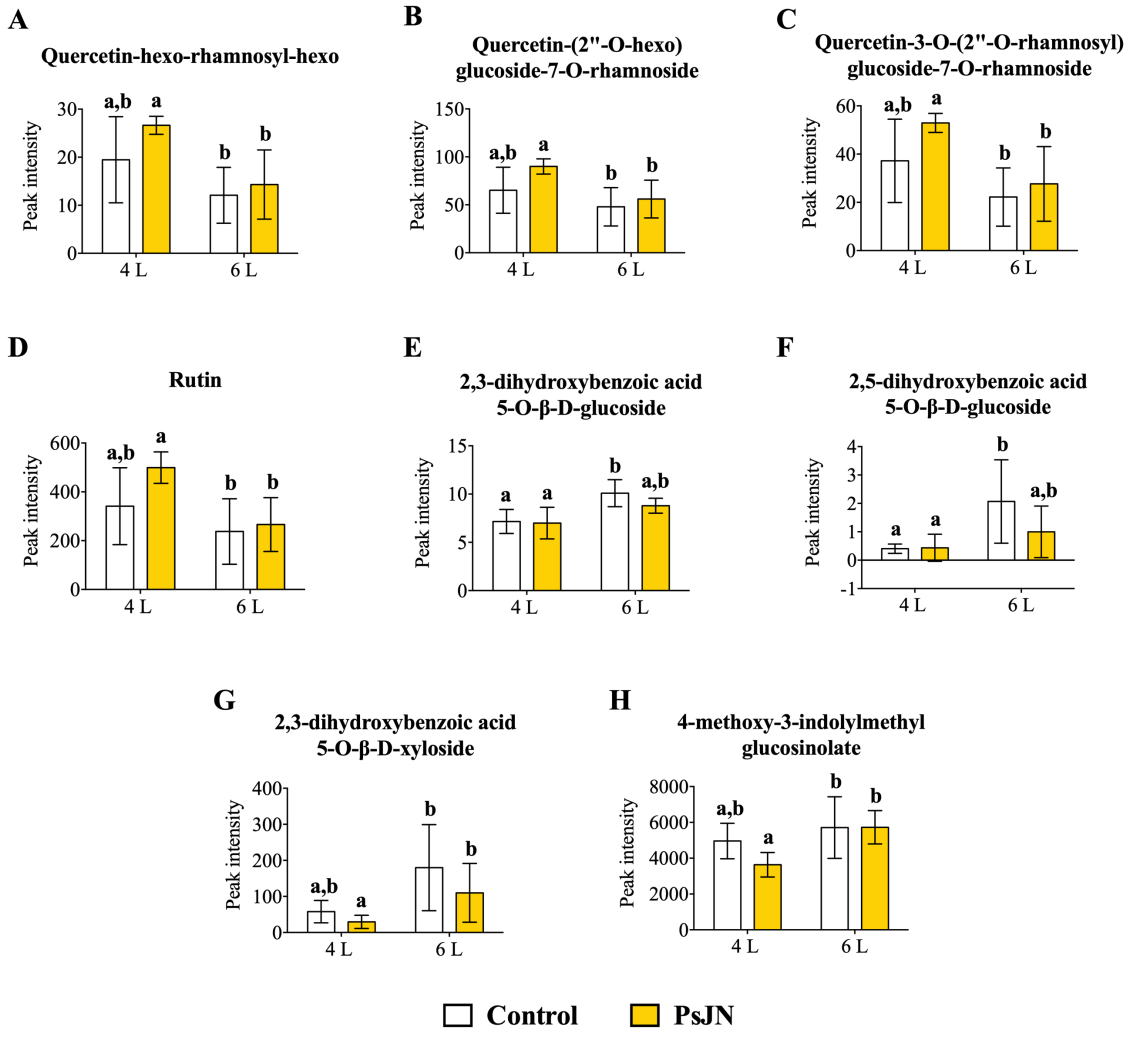


FIGURE 12. Changes in targeted secondary metabolites compounds of Arabidopsis in response to PsJN at the end of the day (ED). (A-H) Secondary metabolites were detected by targeted metabolomics approach using Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS). The analysis was conducted in Arabidopsis rosettes from plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated condition grown under 8:16 h photoperiod and harvested at ED. Five biological replicates were analysed per developmental stage. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

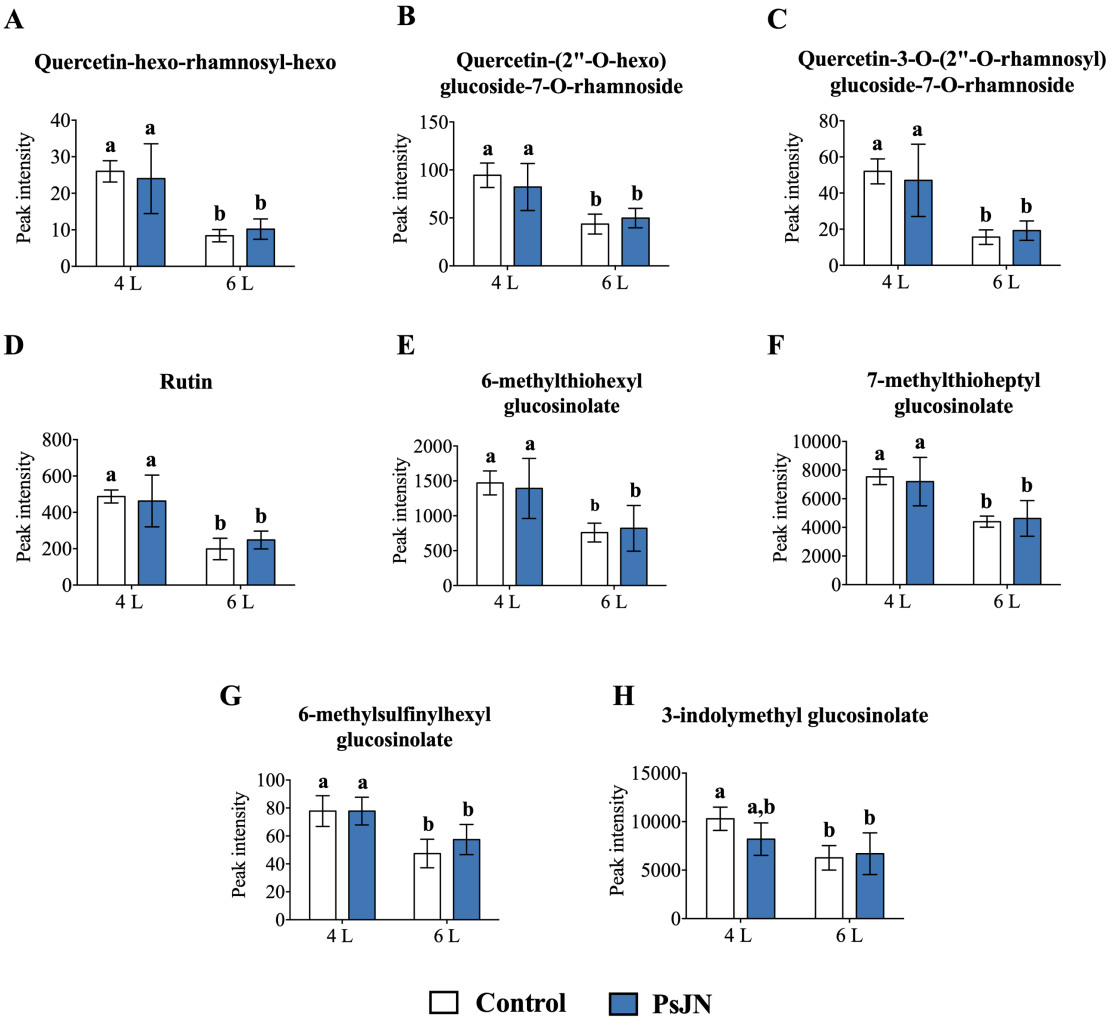


FIGURE 13. Changes in targeted secondary metabolites compounds of Arabidopsis in response to PsJN at the end of the night (EN). (A-H) Secondary metabolites were detected by targeted metabolomics approach using Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS). The analysis was conducted in Arabidopsis rosettes form plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated for control condition grown under 8:16 h photoperiod and harvested at EN. Five biological replicates were analysed per developmental stage. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

By contrast, the compounds 2,3-dihydroxybenzoic acid 5-O-β-D-glucoside and 2,5-dihydroxybenzoic acid 5-O-β-D-glucoside increase their levels in response to the switching of developmental stage in non-inoculated conditions, but in PsJN condition such change do not occur. This suggest that PsJN somehow limits their accumulation in 6 L rosettes.

Eight targeted secondary metabolites responded to the switch of developmental stage at EN of which four also presented changes at ED (FIGURE 13A-D), and four changed exclusively at EN. From the first group, quercetin-hexo-rhamnosyl-hexo, quercetin-(2’’-O-hexo) glucoside-7-O-rhamnoside, quercetin-3-O-(2’’-O-hexo) glucoside-7-O-rhamnoside and rutin, exhibited a significant decrease on their levels from 4 L to 6 L stages in both, PsJN and non-inoculated conditions (FIGURE 13A-D), same pattern observed at ED (FIGURE 12A-D). The glucosinolate derivatives that changed exclusively at EN correspond to 6-methoxythiohexyl glucosinolate, 7-methoxythioheptyl glucosinolate, 6-methoxysulfinylhexyl glucosinolate and 3-indolymethyl glucosinolate. The levels of the three first compounds decreased when transitioning from 4 L to 6 L in both conditions, whereas the last one only showed a significant decrease from 4 L to 6 L in rosettes from the untreated condition (FIGURES 13E-H).

Unknown secondary metabolites explain the variability observed in the global metabolomic analysis of Arabidopsis rosettes in response to PsJN treatment

Using an untargeted-metabolomic approach, variations in some unknown metabolites were detected in response to the plant developmental stage, treatment, and to the interaction of these two factors. A total of 36 and 106 of 671 metabolites changed at ED and EN, respectively (FIGURES 14A and 15A, respectively).

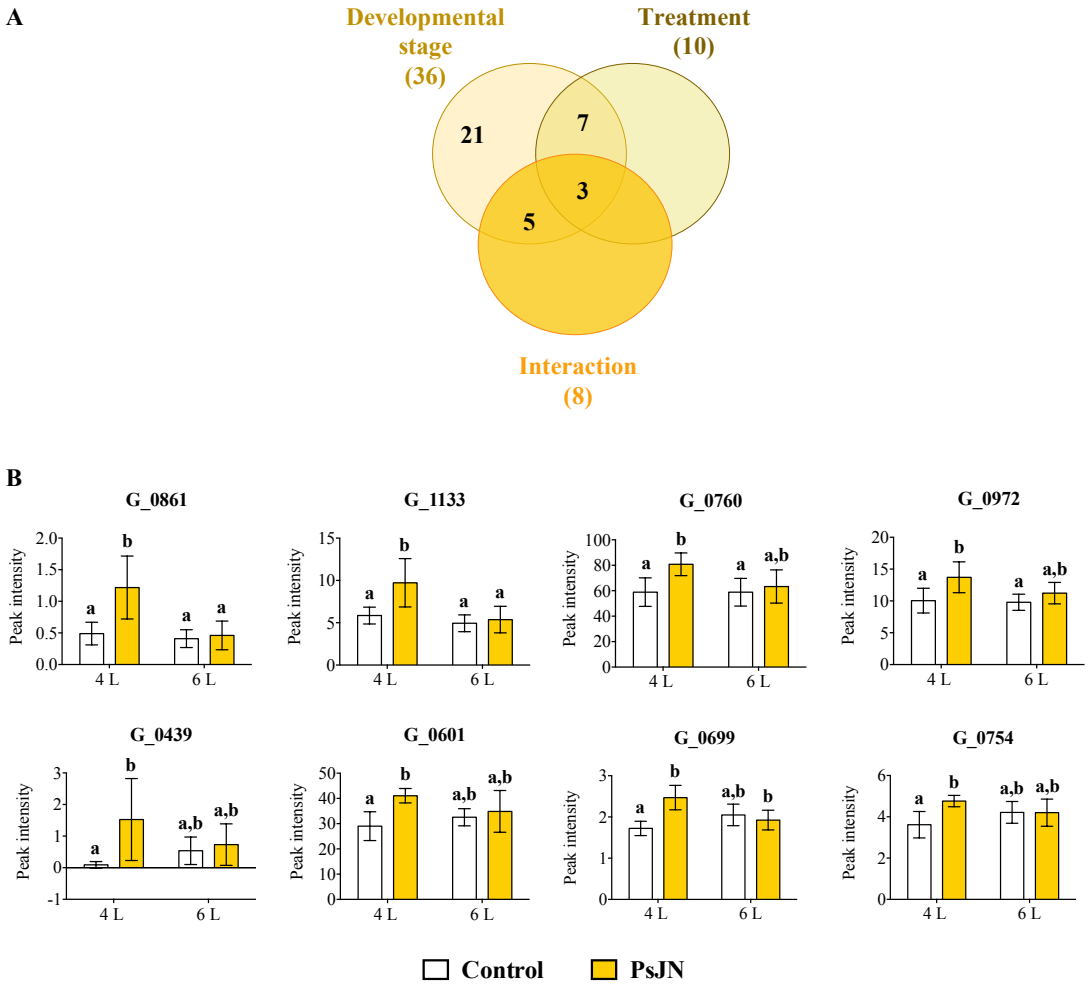


FIGURE 14. Untargeted metabolites differentially accumulated at the end of the day (ED) in 4 L rosettes. Secondary metabolites were detected by the untargeted metabolomics approach using Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS). The analysis was conducted in Arabidopsis rosettes form plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated as control condition grown under 8:16 h photoperiod and harvested at ED. Five biological replicates were analysed per developmental stage. Peak intensity values for each metabolite detected were normalized by sample fresh weight [mg]. **(A)** Venn diagram of compounds with significant changes in rosettes in response to developmental stage, PsJN-treatment, and the interaction of both factors (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5). **(B)** Eight untargeted metabolites were increased in 4 L rosettes in PsJN plants compared with the non-inoculated condition. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

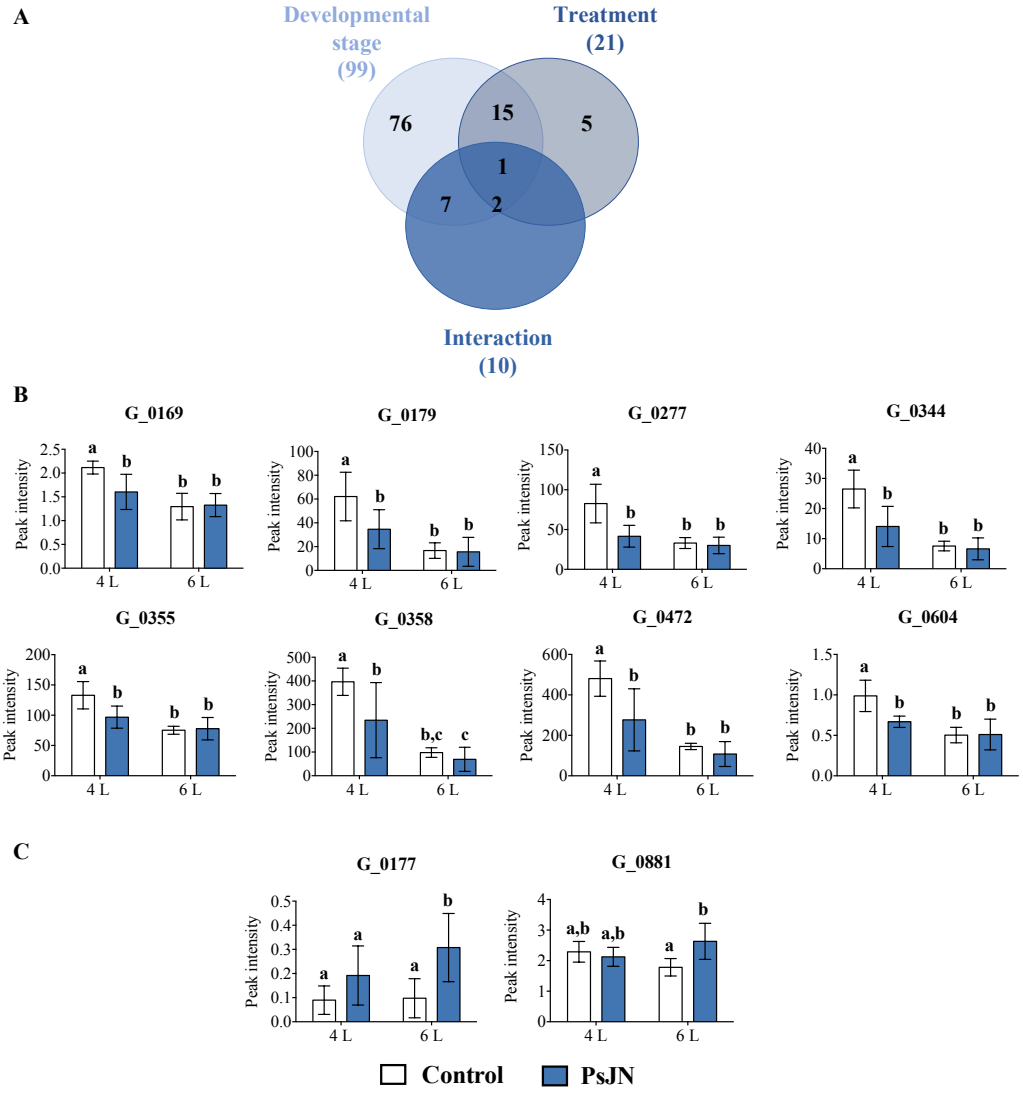


FIGURE 15. Untargeted metabolites differentially accumulated at the end of the night (EN) in 4 L and 6 L rosettes. Phenylpropanoid derivative metabolites were detected by the untargeted metabolomics approach using Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS). The analysis was conducted in Arabidopsis rosettes from plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated condition grown under 8:16 h photoperiod and harvested at EN. Five biological replicates were analysed per developmental stage. Peak intensity values for each metabolite detected were normalized by sample fresh weight [mg]. **(A)** Venn diagram of compounds with significant changes in rosettes as triggered by developmental stage, PsJN-treatment, and/or the interaction of both factors (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5). **(B)** Eight untargeted metabolites were decreased in 4 L rosettes in PsJN plants compared with the non-inoculated condition **(C)** Two untargeted metabolites were increased in 6 L rosettes in PsJN plants compared with the non-inoculated condition. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

At ED a set of 8 metabolites exhibited an increase in the interaction of developmental stage and PsJN treatment factors (FIGURE 14A). These compounds were: G_0861, G_1133, G_760, G_0972, G_0439, G_0601, G_0699 and G_0754, which showed a significant increase on their levels in 4 L rosettes in response to PsJN. The levels of G_0861 and G_1133 were significantly higher in 4 L rosettes from PsJN plants than in 4 L rosettes from non-inoculated condition and in 6 L rosettes from PsJN and non-inoculated condition as well. The rest of the compounds showed a different trend, where their levels remained unchanged in 6 L rosettes in both, PsJN and non-inoculated conditions (FIGURE 14B). At EN a different set of eight peaks (G_0169, G_0179, G_0277, G_0344, G_0355, G_0358, G_0472 and G_0604) showed significant decrease on their levels in 4 L rosettes from the PsJN condition compared with the non-inoculated condition (FIGURE 15B). Their amounts were as low as observed in 6 L rosettes from both conditions at the same day time. These observations suggest PsJN has an effect by decreasing the amount of these metabolites in 4 L rosettes to their amount present in 6 L. The peaks G_0177 and G_0881 were the only metabolites that changed at 6 L, showing higher levels in rosettes from plants inoculated with PsJN condition (FIGURE 15C). G_0177 levels were similar between 4 L and 6 L rosettes form non-inoculated condition, as well as 4 L rosettes form PsJN condition, however it was significantly increased in 6 L rosettes in PsJN condition. Therefore, PsJN drives to a higher accumulation of this metabolite in the transition from 4 L to 6 L rosettes. Contrary, G_0881 levels in 6 L rosettes form PsJN condition did not show differences with its levels in 4 L rosettes from PsJN and non-inoculated conditions. This suggest that PsJN is increasing its accumulation in 6 L developmental stage, by maintaining comparable amounts of it to what was observed in 4 L rosettes.

Because unknown molecules produced the metabolic differences found between rosettes of PsJN-inoculated and non-inoculated plants, the following step was to decipher the identity of compounds corresponding to the abovementioned 18 compounds. For this, a correlation analysis using mass and peak intensities data of the 18 unknown compounds of interest, with data of well-characterised metabolites of the targeted metabolites' library was performed. In this way, three potential groups to classify the metabolites of interest were determined (SUPPLEMENTARY TABLE 3, APPENDIX). Six compounds correlated with glucosinolate derivatives, three with small molecules, two with flavonoid derivatives, and seven remained unclassified.

Among compounds increased at ED in 4 L rosettes in response to PsJN, four corresponded to glucosinolate derivatives, and one to flavonoid derivatives, whereas compounds that were significantly decreased at EN in 4 L rosettes from PsJN condition were two glucosinolate derivatives, one flavonoid derivatives, and three compounds corresponded to small molecules. A group of small molecules was also detected in 6 L rosettes. These results suggest that the metabolism of glucosinolates and flavonoids of rosettes exhibited changes in PsJN-inoculated plants that could be related directly or indirectly to the plant response to this PGPR.

OBJECTIVE 3: To analyse changes in hormonal pathways in *A. thaliana* rosettes in response to the growth-promotion driven by *P. phytofirmans* PsJN.

PsJN accelerates rosette development through gene expression activation of genes involved in hormonal pathways and cell growth.

To further study the phenotypic response of Arabidopsis rosettes to the interaction with PsJN, changes in transcript accumulation of a selected group of gene markers involved in hormonal pathways, stress response and cell growth were analysed (FIGURE 16).

By the fold change analysis of relative gene expression of PsJN to non-inoculated condition in 4 L and 6 L rosettes, up and downregulation in transcriptional levels of some genes of hormonal pathways related to positive regulation of plant growth was detected. The IAA pathway genes *ASB1* and *TAA1* (biosynthesis), *ARF7* and *IAA1* (signalling) in 4 L rosettes, as well as *TAA1* and *ARF7* in 6 L rosettes were upregulated at ED and downregulated at EN. These results suggest that PsJN stimulates the activation of IAA pathway during the day by promoting its synthesis in aerial tissue of rosettes during the day.

Transcriptional levels of some selected genes of Cytokinins (Cks) pathway were upregulated in 6 L rosettes at ED and downregulated at EN in PsJN samples relative to non-inoculated condition. These genes were: *IPT3* (Cks biosynthesis), *AHK4* (Cks receptor), *CGA1* (or *ARR20*) and *GNC* (Cks signalling), although, *AHK4* exhibited increased transcriptional levels in 4 L rosettes at ED and EN as well. The genes of Brassinosteroids (BRs) pathway *BR6ox2* and *DWF4* (biosynthesis) were upregulated at ED in 4 L and 6 L rosettes and downregulated at EN, but the *BEE3* gene involved in BRs signalling showed a different expression pattern.

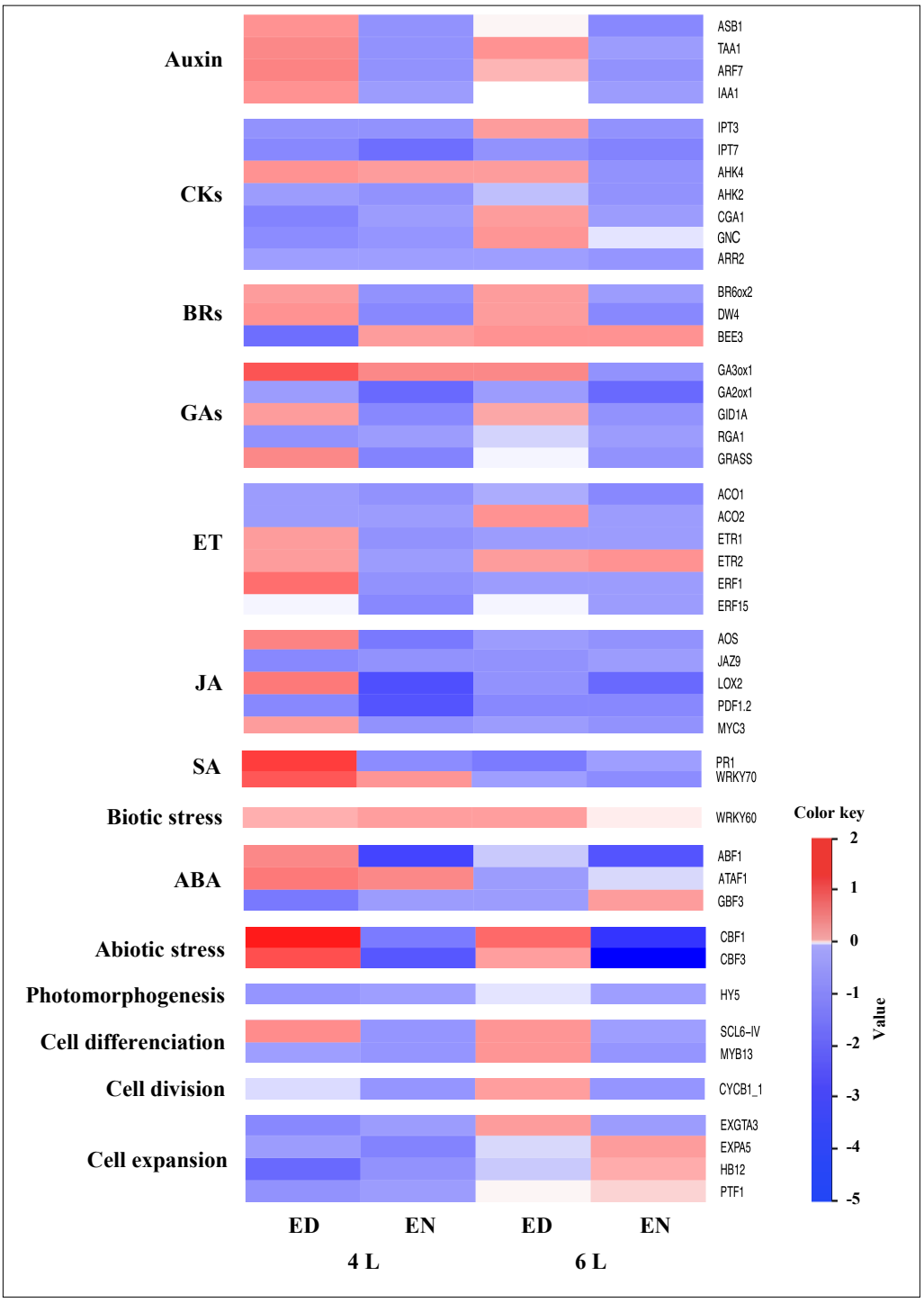


FIGURE 16. PsJN driven changes in expression levels of genes involved in hormone pathways and growth in Arabidopsis rosettes. Gene expression levels in Arabidopsis rosettes inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) were normalised to non-inoculated control plants. Heat map represents log2 of fold change analysis. Two to five biological replicates were analysed per developmental stage at both, at the end of the day (ED) and the end of the night (EN).

The expression of GAs pathway genes was also upregulated in 4 L and 6 L rosettes at ED: *Ga3ox1* (GA4 biosynthesis), *GID1A* (GAs receptor) and *GRASS* (AT3GA9950 signalling). This last gene was induced in 4 L rosettes only. These genes were downregulated at EN in both developmental stages, with the exception of *Ga3ox1*, which was upregulated in 4 L rosettes at EN, but not in 6 L rosettes.

Gene expression analysis in rosettes of PsJN relative to non-inoculated condition related to the hormonal pathways ET, JA and SA was also addressed. These hormones are involved in negative regulation of shoot growth and responses to biotic stress. Some genes of the ET pathway were upregulated in 4 L and 6 L rosettes at ED. The ET signalling genes *ERT1* and *ETR2*, as well as the ET responsive transcription factor ERF1 were upregulated at 4 L rosettes at ED and repressed at EN. However, *ACO1* and *ACO2* genes, which encode the enzyme ACC oxidase catalysing the last step of ET synthesis, were downregulated in these samples. Still, a transcriptional upregulation of *ACO2* and *ETR2* in 6 L rosettes at ED was observed. ERF15, another responsive ET transcription factor did not show changes in gene expression at ED, however its expression levels were downregulated at EN.

Gene expression levels of JA and SA pathway genes tested were upregulated in 4 L rosettes at ED only. Genes *AOS*, *LOX2* and *MYC3* involved in JA biosynthesis and signalling, respectively, as well as *PR1* and *WRKY70* gene markers for SA-mediated response, showed increased transcriptional levels in these conditions. Most of the JA and SA related genes tested were downregulated in 4 L rosettes at EN and 6 L rosettes at ED and EN, with the exception of *WRKY70*, which was induced in 4 L rosettes at EN as well. Concerning other biotic stress genes, it was also observed increased expression levels of *WRKY60*, a pathogen-induced transcription factor. Interestingly, this was the only gene induced in all conditions.

Changes in transcriptional levels in PsJN condition relative to non-inoculated condition of Abscisic Acid pathway (ABA) genes were detected at ED and EN. ABA signalling (*ABF1*) and response (*ATAF1*) genes were upregulated in 4 L rosettes at ED. Gene *ABF1* was solely induced at ED and downregulated at EN in 4 L, as well as in 6 L rosettes at ED and EN. *ATAF1* was equally upregulated in 4 L rosettes and downregulated in 6 L rosettes at ED and EN, respectively. In contrast, *GBF3,* another ABA-response gene, was upregulated only in 6 L rosettes at EN and downregulated in the other conditions. The expression of the transcription factors *CBF1* and *CBF3,* also involved in abiotic stress responses, were strongly upregulated in 4 L and 6 L rosettes at ED and downregulated at EN.

Finally, gene expression changes in a group of genes associated with plant growth and development were analysed. Increased transcriptional levels in genes involved in cell division, expansion and differentiation were found. The expression of the genes *CYCB1* (cell division), *EXGTA3* (cell expansion), *SCL6-IV* and *MYB13* (cell differentiation) was upregulated in 6 L rosettes at ED and downregulated at EN, as well as in 4 L developmental stage at ED and EN. Only transcriptional levels of *SCL6-IV* were increased in both, 4 L and 6 L rosettes at ED. The genes related to cell expansion analysed (*EXPA5*, *HB12* and *PTF1*) exhibited increased transcriptional levels in 6 L rosettes at EN and decreased at ED. Their transcriptional levels were also downregulated in 4 L rosettes in both, ED and EN. Transcriptional levels of the gene *HY5* (involved in photomorphogenesis) were downregulated in 4 L and 6 L rosettes at ED and EN.

These results suggest that PsJN is driving changes in transcriptional levels of genes involved in hormonal pathways and cell processes that positively modulate plant growth, as well as in genes associated with biotic and abiotic stress responses.

DISCUSSION

This study aimed to characterise the metabolic and hormonal changes underlying the phenotypic effects of vegetative growth-promotion of PsJN in Arabidopsis aerial tissue. Through the implementation of an accurate experimental design, it was possible to determine that PsJN promotes the acceleration of leaf development in Arabidopsis. Moreover, its effects in aerial vegetative growth are stronger when plants are subjected to low carbon availability (8:16 h photoperiod). In this regard, in this thesis it was decided to conduct the characterisation of all Arabidopsis' rosette molecular responses by comparing PsJN-inoculated plants v/s non-inoculated condition at the same developmental stage, becoming to the first study in applying this type of experimental design. The results obtained in this thesis showed that PsJN also conduces to changes in transcript accumulation related to hormonal pathways and in the accumulation of secondary metabolites. More importantly, these changes can be associated with cell growth, leaf development, and biotic and abiotic stress, which could be related to priming for further activation of ISR and IST.

PsJN promotes vegetative plant growth without affecting seed germination.

The setting of culture conditions to study Arabidopsis-PsJN interaction system was crucial to standardise one unique experimental condition to test the hypothesis proposed in this thesis. It was found that a period of five days was the best stratification time for full synchronisation of seed germination and to observe the stronger effect of PsJN. This showed that PsJN does not affect the germination process of Arabidopsis seeds. Therefore, its effect on seed-to-plant development is not given by a variation in the germination rate. Some authors have reported for other PGPR models, the capacity to modify the germination parameters in plants

such as garden cress, maise and quinoa, promoting early seed germination (Mahdi et al., 2020; Peng et al., 2019; Sobariu et al., 2017). In these reports, the bacterial treatment influenced positively early plant growth by increasing the germination rate.

PsJN promotes early vegetative development in Arabidopsis rosette.

A more detailed characterisation of the phenotypic aspects of vegetative aerial growth in response to PsJN was assessed. Previous reports have shown that this strain promotes the development of the root system and rosette growth along with the vegetative development, as well as other plant growth-parameters like plant FW and DW (Poupin et al., 2013; Su et al., 2016; Su et al., 2015; Zúñiga et al., 2013).

To understand how PsJN was changing the dynamic of rosette size augmentation in time, AGR and RGR were determined. PsJN-treatment showed an effect in the AGR of rosettes, but not in RGR, meaning that PsJN drives to a major growth velocity of rosettes. PsJN inoculation also conduced to the early emergence of true leaves with one to two days of anticipation in treated plants but, did not produce differences in the number of true leaves when plants started their reproductive development. Therefore, PsJN accelerates vegetative development in a temporal, but not in a spatial dimension. These observations were consistent, in part, with previous reports where it was determined that PsJN leads to an acceleration of the whole life cycle of Arabidopsis plants (Poupin et al., 2013). Still, until now, there was no evidence of significant changes in the number of leaves per day, nor variations in the time needed to reach vegetative developmental stages as a consequence of PsJN exposure. Interestingly, it was observed that PsJN-treated plants had a smaller rosette area than non-inoculated plants when compared at the same developmental stage (SUPPLEMENTARY FIGURE 3, APPENDIX).

These results were crucial for this research and allowed to establish the proper experimental design to test the hypothesis proposed in this thesis.

Up to date, several authors have described some of the hormonal, transcriptional, biochemical and metabolic changes in Arabidopsis plants in response to PsJN at the same plant chronological age (Park & Lazarovits, 2014; Poupin et al., 2013; 2016; Su et al., 2016, 2015), as well as in other plants as potato, switchgrass and grapevine (Da et al., 2012; Fernandez et al., 2012; Theocharis et al., 2012; Wang et al., 2015). But none of these studies has taken into account conducting the characterisation of PsJN effects in plants at the same morphological age (developmental stage).

The latter is a relevant issue, because the results obtained in this thesis are not fully comparable with those previously reported. On one hand, because they describe some of the phenotypical and molecular plant's responses to PsJN in the same chronological age, and do not consider that analysed plants (inoculated and non-inoculated) in the same time point are in different developmental stages, therefore in different physiological states. On the other hand, and based on the observations made in this thesis, it is difficult to dissect if the previous characterisation of PsJN effects in plant-growth promotion are influenced by the differences in the developmental state that plants are going through, or if they are in part biased because of it.

PsJN stimulates rosette growth through cell and leaf enlargement.

Growth and development are the results of cell division, expansion and differentiation, as well as organogenesis (Doerner, 2008; Tessmer et al., 2013). Therefore, to understand how PsJN promotes rosette growth and accelerates aerial vegetative development, phenotypic and molecular aspects related to cell division, expansion and differentiation were analysed.

Rosette area growth exhibited a significant increase since the emergence of the first pair of true leaves in PsJN-inoculated plants, as well as rosette fresh and dry weight. This last, is a key result, since dry weight is the indicator of a major amount of tissue. On the other hand, it was also determined that the main contribution to rosette area augmentation was given by an increase of leaf area and length. This result was consistent with cell size, where cells from the adaxial and abaxial epidermis, as well as mesophyll layers, had a significant area increase in samples inoculated with PsJN than non-inoculated condition. Leaf and cell phenotypes suggest that a possible model to explain how PsJN promotes rosette growth might be linked, in part, to cell expansion by producing larger cells and leaves in Arabidopsis. This hypothesis was supported by gene expression analysis in rosettes of PsJN condition relative to non-inoculated condition, where it was observed an increase in *EXGTA3* and *EXPA5* transcriptional levels at 6 L rosettes from PsJN condition at ED and EN, respectively. These genes encode for the endoxyloglucan transferase A3 (*EXGTA3*) and expansin A5 (*EXPA5*), proteins actively involved in cell expansion through cell wall biogenesis (Matsui et al., 2005) and loosening (Park et al., 2010), respectively. Moreover, *HB12* (or *ATHB12*), a transcription factor regulates leaf development by promoting cell expansion through the activation of genes as *EXPA5* (Hur et al., 2019), was upregulated in 6 L rosettes at EN as well. Intriguingly, *PTF1* (or *TCP13*), an upstream inhibitor of *HB12* (Hur et al., 2019) was also slightly induced in 6 L rosettes at EN. This suggests that *PTF1* level may not be sufficient to supress *HB12* expression, and therefore, to downregulate cell expansion in these conditions. Another gene which exhibited upregulation in 6 L rosettes at ED was *CYCB1* (Cyclin-dependent protein kinase B1). This gene has been widely used as a cell proliferation marker (Schnittger & De Veylder, 2018). Thus, it is possible that PsJN enhances rosette growth by inducing cell division and expansion in Arabidopsis aerial

vegetative tissue. Remarkably, all of these genes were downregulated in 4L rosettes in both, at ED and EN.

Likewise, augmented transcriptional levels of *SCL6-IV* (or *LOM3*), which belongs to *LOST MERISTEMS* (*LOM*) genes, were detected in 4 L and 6 L rosettes at ED and decreased at EN in PsJN condition. *LOM* genes are involved in the promotion of cell differentiation at the periphery of shoot meristems, as well as maintaining their polar organisation (Schulze et al., 2010; Wang et al., 2010). Transcriptional levels of *HY5* were downregulated in rosettes of PsJN condition relative to non-inoculated condition in both developmental stages and timepoints tested. HY5 is a central regulator of seedling development by promoting photomorphogenesis (Gangappa & Botto, 2016; Zhang et al., 2017). HY5 supresses IAA, GAs and BRs signalling, by promoting expression of auxin signalling inhibitors, inducing the expression of the GAs catabolic enzyme *Ga2ox2*, and by interfering with the expression and protein stability of BZR1, BRs signalling protein (Gangappa & Botto, 2016). *HY5* transcriptional downregulation correlates with the upregulation observed in IAA, GAs and BRs pathways. However, in which way PsJN could be involved in HY5 downregulation is still unknown.

Hence, these results show that PsJN promotes rosette growth by inducing a well-coordinated gene upregulation of cell division and differentiation at ED and cell expansion mostly at EN. These processes together contribute to plant growth-promotion, which can be evidenced mainly at the 6 L developmental stage. Noteworthy, PsJN drove downregulation of cell growth genes in 4 L rosettes (with the exception of *SCL6-IV*). Another interesting observation is that PsJN inoculation had a contrary effect in gene expression levels in most of these genes, depending on the time of the day. Therefore, if some gene exhibited upregulation at ED, its expression was downregulated at EN, and vice versa. As expected, these results were

directly correlated with changes in gene expression of plant-growth hormonal pathways, as it is discussed later.

PsJN enhances aerial plant growth in short-day photoperiod.

Day length variates during every year, going from long days in summer to short days in winter (Nagano et al., 2019). These variations implicate great changes in carbon availability in plants, to which they respond by producing metabolic adjustments to buffer this carbon supply imbalance to avoid affecting growth (Gibon et al., 2009).

In this thesis was observed that PsJN-treated plants exhibited a significant increase in rosette area every day since the emergence of the first two true leaves under 8:16 h regime, which was not observed under long or neutral day. In long days, carbon availability is higher; thus, the control of carbon use efficiency is at a relaxed state (Fernandez et al., 2017; Gibon et al., 2009). By contrast, in short-days plants respond by adjusting starch metabolism, increasing its synthesis and decreasing its degradation during day and night, respectively (Fernandez et al., 2017). Therefore, carbon use efficiency and growth are maximised in short days. These observations suggest that PsJN effect on plant-growth promotion could be enhancing those mechanisms involved in carbon use efficiency. Nevertheless, significant changes in starch, soluble sugars, or maximum enzymatic activities related to central carbon metabolism were not detected, neither at ED nor at EN, between rosettes of PsJN and non-inoculated conditions. Hence, PsJN does not affect the net balance of sugars at the end of the light and darkness periods, suggesting that its possible function could be evidenced during these periods, but not at the end of them. Regarding this, time-course experiments along day and night could be useful to unveil how PsJN could be modulating carbon use efficiency to support and enhance growth.

Long cells and leaves, and early leaf emergence strongly suggest that PsJN could be affecting Arabidopsis phyllochron. The phyllochron is defined as the time interval between the appearance of successive leaves, and it is strongly correlated with leaf elongation (Fournier et al., 2005). It has been proved to be driven mainly by thermal time and it is usually expressed as the accumulated growing degree days (Viaud et al., 2017). Interestingly, plant growth-promotion driven by PsJN is enhanced under short-day photoperiod. Therefore, phyllochron determination in Arabidopsis plants appears to be promising to model plant growth and productivity in response to PGPR. This could be addressed by taking daily consecutive images of rosettes since cotyledons are fully opened until bolting as described in Viaud et al. (2017). In this way, it could be possible to model the dynamics of leaf growth and development of the whole plant.

PsJN induces plant growth-promoting hormone pathways.

PsJN leads to the induction of gene expression of some genes of the major classes of hormones that promote plant growth and development: IAA, BRs, Cks and GAs. These phytohormones and their crosstalk regulate pivotal aspects of the vegetative development. They modulate several processes as cell division, expansion and differentiation, morphogenesis, chloroplast development and growth, leaf expansion, assimilate partitioning, among others (Davies, 1987).

Upregulation of gene expression related to IAA biosynthesis and signalling and BRs biosynthesis in 4 L and 6 L rosettes at ED was observed in response to PsJN inoculation. Interestingly, *BEE3* involved in BRs signalling, was induced mainly at EN, contrary to what was observed in the biosynthetic genes. In this regard, other BRs response genes such as *BRI1*

(perception), *BZR1* and *BES1* (signalling) (Planas-Riverola et al., 2019) could be analysed to determine if their expression matches with the expression of BRs synthesis genes at ED. In addition, measurements of IAA and BRs content must be done, to determine if the gene expression variations observed are directly correlated with their levels in rosettes.

Furthermore, Cks are known as an essential factor, as well as IAA, in control of *de novo* organogenesis in shoots and roots (Skoog & Miller, 1957). In this regard, some genes of Cks pathway were induced mainly al 6 L developmental stage in PsJN condition. The Cks biosynthetic gene *IPT3* was induced at ED. This gene encodes to the phosphate-isopentenyl transferase 3, which is transcriptionally active in photosynthetic tissues. In contrast, the biosynthetic gene *IPT7* was downregulated in all conditions tested. This can be explained because *IPT7* has been found being active mainly in the root system (Takei et al., 2004). Upregulation of *AHK4*, *CGA* and *GNC* was also observed; these genes are involved in perception and signalling of Cks, respectively. *AHK4* a cytokinin-binding receptor which transduces Cks signal across plasmatic membrane (Li et al., 2013; Pernisova et al., 2018), exhibited induction in 6 L rosettes at ED, as well as, in 4 L rosettes at ED and EN. This receptor has been implicated in the nuclear activation of Cks signalling genes as *CGA*, involved in leaf pavement cells (PCs) morphogenesis (Li et al., 2013). Recently, an essential role of this receptor linked to Ck synthesis in the control of *de novo*-induced organ identity has also been proposed (Pernisova et al., 2018). CGA and GNC are transcription factors that have been recently described as co-regulators implicated in several aspects of plant growth and development and act as key components of IAA and GAs signalling pathways (Xu et al., 2017). Their function is related to the coordination of chlorophyll synthesis and chloroplast biogenesis in shoots (Zubo

et al., 2018). These results suggest that PsJN could be promoting the early development of leaves through Cks pathway.

Additionally, induction of genes related to GAs pathway, another plant growth-regulating hormone, was also detected in 4 L and 6 L rosettes at ED. GAs are involved in leaf expansion by upregulating genes of cell expansion processes (Griffiths et al., 2006; Xu et al., 2017), as well as in plant developmental transitions (Hauvermale & Steber, 2020). Induction of the gene *Ga3ox1,*which encodes the enzyme involved in later steps of the synthesis pathway of the bioactive gibberellin 4 (GA4) (Eriksson et al., 2006) was observed. Also, upregulation of *GID1A*, that encodes a GA receptor with affinity for GA4 (Iuchi et al., 2007), was observed in the same condition. The GA-GID1 complex participates in the degradation of the proteins DELLAs, the repressors of GA-signalling (Griffiths et al., 2006). GDI1 receptors have an essential role in the promotion of leaf elongation in Arabidopsis (Griffiths et al., 2006). These results suggest that the GA4 pathway is active during early rosette development and could be related to the leaf elongation phenotype observed in response to PsJN. Also, GRASS, a transcription factor involved in lamina maturation mainly active in young leaf lamina (Efroni et al., 2008), was only induced at 4 L rosettes. However, in 6 L rosettes, its transcriptional levels did not show differences with the control condition. The quantification of GA4 levels could be addressed to determine if gene expression induction correlates with the corresponding hormonal levels.

Taken together, these results strongly suggest that PsJN produces the induction of cell, leaf and rosette growth, as well as the initiation of the development of new leaves. This could be explained by a modulatory effect of PsJN on gene expression coordination of hormonal pathways related to plant growth during vegetative development.

Secondary metabolites and stress hormones respond to PsJN during early vegetative development.

In this thesis, the analysis of primary and secondary metabolism was carried out in order to determine plant metabolic changes driven by the interaction with PsJN, and if there was a direct influence on plant growth-promotion. The exploratory analysis of a total of 751 compounds from both metabolisms showed differences between diurnal and nocturnal metabolic output in all conditions, as expected. The variations in day and night metabolite accumulation were mainly driven by a set of unknown secondary metabolite derivatives of the phenylpropanoid pathway in response to PsJN.

These responses occurred mainly at ED, where changes were detected in eight unknown compounds, which were significantly increased in 4 L rosettes from PsJN-inoculated plants. Four were classified as glucosinolate derivatives and one as a flavonoid derivative. Both types of molecules have been mainly related to plant defence, but new evidence has involved them into plant growth and development regulation (Burow & Halkier, 2017; Erb & Kliebenstein, 2020; Katz et al., 2020; Malinovsky et al., 2017). Furthermore, at EN another group of eight different compounds also classified as glucosinolate and flavonoid derivatives and small molecules, was decreased in 4 L rosettes of PsJN-plants compared with the control condition. Rosettes at the 6 L developmental stage showed a significant increase in two compounds, where only one of them could be classified as a small molecule. To which type of glucosinolate and flavonoid derivatives, as well as the small molecules, may correspond the compounds that changed in response to PsJN, is a relevant matter of study. In recent years, it has been proposed that the role of secondary metabolites as plant growth and development regulators is beyond their role in response to environmental cues (Zhang et al., 2017; Erb & Kliebenstein, 2020). In

this regard, it could be interesting to conduct the characterisation of the chemical structure of those metabolites that exhibited significant changes in response to PsJN.

The synthesis of phenylpropanoid derivatives, as glucosinolates and flavonoids have been related to plant responses to the detection of pathogens as the innate immune system for plant defence (Burow & Halkier, 2017), although plant defence responses have been associated with negative effects on plant growth. Stress conditions cause a redirection of energy, that is normally invested in growth and developmental processes, to transcriptional and metabolic reprogramming to face the stressing condition (Caldana et al., 2019; Pieterse et al., 2012; Singh et al., 2002). In this regard, the analysis of gene expression related to plant-defence responses was conducted, finding transcriptional changes in a set of genes involved in the hormonal pathways ET, JA, SA and ABA. These hormones are related to the regulation of plant stress-responses, priming ISR, IST and development (Pieterse et al., 2012, 2014; Thain et al., 2004). Priming is defined as "the sensibilisation of the whole plant for enhanced defence, which is characterised by faster and stronger activation of cellular defences upon pathogen invasion" (Pieterse et al., 2014), and has been proposed that the activation of ISR by PGPR is based on it. In this sense, it was observed upregulation of genes related to these four phytohormones mainly in 4 L rosettes at ED. Moreover, *PR1* and *WRKY70* gene markers for SA-mediated response (Pieterse et al., 2012), showed changes in expression levels in PsJN condition relative to non-inoculated condition, exhibiting upregulation in 4 L rosettes and downregulation in 6 L rosettes. The protein WRKY60, a stress-induced transcription factor activated by pathogen attack, abiotic stress and ABA (Chen et al., 2010; Xu et al., 2006), showed upregulation in all conditions, with the exception of 6 L rosettes at EN, where its transcriptional levels were slightly higher than the non-inoculated condition. Despite these results, it was not observed any evidence of stress-

associated phenotype in plants in none of the conditions studied. Therefore, these transcriptional changes might be related to PsJN-triggered priming of the plant.

Transcriptional induction of some genes of ABA signalling (*ABF1*) and response (*ATAF1*) genes was detected in 4 L rosettes. Likewise, genes encoding the transcription factors *CBF1* and *CBF3*, related to low temperature and ABA responses (Knight et al., 2004), were also upregulated in both 4 L and 6 L rosettes at ED, and strongly downregulated at EN. ABA is a well-known hormone with crucial roles in seed and embryo development, stomatal closure, and abiotic stress responses (Yoshida, et al. 2019). This hormone has also been proposed as part of priming response involved in ISR phenotype in Arabidopsis, through the promotion of callose deposition and stomatal closure (Pieterse et al., 2014).

These observations suggest that PsJN drives to the activation of biotic and abiotic stress signalling in early stages of rosette development, which could be associated with priming, mainly for ISR, but also for IST activation. This phenomenon was mainly observed at ED, meanwhile at EN it was down regulated. Nevertheless, more studies are necessary to unveil how these responses could be interplaying with PsJN plant growth-promotion effects.

FIGURE 16 summarises phenotypical and molecular responses in Arabidopsis' vegetative aerial tissues to the interaction with PsJN.

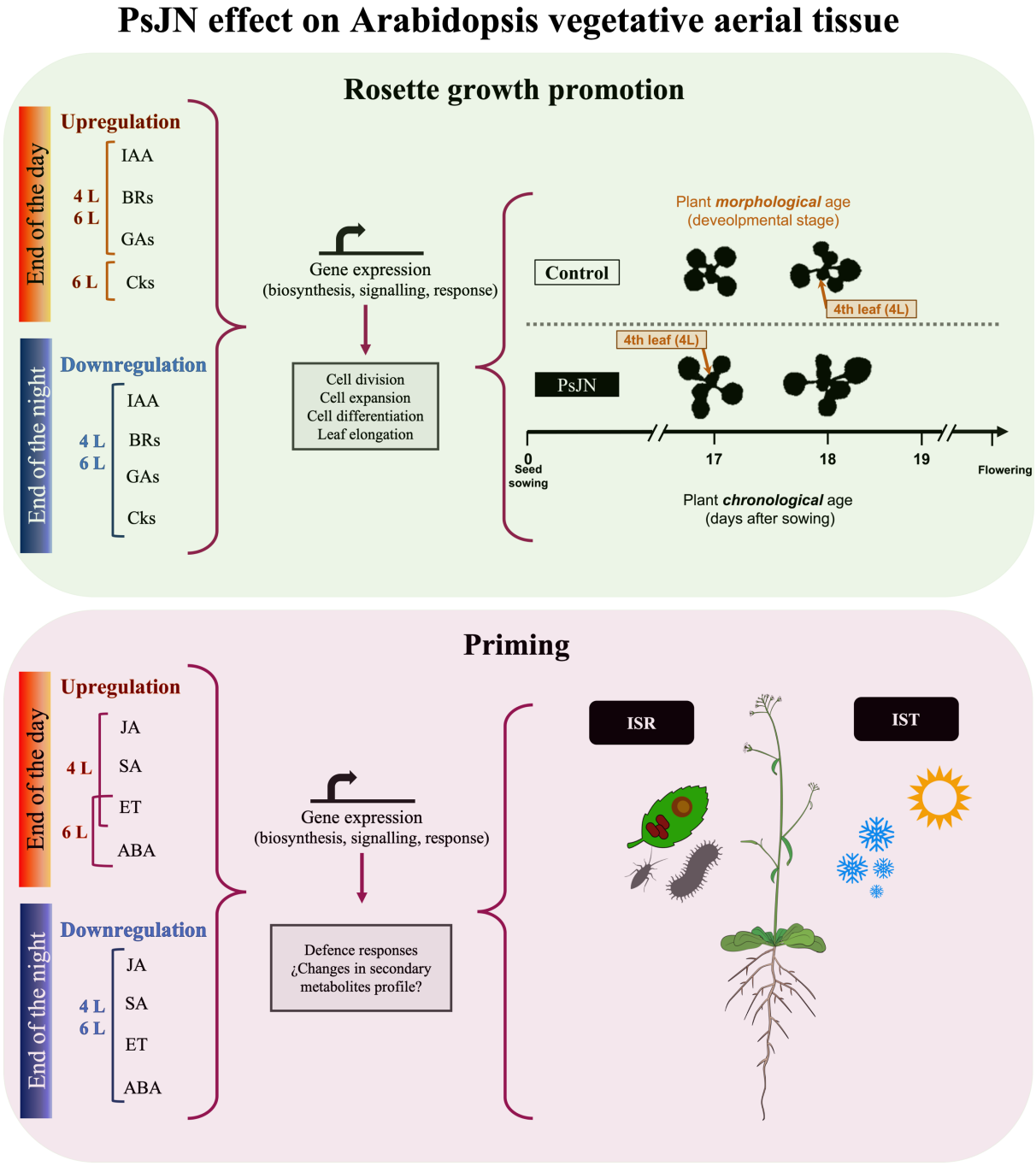


FIGURE 16. Effects of plant-PsJN interaction system in rosette growth, development and priming.

Final considerations.

Finally, it is important to highlight that transcriptional and metabolic changes reported in this thesis shows that PsJN drives to significant differences in Arabidopsis rosettes in two vegetative developmental stages in comparison with non-inoculated plants. These changes correspond not only to the activation of hormonal pathways and accumulation of secondary metabolites at one time point of the day, for instance, at ED, but also that PsJN also drives a strong downregulation of them in the opposite time of the day. In addition, some of the results observed in enzymatic activities related to primary metabolism, as well as in the accumulation of targeted secondary metabolites, suggest that PsJN may have an effect in the regulation of these, which is associated with producing changes in plants in the transitioning of developmental stages.

CONCLUSIONS

- The growth conditions to study Arabidopsis rosette's growth-promotion driven by PsJN is a pivotal aspect to be set before conducting a fine- phenotypical and molecular characterisation of plant's responses. In this thesis, PsJN's effects were enhanced using a long period of seeds stratification, an inoculum of 10^6 CFU/mL and 8:16 h light/darkness regime. In this regard, it was allowed to determine:

- PsJN promotes early vegetative growth of Arabidopsis after germination, by accelerating leaf emergence. Therefore, in this thesis was described some of the Arabidopsis' molecular mechanisms involved in the growth promotion of the aerial vegetative tissues of Arabidopsis triggered by PsJN, by comparing plants at the same morphological age. This last, makes this study the first report using this methodological approach.

- PsJN promotes rosette growth in short-day photoperiod, which could be linked to the carbon use efficiency exhibited by plants under low carbon availability. Besides, PsJN affects the profile of secondary metabolites, which could be associated with their recycling back to primary metabolism as an energy reservoir.

- PsJN also activates hormonal pathways related to defence responses during the early vegetative development, that could be linked to a priming state for further activation of induced systemic resistance and tolerance.

- The effects of PsJN seemed to be closely related to the induction of cell division, expansion and differentiation, as well as leaf elongation, through the transcriptional activation of IAA, BRs, Cks and GAs hormonal pathways.

REFERENCES

Ali, S., Duan, J., Charles, T. C., & Glick, B. R. (2014). A bioinformatics approach to the determination of genes involved in endophytic behavior in *Burkholderia* spp. *Journal of Theoretical Biology*, *343*, 193–198. https://doi.org/10.1016/j.jtbi.2013.10.007

Andersen, C. L., Jensen, J. L., & Ørntoft, T. F. (2004). Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research*, *64*(15), 5245–5250. https://doi.org/10.1158/0008-5472.CAN-04-0496

Ap Rees, T., & Hill, S. A. (1994). Metabolic control analysis of plant metabolism. *Plant, Cell and Environment*, *17*(5), 587–599. https://doi.org/10.1111/j.1365-3040.1994.tb00151.x

Badri, D. V., Weir, T. L., van der Lelie, D., & Vivanco, J. M. (2009). Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology*, *20*(6), 642–650. https://doi.org/10.1016/j.copbio.2009.09.014

Bantan-Polak, T., Kassai, M., & Grant, K. B. (2001). A comparison of fluorescamine and naphthalene-2, 3-dicarboxaldehyde fluorogenic reagents for microplate-based detection of amino acids. *Analytical Biochemistry*, *297*(2), 128–136. https://doi.org/10.1006/abio.2001.5338

Bordiec, S., Paquis, S., Lacroix, H., Dhondt, S., Ait Barka, E., Kauffmann, S., Jeandet, P., Mazeyrat-Goubeyre, F., Clèment, C., Baillieul, F., & Dorey, S. (2011). Comparative analysis of defence responses induced by the endophytic plant growth-promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN and the non-host bacterium *Pseudomonas syringae* pv. pisi in grapevine cell suspensions. *Journal of Experimental Botany*, *62*(2), 595–603. https://doi.org/10.1093/jxb/erq291

Boyes, D. C. (2001). Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. *The Plant Cell Online*, *13*(7), 1499–1510. https://doi.org/10.1105/tpc.13.7.1499

Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B., & Vile, D. (2013). The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in Arabidopsis. *New Phytologist*, *200*(2), 558–569. https://doi.org/10.1111/nph.12383

Burow, M., & Halkier, B. A. (2017). How does a plant orchestrate defense in time and space? Using glucosinolates in Arabidopsis as case study. *Current Opinion in Plant Biology*, *38*, 142–147. https://doi.org/10.1016/j.pbi.2017.04.009

Burrell, M. M., Mooney, P. J., Blundy, M., Carter, D., Wilson, F., Green, J., Blundy, K. S., & ap Rees, T. (1994). Genetic manipulation of 6-phosphofructokinase in potato tubers. *Planta*, *194*(1), 95–101. https://doi.org/10.1007/BF00201039

Caldana, C., Martins, M. C. M., Mubeen, U., & Urrea-castellanos, R. (2019). The magic 'hammer' of TOR : the multiple faces of a single pathway in the metabolic regulation of plant growth and development. *Journal of Experimental Botany*, *70*(8), 2217–2225. https://doi.org/10.1093/jxb/ery459

Casson, S., & Gray, J. E. (2008). Influence of environmental factors on stomatal development. *New Phytologist*, *178*(1), 9–23. https://doi.org/10.1111/j.1469-8137.2007.02351.x

Chen, Han, Lai, Z., Shi, J., Xiao, Y., Chen, Z., & Xu, X. (2010). Roles of arabidopsis WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. *BMC Plant Biology*, *10*(1), 281. https://doi.org/10.1186/1471-2229-10-281

Chen, H., Li, Z., & Xiong, L. (2012). A plant microRNA regulates the adaptation of roots to drought stress. *FEBS Letters*, *586*(12), 1742–1747. https://doi.org/10.1016/j.febslet.2012.05.013

Chong, J., Wishart, D. S., & Xia, J. (2019). Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Current Protocols in Bioinformatics*, *68*(1). https://doi.org/10.1002/cpbi.86

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024

Compant, S., Kaplan, H., Sessitsch, A., Nowak, J., Ait Barka, E., & Clèment, C. (2008). Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: From the rhizosphere to inflorescence tissues. *FEMS Microbiology Ecology*, *63*(1), 84–93. https://doi.org/10.1111/j.1574-6941.2007.00410.x

Compant, S.e, Reiter, B., Sessitsch, A., Nowak, J., Clément, C., & Barka, E. A. (2005). Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia sp*. strain PsJN. *Applied and Environmental Microbiology*, *71*(4), 1685–1693. https://doi.org/10.1128/AEM.71.4.1685-1693.2005

Cross, J. M., Von Korff, M., Altmann, T., Bartzetko, L., Sulpice, R., Gibon, Y., Palacios, N., & Stitt, M. (2006). Variation of enzyme activities and metabolite levels in 24 arabidopsis accessions growing in carbon-limited conditions. *Plant Physiology*, *142*(4), 1574–1588. https://doi.org/10.1104/pp.106.086629

Czechowski, T., Bari, R. P., Stitt, M., Scheible, W. R., & Udvardi, M. K. (2004). Real-time RT-PCR profiling of over 1400 Arabidopsis transcription factors: Unprecedented sensitivity reveals novel root-and shoot-specific genes. *Plant Journal*, *38*(2), 366–379. https://doi.org/10.1111/j.1365-313X.2004.02051.x

Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K., & Scheible, W. R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in arabidopsis. *Plant Physiology*, *139*(1), 5–17. https://doi.org/10.1104/pp.105.063743

Da, K., Nowak, J., & Flinn, B. (2012). Potato cytosine methylation and gene expression changes induced by a beneficial bacterial endophyte, *Burkholderia phytofirmans* strain PsJN. *Plant Physiology and Biochemistry*, *50*, 24–34. https://doi.org/10.1016/j.plaphy.2011.09.013

Davies, P. J. (1987). The Plant Hormones: Their nature, occurrence, and functions. In *Plant Hormones and their Role in Plant Growth and Development* (pp. 1–11). Springer Netherlands. https://doi.org/10.1007/978-94-009-3585-3_1

Dimkpa, C., Weinand, T., & Asch, F. (2009). Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment*, *32*(12), 1682–1694. https://doi.org/10.1111/j.1365-3040.2009.02028.x

Doerner, P. (2008). Signals and mechanisms in the control of plant growth. *Plant Cell Monographs*, *10*, 1–23. https://doi.org/10.1007/7089_2007_142

Donoso, R., Leiva-Novoa, P., Zúñiga, A., Timmermann, T., Recabarren-Gajardo, G., & González, B. (2017). Biochemical and genetic bases of indole-3-acetic acid (auxin phytohormone) degradation by the plantgrowth- promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN. *Applied and Environmental Microbiology*, *83*(1). https://doi.org/10.1128/AEM.01991-16

Dorn, E., Hellwig, M., Reineke, W., & Knackmuss, H. J. (1974). Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. *Archives of Microbiology*, *99*(1), 61–70. https://doi.org/10.1007/BF00696222

Efroni, I., Blum, E., Goldshmidt, A., & Eshed, Y. (2008). A protracted and dynamic maturation schedule underlies Arabidopsis leaf development. *The Plant Cell*, *20*(9), 2293–2306. https://doi.org/10.1105/tpc.107.057521

Erb, M., & Kliebenstein, D. J. (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites : The blurred functional thrichotomy. *Plant Physiology, 184*, 39–52. https://doi.org/10.1104/pp.20.00433

Eriksson, S., Böhlenius, H., Moritz, T., & Nilsson, O. (2006). GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. *The Plant Cell*, *18*(9), 2172–2181. https://doi.org/10.1105/tpc.106.042317

Eveland, A. L., & Jackson, D. P. (2012). Sugars, signalling, and plant development. *Journal of Experimental Botany*, *63*(9), 3367-3377. https://doi.org/10.1093/jxb/err379

Fernandez, O., Ishihara, H., George, G. M., Mengin, V., Flis, A., Sumner, D., Arrivault, S., Feil, R., Lunn, J. E., Zeeman, S. C., Smith, A. S., & Stitt, M. (2017). Leaf starch turnover occurs in long days and in falling light at the end of the day. *Plant Physiology*, *174*(4), 2199–2212. https://doi.org/10.1104/pp.17.00601

Fernandez, O., Theocharis, A., Bordiec, S., Feil, R., Jacquens, L., Clément, C., Fontaine, F., & Barka, E. A. (2012). *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. *Molecular Plant-Microbe Interactions*, *25*(4), 496–504. https://doi.org/10.1094/MPMI-09-11-0245

Fernandez, O., Vandesteene, L., Feil, R., Baillieul, F., Lunn, J. E., & Clément, C. (2012). Trehalose metabolism is activated upon chilling in grapevine and might participate in *Burkholderia phytofirmans* induced chilling tolerance. *Planta*, *236*(2), 355–369. https://doi.org/10.1007/s00425-012-1611-4

Figueroa, C. M., & Lunn, J. E. (2016). A tale of two sugars: Trehalose 6-phosphate and sucrose. *Plant Physiology*, *172*(1), 7–27. https://doi.org/10.1104/pp.16.00417

Fournier, C., Durand, J. L., Ljutovac, S., Schäufele, R., Gastal, F., & Andrieu, B. (2005). A functional-structural model of elongation of the grass leaf and its relationships with the phyllochron. *New Phytologist*, *166*(3), 881–894. https://doi.org/10.1111/j.1469-8137.2005.01371.x

Frommel, M. I., Nowak, J., & Lazarovits, G. (1991). Growth enhancement and developmental modifications of in vitro grown potato (*Solanum tuberosum* spp. *tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp. *Plant Physiology*, *96*(3), 928–936. https://doi.org/10.1104/pp.96.3.928

Gangappa, S. N., & Botto, J. F. (2016). The Multifaceted Roles of HY5 in Plant Growth and Development. *Molecular Plant*, *9*, 1353-1365. https://doi.org/10.1016/j.molp.2016.07.002

Giavalisco, P., Köhl, K., Hummel, J., Seiwert, B., & Willmitzer, L. (2009). ^{13}C isotope-labeled metabolomes allowing for improved compound annotation and relative quantification in liquid chromatography-mass spectrometry-based metabolomic research. *Analytical Chemistry*, *81*(15), 6546–6551. https://doi.org/10.1021/ac900979e

Gibon, Y., Vigeolas, H., Tiessen, A., Geigenberger, P., & Stitt, M. (2002). Sensitive and high throughput metabolite assays for inorganic pyrophosphate, ADPGlc, nucleotide phosphates, and glycolytic intermediates based on a novel enzymic cycling system. *The Plant Journal : For Cell and Molecular Biology*, *30*(2), 221–235. https://doi.org/10.1046/J.1365-313X.2001.01278.X

Gibon, Y., Blaesing, O. E., Hannemann, J., Carillo, P., Höhne, M., Hendriks, J. H. M., Fisahn, J., Höhne, M., Guenther, M., & Stitt, M. (2004). A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: Comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. *The Plant Cell*, *16*(12), 3304–3325. https://doi.org/10.1105/tpc.104.025973

Gibon, Y., Pyl, E. T., Sulpice, R., Lunn, J. E., Höhne, M., Günther, M., & Stitt, M. (2009). Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. *Plant, Cell and Environment*, *32*(7), 859–874. https://doi.org/10.1111/j.1365-3040.2009.01965.x

Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, *2012*. https://doi.org/10.6064/2012/963401

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z., Powers, S. J., Gong, F., Phillips, A. L., Hedden, P., Sun, T. P ., & Thomas, S. G. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. *The Plant Cell*, *18*, 3399–3414. https://doi.org/10.1105/tpc.106.047415

Hansen, L. D., Church, J. N., Matheson, S., McCarlie, V. W., Thygerson, T., Criddle, R. S., & Smith, B. N. (2002). Kinetics of plant growth and metabolism. *Thermochimica Acta*, *388*(1–2), 415–425. https://doi.org/10.1016/S0040-6031(02)00021-7

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293–320. https://doi.org/10.1128/MMBR.00050-14

Hauvermale, A. L., & Steber, C. M. (2020). GA signaling is essential for the embryo-to-seedling transition during Arabidopsis seed germination , a ghost story seed germination , a ghost story. *Plant Signalling and Behavior*, *15*(1). https://doi.org/10.1080/15592324.2019.1705028

Hendriks, J. H. M., Kolbe, A., Gibon, Y., Stitt, M., & Geigenberger, P. (2003). ADP-Glucose pyrophosphorylase is activated by posttranslational redox-modification in response to light and to sugars in leaves of Arabidopsis and other plant species. *Plant Physiology, 133(2),* 838-849. https://doi.org/10.1104/pp.103.024513

Hughes, D. T., & Sperandio, V. (2008). Inter-kingdom signalling: Communication between bacteria and their hosts. *Nature Reviews Microbiology*, *6*(2), 111–120. https://doi.org/10.1038/nrmicro1836

Hur, Y.-S., Kim, J., Kim, S., Son, O., Kim, W.-Y., Kim, G.-T., Ohme-Takagi, M., & Cheon, C.-I. (2019). Identification of TCP13 as an upstream regulator of ATHB12 during leaf development. *Genes*, *10*(9), 644. https://doi.org/10.3390/genes10090644

Iuchi, S., Suzuki, H., Kim, Y. C., Iuchi, A., Kuromori, T., Ueguchi-Tanaka, M., Asami, T., Yamaguchi, Y., Matsuoka, M., Kobayashi, M., & Nakajima, M. (2007). Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. *Plant Journal*, *50*(6), 958–966. https://doi.org/10.1111/j.1365-313X.2007.03098.x

Jaillais, Y., & Chory, J. (2010). Unraveling the paradoxes of plant hormone signaling integration. *Nature Structural and Molecular Biology*, *17*(6), 642–645. https://doi.org/10.1038/nsmb0610-642

Katz, E., Bagchi, R., Jeschke, V., Rasmussen, A. R. M., Hopper, A., Burow, M., Estelle, M., & Kliebenstein, D. J. (2020). Diverse allyl glucosinolate catabolites independently influence root growth and development. *Plant Physiology*, *183*, 1376–1390. https://doi.org/10.1104/pp.20.00170

Kim, S., Lowman, S., Hou, G., Nowak, J., Flinn, B., & Mei, C. (2012). Growth promotion and colonization of switchgrass (Panicum virgatum) cv. alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN. *Biotechnology for Biofuels*, *5*, 1–10. https://doi.org/10.1186/1754-6834-5-37

Knight, H., Zarka, D. G., Okamoto, H., Thomashow, M. F., & Knight, M. R. (2004). Abscisic acid induces CBF gene transcription and subsequent induction of cold-regulated genes via the CRT promoter element. *Plant Physiology*, *135*(3), 1710–1717. https://doi.org/10.1104/pp.104.043562

Kost, T., Stopnisek, N., Agnoli, K., Eberl, L., & Weisskopf, L. (2013). Oxalotrophy, a widespread trait of plant-associated *Burkholderia* species, is involved in successful root colonization of lupin and maize by *Burkholderia phytofirmans*. *Frontiers in Microbiology*, *4*, 1–9. https://doi.org/10.3389/fmicb.2013.00421

Lepistö, A., & Rintamäki, E. (2011). Coordination of plastid and light signaling pathways upon development of Arabidopsis leaves under various photoperiods. *Molecular Plant*, *5*(4), 799–816. https://doi.org/10.1093/mp/ssr106

Li, B., Förster, C., Robert, C. A. M., Züst, T., Hu, L., Machado, R. A. R., Berset, J. D., Handrik, V., Knauer, T., Hensel, G., Chen, W., Kumlehn, J., Yang, P., Keller, B., Gershenzon, J., Jander, G., Köllner, T.G., & Erb, M. (2018). Convergent evolution of a metabolic switch between aphid and caterpillar resistance in cereals. *Science Advances*, *4*(12), eaat6797. https://doi.org/10.1126/sciadv.aat6797

Li, H., Xu, T., Lin, D., Wen, M., Xie, M., Duclercq, J., Bielach, A., Kim, J., Redy, G. V., Zuo, J., Bencovà, B., Friml, J., Guo, H., & Yang, Z. (2013). Cytokinin signaling regulates pavement cell morphogenesis in Arabidopsis. *Cell Research*, *23*(2), 290–299. https://doi.org/10.1038/cr.2012.146

Li, L., & Sheen, J. (2016). Dynamic and diverse sugar signaling. *Current Opinion in Plant Biology*, *33*, 116–125. https://doi.org/10.1016/j.pbi.2016.06.018

Lilley, R. M., & Walker, D. A. (1975). Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose bisphosphate carboxylase from spinach. *Plant Physiology*, *55*(6), 1087–1092. https://doi.org/10.1104/pp.55.6.1087

Lommen, A., & Kools, H. J. (2012). MetAlign 3.0: Performance enhancement by efficient use of advances in computer hardware. *Metabolomics*, *8*(4), 719–726. https://doi.org/10.1007/s11306-011-0369-1

Lunn, J. E., Feil, R., Hendriks, J. H. M., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W. R., Carillo, P., Hajirezaei, M. R., & Stitt, M. (2006). Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. *Biochemical Journal*, *397*(1), 139–148. https://doi.org/10.1042/BJ20060083

Lunn, J. E., Delorge, I., Mar, C., Dijck, P. Van, & Stitt, M. (2014). Trehalose metabolism in plants. *The Plant Journal, 79,* 544–567. https://doi.org/10.1111/tpj.12509

Mahdi, I., Fahsi, N., Hafidi, M., Allaoui, A., & Biskri, L. (2020). Plant growth enhancement using rhizospheric halotolerant phosphate solubilizing bacterium *Bacillus licheniformis* qa1 and *Enterobacter asburiae* qf11 isolated from chenopodium quinoa willd. *Microorganisms*, *8*(6), 1–21. https://doi.org/10.3390/microorganisms8060948

Malinovsky, F. G., Thomsen, M. F., Nintemann, S. J., Jagd, L. M., Bourgine, B., Burow, M., & Kliebenstein, D. J. (2017). An evolutionarily young defense metabolite influences the root growth of plants via the ancient TOR signaling pathway. *eLife, 6*, e29353. https://doi.org/10.7554/eLife.29353

Manjunath, S., Lee, C. H. K., Vanwinkle, P., & Bailey-Serres, J. (1998). Molecular and biochemical characterization of cytosolic phosphoglucomutase in maize: Expression during development and in response to oxygen deprivation. *Plant Physiology*, *117*(3), 997–1006. https://doi.org/10.1104/pp.117.3.997

Matsui, A., Yokoyama, R., Seki, M., Ito, T., Shinozaki, K., Takahashi, T., Komeda, Y., & Nishitani, K. (2005). AtXTH27 plays an essential role in cell wall modification during the development of tracheary elements. *The Plant Journal*, *42*(4), 525–534. https://doi.org/10.1111/j.1365-313X.2005.02395.x

Meyer, R. C., Steinfath, M., Lisec, J., Becher, M., Witucka-wall, H., Willmitzer, L., Willmitzer, L., Selbig, J., & Fiehn, O. (2007). Metabolic signature related to high plant growth. *Proceedings of National Academy of Sciences*, *104*(11), 4759–4764. www.pnas.org/cgi/doi/10.1073/pnas.0609709104

Mitter, B., Petric, A., Sg Chain, P., Trognitz, F., Nowak, J., Compant, S., & Sessitsch, A. (2013a). Genome analysis, ecology, and plant growth promotion of the endophyte *Burkholderia phytofirmans* strain PsJN. *Molecular Microbial Ecology of the Rhizosphere*, *2*, 865–874. https://doi.org/10.1002/9781118297674.ch81

Mitter, B., Petric, A., Shin, M. W., Chain, P. S. G., Hauberg-Lotte, L., Reinhold-Hurek, B., Nowak, J., & Sessitsch, A. (2013b). Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Frontiers in Plant Science*, *4*, 1–15. https://doi.org/10.3389/fpls.2013.00120

Munné-Bosch, S., & Müller, M. (2013). Hormonal cross-talk in plant development and stress responses. *Frontiers in Plant Science*, *4*, 529. https://doi.org/10.3389/fpls.2013.00529

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio agsays with tobacco tissue cultures. *Physiologia Plantarum*, *15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nagano, A. J., Kawagoe, T., Sugisaka, J., Honjo, M. N., Iwayama, K., & Kudoh, H. (2019). Annual transcriptome dynamics in natural environments reveals plant seasonal adaptation. *Nature Plants*, *5,* 74-83. http://doi.org/10.1038/s41477-018-0338-z

Naveed, M., Mitter, B., Reichenauer, T. G., Wieczorek, K., & Sessitsch, A. (2014). Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. *Environmental and Experimental Botany*, *97*, 30–39. https://doi.org/10.1016/j.envexpbot.2013.09.014

Park, C. H., Kim, T. W., Son, S. H., Hwang, J. Y., Lee, S. C., Chang, S. C., Kim, S. H., Kim, S. W., & Kim, S. K. (2010). Brassinosteroids control *AtEXPA5* gene expression in *Arabidopsis thaliana*. *Phytochemistry*, *71*(4), 380–387. https://doi.org/10.1016/j.phytochem.2009.11.003

Park, J. M., & Lazarovits, G. (2014). Involvement of hexokinase1 in plant growth promotion as mediated by *Burkholderia phytofirmans*. *Canadian Journal of Microbiology*, *354*, 343–354. https://doi.org/10.1139/cjm-2014-0053

Peng, J., Wu, D., Liang, Y., Li, L., & Guo, Y. (2019). Disruption of *acdS* gene reduces plant growth promotion activity and maize saline stress resistance by *Rahnella aquatilis* HX2. *Journal of Basic Microbiology*, 1–10. https://doi.org/10.1002/jobm.201800510

Pernisova, M., Grochova, M., Konecny, T., Plackova, L., Harustiakova, D., Kakimoto, T., Heisler, M. G., Novak, O., & Hejatko, J. (2018). Cytokinin signalling regulates organ identity via the AHK4 receptor in Arabidopsis. *Development (Cambridge)*, *145*(14). https://doi.org/10.1242/dev.163907

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper-Excel-based tool using pair-wise correlations. *Biotechnology Letters*, *26,* 509-515. http://www.wzw.tum.de/gene-quantification/bestkeeper.html

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, *28*(1), 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, *52*, 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pillay, V. K., & Nowak, J. (1997). Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. *Canadian Journal of Microbiology*, *43*(4), 354–361. https://doi.org/10.1139/m97-049

Pinedo, I., Ledger, T., Greve, M., & Poupin, M. J. (2015). *Burkholderia phytofirmans* PsJN induces long-term metabolic and transcriptional changes involved in *Arabidopsis thaliana* salt tolerance. *Frontiers in Plant Science*, *6*, 1–17. https://doi.org/10.3389/fpls.2015.00466

Planas-Riverola, A., Gupta, A., Betego, I., Bosch, N., & Iban, M. (2019). Brassinosteroid signaling in plant development and adaptation to stress. *Development*, *1*, 1–11. https://doi.org/10.1242/dev.151894

Poupin, M. J., Greve, M., Carmona, V., & Pinedo, I. (2016). A complex molecular interplay of auxin and ethylene signaling pathways is involved in Arabidopsis growth promotion by *Burkholderia phytofirmans* PsJN. *Frontiers in Plant Science*, *7*, 1–16. https://doi.org/10.3389/fpls.2016.00492

Poupin, M. J., Timmermann, T., Vega, A., Zuñiga, A., & González, B. (2013). Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. *PLoS ONE*, *8*(7), 22–24. https://doi.org/10.1371/journal.pone.0069435

Quiza, L., St-Arnaud, M., & Yergeau, E. (2015). Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. *Frontiers in Plant Science*, *6*, 1–11. https://doi.org/10.3389/fpls.2015.00507

Rohrmann, J., Tohge, T., Alba, R., Osorio, S., Caldana, C., McQuinn, R., Arvidson, S., Van Der Marwe, M. J., Riaño-Pachon, D. M., Nesi, A. M., Giovannoni, J. J., & Fernie, A. R. (2011). Combined transcription factor profiling, microarray analysis and metabolite profiling reveals the transcriptional control of metabolic shifts occurring during tomato fruit development. *Plant Journal*, *68*(6), 999–1013. https://doi.org/10.1111/j.1365-313X.2011.04750.x

Ruan, Y.-L. (2014). Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. *Annual Review of Plant Biology*, *65*(1), 33–67. https://doi.org/10.1146/annurev-arplant-050213-040251

Sairanen, I., Novák, O., Pěnčík, A., Ikeda, Y., Jones, B., Sandberg, G., & Ljung, K. (2013). Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. *Plant Cell*, *24*(12), 4907–4916. https://doi.org/10.1105/tpc.112.104794

Sakr, S., Wang, M., Dédaldéchamp, F., Perez-Garcia, M. D., Ogé, L., Hamama, L., & Atanassova, R. (2018). The sugar-signaling hub: Overview of regulators and interaction with the hormonal and metabolic network. *International Journal of Molecular Sciences*, *19*(9), 2506. https://doi.org/10.3390/ijms19092506

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, *183*, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Schnittger, A., & De Veylder, L. (2018). The Dual Face of Cyclin B1. *Cell Press Reviews*, *23*(6), 475–478.

Schulze, S., Schäfer, B. N., Parizotto, E. A., Voinnet, O., &Theres, K. (2010). LOST MERISTEMS genes regulate cell differentiation of central zone descendants in Arabidopsis shoot meristems. *The Plant Journal, 64*, 668–678. https://doi.org/10.1111/j.1365-313X.2010.04359.x

Singh, K. B., Foley, R. C., & Oñate-Sánchez, L. (2002). Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology*, *5*(5), 430-436. https://doi.org/10.1016/S1369-5266(02)00289-3

Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in Environmental Science and Biotechnology*, *14*(3), 407–426. https://doi.org/10.1007/s11157-015-9372-8

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology*, *11*, 118–130. https://pubmed.ncbi.nlm.nih.gov/13486467/

Skylar, A., Sung, F., Hong, F., Chory, J., & Wu, X. (2011). Metabolic sugar signal promotes Arabidopsis meristematic proliferation via G2. *Developmental Biology*, *351*(1), 82–89. https://doi.org/10.1016/j.ydbio.2010.12.019

Sobariu, D. L., Fertu, D. I. T., Diaconu, M., Pavel, L. V., Hlihor, R. M., Drăgoi, E. N., Curteanu, S., Lenz, M., Corvini, P. F. X., & Gavrilescu, M. (2017). Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. *New Biotechnology*, *39*, 125–134. https://doi.org/10.1016/j.nbt.2016.09.002

Soeno, K., Goda, H., Ishii, T., Ogura, T., Tachikawa, T., Sasaki, E., Yoshida, S., Fujioka, S., Asami, T., & Shimada, Y. (2010). Auxin biosynthesis inhibitors, identified by a genomics-based approach, provide insights into auxin biosynthesis. *Plant and Cell Physiology*, *51*(4), 524–536. https://doi.org/10.1093/pcp/pcq032

Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*, *31*(4), 425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Stitt, M., & Gibon, Y. (2014). Why measure enzyme activities in the era of systems biology? *Trends in Plant Science*, *19*(4), 256–265. https://doi.org/10.1016/j.tplants.2013.11.003

Stitt, M., Lilley, R. M. C., Gerhardt, R., & Heldt, H. W. (1989). Metabolite levels in specific cells and subcellular compartments of plant leaves. *Methods in Enzymology*, *174*(C), 518–552. https://doi.org/10.1016/0076-6879(89)74035-0

Su, F., Gilard, F., Guérard, F., Citerne, S., & Clément, C. (2016). Spatio-temporal responses of Arabidopsis leaves in photosynthetic performance and metabolite contents to *Burkholderia phytofirmans* PsJN. *Frontiers in Plant Science 7*, 1–15. https://doi.org/10.3389/fpls.2016.00403

Su, F., Jacquard, C., Villaume, S., Michel, J., Rabenoelina, F., Clément, C., Barka, E. A., Dhondt-Cordelier, S., & Vaillant-Gaveau, N. (2015). *Burkholderia phytofirmans* PsJN reduces impact of freezing temperatures on photosynthesis in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *6*, 1–13. https://doi.org/10.3389/fpls.2015.00810

Su, F., Villaume, S., Rabenoelina, F., Crouzet, J., Clément, C., Vaillant-Gaveau, N., & Dhondt-Cordelier, S. (2017). Different *Arabidopsis thaliana* photosynthetic and defense responses to hemibiotrophic pathogen induced by local or distal inoculation of *Burkholderia phytofirmans*. *Photosynthesis Research*, *134*(2), 201–214. https://doi.org/10.1007/s11120-017-0435-2

Sulpice, R., Trenkamp, S., Steinfath, M., Usadel, B., Gibon, Y., Witucka-Wall, H., Pyl, E. T., Tschoep, H., Steihauser, M. C., Guenther, M., Hoenhe, M., Rohwer, J. M., Altman, T., Fernie, A., & Stitt, M. (2010). Network analysis of enzyme activities and metabolite levels and their relationship to biomass in a large panel of Arabidopsis accessions. *The Plant Cell*, *22*(8), 2872–2893. https://doi.org/10.1105/tpc.110.076653

Sulpice, R., & McKeown, P. C. (2015). Moving toward a comprehensive map of central plant metabolism. *Annual Review of Plant Biology*, *66*(1), 187–210. https://doi.org/10.1146/annurev-arplant-043014-114720

Sun, Y., Cheng, Z., & Glick, B. R. (2009). The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. *FEMS Microbiology Letters*, *296*(1), 131–136. https://doi.org/10.1111/j.1574-6968.2009.01625.x

Takei, K., Ueda, N., Aoki, K., Kuromori, T., Hirayama, T., Shinozaki, K., Yamaya, T., & Sakakibara, H. (2004). AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis. *Plant and Cell Physiology*, *45*(8), 1053–1062. https://doi.org/10.1093/pcp/pch119

Tessmer, O. L., Jiao, Y., Cruz, J. A., Kramer, D. M., & Chen, J. (2013). Functional approach to high-throughput plant growth analysis. *BMC Systems Biology*, *7*(Suppl 6), S17. https://doi.101186/1752-0509-7-S6-S17

Thain, S. C., Vandenbussche, F., Laarhoven, L. J. J., Dowson-Day, M. J., Wang, Z. Y., Tobin, E. M., Harren, F. J. M., Millar, A. J., & Van Der Straeten, D. (2004). Circadian rhythms of ethylene emission in Arabidopsis. *Plant Physiology*, *136*(3), 3751–3761. https://doi.org/10.1104/pp.104.042523

Theocharis, A., Bordiec, S., Fernandez, O., Paquis, S., Dhondt-Cordelier, S., Baillieul, F., Clèment, S., & Barka, E. A. (2012). *Burkholderia phytofirmans* PsJN primes *Vitis vinifera* L. and confers a better tolerance to low nonfreezing temperatures. *Molecular Plant-Microbe Interactions*, *25*(2), 241–249. https://doi.org/10.1094/MPMI-05-11-0124

Timmermann, T., Armijo, G., Seguel, A., Holuigue, L., & González, B. (2017). *Paraburkholderia phytofirmans* PsJN protects *Arabidopsis thaliana* against a virulent strain of *Pseudomonas syringae* through the activation of induced resistance, *Molecular Plant-Microbe Interactions*, *30*(3), 215–230. http://dx.doi.org/10.1094/MPMI-09-16-0192-R

Timmermann, T., Poupin, J. M., Vega, A., Urrutia, C., Ruz, G. A., & González, B. I. (2019). Gene networks underlying the early regulation of *Paraburkholderia phytofirmans* PsJN induced systemic resistance in Arabidopsis. *PLoS ONE*, *14*(8), e0221358. https://doi.org/10.1371/journal.pone.0221358

Tohge, T., & Fernie, A. R. (2009). Web-based resources for mass-spectrometry-based metabolomics: A user's guide. *Phytochemistry*, *4*, 450-456. https://doi.org/10.1016/j.phytochem.2009.02.004

Tohge, T., & Fernie, A. R. (2010). Combining genetic diversity, informatics and metabolomics to facilitate annotation of plant gene function. *Nature Protocols*, *5*(6), 1210–1227. https://doi.org/10.1038/nprot.2010.82

Vacheron, J., Desbrosses, G., Bouffaud, M.-L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyè, F., & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*, *4*, 1–19. https://doi.org/10.3389/fpls.2013.00356

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), 1–12. https://doi.org/10.1186/gb-2002-3-7-research0034

Vanhaeren, H., Gonzalez, N., & Inzé, D. (2015). A journey through a leaf: phenomics analysis of leaf growth in *Arabidopsis thaliana*. *The Arabidopsis Book*, *13*, e0181. https://doi.org/10.1199/tab.0181

Viaud, G., Loudet, O., & Cournède, P. H. (2017). Leaf segmentation and tracking in *Arabidopsis thaliana* combined to an organ-scale plant model for genotypic differentiation. *Frontiers in Plant Science*, *7*, 2057. https://doi.org/10.3389/fpls.2016.02057

Wang, B., Mei, C., & Seiler, J. R. (2015). Early growth promotion and leaf level physiology changes in *Burkholderia phytofirmans* strain PsJN inoculated switchgrass. *Plant Physiology and Biochemistry*, *86*, 16–23. https://doi.org/10.1016/j.plaphy.2014.11.008

Wang, L., Mai, Y. X., Zhang, Y. C., Luo, Q., & Yang, H. Q. (2010). MicroRNA171c-targeted *SCL6-II, SCL6-III*, and *SCL6-IV* genes regulate shoot branching in Arabidopsis. *Molecular Plant*, *3*(5), 794–806. https://doi.org/10.1093/mp/ssq042

Wang, L., & Ruan, Y. (2013). Regulation of cell division and expansion by sugar and auxin signaling. *Frontiers in Plant Sciences*, *4*, 1–9. https://doi.org/10.3389/fpls.2013.00163

Weeden, N. F., & Gottlieb, L. D. (1982). Dissociation, reassociation, and purification of plastid and cytosolic phosphoglucose isomerase isozymes. *Plant Physiology*, *69*(3), 717–723. https://doi.org/10.1104/pp.69.3.717

Weilharter, A., Mitter, B., Shin, M. V., Chain, P. S. G., Nowak, J., & Sessitsch, A. (2011). Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. *Journal of Bacteriology*, *193*(13), 3383–3384. https://doi.org/10.1128/JB.05055-11

Xu, X., Chen, C., Fan, B., & Chen, Z. (2006). Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. *The Plant Cell*, *18*(5), 1310–1326. https://doi.org/10.1105/tpc.105.037523

Xu, Z., Casaretto, J. A., Bi, Y.-M., & Rothstein, S. J. (2017). Genome-wide binding analysis of AtGNC and AtCGA1 demonstrates their cross-regulation and common and specific functions. *Plant Direct*, *1*(4), e00016. https://doi.org/10.1002/pld3.16

Yoshida, T., Obata, T., Feil, R., Lunn, J. E., & Fujita, Y. (2019). The role of abscisic acid signaling in maintaining the metabolic balance required for Arabidopsis growth under nonstress conditions. *The Plant Cell*, *31*, 84–105. https://doi.org/10.1105/tpc.18.00766

Zhang, W., Corwin, J. A., Copeland, D., Feusier, J., Eshbaugh, R., Chen, F., Atwell, S., & Kliebenstein, D. J. (2017). Plastic transcriptomes stabilize immunity to pathogen diversity : The jasmonic acid and salicylic acid networks within the Arabidopsis / Botrytis pathosystem. *The Plant Cell*, *29*, 2727–2752. https://doi.org/10.1105/tpc.17.00348

Zhang, X., Huai, J., Shang, F., Xu, G., Tang, W., Jing, Y., & Lin, R. (2017). A PIF1/PIF3-HY5-BBX23 transcription factor cascade affects photomorphogenesis. *Plant Physiology*, *174*(4), 2487–2500. https://doi.org/10.1104/pp.17.00418

Zubo, Y. O., Blakley, I. C., Franco-Zorrilla, J. M., Yamburenko, M. V., Solano, R., Kieber, J. J., Loraine, A. E., & Schaller, G. E. (2018). Coordination of chloroplast development through the action of the GNC and GLK transcription factor families. *Plant Physiology*, *178*(1), 130–147. https://doi.org/10.1104/pp.18.00414

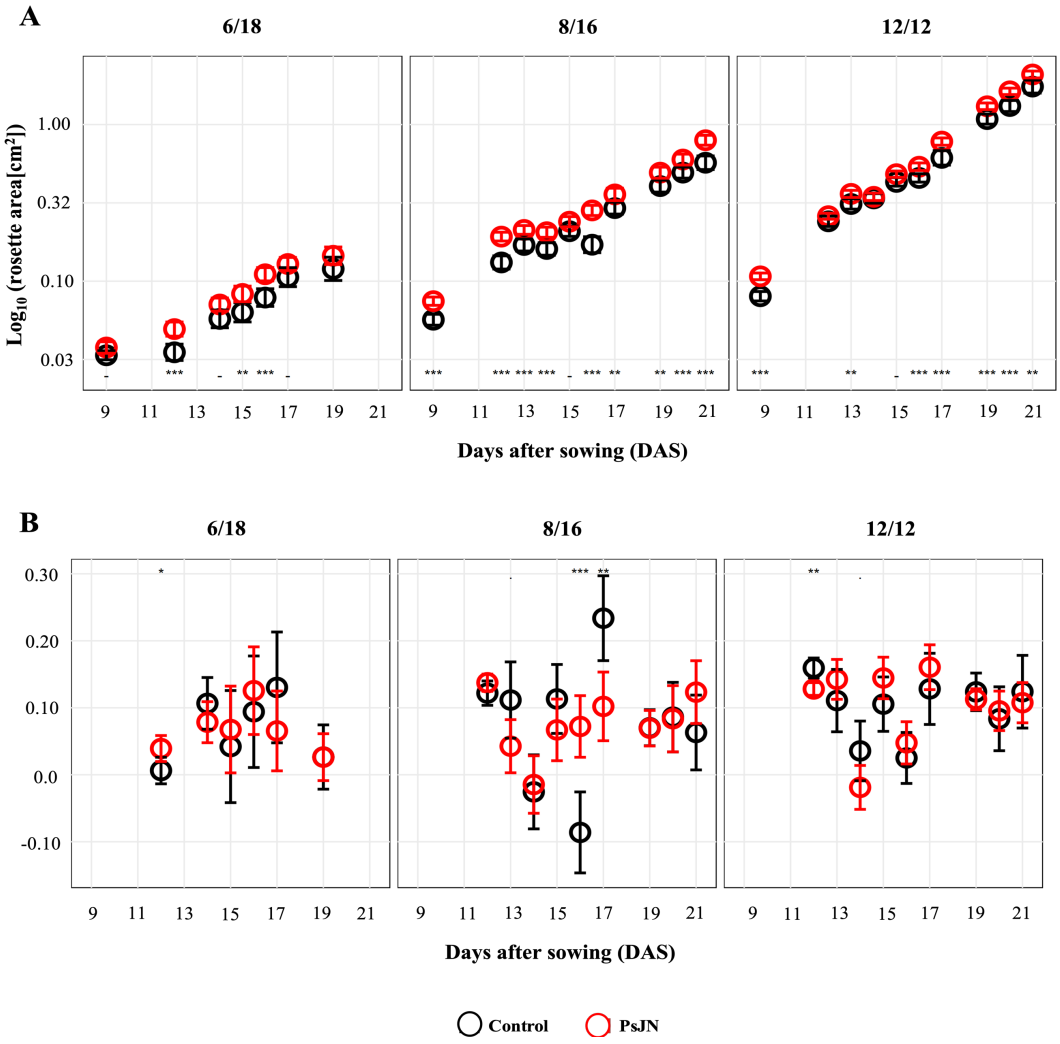
Zúñiga, A., Poupin, M. J., Donoso, R., Ledger, T., Guiliani, N., Gutiérrez, R. A., & González, B. (2013). Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of *Arabidopsis thaliana* by *Burkholderia phytofirmans* PsJN. *Molecular Plant-Microbe Interactions*, *26*(5), 546–553. https://doi.org/10.1094/MPMI-10-12-0241-R

APPENDIX

SUPPLEMENTARY TABLE 1. Data of statistical analysis of rosettes growth curves under neutral and short days in Arabidopsis plants. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation as control condition. Plants were grown under three different photoperiods 12:12 h, 8:16 h and 6:18 h, for 19 days. Rosette area was measured from 9 to 19 DAS. Multiple t-tests ($p<0.05$); Holm-Sidak multiple comparisons method $n_{12/12}$= 30 $n_{8/16}$=28-30 $n_{6/18}$=31.

		12:12 h		**8:16 h**		**6:18 h**	
Time (DAS)	**Multiple t-Tests parameters**	**Control**	**PsJN**	**Control**	**PsJN**	**Control**	**PsJN**
9	Mean	0.082	0.108	0.058	0.075	0.0342	0.0388
	p-value	**8.585×10^{-9} (*)**		**4.894×10^{-6} (*)**		**0.0286 (ns)**	
	Difference	-0.0266		-0.0174		-0.0045	
	SE of difference	0.004		0.003		0.00202	
12	Mean	0.246	0.262	0.136	0.196	0.0371	0.0513
	p-value	**0.1641 (ns)**		**8.935×10^{-9} (*)**		**4.166×10^{-5} (*)**	
	Difference	-0.0154		-0.0596		-0.014	
	SE of difference	0.0109		0.009		0.003	
14	Mean	0.344	0.349	0.165	0.209	0.061	0.073
	p-value	**0.7216 (ns)**		**6.139×10^{-5} (*)**		**0.0087 (*)**	
	Difference	-0.006		-0.044		-0.013	
	SE of difference	0.0154		0.010		0.005	
15	Mean	0.437	0.486	0.213	0.249	0.068	0.087
	p-value	**0.0112 (*)**		**0.0011 (*)**		**0.0026 (*)**	
	Difference	-0.0489		-0.036		-0.019	
	SE of difference	0.019		0.011		0.006	
16	Mean	0.463	0.545	0.174	0.294	0.083	0.115
	p-value	**4.121×10^{-4} (*)**		**7.316×10^{-13} (*)**		**9.549×10^{-5} (*)**	
	Difference	-0.082		-0.120		-0.031	
	SE of difference	0.0218		0.013		0.008	
17	Mean	0.635	0.787	0.292	0.376	0.110	0.133
	p-value	**6.338×10^{-5} (*)**		**1.267×10^{-5} (*)**		**0.0113 (*)**	
	Difference	-0.1519		-0.084		-0.023	
	SE of difference	0.0353		0.017		0.009	
19	Mean	1.800	2.109	0.4156	0.521	0.128	0.150
	p-value	**0.00138 (*)**		**3.909×10^{-5} (*)**		**0.0829 (ns)**	
	Difference	-0.3090		-0.1050		-0.0225	
	SE of difference	0.0919		0.0234		0.0127	

Asterisks indicate statistical significance; ns: indicate non-significant.

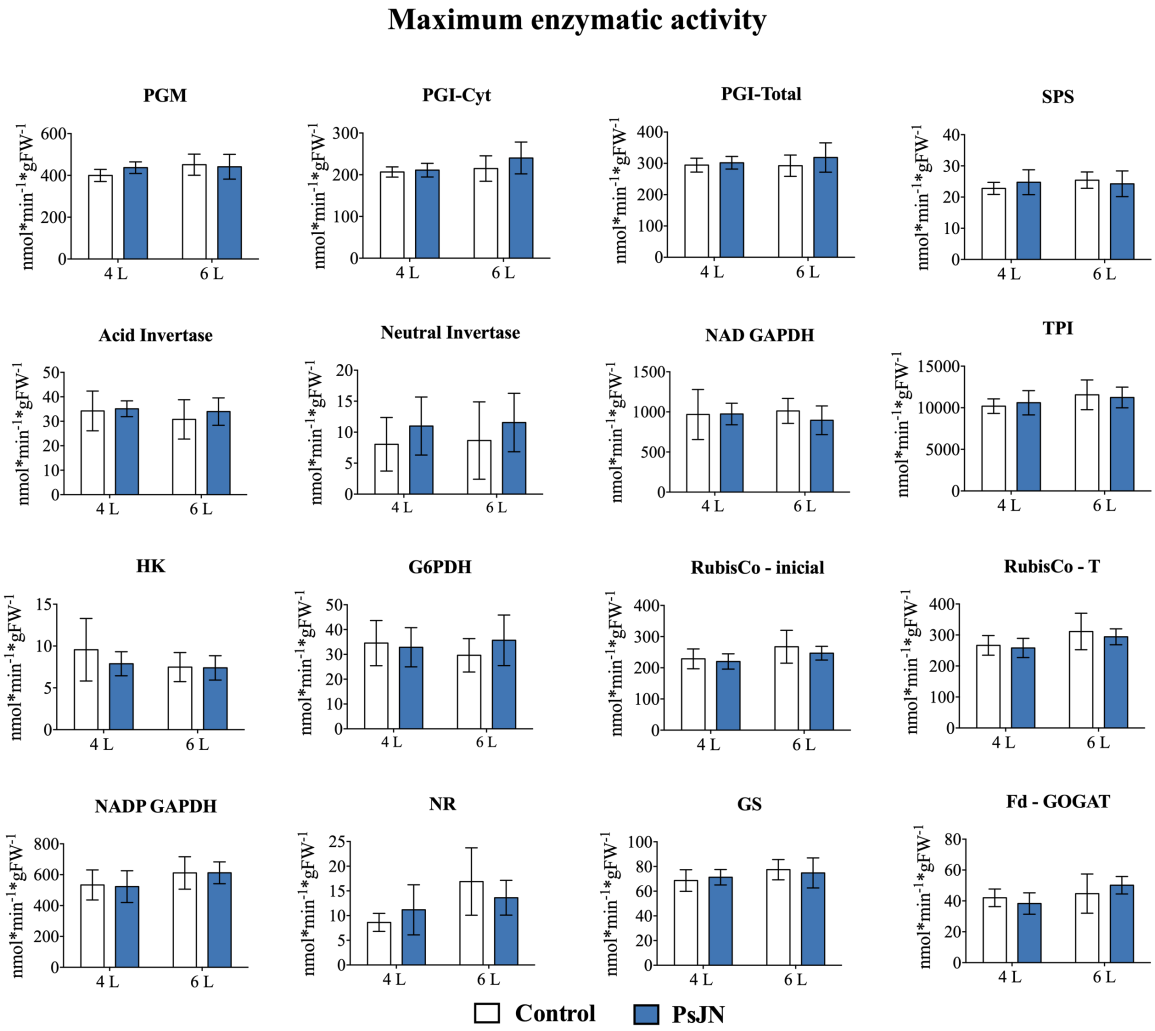


SUPPLEMENTARY FIGURE 1. **Effect of short days on plant growth-promotion of PsJN in Arabidopsis aerial tissue**. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation as control condition. Plants were grown under three different photoperiods 12:12 h, 8:16 h and 6:18 h for 21 days. The rosette area curves were plotted using $\log_{10}$ transformed data. **(A)** Absolute growth rate (AGR)**. (B)** Relative growth rate (RGR). Symbols represent mean and standard deviation (SD). Asterisks indicate statistical significance (two-way ANOVA, Tukey's multiple comparisons test (p-value>0.05).

SUPPLEMENTARY TABLE 2. Effect of PsJN and photoperiod in rosettes growth rates in Arabidopsis plants. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation as control condition. Plants were grown under three different photoperiods 12:12 h, 8:16 h and 6:18 h, for 21 days. ANCOVA analysis was carried out to test differences between growth rates calculated using the whole period (9-21 DAS), as well as 9-16 DAS and 16-21 DAS.

		12:12 h		**8:16 h**		**6:18 h**	
Time period	**ANCOVA parameters**	**Control**	**PsJN**	**Control**	**PsJN**	**Control**	**PsJN**
9 – 21 DAS	Slope	0.1351	0.1647	0.0434	0.0558	0.0105	0.0124
	Standard error	0.0043	0.0046	0.0015	0.0017	0.0007	0.0006
	p-value	0.734 (ns)		0.492 (ns)		0.685 (ns)	
9 – 16 DAS	Slope	0.0557	0.0620	0.0197	0.0278	0.0069	0.0101
	Standard error	0.0020	0.0020	0.0014	0.0014	0.0006	0.0007
	p-value	**0.025 (*)**		0.627 (ns)		0.115 (ns)	
16 – 21 DAS	Slope	0.2572	0.3033	0.0779	0.0961	0.0156	0.0127
	Standard error	0.0114	0.0093	0.0046	0.0049	0.0036	0.0032
	p-value	0.974 (ns)		**0.0716 (ns)**		0.3249 (ns)	

Asterisks indicate statistical significance; ns: indicate non-significant.

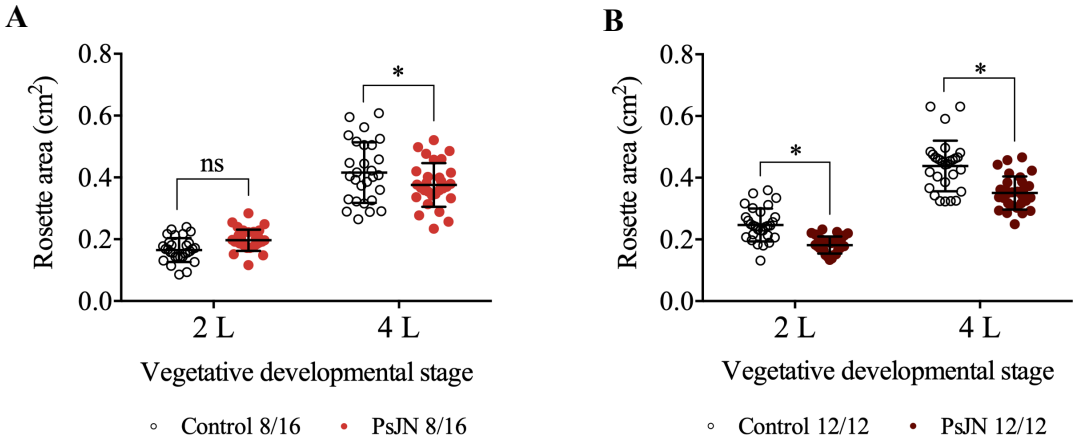


SUPLEMENTARY FIGURE 2. Effect of PsJN in enzymatic activity of enzymes from carbon – nitrogen metabolism in Arabidopsis at the end of the night (EN). Sixteen maximum enzymatic activities were determined for enzymes involved in primary metabolism of Arabidopsis. The measurements were carried out in rosettes form plants inoculated with PsJN (10^6 colony forming units per millilitre [CFU/mL]) and non-inoculated as control condition grown under 8:16 h photoperiod and harvested at EN. Five biological replicates were analysed per developmental stage. Maximum enzymatic activities were normalised to fresh weight (FW) and total protein content of each sample. Bar plots represent mean and SD values. Letters indicate statistical significance (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).

SUPPLEMENTARY TABLE 3. Molecular classification of significative unknown peaks that responded to treatment with PsJN.

Metabolite class	Peak	m/z[a]	RT[b]	Time point/ DS[c]	Change levels[d]
Glucosinolate derivative	G_0439	441.92	4.89	ED/4 L	Increase
	G_0601	661.19	6.75		
	G_0699	387.14	7.8		
	G_0760	457.2	8.41		
	G_0344	295.1	3.63	EN/4 L	Decrease
	G_0604	421.13	6.78		
Flavonoid derivative	G_1133	623.27	12.24	ED/4 L	Increase
	G_0472	347.09	5.28	EN/ 4L	Decrease
Small molecules	G_0169	314.08	1.78	EN/4 L	Decrease
	G_0179	35	1.88		
	G_0177	2422.08	1.87	EN/6 L	Increase
Unclassified	G_0754	565.15	8.34	ED/4 L	Increase
	G_0861	541.14	9.49		
	G_0972	439.21	10.63		
	G_0277	297.11	2.9	EN/4 L	Decrease
	G_0355	329.08	3.77		
	G_0358	305.08	3.81		
	G_0881	523.34	9.7	EN/6 L	Increase

[a]m/z: mass-to-charge ratio; [b]RT: retention time; [c]Developmental stage; [d]Significative changes in PsJN v/s non-inoculated condition (two-way ANOVA; Tukey's multiple comparisons test (p-value >0.05) n= 5).



SUPPLEMENTARY FIGURE 3. Rosettes of Arabidopsis PsJN-treated plants exhibit smaller rosette area than control condition at the same developmental stage. Arabidopsis plants were grown in presence of PsJN (10^6 colony forming units per millilitre [CFU/mL]) and without inoculation for control condition. Plants were grown under 8:16 h and 12:12h light/ darkness cycles. **(A)** Rosette area of plants grown under 8:16 h light/ darkness cycle. Measurements were done at 12 and 17 days after sowing (DAS) in rosettes of two (2 L) and 4 true leaves (4 L), respectively for PsJN condition, and at 14 and 18 DAS (2 L and 4 L, respectively) for control condition. **(B)** Rosette area of plants grown under 12:12 h light/ dar12 and 15 DAS (2 L and 4 L, respectively) for control condition. Scatter plots represent mean and standard deviation (SD). Asterisks indicate statistical significance (two-way ANOVA, Tukey's multiple comparisons test (p-value>0.05).