UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

INSTITUTO DE INVESTIGACIONES QUÍMICO-BIOLÓGICAS

Laboratorio de Biología del Desarrollo Vegetal

“Participación de los genes *FEZ* y *SOMBRERO* en el crecimiento de la raíz de *Arabidopsis* en interacción con *Achromobacter* sp. 5B1”

Que presenta:
M.C. Kirán Rubí Jiménez Vázquez

Para obtener el grado de:
DOCTORA EN CIENCIAS EN BIOLOGÍA EXPERIMENTAL

Director de tesis:
D. C. José López Bucio

INSTITUTO DE INVESTIGACIONES QUÍMICO-BIOLÓGICAS
UMSNH

Morelia, Michoacán, Mayo 2025

El presente trabajo fue realizado en el

Laboratorio de Biología del Desarrollo Vegetal del

Instituto de Investigaciones Químico Biológicas de la

Universidad Michoacana de San Nicolás de Hidalgo

bajo la asesoría del D. C. José López Bucio

A la memoria de Angelina Vázquez Medina, mi abuelita.
Deseo que en cualquier espacio-tiempo donde se situé su alma,
sea un lugar lleno de amor.

AGRADECIMIENTOS

Al D. C. José López Bucio

Por su guía, consejos y apoyo desde el inicio de mi camino en la investigación. Sus enseñanzas a través de conversaciones en el laboratorio y en las convivencias, así como su excelencia y pasión por la ciencia son una fuente de inspiración para cada uno de sus aprendices. “Agradecida y orgullosa de ser su aprendiz “.

Al comité sinodal

A los D.C. Ernesto García Pineda, Ángel Arturo Guevara García, Ramón Pelagio Flores y Homero Reyes de la Cruz por sus aportaciones y sugerencias en cada una de las evaluaciones semestrales, guiaron y reforzaron mi proyecto de investigación.

A mis amigos

Los logros no son individuales, definitivamente la convivencia, el apoyo y la colaboración con mis compañeros y amigos de laboratorio es enriquecedora. Así que agradezco a mis amigos por cada momento que pase con ustedes y el apoyo que siempre me brindaron: Gus, Ramón, Adrián, Liz, Sara, Aaron, Marina, Lupita, Juanito, Viry, Edith, Hano, Javier, Pedro, Jos, Pepe, y Fer.

A mis amigos del instituto con los que pase grandes momentos que atesorare por siempre. Eli, Hana, Mandy, Albert, Sergio y Luis.

A mis amigas Kari, Brenda, Yesi, Moni y Candy por su apoyo, por los momentos increíbles que hemos vivido y sobre todo por su amistad incondicional.

A mi familia

A mi mamá Irma por su cariño incondicional, por permitirme aprender de ella de la manera más honesta y, sobre todo, por ser una motivación cada día. A mi hermana Ámbar por ser mi apoyo y cómplice en cada decisión, las lecciones y aprendizajes de la vida, las hemos transcurrido juntas. A otro miembro de la familia que siempre nos alegra con sus travesuras y muestras de cariño, mi perrito Yodi. Especialmente, a mi novio Adrián le agradezco por su cariño y apoyo incondicional en cada decisión, en cada momento de frustración, en cada momento de felicidad, en fin, en cada momento que compartimos. Definitivamente eres mi persona-lugar favorito.
A mis tíos Sergio y Otilia que siempre nos mostraron su cariño y apoyo, así como a mis primos Emily y Arik por su cariño y por ser personitas increíbles.

ÍNDICE DE CONTENIDO

1. RESUMEN 1
2. ABSTRACT 2
3. INTRODUCCIÓN 3
4. ANTECEDENTES 6
   4.1. La planta modelo *Arabidopsis thaliana* 6
      4.1.1. El sistema radicular de *Arabidopsis thaliana* 7
      4.1.1.1. La raíz primaria 8
      4.1.1.2. Las raíces laterales 8
      4.1.1.3. La cofia 11
      4.1.1.3.1. Estructura y morfología de la cofia 11
      4.1.1.3.2. Regulación molecular en el proceso de desarrollo de la cofia 12
   4.2. Función de la cofia en la adaptación de la raíz en el suelo 15
      4.2.1. Gravitropismo 16
      4.2.2. Halotropismo 18
      4.2.3. Tigmotropismo 19
      4.2.4. Hidrotropismo 19
      4.2.5. Percepción y absorción de los nutrientes 20
   4.3. La interrelación de la cofia con los microorganismos del suelo 21
      4.3.1. La secreción de mucilago y las células derivadas de la cofia configuran el microbioma de la rizosfera 22
      4.3.2. Las raíces reconocen los compuestos producidos por los microorganismos en la rizosfera 23
   4.4. Las rizobacterias promotoras del crecimiento vegetal 24
      4.4.1. Efecto de las rizobacterias promotoras del crecimiento vegetal en el desarrollo del sistema radicular 25
      4.4.2. El género *Achromobacter* 26
      4.4.2.1. *Achromobacter* sp. 5B1 28
5. JUSTIFICACIÓN 31
6. HIPÓTESIS 31
7. OBJETIVOS 31
   7.1. Objetivo general 31
   7.2. Objetivos específicos 31
8. RESULTADOS 32

8.1. Capítulo I. 33
8.2. Capítulo II. 57
8.3. Capítulo III 72
8.4. Capítulo IV 90
8.5. Capítulo V 105
9. DISCUSIÓN Y CONCLUSIONES 113
10. REFERENCES 118

ÍNDICE DE FIGURAS

Figura 1. El ciclo de vida de *Arabidopsis* ……..……..……..7

Figura 2. Estructura de la raíz primaria y las etapas de desarrollo de las raíces laterales en *Arabidopsis* ……..9

Figura 3. Regulación molecular en el proceso de desarrollo de la cofia……..…13

Figura 4. Interacción de la cofia con la rizosfera……...15

Figura 5. Respuesta gravitrópica de la punta de la raíz de *Arabidopsis* ……..17

Figura 6. La comunicación química entre la cofia y la rizosfera estimula el desarrollo del sistema radicular……..……...21

Figura 7. Las rizobacterias promotoras del crecimiento vegetal modulan el desarrollo del sistema radicular……..27

Figura 8. Efecto de la co-inoculación de *Arabidopsis* con la rizobacteria benéfica *Achromobacter* sp. 5B1……..…29

1. RESUMEN

La cofia protege el meristemo de la raíz y percibe los estímulos ambientales como la gravedad y la disponibilidad de nutrientes. Aunque por su posición anatómica se establece una estrecha relación con la rizosfera, su función en la percepción de los microrganismos del suelo se desconoce. En este trabajo, a través de la inoculación directa de la raíz de *A. thaliana* con la rizobacteria promotora del crecimiento vegetal *Achromobacter* sp. 5B1, descubrimos una función crucial y opuesta de dos factores de transcripción, FEZ y SOMBRERO en el crecimiento, ondulación y formación de giros de la raíz primaria como una respuesta a la bacteria. En plántulas de *A. thaliana* con mutaciones en el *FEZ,* la rizobacteria incrementa la formación de giros, mientras en plántulas que albergan mutaciones en el gen *SOMBRERO*, la formación de giros no ocurre y la raíz mantiene la respuesta normal a la gravedad. Esto se correlaciona con la elongación de las células en el lado derecho o convexo, aspecto que se incrementa en las mutantes *fez-2* pero no se manifiesta en las mutantes *smb-3*. La interacción con *Achromobacter* sp. 5B1 incrementó el tamaño de la cofia, lo que coincidió con la disminución o incremento de la detección *in vivo de FEZ::FEZ:GFP y SMB::SMB:GFP,* respectivamente, y con el fenotipo de la mutante *smb-3*, que desarrolla raíces con una con cofia robusta independientemente de la bacterización. La expresión de los transportadores de auxinas PIN1, PIN2, PIN3, PIN4 y PIN7, en general disminuyó tanto en plantas tipo silvestre, como en las mutantes *fez-2* y *smb-3* con la inoculación bacteriana, lo que sugiere que el comportamiento agravitrópico de las raíces puede estar relacionado con la alteración de la distribución de las auxinas en la punta de la raíz. Los datos indican que la cofia percibe la rizobacteria *Achromobacter* sp. 5B1 a través de un mecanismo molecular que involucra a FEZ y SOMBRERO, indicando un papel directo del microbioma en el crecimiento direccional de la raíz, y la exploración del suelo.

Palabras clave: Cofia, factores de transcripción, *Achromobacte*r sp. 5B1, crecimiento radicular, distribución auxínica.

2. ABSTRACT

The root cap protects the meristem as the root grows and senses stimuli such as gravity and nutrient availability. Although it enables tight interaction with the rhizosphere, a role in perceiving microorganisms is unknown. Here, through *Arabidopsis* direct root inoculation with the plant growth promoting rhizobacterium *Achromobacter* sp. 5B1, we unveiled a critical, opposite role of two transcription factors, FEZ and SOMBRERO in mediating growth, waving and coiling of primary roots as a response to the bacterium. In *Arabidopsis* seedlings with mutations in the gen *FEZ*, the rhizobacterium had increased formation of coils, whereas in seedlings harboring mutations in the gen *SOMBRERO*, the formation of coils did not occur and the root maintains normal gravity response. This correlated with elongation of the cells on the right or convex side, an aspect that is increased in *fez-2* mutants but does not manifest itself in *smb-3* mutants. Interaction with *Achromobacter* sp. 5B1 increased the size of the root cap, which coincided with decreased or increased detection in vivo of *FEZ:FEZ:GFP* and *SMB:SMB:GFP*, respectively and with the phenotype of *smb-3* mutants, which develop roots with a huge cap irrespective of bacterization. Expression of auxin transporters PIN1, PIN2, PIN3, PIN4 and PIN7 overall decreased in the WT, *fez-2* and *smb-3* upon bacterial inoculation, suggesting that the agravitropic behavior of roots may be linked to disturbance of auxin distribution within the root tip. The data indicate that the root cap senses the rhizobacterium *Achromobacter* sp. 5B1 through FEZ and SOMBRERO for the directional growth and soil exploration.

Keywords: Root cap, transcription factors, *Achromobacter* sp. 5B1, root growth, auxin distribution.

3. INTRODUCCIÓN

La posición de la cofia cubriendo la punta de la raíz provee protección al meristemo, y le permite realizar funciones únicas como la percepción de las señales ambientales y la interacción con la rizosfera (Arnaud *et al*., 2010; Kumpf y Nowack, 2015). En *Arabidopsis thaliana*, la cofia se compone de dos tipos de tejidos: la columnela y la cofia lateral (Dolan *et al*., 1993). En conjunto, estos tejidos forman de 5-6 capas de células en la parte distal de la raíz, y en este órgano se mantiene un balance homeostático entre los procesos de división, diferenciación, recambio y muerte celular, para preservar la estructura, tamaño y funcionamiento adecuado. Estos procesos involucran proteínas con funciones señalizadoras y regulatorias incluyendo receptores, cinasas, fosfatasas y factores transcripcionales que actúan en conjunto con las hormonas (Willemsem *et al*., 2008; Fedryinch *et al*., 2014; Shi *et al*., 2018; Kumar y Lyer-Pascuzzi, 2020).

Los factores de transcripción de la familia NAC (NO APICAL MERISTEM, ARABIDOPSIS THALIANA ACTIVATING FACTOR, CUP-SHAPED COTYLEDON) que incluyen FEZ y SOMBRERO (SMB) coordinan los procesos de desarrollo de la cofia. FEZ se expresa en las células iniciales de la columnela y la cofia lateral, donde induce la división celular, mientras que SMB reprime la actividad de FEZ en las células proliferativas de la cofia, regulando la diferenciación, la maduración y la muerte celular programada (MCP). Las mutantes *fez-2* y *smb-3* exhiben fenotipos contrastantes; la mutante *fez-2* muestra un tamaño reducido, mientras que *smb-3* presenta un incremento en el número de capas de células de la cofia (Willemsen *et al*., 2008; Bennett *et al*., 2014; Fendrych *et al*., 2014). Adicionalmente, el desarrollo de este órgano está regulado por gradientes de auxinas y citocininas (Dubreuil *et al*., 2018).

La cofia reconoce las señales ambientales como la gravedad, el potencial hídrico, los obstáculos físicos, las concentraciones de sal, así como la disponibilidad de nutrientes circundantes, redirigiendo el crecimiento de la raíz hacia o lejos de los estímulos ambientales (tropismos), para adaptarse al entorno heterogéneo (Muthert *et al*., 2020; Ganesh *et al*., 2022). En plantas desprovistas de la cofia mediante ablación láser o la muerte genéticamente programada de las células de la columela,

las raíces manifiestan un crecimiento agravitrópico. Estas evidencias revelaron el sitio específico de la percepción de la gravedad (Blancaflor *et al*., 1999; Tsugeki y Fedoroff, 1999).

El gravitropismo de la raíz consta de tres etapas secuenciales: la percepción de la gravedad, la transducción de la señal y el crecimiento hacia el estímulo (Sato *et al*., 2015). En raíces graviestimuladas, los amiloplastos se sedimentan en las células de la columnela, lo que desencadena la relocalización de las proteínas de transporte de eflujo de las auxinas PIN3 y PIN7, en la membrana plasmática. Esto redirige el flujo de las auxinas hacia la parte inferior de la raíz mediante la acción de la proteína PIN2, localizada en las células de la cofia lateral y la epidermis, causando una distribución asimétrica de las auxinas entre la parte superior e inferior de la raíz. Finalmente, la curvatura gravitrópica de la raíz ocurre a través de la elongación diferencial de las células en la zona de elongación (Blancaflor, 2013; Su *et al*., 2017).

En la rizosfera se establecen interacciones entre las raíces y los microorganismos mediante un lenguaje químico, crucial para el crecimiento y la adaptación de las plantas. Las raíces secretan mucilago y liberan células derivadas de la cofia, que representan una fuente de carbono esencial para el establecimiento de las interacciones simbióticas con los microorganismos de la rizosfera. Los exudados radiculares contienen polisacáridos, glicoproteínas, aminoácidos, ácidos orgánicos, ADN extracelular y enzimas que atraen a los microorganismos (Badri y Vivanco, 2009; Driouich *et al*., 2021; Ganesh *et al*., 2022). Por otra parte, las raíces también poseen la capacidad de percibir moléculas que secretan las bacterias, principalmente *N*-acil-*L*-homoserina lactonas y ciclo dipéptidos, así como fitohormonas que regulan el crecimiento vegetal (auxinas y citocininas) y mezclas de compuestos orgánicos volátiles emitidas por los microrganismos rizosféricos (Ortiz-Castro *et al*., 2019; Ravelo-Ortega *et al*., 2023). Sin embargo, a la fecha se desconoce el sitio específico de la raíz involucrado en la percepción de los microorganismos o los metabolitos secretados en su interacción con la raíz.

Las rizobacterias promotoras del crecimiento vegetal (PGPR, por las siglas del inglés, *Plant Growth Promoting Rhizobacteria*), influyen en la arquitectura del sistema radicular, generalmente a través de la biosíntesis, la modulación del

transporte y la señalización de las auxinas (Perez-Flores *et al*., 2017; Méndez-Gómez *et al*., 2020; Li *et al*., 2021: García-Cárdenas *et al*., 2023). En un trabajo previo del laboratorio, se reportó que la rizobacteria benéfica *Achromobacter* sp. 5B1 influye sobre el sistema radicular de *Arabidopsis*, e interfiere en la respuesta gravitrópica, desencadenando la formación de ondulaciones y giros (Jiménez-Vázquez *et al*., 2020). Más recientemente, se demostró que la inoculación con esta rizobacteria promueve el crecimiento de las plántulas en condiciones de alcalinidad, mediante la inducción de la respuesta auxínica (Jiménez-Vázquez *et al*., 2023). En el presente trabajo, se presenta evidencia de que los factores de transcripción FEZ y SMB regulan de forma opuesta el comportamiento agravitrópico manifestado por la raíz primaria de *Arabidopsis* en contacto con *Achromobacter* sp. 5B1, probablemente actuando rio abajo de los transportadores de auxinas PIN. Específicamente, se demostró que se requiere de la actividad de SMB para el crecimiento direccional de la raíz mediante la elongación diferencial de las células en respuesta a la rizobacteria. Estos hallazgos amplían el conocimiento sobre la función de las rizobacterias en los movimientos radiculares, lo que podría ayudar a las plantas a ampliar su capacidad exploratoria y adaptativa a las necesidades del entorno, y para un mejor aprovechamiento de los recursos disponibles.

4. ANTECEDENTES

4.1. La planta modelo *Arabidopsis thaliana*

Hace cuatro décadas, *A. thaliana* se estableció como el organismo modelo por excelencia para el estudio de la fisiología, bioquímica y el desarrollo de las plantas, así como el entendimiento de los procesos biológicos en respuesta a los estímulos ambientales bióticos y abióticos. Esta angiosperma simple pertenece a la familia *Brassicaceae y* ofrece ventajas en la investigación de la biología vegetal. Rasgos que incluyen un tamaño pequeño, un ciclo de vida corto de 6 a 8 semanas (**Fig. 1**), producción prolífica de semillas, la facilidad para realizar cruzas dirigidas, así como un genoma pequeño, de aproximadamente 25,000 genes organizados en 5 cromosomas, lo que, aunado a la disponibilidad de mutantes para muchos de sus genes, han convertido a *Arabidopsis* en el modelo vegetal por excelencia para el estudio de los procesos biológicos fundamentales (Woodward y Bartel, 2018).

La secuenciación del genoma de *Arabidopsis* en el año 2000 (*The Arabidopsis Genome Initiative*, 2000), expandió la posibilidad para realizar estudios genómicos y moleculares detallados. Permitiendo ir en sentido inverso (genética inversa) e identificar mutaciones en función de su secuencia y no solo de acuerdo a su fenotipo, como ocurre en la genética clásica (Provart *et al*., 2016). El uso de *A. thaliana* en el laboratorio ha permitido realizar análisis de expresión génica bajo diferentes condiciones, visualizar proteínas reporteras bajo la regulación de los promotores de genes de interés e incluso alterar la expresión de genes por transgénesis y edición genética precisa aplicando la novedosa estrategia de CRISPR/Cas9 (Li *et al*., 2013; Cheng *et al*., 2017). Sin duda, *A. thaliana* ha revolucionado la investigación en biología vegetal, facilitando el estudio de mecanismos complejos como la genética del desarrollo, las respuestas a estrés, las vías de señalización hormonales, y las interacciones planta-microorganismo.

4.1.1. El sistema radicular de *Arabidopsis thaliana*

Las raíces crecen generalmente bajo el suelo, proporcionan soporte estructural y son determinantes en la absorción de agua y nutrientes necesarios para el crecimiento y el desarrollo de la planta. Además, responden y se adaptan a los estímulos ambientales como la gravedad, la salinidad o la sequía, así como la presencia de microrganismos (Petricka *et al*., 2012). Por tanto, la arquitectura del sistema radicular depende de los factores ambientales tanto bióticos como abióticos de su entorno, como de los rasgos particulares de cada especie vegetal, para ajustar la distribución de las raíces en el suelo (Smith y De Smet, 2012; Ranjan *et al*., 2022).

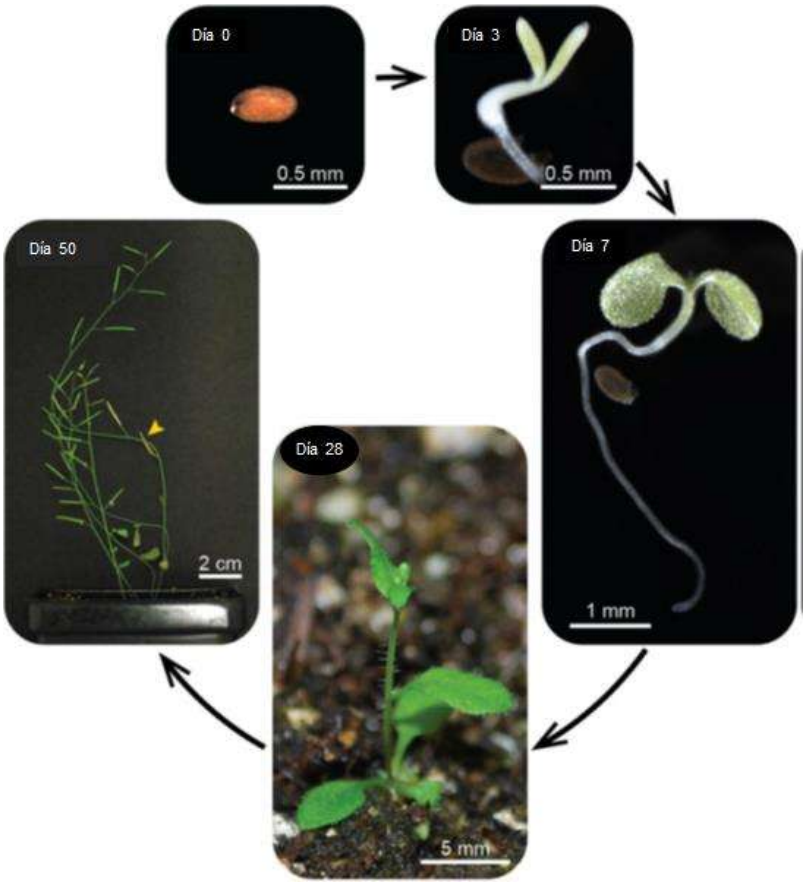


Figura 1. El ciclo de vida *Arabidopsis*. El desarrollo desde las semillas hasta las plantas maduras ocurre en un lapso de tiempo de 6 a 8 semanas. Las semillas germinan en las primeras 48 horas, mientras que los cotiledones emergen completamente en la semana 1. La formación de las primeras hojas de la roseta, el crecimiento del tallo y el desarrollo de las inflorescencias transcurre entre las semanas 2 a 5. Finalmente, partir de la semana 7 se completa el crecimiento de las plantas e inicia el proceso de senescencia (Modificada de Woodward y Bartel, 2018).

El crecimiento del sistema radicular manifiesta alta plasticidad en respuesta a las señales dinámicas de desarrollo endógenas y ambientales (externas). En este sentido, las fitohormonas que incluyen auxinas, citocininas, giberelinas, etileno, ácido jasmónico, ácido salicílico, estringolactonas y brasinoesteroides, actúan como los reguladores centrales de los procesos de crecimiento y desarrollo de la raíz (Waidmann *et al*., 2020).

4.1.1.1. La raíz primaria

La raíz primaria de *A. thaliana* emerge de la semilla durante la germinación (De Smet *et al*., 2010). El crecimiento de la raíz primaria y las raíces laterales es continuo durante su ciclo vital y depende del balance entre la proliferación, la elongación y la diferenciación celular. La raíz primaria consta de varias regiones de desarrollo a lo largo de su eje longitudinal; la cofia, la zona meristemática, la zona de elongación y la zona de diferenciación (**Fig. 2a**) (Dolan *et al*., 1993). En la parte más distal de la punta de la raíz, la cofia realiza la doble función de percibir y transmitir las señales externas al follaje, además protege el meristemo de la abrasión al crecer a través del suelo (Kumpf y Novack, 2015). En la zona meristemática, las células hijas derivadas del nicho de células iniciales están en constante división, hasta que progresan a la zona de elongación donde se detiene su proliferación y comienza su expansión, para finalmente alcanzar su identidad anatómica y funcional en la denominada zona de diferenciación (Dolan *et al*., 1993; Motte *et al*., 2019). Asimismo, la raíz presenta una organización radial que consiste de anillos concéntricos de células de la epidermis, el córtex, la endodermis y el periciclo que rodean el tejido vascular central (**Fig. 2b-c**) (Dolan *et al*., 1993; Luijten y Heidstra, 2009).

4.1.1.2. Las raíces laterales

A diferencia de la raíz primaria que tiene un origen embrionario, la formación de las raíces laterales es un proceso postembrionario. Las raíces laterales se originan a partir de la raíz primaria, en las células del periciclo adyacentes a los po-

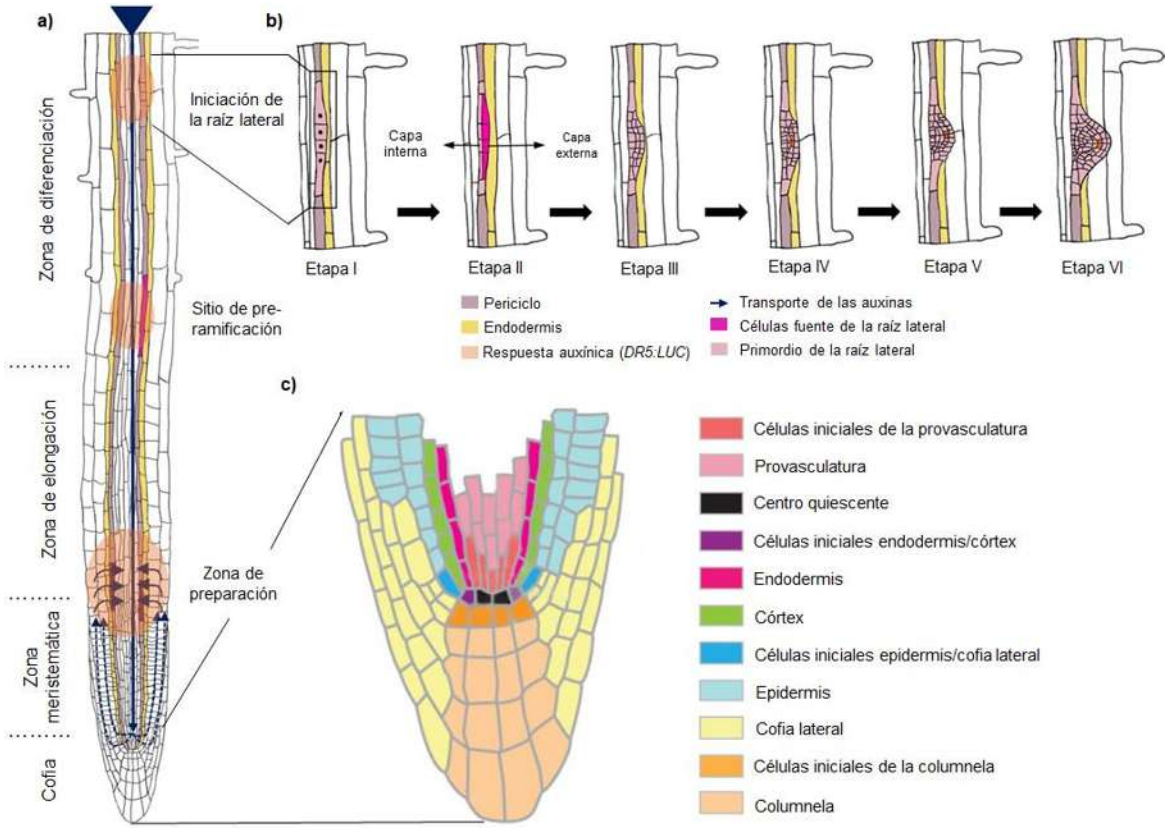


Figura 2. La estructura de la raíz primaria y las etapas de desarrollo de las raíces laterales en *Arabidopsis*. a) La raíz primaria consta de cuatro zonas de desarrollo: la cofia, la zona meristemática, la zona de elongación y la zona de diferenciación. El transporte de las auxinas desempeña una función fundamental en el proceso de desarrollo del sistema radicular. b) La formación de las raíces laterales inicia en la zona de preparación y se caracteriza por variaciones periódicas en la señalización auxínica. En la zona de elongación se establecen los sitios de pre-ramificación, mientras que, en la zona de diferenciación, estos sitios adquieren la identidad de células fuente e inicia la formación de la raíz lateral a través de la formación del primordio y, eventualmente, emerge la nueva raíz lateral. c) El centro quiescente controla la actividad del nicho de células iniciales que dan origen a los diferentes tejidos que conforman la raíz (Adaptado de Motte *et al*., 2019; Yalamanchili *et al*., 2024).

los del xilema (De Smet *et al*., 2010; Tian *et al*., 2014). En *Arabidopsis*, la organogénesis de las raíces laterales es un proceso que involucra múltiples etapas de desarrollo, reguladas principalmente por la señalización de las auxinas: i) la activación y especificación de las células fuente del pericicio o células madre de la

raíz lateral, (ii) la iniciación del primordio de la raíz lateral (PRL), (iii) el desarrollo del primordio y (iv) la emergencia de la raíz lateral (**Fig. 2b**) (Malamy y Benfey, 1997, Dubrovsky *et al*., 2000; Stoeckle *et al*., 2018; Du y Scheres 2018; Teixeira y Tusscher 2019; Hu *et al*., 2024).

El primer evento en la formación de las raíces laterales ocurre en el meristemo basal de la raíz (zona de preparación), comprendida por la zona de transición y el inicio de la zona de elongación (Dubrovsky *et al*., 2000; De Smet *et al*., 2007; Ovecka *et al*., 2020). La especificación de las células madre de la raíz lateral está determinada por la acumulación o biosíntesis local de auxinas y cambios subsecuentes en la expresión génica. Este mecanismo se puede visualizar mediante la actividad del promotor sintético DIRECT REPEAT5 (DR5) fusionado al gen reportero LUCIFERASE (*DR5:LUC*), que marca la respuesta transcripcional de las auxinas en un subconjunto de células del protoxilema, a través de la oscilación de la expresión génica. En este proceso de preparación se especifican los sitios en la raíz primaria donde se producirán las futuras raíces laterales (De Smet *et al*., 2007; De Rybel *et al*., 2010; Xuan *et al*., 2020).

Una vez que las células establecen su identidad, inicia la morfogénesis de la raíz lateral. Los núcleos de las células iniciales del primordio emigran hacia los polos y se expanden radialmente de manera asimétrica creando una apariencia en forma de domo (Go *et al*., 2012; Banda *et al*., 2019). La proliferación celular está coordinada con un incremento en la acumulación de las auxinas, que se reduce gradualmente hacia la periferia (Du y Scheres, 2018; Texeira y Tusscher, 2019; Yalamanchili *et al*., 2024).

El desarrollo del primordio hasta alcanzar un crecimiento independiente comprende una serie de divisiones periclinales y anticlinales que conducen a la formación de un meristemo con características anatómicamente similares al meristemo de la raíz primaria (von Wangenheim *et al*., 2016). La morfogénesis del transcurre durante siete fases de desarrollo, caracterizadas por el número de capas de células que se forman. En las fases I y II se establece el eje de crecimiento y se confiere la identidad asimétrica a las capas internas y externas. En la transición de la fase IV a V se establece el meristemo *de novo* asociado con un incremento en la

respuesta de las auxinas, que coincide con el paso del PRL por la endodermis. En las fases V a VII ocurre un crecimiento acelerado. La progresión del primordio a través de las capas de córtex y epidermis, depende del transportador de auxinas LIKE-AUXIN 3 (LAX3), se elevan los niveles de auxinas y se induce la síntesis de pared celular. Adicionalmente, estos eventos involucran la modulación en la expresión y localización polar de los transportadores de eflujo de las auxinas, las proteínas PIN-FORMED (PIN) (Benkova *et al*., 2003: Marhavý y Benková 2015; Du y Scheres *et al*., 2018).

Finalmente, el proceso de emergencia de las raíces laterales está coordinado por una serie de respuestas bioquímicas y biomecánicas entre los tejidos adyacentes, los primordios emergen perpendicularmente al eje de crecimiento de la raíz primaria en ángulos específicos (Roychoudhry y Kepinski, 2022; Yalamanchili *et al*., 2024). La formación de raíces laterales durante el ciclo de vida permite la exploración del suelo y afianzar el anclaje en función del crecimiento del follaje.

4.1.1.3. La cofia

Charles y Francis Darwin en su obra "*The power of movement in plants*" atribuyeron funciones sensoriales a la punta de la raíz (Darwin y Darwin, 1888). En la actualidad, se conoce que estas funciones se realizan en la cofia o caliptra. Su disposición en la parte más distal de la raíz, le permite proteger el meristemo y percibir las señales ambientales como la gravedad y la disponibilidad de los nutrientes en el suelo (Kumpf y Novack, 2015; Hetherington y Dolan, 2018b).

4.1.1.3.1. Estructura y morfología de la cofia

En *Arabidopsis*, la cofia consiste de varias capas de células denominadas columnela, flanqueadas por la cofia lateral (**Fig. 3a**). Estos tejidos son producidos por distintos grupos de células iniciales, la columnela surge a partir de las células iniciales ubicadas en la parte inferior del centro quiescente mediante divisiones periclinales, mientras que, las células iniciales de la cofia lateral tienen su propia fuente. La división de estas células ocurre de manera coordinada, lo que permite

que se formen nuevas capas de manera iterativa (Dolan *et al.,* 1993; Arnaud *et al.,* 2010). Asimismo, la columnela está organizada en diferentes tipos de células que incluyen las células que contienen amiloplastos (gránulos llenos de almidón involucrados en la percepción de la gravedad), las células secretoras que incluyen la capa externa de la columnela y las células de la cofia lateral (Nakamura *et al*., 2019). Estas últimas, secretan mucilago que facilita el crecimiento de la raíz, disminuyendo la fricción y la resistencia mecánica del suelo (Lijima *et al.,* 2008).

4.1.1.3.2. Regulación molecular en el proceso de desarrollo de la cofia

El crecimiento de la raíz está determinado por la generación constante de células nuevas a partir del meristemo. De manera similar, la cofia preserva su estructura y funcionamiento contando con su propio meristemo, así se mantiene la homeostasis entre los procesos de división, diferenciación, recambio y muerte celular, en respuesta al ambiente, el estado hormonal y la expresión génica (Willemsem *et al*., 2008; Fedryinch *et al*., 2014; Shi *et al*., 2018; Kumar y Lyer-Pascuzzi, 2020).

En *Arabidopsis*, los factores de transcripción NAC (NO APICAL MERISTEM, ARABIDOPSIS THALIANA ACTIVATING FACTOR, CUP-SHAPED COTYLEDON) coordinan el desarrollo de la cofia (Willemsen *et al*., 2008; Benett *et al*., 2010; Huysmans *et al*., 2018). El factor de transcripción ANAC009/FEZ promueve las divisiones formativas de las células iniciales de la columnela y la cofia lateral/epidermis, y su mutación provoca la reducción en el número de capas de células que conforman la cofia (Willemsem *et al*., 2008). Por otra parte, el factor de transcripción ANAC033/SOMBRERO promueve la diferenciación y maduración de las células de la columnela, y activa la muerte celular programada en las capas de cofia lateral que tienen que ser reemplazadas (**Fig. 3b**), las mutantes con pérdida de función en *smb-3*, muestran retraso en el proceso de diferenciación celular, lo que resulta en una cofia robusta con incremento en el número de capas de células de la columnela y la cofia lateral (Willemsem *et al*., 2008). FEZ se expresa en las células iniciales de la cofia y activa la expresión de SMB en las células hijas,

mientras que SMB reprime la actividad de FEZ en las células hijas de la cofia, induciendo la diferenciación de estas células (Willemsem *et al*., 2008; Bennet *et al*., 2014).

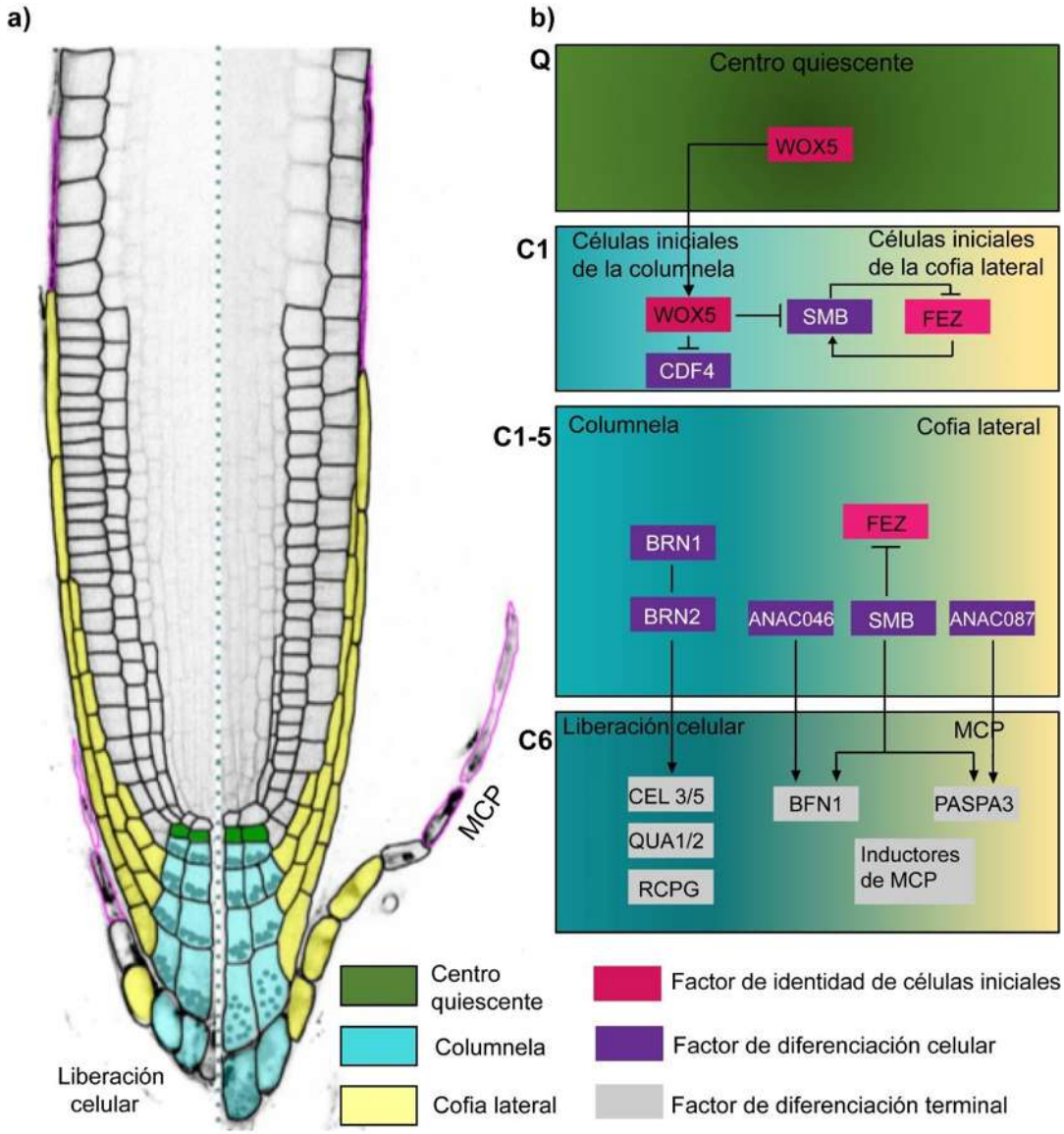


Figura 3. Regulación molecular en el proceso de desarrollo de la cofia. a) La cofia se compone de varias capas de células de la columnela rodeadas por la cofia lateral. b) Los procesos de identidad celular, división, diferenciación, recambio y muerte celular, están coordinados por factores transcripcionales tanto en las células de la columnela (azul) como en las células de la cofia lateral (amarillo). Las leyendas indican la función de cada uno de los factores transcripcionales involucrados en el proceso de desarrollo (Adaptado de Feng *et al*., 2022).

El factor de transcripción WUSCHEL HOMEOBOX 5 (WOX5) que se expresa en el centro quiescente y mantiene el estado indiferenciado de las células iniciales, reprime la expresión de SMB y CYCLIN DOF FACTOR4 (CDF4), factores de transcripción implicados en la diferenciación de las células de la columnela, impidiendo la diferenciación de estas células iniciales (Bennet *et al*., 2014; Pi *et al*., 2015). Dos factores de transcripción adicionales BEARSKIN 1 (BRN1) y BRN2 promueven la diferenciación y el recambio celular. BRN1 y BRN2 se expresan en las capas externas de la cofia, donde promueven el recambio celular a través de la expresión de las enzimas que degradan la pared celular CELLULASE3 (CEL3), CEL5, ROOT CAP POLYGALACTURONASE (RCPG), QUASIMODO 1 (QUA1) y QUA2 (**Fig. 3b**) (Bennet *et al*., 2010; Kamiya *et al*., 2016).

Las capas más externas de la cofia se desprenden cuando han alcanzado su vida media. Las células de la cofia lateral pasan por un proceso de muerte celular programada controlado por SMB (Frendych *et al.,* 2014). SMB, en conjunción con los factores ANAC046 y ANAC087 regulan positivamente las enzimas hidrolíticas BFN1 y PASPA3, claves en la degradación celular (Frendych *et al*., 2014; Olvera-Carrillo *et al*., 2015; Frendych *et al*., 2014; Huysmans *et al*., 2018).

Adicionalmente, el proceso de desarrollo de este órgano está coordinado por gradientes de auxinas. De acuerdo con Dubreuil y col. (2018), la distribución de las auxinas con una mayor acumulación en el centro quiescente y un gradiente decreciente hacia las capas externas de la columnela es crucial para el recambio celular, lo cual se relaciona con la represión de los transportadores de eflujo de las auxinas PIN3, PIN4 y PIN7 en las capas más externas de la cofia. En este sentido, los tratamientos en las raíces con la auxina sintética ácido-1-naftalenacético, estimulan la diferenciación de las células iniciales de la columela (Ding y Friml, 2010). Sin embargo, el mecanismo de regulación implicado en cada uno de los procesos de desarrollo de la cofia está en espera ser dilucidado.

4.2. Función de la cofia en la adaptación de la raíz en el suelo

La cofia es el primer sitio en contacto de la raíz con el suelo y desempeña un papel importante en la coordinación de los movimientos de la raíz conocidos como tropismos (Muthert *et al*., 2020; Ganesh *et al*., 2022; (**Fig. 4**). Los tropismos muestran de una forma fascinante como las plantas perciben y reaccionan ante las señales ambientales, así como su capacidad adaptativa a través del crecimiento dinámico de los órganos. En los siguientes apartados se describen los principales factores ambientales de tipo abiótico, que percibe la cofia y como se integran estas señales para ajustar la arquitectura del sistema radicular.

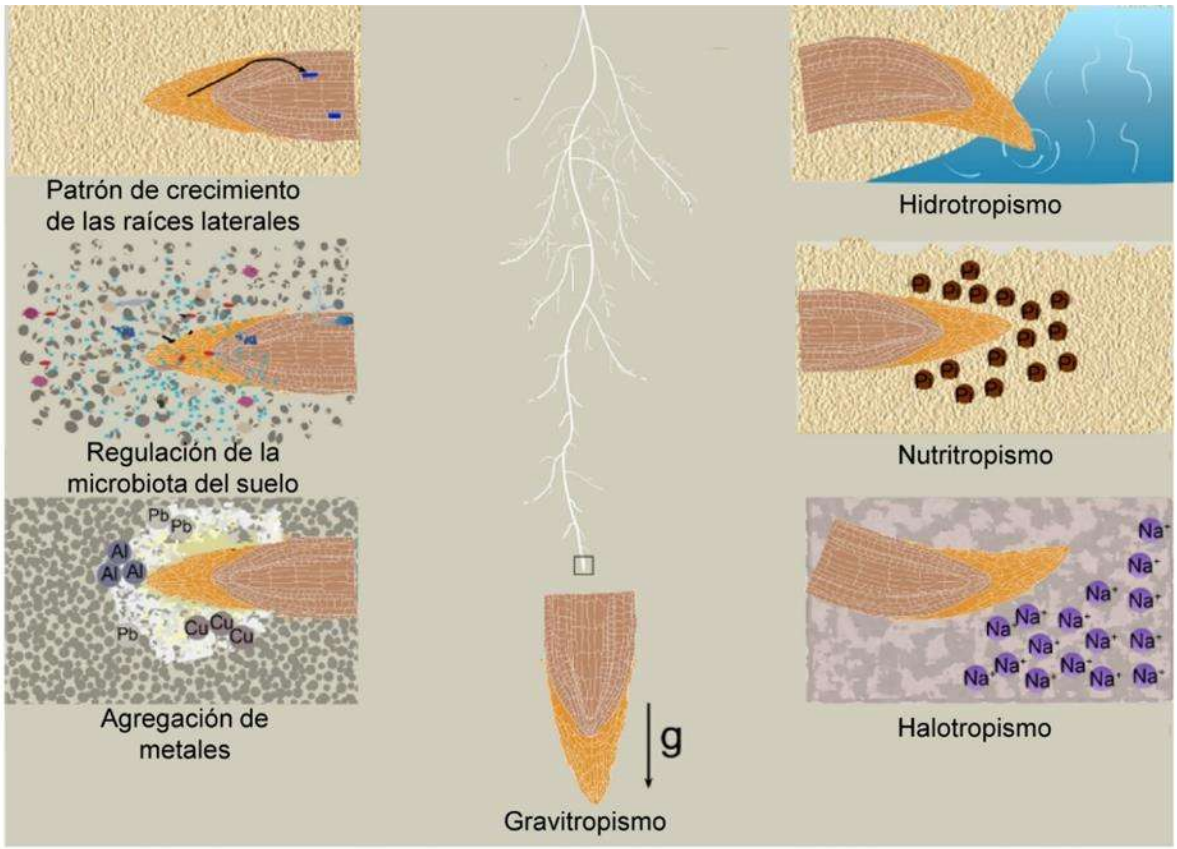


Figura 4. Interacción de la cofia con la rizosfera. Las raíces adaptan su crecimiento hacia o lejos de los estímulos ambientales, a través de los tropismos: gravitropismo, halotropismo, nutritropismo, hidrotropismo y tigmotropismo. Por otra parte, la liberación de las células derivadas de la cofia y las secreciones, actúan como una trampa para los metales pesados en el suelo. La cofia tiene una función central en la regulación del microbioma de la rizosfera. Adicionalmente, las auxinas derivadas de la cofia contribuyen en el patrón de crecimiento de las raíces laterales (Modificado de Ganesh *et al*., 2022).

4.2.1. Gravitropismo

La evolución de la respuesta gravitrópica de la raíz, contribuyó a la colonización de la tierra por las plantas (Rakusova *et al.,* 2015). Capacidad que adquirieron las raíces simultáneamente con la innovación de la cofia. En experimentos que incluyen la eliminación de la cofia mediante ablación láser o la muerte genéticamente programada de las células de la columela, las raíces revelaron un comportamiento agravitrópico (Blancaflor *et al*., 1999; Tsugeki y Fedoroff, 1999). Estos resultados demostraron que específicamente las células de la columnela son el sitio de percepción de la gravedad en la raíz (**Fig. 5a-c**).

Hace más de un siglo surgió “la hipótesis de los estatolitos de almidón” que explica la percepción de la gravedad. La hipótesis establece que los amiloplastos o estatolitos densos llenos de almidón dentro de las células de la columnela se sedimentan en la dirección de la gravedad, lo que resulta en la generación de una señal que provoca el crecimiento asimétrico (Haberlandt *et al*., 1900; Němec, 1900; Blancaflor *et al*., 2003; Su *et al*., 2017). Esta hipótesis está respaldada por múltiples evidencias experimentales. Plantas con mutaciones en genes involucrados en la síntesis de almidón, muestran alteraciones en el gravitropismo tanto de las raíces como de los tallos (Kiss *et al*., 1989, 1997; Weise y Kiss, 1999). En contraste, en las plantas mutantes que presentan amiloplastos más grandes se favorece el gravitropismo (Vitha *et al*., 2007).

En general, el gravitropismo de la raíz comprende la percepción de la gravedad, y la conversión del estímulo en una señal fisiológica para el crecimiento diferencial de las células (Sato *et al*., 2015). En las raíces graviestimuladas, la percepción de la gravedad ocurre en las células de la columnela, donde los amiloplastos se sedimentan en la nueva base de las células. Este estímulo físico transduce una señal gravitrópica que desencadena la relocalización de las proteínas del transporte de eflujo de las auxinas PIN3 y PIN7 en la membrana plasmática, hacia el vector de la gravedad. Esto redirige el flujo de las auxinas hacia la parte inferior mediante la actividad de la proteína PIN2, presente en las células de la cofia lateral y la epidermis, generando una distribución asimétrica de las auxinas entre la parte superior e inferior de la raíz. La disminución de las auxinas en la parte superior,

estimula la elongación celular, mientras que la acumulación de las auxinas en la parte inferior de la raíz inhibe el crecimiento de las células. Finalmente, la elongación diferencial de las células en la zona de elongación, conduce al crecimiento gravitrópico de la raíz (Blancaflor, 2013; Baldwin *et al*., 2013; Su *et al*., 2017).

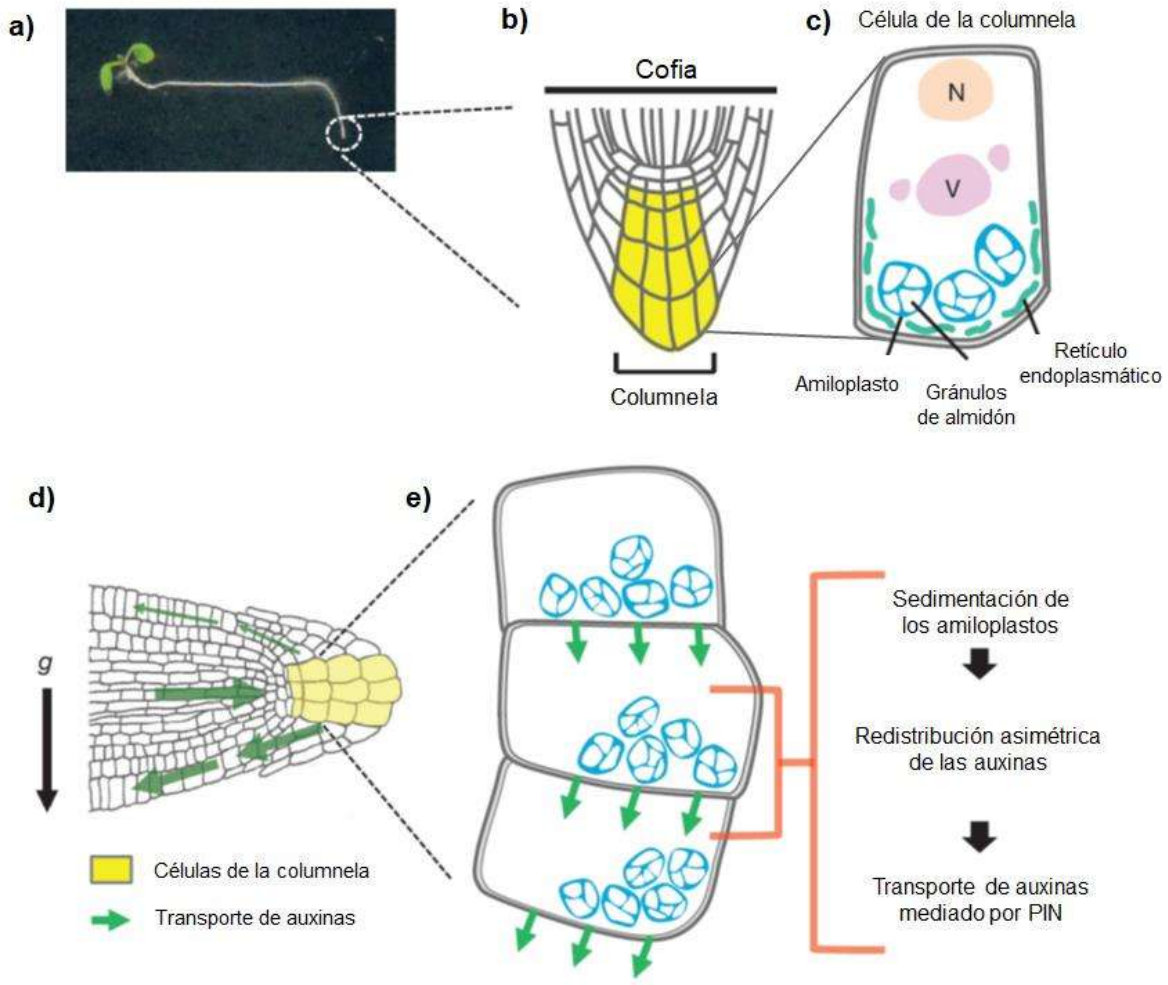


Figura 5. Respuesta gravitrópica de la punta de la raíz de *Arabidopsis*. a) Plántulas de *Arabidopsis* graviestimuladas redirigen el crecimiento de la raíz hacia la gravedad. b) Las células de la cofia son el sitio de percepción de la gravedad, c) los amiloplastos que son gránulos llenos de almidón, se sedimentan hacia el vector de la gravedad. d, e) La sedimentación de los amiloplastos cambia la relocalización de las proteínas PIN en las células de la columnela, redireccionando el flujo de las auxinas hacia la parte inferior de la raíz. Flujo alimentado por el transportador de auxinas PIN2, desencadenando la redistribución asimétrica de las auxinas, lo que influyen en la elongación diferencial de las células en la zona de elongación que lleva al crecimiento gravitrópico de la raíz (Modificado de Nakamura *et al*., 2019).

4.2.2. Halotropismo

Actualmente, la salinidad representa uno de los agobios ambientales más perjudiciales en la producción agrícola a nivel mundial (Galván-Ampudia *et al*., 2013). Aproximadamente el 8.7% de la superficie del suelo del planeta está afectada por la salinidad debido al incremento del nivel del mar, las sequias y las inundaciones con agua salobre en tierras de cultivo, problemáticas que empeoran con el cambio climático (Rengasamy, 2006a; Kader y Lindberg, 2008; Mazumder *et al*., 2024). Para asegurar la supervivencia de la planta y adaptar el crecimiento a las condiciones de estrés salino, la raíz cambia la dirección de crecimiento lejos de las regiones con concentraciones elevadas de sal, proceso conocido como halotropismo (Galván-Ampudia *et al*., 2013). La respuesta halotrópica negativa, es específica a la abundancia de sodio, puesto que la adición de $CaCl_2$ al medio, no activa la curvatura de la raíz para evadir el gradiente salino (Galván-Ampudia *et al*., 2013).

Previamente se mostró que la salinidad del suelo impacta la estructura de la cofia, especialmente la distribución de los estatolitos (Hodson y Mayer, 1987). En los años posteriores, el halotropismo se atribuyó a la distribución dinámica de las auxinas en la punta de la raíz. En las células de la cofia y la epidermis, la fitohormona se transloca hacia el lado opuesto del gradiente salino, desencadenando la distribución asimétrica de las auxinas y la curvatura de la raíz (Galván-Ampudia *et al*., 2013). Los transportadores de eflujo de las auxinas regulan la movilización de las auxinas, especialmente la exposición a concentraciones salinas induce la internalización de la proteína PIN2 en el lado de la raíz próximo a la región salina mediada por las fosfolipasas PLD1 y PLD2 (Korver *et al*., 2020). Adicionalmente, el transportador de influjo de las auxinas AUX1 contribuye en los cambios en el flujo de las auxinas durante la respuesta halotrópica (van den Berg *et al*., 2016). Recientemente, se reveló la función crucial del factor de transcripción SMB localizado en la cofia en el establecimiento del gradiente lateral de las auxinas en este órgano para dirigir la curvatura halotrópica (Zheng *et al*., 2024).

4.2.3. Tigmotropismo

Las raíces tienen la habilidad de detectar estímulos mecánicos en el suelo y evitar los obstáculos a través de cambios en la dirección del crecimiento. Esta capacidad de curvar la raíz para evitar los obstáculos, que se conoce como evasión de obstáculos o tigmotropismo, requiere de la supresión de la respuesta gravitrópica para cambiar la dirección del crecimiento de la raíz y rodear la barrera física. La cofia es el primer órgano que se enfrenta a los obstáculos, por lo tanto, conlleva la capacidad de percibir las señales mecánicas y desencadenar la respuesta de crecimiento para adaptarse a las condiciones heterogéneas de su entorno (Massa y Gilroy, 2003; Tanaka *et al*., 2010).

De acuerdo con reportes previos, cuando la raíz entra en contacto con el obstáculo, se incrementan los niveles de Ca^{2+}, desencadenando una cascada de señalización rio abajo que implica modificaciones en el pH y la elevación de la concentración de especies reactivas de oxígeno. Durante la curvatura, se incrementan los niveles de Ca^{2+} en el citosol de las células epidérmicas en el lado convexo de la raíz (Monshausen y Gilroy, 2009). Este pico en los niveles de Ca^{2+} activa la producción de especies reactivas de oxígeno y la acidificación, procesos asociados con el crecimiento asimétrico de las células (Ponce *et al*., 2017).

Como en la mayoría de los tropismos, en el tigmotropismo también interviene la distribución asimétrica de las auxinas, que se acumulan en el lado cóncavo de las raíces, desencadenando la curvatura de la raíz lejos de la barrera física. Después de pocas horas, la raíz experimenta una segunda curvatura para restablecer su crecimiento hacia el vector de la gravedad (Lee *et al*., 2020).

4.2.4. Hidrotropismo

El hidrotropismo es el crecimiento de la raíz hacia las regiones con potencial hídrico, que permite la adaptación de las plantas a la disponibilidad del agua. La respuesta hidrotrópica se ha estudiado en varias especies vegetales, con la finalidad de determinar el sitio de percepción y la respuesta al agua (Miyazawa y Takahashi, 2020). Por ejemplo, plantas de frijol y maíz a las que se les removieron las cofias, perdieron la habilidad de modificar su crecimiento hacia zonas húmedas, mientras

que, las raíces de arroz y pepino desprovistas de la cofia respondieron al potencial hídrico sin dificultad (Fujii *et al*., 2018). Lo que demuestra que la función de la cofia en la percepción de fuentes de agua disponibles en el entorno, depende de la especie vegetal.

Particularmente en *Arabidopsis*, se demostró que la cofia no es necesaria para percibir el hidrotropismo. En cambio, este tropismo, la expresión especifica de MIZU-KUSSEI1 dependiente de ácido abscísico en las células del córtex es suficiente para detectar la señal hídrica y activar la curvatura de la raíz (Dietrich, 2018). Sin embargo, en un estudio posterior, se indicó que la expresión asimétrica mediada por MIZ1 de los reguladores de respuesta a citocininas (ARR) ARR16 y ARR17 provoca la curvatura de la raíz hacia la señal hídrica, por lo tanto, las citocininas localizadas en la cofia influyen en el hidrotropismo (Chang *et al*., 2019).

4.2.5. Percepción y absorción de los nutrientes

Recientemente se asignó el término “nutritropismo” para referirse al crecimiento direccional de la raíz hacia las regiones del suelo con altas concentraciones de nutrientes. Yamazaki y col. (2020), reportaron que las raíces laterales de arroz cambiaron la dirección de crecimiento hacia la zona del medio con una mayor concentración de amonio (NH_4^+), sin embargo, este comportamiento no se presentó con otras formas de nitrógeno como nitrato (NO_3^-) o nitrito (NO_2^-), lo que indica que la forma química del nitrógeno es determinante para desencadenar la respuesta de crecimiento hacia el nutriente. Específicamente, la cofia parece contribuir en la percepción y absorción de los nutrientes.

En concordancia con lo anterior, los transportadores de alta afinidad a fosfato inorgánico (Pi) PHOSPHATE TRANSPORTER 1.1 (PHT1.1) y (PHT1.2) se expresan en las células de la cofia, lo que correlaciona directamente a este órgano con la absorción de Pi. De hecho, la absorción de Pi por la cofia representó el 20% del Pi total en la plántula (Kanno *et al*., 2016). Adicionalmente, se mostró que el factor de transcripción SMB actúa como un modulador negativo de la señalización auxínica en deficiencia de nutrientes, particularmente en condiciones de bajo nivel de fosfato (Ravelo-Ortega *et al*., 2022). A pesar de las evidencias sobre la función

de la cofia en respuesta a los nutrientes, se requiere más investigación para explorar los mecanismos de percepción y absorción responsables de las respuestas de crecimiento de la raíz, así como la contribución específica de la cofia.

4.3. La interrelación de la cofia con los microorganismos del suelo

La posición de la cofia al frente de la raíz, la convierte en el primer órgano vegetal en interactuar con el microbioma del suelo. La cofia tiene una función central en las interacciones simbióticas planta-microorganismo (Driouich *et al*., 2013; Hawes *et al*., 2016; Driouich *et al*., 2021; Ganesh *et al*., 2022). Las células derivadas de la cofia, así como la secreción de mucílago, son esenciales para la interacción con los microorganismos en la rizosfera (Driouich *et al*., 2021). Por otro lado, los microorganismos del suelo producen metabolitos, reguladores del crecimiento vegetal, y compuestos orgánicos volátiles que pueden ser percibidos por las raíces (**Fig. 6**) (Ortiz-Castro *et al*., 2011; Ortiz-Castro y López-Bucio, 2019; Ortiz-Castro *et al*., 2020; Ravelo-Ortega *et al*., 2023). Este ciclo de retroalimentación entre la raíz y el suelo es fundamental para la adaptación, y el crecimiento óptimo de las plantas.

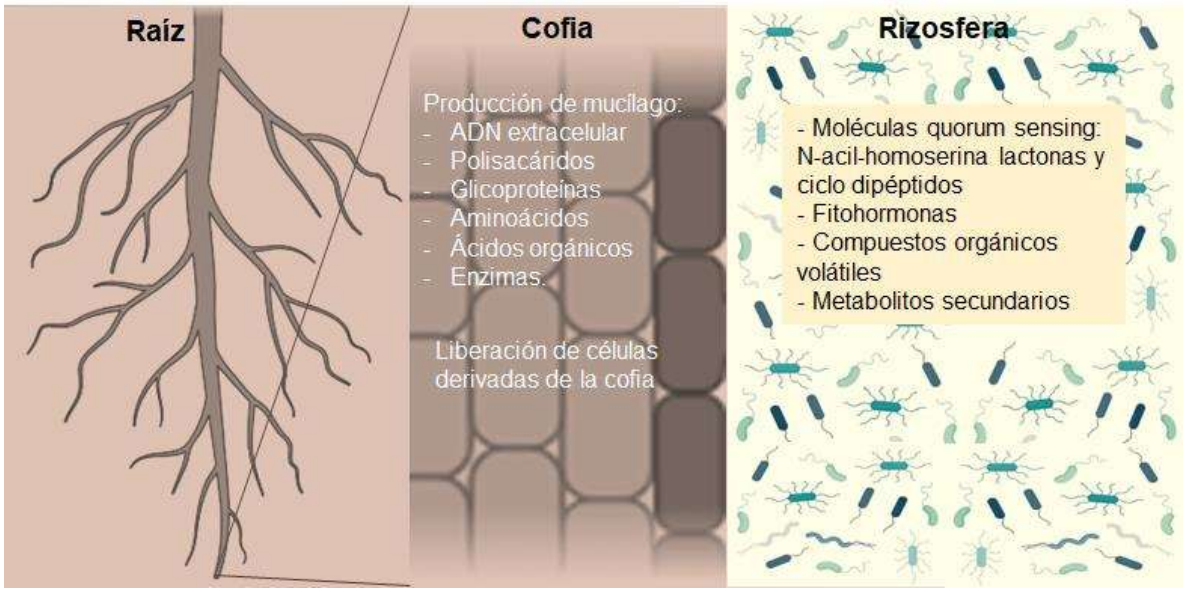


Figura 6. La comunicación química entre la cofia y la rizosfera estimula el desarrollo del sistema radicular. Los exudados radiculares y las células de la cofia asociadas a la raíz, actúan como atrayentes para los microorganismos benéficos y como barrera protectora para los patógenos. Por su parte, los microorganismos en la rizosfera producen fitohormonas, compuestos orgánicos volátiles y metabolitos secundarios que contribuyen en el establecimiento de las interacciones simbióticas para promover el crecimiento del sistema radicular.

4.3.1. La secreción de mucilago y las células derivadas de la cofia configuran el microbioma de la rizosfera

La cofia permite la penetración de la raíz a través del suelo mediante la secreción de metabolitos y substancias lubricantes, que reducen la resistencia mecánica. El proceso de biosíntesis del mucilago inicia desde la transición funcional de las células de la columnela que perciben la gravedad a células secretoras, que involucra la degradación de los amiloplastos (Driouich *et al*., 2013; Hawes *et al*., 2016; Maeda *et al*., 2019). Cuando las vesículas secretoras derivadas del aparato de Golgi alcanzan la membrana plasmática de las células de la última capa de la cofia, se fusionan a esta y liberan el contenido (Badri y Vivanco, 2009). Los exudados radiculares producidos contienen polisacáridos, glicoproteínas, aminoácidos, ácidos orgánicos, ADN extracelular y enzimas que atraen microorganismos benéficos que promueven el crecimiento radicular, en consecuencia, las plantas ejercen efectos selectivos sobre las bacterias de la rizosfera para adquirir rasgos funcionales específicos necesarios para su adaptación (Badri y Vivanco, 2009; Ganesh *et al*., 2022; Driouich *et al*., 2019). Hasta el 25% del carbono fotosintetizado es liberado a la rizosfera, por ejemplo: en forma de flavonoides para atraer a los microorganismos o mediante producción de arabinogalactanos, esenciales en la formación de biopelículas bacterianas benéficas (Odell *et al*., 2008; Jones *et al*., 2009; Beauregard *et al*., 2015). Estas fuentes de carbono son cruciales en las interacciones simbióticas con microorganismos benéficos. Recientemente, se demostró que las plantas de maíz desprovistas de la cofia modifican la composición de su microbioma, indicando que la cofia tiene una función esencial en la configuración del microbioma en la rizosfera (Ruger *et al*., 2023).

La secreción de metabolitos y la liberación de células a partir de la cofia, activan las respuestas de defensa contra microorganismos patógenos, en las que se estimula la secreción de proteínas antimicrobianas y ADN extracelular que funcionan como una barrera protectora para la raíz, llamada “trampa extracelular” (Hawes *et al*., 2016; Weiller *et al*., 2017). En plantas de chícharo, las células de la cofia secretan más de 100 proteínas, de las que 80% son glicoproteínas, extensinas, quitinasas, glucanasas y endoxiloglucano transferasas que participan en la respuesta de defensa activada por patógenos (Wen *et al*., 2007). En soja, los exudados de las células derivadas de la cofia limitaron la infección por *Phytophthora parasítica* (Ropitaux *et al*., 2020). Adicionalmente, las células de la cofia asociadas a la raíz de chícharo liberaron trampas extracelulares en interacción con la bacteria patógena *Ralstonia solanacearum*, que en conjunto con la histona 14 destruyeron parcialmente las células del patógeno (Tran *et al*., 2016). Esta evidencia sugiere que los exudados y las células derivadas de la raíz proporcionan una doble funcionalidad, por una parte, se comportan como quimioatrayentes para los microrganismos beneficios y, por otra, actúan como un escudo protector contra los patógenos.

4.3.2. Las raíces reconocen los compuestos producidos por los microorganismos en la rizosfera

En la rizosfera se establecen complejas redes de comunicación entre las diferentes especies microbianas y las plantas, como la interacción planta-bacteria, que involucra el reconocimiento mutuo y la respuesta adaptativa (Chialva *et al*., 2022). Al igual que los microorganismos del suelo que perciben los exudados radiculares, las raíces reaccionan ante la presencia de *N*-acil-*L*-homoserina lactonas y ciclo dipéptidos, así como fitohormonas que regulan el crecimiento vegetal (auxinas y citocininas) y mezclas de compuestos orgánicos volátiles emitidas por especies bacterianas y fúngicas (Ortiz-Castro *et al*., 2019; Ravelo-Ortega *et al*., 2023).

Los microorganismos de la rizosfera incluyen patrones moleculares asociados a microbios (MAMPs, por sus siglas en ingles *Microbe-Associated Molecular Patterns*), que incluyen lipopolisacáridos, peptidoglicanos, flagelina, quitina, entre otros (Millet *et al*., 2010; Pantigoso *et al*., 2022). Adicionalmente, estos elicitores activan la respuesta de defensa sistémica tanto a estrés biótico como abiótico. En este sentido, bacterias del género *Rhizobium* y los hongos micorrízicos, producen factores Nod y Myc, respectivamente, necesarios para iniciar la simbiosis con las plantas y la formación de nódulos en la raíz o para permitir la proliferación fúngica (Oldroyd, 2013). Por otra parte, las *N*-acil-*L*-homoserina lactonas y los ciclo dipéptidos de origen bacteriano influyen en la expresión de genes relacionados con el desarrollo de la planta, las respuestas a estrés y la inmunidad vegetal (Ortiz -Castro *et al*., 2008;2011; Palmer *et al*., 2014; Ortiz-Castro *et al*., 2020).

Los microrganismos rizosféricos también poseen la capacidad de biosintetizar fitohormonas, principalmente auxinas y citocininas e influir en los procesos de desarrollo de la raíz, y la producción de biomasa (Spaepen *et al*., 2014; Egamberdieva *et al*., 2017). Adicionalmente, la producción de compuestos orgánicos volátiles emitidos por las bacterias influyen en la promoción del crecimiento vegetal (Bailly *et al*., 2014; Sánchez-López *et al*., 2016; Ling *et al*., 2022). Sin embargo, se desconocen los sitios específicos de la raíz involucrados en la percepción de los microorganismos del suelo.

4.4. Las rizobacterias promotoras del crecimiento vegetal

El surgimiento de la Revolución Verde en el siglo XX, permitió la expansión de la productividad agrícola a nivel mundial. Sin embargo, el panorama actual es diferente, los suelos cultivables se encuentran en franco deterioro por el abuso de fertilizantes químicos y pesticidas, lo que resulta en un declive en la producción agrícola mundial (Kesavan y Swaminathan, 2018; Basu *et al*., 2021). Esta problemática hace necesarias alternativas ecológicas que preserven la diversidad del suelo y sean respetuosas con el medio ambiente, como el uso de microorganismos benéficos. Las rizobacterias que colonizan la raíz son

herramientas potenciales que promueven el crecimiento vegetal y actúan como agentes de control biológico (Hartman y Tringe, 2019; Atieno *et al*., 2020; Khoso *et al*., 2024; Hasan *et al*., 2024).

Algunas bacterias mejoran el crecimiento vegetal y, por ende, benefician a los cultivos, mediante la producción de fitohormonas (auxinas, citocininas y giberelinas), la adquisición y solubilización de los nutrientes, o el control de los fitopatógenos, la inducción de la tolerancia a factores abióticos, la biorremediación de metales pesados y la degradación de residuos potencialmente tóxicos como los pesticidas (Backer *et al*., 2018; Hasan *et al*., 2024).

4.4.1. Efecto de las rizobacterias promotoras del crecimiento vegetal en el desarrollo del sistema radicular

Las bacterias estimulan el desarrollo del sistema radicular a través de cambios morfológicos que comprenden modificaciones en la longitud de la raíz primaria, la frecuencia en la ramificación, y la elongación de los pelos radiculares, e incluso provocan cambios en la dirección del crecimiento de las raíces para adaptarse al entorno dinámico y mejoran así la nutrición de la planta (Méndez-Gómez *et al*., 2020; Jiménez-Vázquez *et al*., 2020; García-Cárdenas *et al*., 2023).

Azospirillum brasilense es un rizobacteria que promueve el crecimiento de las raíces laterales y la formación de pelos radiculares. Estos cambios están relacionados con la producción de las auxinas (Spaepen *et al*., 2014; Cassan y Diaz-Zorita, 2016). De acuerdo con Méndez-Gómez y col. (2020), el efecto de *A. brasilense* en *Arabidopsis* involucra elementos de las vías de señalización de auxinas y en menor medida del etileno.

Las rizobacterias *Bacillus methylotrophicus* M4-96 y *B. amyloliquefaciens* SQR9 producen compuestos orgánicos volátiles como parte de la comunicación química. La emisión de los volátiles bacterianos incrementó hasta 3 veces la biomasa del follaje y las raíces, lo que coincidió con un aumento en el contenido endógeno de ácido indol-3-acético (AIA) y la inducción en la expresión del promotor sintético inducible por auxinas *DR5:GUS* en la punta de la raíz primaria y los primordios de las raíces laterales (Pérez-Flores *et al*., 2017; Li *et al*., 2021; **Fig. 7**).

Estas bacterias además incrementan el contenido de clorofila en las hojas. Los efectos por la interacción planta-bacteria y el incremento de la respuesta local de las auxinas en los primordios de las raíces laterales, revelaron una función crucial de los elementos de señalización de las auxinas y el fitocromo como elementos claves en la fitoestimulación (García-Cárdenas *et al*., 2023).

Por otra parte, *Micrococcus luteus* LS570 estimula el crecimiento vegetal, si bien detiene la elongación de la raíz primaria, en compensación, potencia la capacidad de ramificación para facilitar la absorción de los nutrientes de su entorno (**Fig. 7**). El incremento en la biomasa de la raíz, los cambios en la estructura y en las células de la zona de crecimiento de la raíz se relacionan con la inducción en la expresión de los marcadores inducibles por las auxinas (García-Cárdenas *et al*., 2022). En conjunto, esta información nos indica que el microbioma es crucial en el desarrollo de los rasgos de la raíz a través de la respuesta hormonal, lo que finalmente, optimiza la producción agrícola sostenible y contribuiría a una segunda Revolución Verde para preservar la seguridad alimentaria.

4.4.2. El género *Achromobacter*

Las especies del género *Achromobacter* son bacilos Gram-negativos aerobios de la familia *Alcaligenacea*. La mayoría de las especies bacterianas identificadas de este género habitan en el intestino humano, sin embargo, también presentan una amplia distribución en el suelo. Varios reportes indican que las cepas de *Achromobacter* son miembros comunes del microbioma de las plantas (Jha y Kumar, 2009; Nascimento *et al*., 2019; Raj *et al*., 2019; Jiménez-Vázquez *et al*., 2020; Nascimento *et al*., 2021).

De acuerdo con Jha y Kumar (2009), la bacteria endofítica *Achromobacter xylosoxidans* WM234C-3 aislada de los tejidos de plantas de trigo, actúa como agente promotor del crecimiento vegetal, mediante su actividad nitrogenasa, la producción de AIA y la capacidad de solubilizar fosfato (P). Otra bacteria identificada como EMCC1936 pertenece al género *Achromobacter* y produce AIA y giberelinas, además, muestra la capacidad de solubilizar P y proveer formas asequibles de nitrógeno a las plantas mediante la actividad nitrogenasa (Abdel-Rahman *et al*.,

2017). Adicionalmente, los aislados rizosféricos *Achromobacter xylosoxidans* SOLR10 y *Achromobacter insolitus* AB2 son capaces de catabolizar las auxinas, el etileno y el ácido salicílico, y utilizarlas como fuente de carbono (Nascimento *et al*., 2021). En resumen, las bacterias del género *Achromobacter* influyen en el crecimiento y el desarrollo de las plantas a través de la adquisición de nutrientes y la modulación de los niveles de fitohormonas.

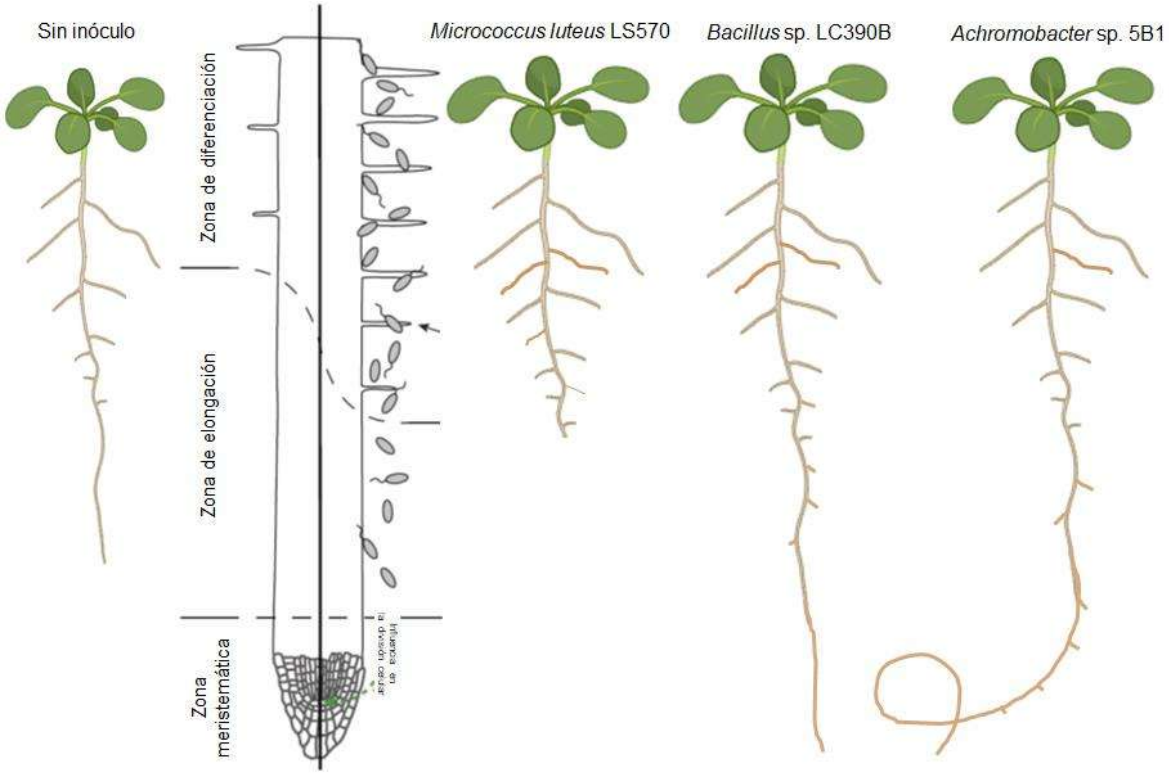


Figura 7. Las rizobacterias promotoras del crecimiento vegetal modulan el desarrollo del sistema radicular. Las rizobacterias benéficas modifican los procesos de desarrollo de la raíz que incluyen la división, elongación y diferenciación celular. Algunas rizobacterias inhiben la elongación de la raíz primaria y promueven la formación de las raíces laterales como *Micrococcus luteus* LS570. Mientras que, rizobacterias como *Bacillus* sp. LC390B y *Achromobacter* sp. 5B1 incrementan el crecimiento de la raíz primaria, así como la ramificación de la raíz, incluso esta última desencadena cambios en la dirección del crecimiento de la raíz a través de la interrupción de la respuesta gravitrópica (Adaptado de Verbon y Liberman, 2016).

4.4.2.1. *Achromobacter* sp. 5B1

En el desierto de Chihuahua se localiza un sistema de pozos, marismas y manantiales rodeados de áreas extensas de suelos calcáreos alcalinos. Dentro de esta área natural protegida se ubica la “Poza Salada”, cuyo nombre hace alusión a su alta concentración de sal. A pesar del suelo salino y el pH alcalino, diversas especies vegetales que incluyen pastizales, matorrales y arbustos crecen en la proximidad de la poza. Investigaciones recientes, atribuyen la resistencia de estas plantas a dichas condiciones, al microbioma de la rizosfera. Los aislados rizobacterianos *Bacillus* sp. y *Pseudomonas lini* mostraron la capacidad de tolerar concentraciones elevadas de sal y proveer beneficios al pasto *Distichlis spicata* (Palacio-Rodríguez *et al.,* 2017). A partir de la rizosfera de los arbustos de mezquite (*Prosopis* sp.), se aisló la rizobacteria *Achromobacter* sp. 5B1 que induce el crecimiento vegetal y la resistencia al estrés salino (Jiménez-Vázquez *et al*., 2020).

En nuestro grupo de trabajo, se demostró que el co-cultivo de plántulas de *Arabidopsis* con la cepa bacteriana *Achromobacter* sp. 5B1 reconfigura la arquitectura del sistema radicular. Los datos mostraron que la rizobacteria mejora el crecimiento de la raíz primaria, y simultáneamente, incrementa la ramificación a través de la estimulación de la maduración de los primordios de las raíces laterales (**Fig. 8 a, b**). Este efecto probiótico se mostró en tres sistemas de co-inoculación planta-bacteria *in vitro,* y en sustrato. En uno de los sistemas de interacción planta-bacteria, las raíces fueron colocadas sobre la estría bacteriana, además, se empleó un sistema de comunicación vía volátiles, donde las plantas y la bacteria crecieron en lados separados de la placa de Petri, adicionalmente, la estría bacteriana fue colocada en frente de la raíz primaria en crecimiento (Jiménez-Vázquez *et al*., 2020). Sorprendentemente, las raíces colonizadas con la rizobacteria benéfica mostraron un comportamiento agravitrópico, que desencadena la formación de ondulaciones y la formación de giros en la raíz primaria. La distribución asimétrica de las auxinas en la punta de la raíz mediada por la modulación en el transporte de las auxinas, correlaciona con la desviación en el crecimiento direccional de la raíz (**Fig. 8 c-d**). Los patrones de crecimiento de la raíz en respuesta a la interacción con *Achromobacter* sp. 5B1 podrían ayudar a la planta a explorar y adaptarse a las

condiciones ambientales de su entorno, y crecer hacia zonas con mayor disponibilidad de agua y nutrientes (Jiménez-Vázquez *et al*., 2020).

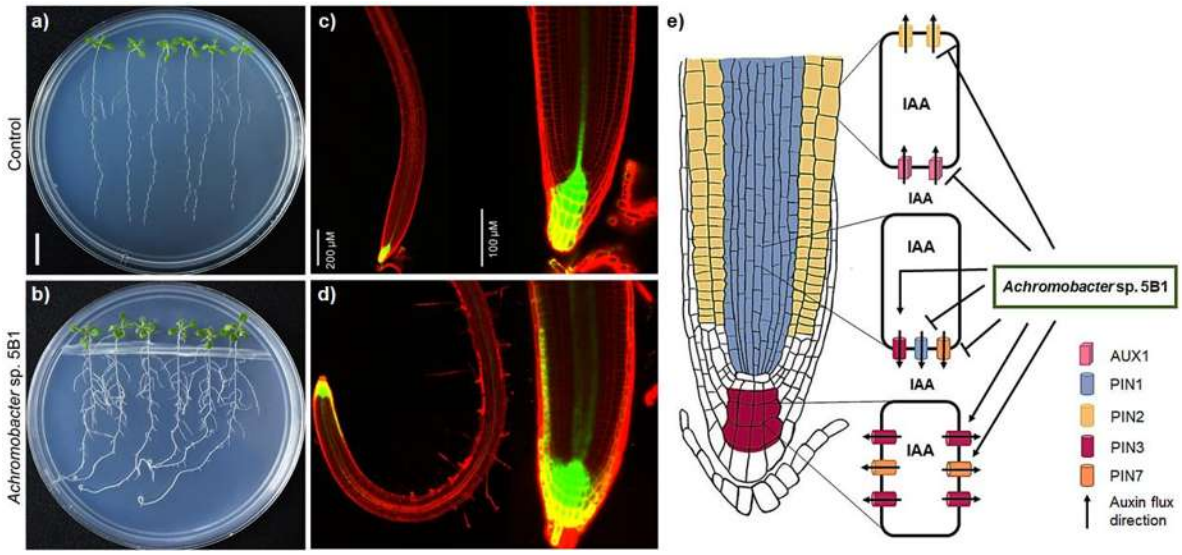


Figura 8. Efecto de la co-inoculación de *Arabidopsis* con la rizobacteria benéfica *Achromobacter* sp. 5B1. a, b) Plántulas de Arabidopsis en condiciones axénicas e inoculadas con la cepa *Achromobacter* sp. 5B1. c, d) En condición control, el patrón de expresión de *DR5:GFP* se observa en el haz vascular, el centro quiescente y las células de la columnela, mientras que, la inoculación con *Achromobacter* sp. 5B1 muestra un comportamiento agravitrópico y la expresión de *DR5:GFP* se redistribuye hacia el lado cóncavo de los giros de la raíz. e) Representación esquemática de la punta de la raíz primaria de *A. thaliana* donde se muestra los dominios de expresión de los transportadores de eflujo de las auxinas PIN. *Achromobacter* sp. 5B1 induce la expresión de PIN3 en las células de la columnela, sin embargo, reprime la expresión de PIN1 y PIN2 en la zona meristemática y la zona de elongación. (Modificado de Jiménez-Vázquez *et al*., 2020; Verhage, 2020).

Recientemente, se reportó como la inoculación con esta rizobacteria rescata a las plantas del estrés alcalino, y mostró que el genoma de esta rizobacteria alberga genes involucrados en la comunicación planta-bacteria y la re-

sistencia a estrés, lo que podría contribuir al crecimiento de las plantas bajo condiciones limitantes y mejorar la producción de biomasa (Jiménez-Vázquez *et al.,* 2023). Sin embargo, cómo la raíz percibe a la rizobacteria benéfica para desencadenar cambios en la dirección de su crecimiento e influir en la respuesta adaptativa de la planta, se desconoce. En este trabajo, se investigó la función de la cofia, específicamente la participación de los factores de transcripción FEZ y SMB

involucrados en el desarrollo del órgano de percepción de las señales ambientales, en la respuesta de crecimiento de la raíz ante la inoculación con la rizobacteria benéfica *Achromobacter* sp. 5B1.

5. JUSTIFICACIÓN

Achromobacter sp. 5B1 influye en el gravitropismo a través de alteraciones en el transporte y la distribución de las auxinas en la raíz. La cofia dirige el crecimiento de la raíz en respuesta a los estímulos ambientales como la gravedad y los nutrientes a través de los factores transcripcionales FEZ y SOMBRERO. Estudiar la participación de los elementos localizados en la cofia en el crecimiento de la raíz y la interacción con *Achromobacter* sp. 5B1, permitirá dilucidar la importancia de esta rizobacteria en la adaptación ambiental y la productividad vegetal.

6. HIPÓTESIS

Los factores de transcripción FEZ y SOMBRERO involucrados en los procesos de desarrollo de la cofia regulan el crecimiento direccional de la raíz en respuesta a *Achromobacter* sp. 5B1.

7. OBJETIVOS

7.1. Objetivo general

Determinar la participación de los factores de transcripción FEZ y SOMBRERO en la respuesta de crecimiento de la raíz de *A. thaliana* a la interacción con *Achromobacter* sp. 5B1.

7.2. Objetivos específicos

1. Evaluar la participación de los genes *FEZ y SOMBRERO* involucrados en el desarrollo de la cofia en respuesta a la interacción con *Achromobacter* sp. 5B1.
2. Analizar el efecto de la inoculación con *Achromobacter* sp. 5B1 sobre la estructura de la cofia en plantas de *A. thaliana* afectadas en los factores de transcripción FEZ y SOMBRERO.
3. Determinar la participación de los elementos de respuesta a auxinas involucrados en el reconocimiento de *Achromobacter* sp. 5B1 a través de los factores de transcripción localizados en la cofia.

8. RESULTADOS

8.1. Capítulo I.

Jiménez-Vázquez, K.R., López-Bucio, J. S., Ruíz-Herrera, L.F., and López-Bucio, J. (2025). Root cap transcription factors control directional root growth in *Arabidopsis* seedlings in response to the plant growth promoting rhizobacterium *Achromobacter* sp. 5B1. *The Plant Journal,* 122 e70226*.*

8.2. Capítulo II

Jiménez-Vázquez, K. R. J., López-Hernández, J., García-Cárdenas, E., Pelagio-Flores, R., López-Bucio, J. S., Texón, A. C., *et al.* (2024). The plant growth promoting rhizobacterium *Achromobacter* sp. 5B1, rescues Arabidopsis seedlings from alkaline stress by enhancing root organogenesis and hormonal responses. *Microbiological Research*, 281, 127594.

8.3. Capítulo III

López-Bucio, J., Ortiz-Castro, R., Magaña-Dueñas, V., García-Cárdenas, E., **Jiménez-Vázquez, K. R.,** Raya-González, J., Pelagio-Flores, R., Ibarra-Laclette, E., & Herrera-Estrella, L. (2023). *Pseudomonas aeruginosa* LasI-dependent plant growth promotion requires the host nitrate transceptor AtNRT1. 1/CHL1 and the nitrate reductases NIA1 and NIA2. *Planta*, 258(4), 80.

8.4. Capítulo IV

López-Hernández, J., García-Cárdenas, E., López-Bucio, J.S., **Jiménez-Vázquez, K.R.,** de la Cruz, H.R., Ferrera-Rodríguez, O., Santos-Rodríguez D.L., Ortiz-Castro, R., & López-Bucio, J. (2023). Screening of phosphate solubilization identifies six *Pseudomonas* species with contrasting phytostimulation properties in Arabidopsis seedlings. *Microbial Ecology*, 86(1), 431-445.

8.5. Capítulo V

Jiménez-Vázquez, K.R., López-Bucio, J., Beltrán-Peña, E. B. (2022). Influencia de las bacterias en la respuesta hormonal y la arquitectura de la raíz. *Biológicas Revista de la DES Ciencias Biológico Agropecuarias Universidad Michoacana de San Nicolás de Hidalgo*, 21(1), 1-8.

8.1. Capítulo I.

the plant journal

The Plant Journal (2025) 122, e70226 doi: 10.1111/tpj.70226

Root cap transcription factors control directional root growth in Arabidopsis seedlings in response to the plant growth-promoting rhizobacterium *Achromobacter* sp. 5B1

Kirán Rubí Jiménez-Vázquez[1], Jesús Salvador López-Bucio[2], León Francisco Ruíz-Herrera[1] and José López-Bucio[1,*]

[1]*Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C.P. 58030, Morelia, Michoacán, México, and*

[2]*CONACYT-Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolas de Hidalgo, Edificio B1, Ciudad Universitaria, C.P. 58030, Morelia, Michoacán, México*

Received 16 January 2025; revised 3 April 2025; accepted 2 May 2025.
*For correspondence (e-mail jbucio@umich.mx).
Kirán Rubí Jiménez Vázquez—first author.

SUMMARY

The root cap protects the meristem as the root grows and senses stimuli such as gravity and nutrient availability. Although it enables tight interaction with the rhizosphere, a role in perceiving microorganisms is unknown. Here, through Arabidopsis direct root inoculation with the plant growth promoting rhizobacterium *Achromobacter* sp. 5B1, we unveiled a critical, opposite role of two transcription factors, FEZ and SOMBRERO, in mediating growth, waving, and coiling of primary roots as a response to the bacterium. In Arabidopsis seedlings with mutations in *FEZ*, the rhizobacterium had increased formation of coils, whereas in seedlings harboring mutations in *SOMBRERO*, the formation of coils did not occur and the root maintains normal gravity response. This correlated with the elongation of the cells on the right or convex side, an aspect that is increased in *fez-2* mutants but does not manifest itself in *smb-3* mutants. Interaction with *Achromobacter* sp. 5B1 increased the size of the root cap, which coincided with decreased or increased detection *in vivo* of *FEZ:FEZ:GFP* and *SMB:SMB:GFP*, respectively, and with the phenotype of *smb-3* mutants, which develop roots with a huge cap irrespective of bacterization. Expression of auxin transporters PIN1, PIN2, PIN3, PIN4, and PIN7 overall decreased in the WT, *fez-2*, and *smb-3* upon bacterial inoculation, suggesting that the agravitropic behavior of roots may be linked to disturbance of auxin distribution within the root tip. Our data indicate that the root cap senses the rhizobacterium *Achromobacter* sp. 5B1 through FEZ and SOMBRERO for the directional growth and soil exploration.

Keywords: root cap, transcription factors, *Achromobacter* sp. 5B1, root growth, auxin distribution.

INTRODUCTION

The root cap, comprised of several layers of mucilage-secreting cells, protects the meristem and is responsible for directional root growth in response to environmental stimuli such as gravity and nutrient patches within the soil (Kumpf & Nowack, 2015). In *Arabidopsis thaliana*, this organ consists of the columella and the lateral root cap (LRC), which covers the meristem (Arnaud et al., 2010; Dolan et al., 1993). The columella is organized into two different types of cells: cells rich in amyloplasts, starch-containing plastids involved in gravity perception, and secretory cells including the outer columella and the LRC layers (Nakamura et al., 2019). Besides its essential role in gravity perception (gravitropism), the root cap senses touch, water, and light, to mention a few examples (Kutschera & Briggs, 2012; Massa & Gilroy, 2003; Sato et al., 2015). Recently, root cap components were involved in the response to nutrient deficiency and root halotropism (Ravelo-Ortega et al., 2022; Zheng et al., 2024) as well as rhizosphere microbiome composition (Rüger et al., 2023) indicating its critical role in adaptation to both biotic and abiotic stimuli.

Root gravitropism comprises three sequential phases: gravity perception, signal transduction, and differential cell growth (Sato et al., 2015). In gravistimulated roots, the perception of gravity occurs in columella cells and the amyloplasts sediment to the new bottom of the cell. This physical stimulus transduces a gravitropic signal to trigger the re-localization of the auxin efflux carriers PIN-FORMED (PIN) proteins PIN3 and PIN7 on the plasma membrane

toward the direction of gravity, redirecting the auxin flow downwards by the action of the PIN2 protein located in the cells of the LRC and epidermis, generating an asymmetric distribution of auxins between the upper and lower sides of the root. Finally, the growth response occurs within the elongation zone leading to gravitropic root growth (Blancaflor, 2013; Su et al., 2017).

Root growth is characterized by continuous cell generation. However, the root cap maintains a constant size through the balance between cell division, differentiation, sloughing, and cell death (Barlow, 2003; Fendrych et al., 2014; Kumar & Iyer-Pascuzzi, 2020). Experiments that included removal of the root cap via laser ablation or genetically programmed death of columella cells resulted in halted growth and short roots with an agravitropic behavior (Blancaflor et al., 1999; Tsugeki & Fedoroff, 1999). The transcription factors NAC (NO APICAL MERISTEM, ARABIDOPSIS THALIANA ACTIVATING FACTOR, CUP-SHAPED COTYLEDON), including FEZ and SOMBRERO (SMB) critically control root cap development (Bennett et al., 2010; Huysmans et al., 2018; Willemsen et al., 2008). FEZ is expressed in columella and epidermis/LRC stem cells and promotes the formative divisions, whereas SMB represses FEZ activity in root cap daughter cells, regulates differentiation and maturation, and activates programmed cell death (PCD) in LRC cells (Bennett et al., 2014; Fendrych et al., 2014; Willemsen et al., 2008). Indeed, the *fez-2* and *smb-3* mutants exhibit contrasting root cap phenotypes; the *fez-2* mutant shows reduced root cap size, while *smb-3* has an increased number of root cap cell layers (Bennett et al., 2014; Fendrych et al., 2014).

The establishment of an auxin gradient with maximum accumulation in the quiescent center and an auxin response minimum in the most apical columella cell layer is critical for cell turnover, which is related to the null expression of auxin efflux transporters PIN3, PIN4, and PIN7 in outermost cap layers (Dubreuil et al., 2018). In this regard, treatments in roots with the synthetic auxin 1-naphthaleneacetic acid (NAA) stimulate the differentiation of the columella stem cells (Ding & Friml, 2010). The microbiota feedback in the soil–plant interface is fundamental for adaptation and optimal plant growth. Root cap-derived cells or border cells and mucilage secretion are essential for interactions with soil microorganisms (Driouich et al., 2021). Root exudates contain sugars, amino acids, organic acids, glycoproteins, extracellular DNA, and enzymes to attract beneficial microorganisms that promote root growth and contribute to the biofilm formation of beneficial bacteria (Badri & Vivanco, 2009; Ganesh et al., 2022). On the other hand, roots perceive quorum-sensing molecules, plant growth regulators, volatile organic compounds (VOCs), and secondary metabolites already released by soil microorganisms, which enables tight communication (Ortiz-Castro et al., 2011, 2020; Ortiz-Castro & López-Bucio, 2019; Ravelo-Ortega et al., 2023). Thus, understanding how the root perceives beneficial bacteria is fundamental in studying plant–microbe interactions.

Beneficial bacteria influence the development of the root system through auxin biosynthesis or modulation of auxin transport or response (García-Cárdenas et al., 2022; Méndez-Gómez et al., 2020). Previous research indicated that the plant-beneficial rhizobacterium *Achromobacter* sp. 5B1 promotes primary root growth and lateral root formation while interfering with the root gravitropic response, root waving, and coiling (Jiménez-Vázquez et al., 2020). Additionally, we showed that plant inoculation with this bacterium enhanced surveillance under alkaline conditions, maintaining root meristems active through an enhanced auxin response (Jiménez-Vázquez et al., 2023). In the present work, we report that the transcription factors FEZ and SMB oppositely regulate the root agravitropic behavior manifested by Arabidopsis primary roots in contact with *Achromobacter* sp. 5B1, likely acting downstream of PIN auxin transporters. More specifically, we show that SMB is required for asymmetric directional root growth through differential cell elongation in response to the bacterium. Our findings extend what is known about the role of rhizobacteria influencing root movements, which may help plants to expand their exploratory and adaptive capacity to the needs imposed by the environment and take advantage of available resources.

RESULTS

Root contact with *Achromobacter* sp. 5B1 determines the deviation of root growth of Arabidopsis seedlings from the gravity vector

Recognizing the diversity of mechanisms involved in plant biostimulation by *Achromobacter* sp. 5B1, it would be expected that the degree of modification in developmental processes is associated with bacterial spread over the root, secretion of phytohormones, or emission of volatile compounds (Jiménez-Vázquez et al., 2020, 2023). Analyses of the interaction between *Achromobacter* sp. 5B1 and Arabidopsis plants were done using three different *in vitro* systems: (i) placing the seedlings with their roots in direct contact with a bacterial streak, (ii) growing the bacterial streak and seedlings on opposite sides of divided Petri plates, which enables the sensing of bacterial VOCs by plants, and (iii) inoculating the bacterium at a distance from the root tip of seedlings growing over the plate, which enables roots to react to diffusible molecules such as bacterial metabolites and phytohormones.

In all three systems, the bacterium promoted primary root growth, lateral root formation, and lateral root density (Figure S1), but only seedlings directly in contact with the bacterial inoculum deviated their growth from the gravity

vector (Figure S1) and had an increased auxin-inducible expression revealed by the *DR5* promoter (Figure S2). In another set of experiments, the bacterial inoculum was placed on one side of Petri plates, and the seedlings were transferred side by side; in this case, the plant response varied with the distance to the inoculum, and only the first plant, which contacted the streak, deviated the growth of its primary root (Figure S3). These data suggest that it is the root contact with *Achromobacter* sp. 5B1, but not bacterial volatiles or diffusible compounds, that determine the deviation of root growth of Arabidopsis seedlings from the gravity vector.

The root cap plays an important role in the deviation of primary root growth of Arabidopsis seedlings in response to *Achromobacter* sp. 5B1 inoculation

The root cap is located at the tip of each root and controls microbiome assembly and gravitropism (Rüger et al., 2023). To test the role of the root cap in the deviation of primary root growth in response to *Achromobacter* sp. 5B1 inoculation, transgenic Arabidopsis seedlings expressing *DR5:GFP* were germinated and grown on 0.2× MS media for 6 days. Subsequently, plants with root caps (Figure S4a–d) or those having removed root caps with a scalpel (Figure S4e–h) were transferred to axenic MS 0.2× media (Figure S4a,b,e,f) or over a bacterial streak (Figure S4c,d,g,h). Representative images of plates and root tips expressing the *DR5* promoter were taken after three additional days. Figure S4 shows that plants with removed root caps did not deviate from their root growth like the WT in response to the root contact with the bacterium and the auxin maximum was also much decreased.

To assess whether bacterial cells could be present in the proximity of the root cap, once the plants have grown and roots are coiled, bacterial growth around Arabidopsis roots co-cultivated with *Achromobacter* sp. 5B1 was monitored at 2, 4, and 6 days after transfer of seedlings over the bacterial inoculum. At these times, the seedlings were carefully removed from the media to detect bacterial presence. Representative images of Petri plates where the plants were co-cultivated with *Achromobacter* sp. 5B1 in direct contact with roots shows the remaining bacteria that mimic the form of the host root, indicative of spread growth around the root system (Figure S5). These data indicate that the bacteria extend over the root system from the streak and that the root cap is the organ responsible for the sensing of the bacterium to direct root growth direction.

FEZ and SOMBRERO control root growth direction in response to *Achromobacter* sp. 5B1

The transcription factors FEZ and SMB drive the formation of the Arabidopsis root cap (Bennett et al., 2010; Willemsen et al., 2008). Considering the potential role of their corresponding genes in sensing environmental stimuli, the growth and development of Arabidopsis wild-type (WT) seedlings and *fez-2* and *smb-3* mutants under axenic conditions and co-cultivated with *Achromobacter* sp. 5B1 was compared. Four days after germination, Arabidopsis seedlings were transferred to Petri plates containing 0.2× MS medium with or without a bacterial streak and grown on plates placed vertically for six additional days. In axenic medium, WT and *fez-2* mutant seedlings exhibited comparable root growth, whereas the *smb-3* mutants showed slightly reduced growth (Figure 1A–C). In the plant–bacteria interaction, WT, *fez-2*, and *smb-3* mutant seedlings displayed notoriously more lateral roots, and WT roots deviated their growth toward the side of the plate, and *fez-2* roots showed an exacerbated root deviation; most of the roots made right-handed coils in a synchronized manner, whereas in *smb-3* roots, no gravitropic deviation was noticed (Figure 1D–I).

It was tested whether these changes in directional root growth influenced total plant biomass, shoot biomass, or root biomass production as well as root architecture traits including primary root growth, lateral root formation, and root coil formation. Bacterization clearly increased total fresh weight, shoot fresh weight, and root fresh weight (Figure 2A–C) and promoted primary root growth and root branching in the WT, *fez-2*, and *smb-3* mutants (Figure 2D, E). However, the most contrasting effect of the rhizobacterium was observed in directional root growth, where 30, 80, and 0% of primary roots of WT, *fez-2*, and *smb-3* seedlings, respectively, formed coils (Figure 2F). The contrasting root coiling phenotypes in *fez-2* and *smb-3* when compared to WT seedlings suggest that transcription factors FEZ and SMB control root growth direction upon Arabidopsis cocultivation with *Achromobacter* sp. 5B1.

Root growth direction response of Arabidopsis seedlings to *Achromobacter* sp. 5B1 is not a general pattern of response to other bacteria

Plant growth-promoting rhizobacteria affect plant morphogenesis in several ways. To determine if the deviation of root growth as a response to *Achromobacter* sp. 5B1 is specific or if it is a general pattern of response to plant growth-promoting bacteria or to any other member of the root microbiome, we compared the root architectural response and deviation of primary root growth from the gravity vector of Arabidopsis WT, *fez-2*, and *smb-3* mutants in response to *Achromobacter* sp. 5B1, *Bacillus* sp. LC390B, and *Micrococcus luteus* LS570. Figure 3 shows representative images of WT, *fez-2*, and *smb-3* mutants transferred 4 days after germination to 0.2× MS axenic media or placed over bacterial streaks and grown for six additional days. It can be appreciated the diverse root phenotypes elicited by each bacterium (Figure 3A–H). *Micrococcus luteus* LS570 repressed primary root growth, but in contrast, *Achromobacter* sp. 5B1 and *Bacillus* sp. LC390B

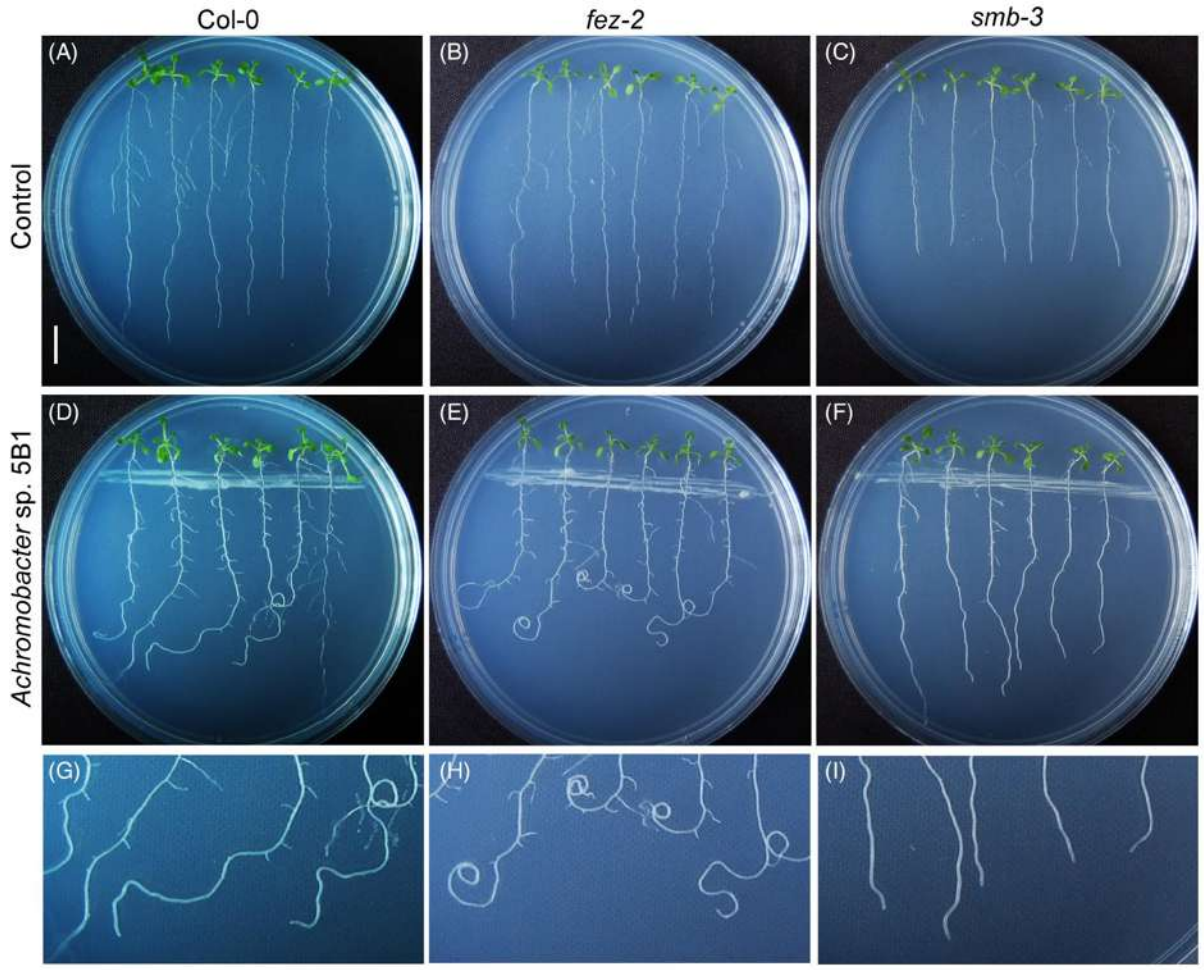


Figure 1. Root response of *Arabidopsis* wild-type, *fez-2*, and *smb-3* to colonization by *Achromobacter* sp. 5B1.
Representative images of WT, and *fez-2* and *smb-3* mutants germinated and grown on agar solidified 0.2× MS media for 6 days. Subsequently, the plants were transferred to fresh, axenic media without bacterial inoculum (A–C) or placed over a bacterial streak (D–F) for six additional days. Close-ups of root tips treated with *Achromobacter* sp. 5B1 (G–I). Scale bar = 1 cm. The experiment was repeated three times with comparable results.

promoted primary root growth (Figure 3I). Despite all three bacteria clearly promoting root branching (Figure 3J,K), only *Achromobacter* sp. 5B1 could deviate WT primary root growth and induce the formation of coils in *fez-2* mutants (Figure 3B,F), which indicates the root growth direction response of Arabidopsis seedlings to *Achromobacter* sp. 5B1 is not a general pattern of response to other bacteria.

Gravity response deviation in response to *Achromobacter* sp. 5B1 is related to cell differential growth at the root elongation zone

Arabidopsis roots growing on inclined agar surfaces exhibit undulations and re-orientation from the gravitational axis. These root behaviors, named waving and skewing, result from circumnutation, gravitropism, and contact with the solidified agar medium (Oliva & Dunand, 2007; Porat et al., 2024). WT, *fez-2*, and *smb-3* mutant seedlings were grown and transferred 4 days after germination to 0.2× MS media with or without bacterial inoculum. After 6 days of growth, axenic seedlings showed the oscillatory movement typical of root growth on the medium, characterized by the frequency and amplitude of waves; in *fez-2* roots, an increased oscillatory frequency was found; by contrast, in *smb-3* roots, waving was very reduced (Figure S6a–c). In plant–bacteria co-cultivation, WT seedlings deviated their roots toward the right side of the plate, and root coiling appeared, a trait exacerbated in the *fez-2* mutant. In contrast, *smb-3* roots exhibited slight sinusoidal growth (Figure S6d–h). The waving generated by bacterial inoculum increased about twice in both wavelength and amplitude in WT and *fez-2* seedlings compared with WT

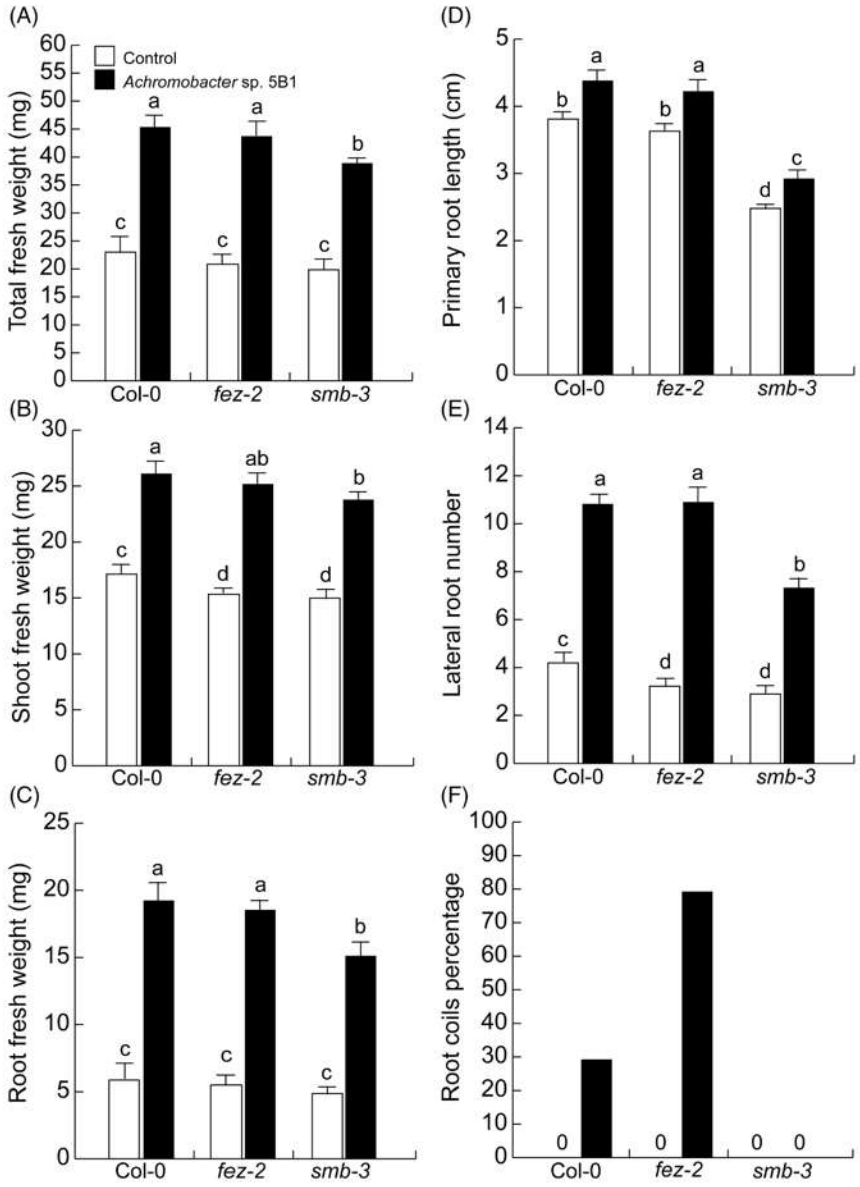


Figure 2. Biomass production and modulation of root architecture in Arabidopsis WT, *fez-2*, and *smb-3* mutants in cocultivation with *Achromobacter* sp. 5B1. WT, *fez-2*, and *smb-3* seedlings were transferred 4 days after germination to 0.2x MS axenic media or over a bacterial inoculum and grown for six additional days. (A) Total fresh weight, (B) shoot fresh weight, (C) root fresh weight, (D) primary root length, (E) lateral root number, and (F) Percentage of primary roots forming coils at the tip. Bars show the mean ± SD. Different letters indicate statistically significant differences ($P < 0.05$; $n = 18$). The experiment was repeated three times with comparable results.

seedlings without bacterial inoculum; however, in *smb-3* roots, this did not occur (Figure S6g,h).

We examined whether agravitropic root growth in response to *Achromobacter* sp. 5B1 is associated with differential cell growth at the root elongation zone. WT, *fez-2*, and *smb-3* seedlings without bacterial streak showed comparable growth of epidermal and cortex cells on both left and right root flanks (Figure 4A–C). In plant–bacteria cocultivation, WT seedlings showed increased cell length on the convex side of the coils compared to cells on the concave side. Arabidopsis *fez-2* and *smb-3* mutant seedlings inoculated with the bacterium showed contrasting cell elongation; *fez-2* mutant cells on the convex side of the coils reached a length three times greater than WT

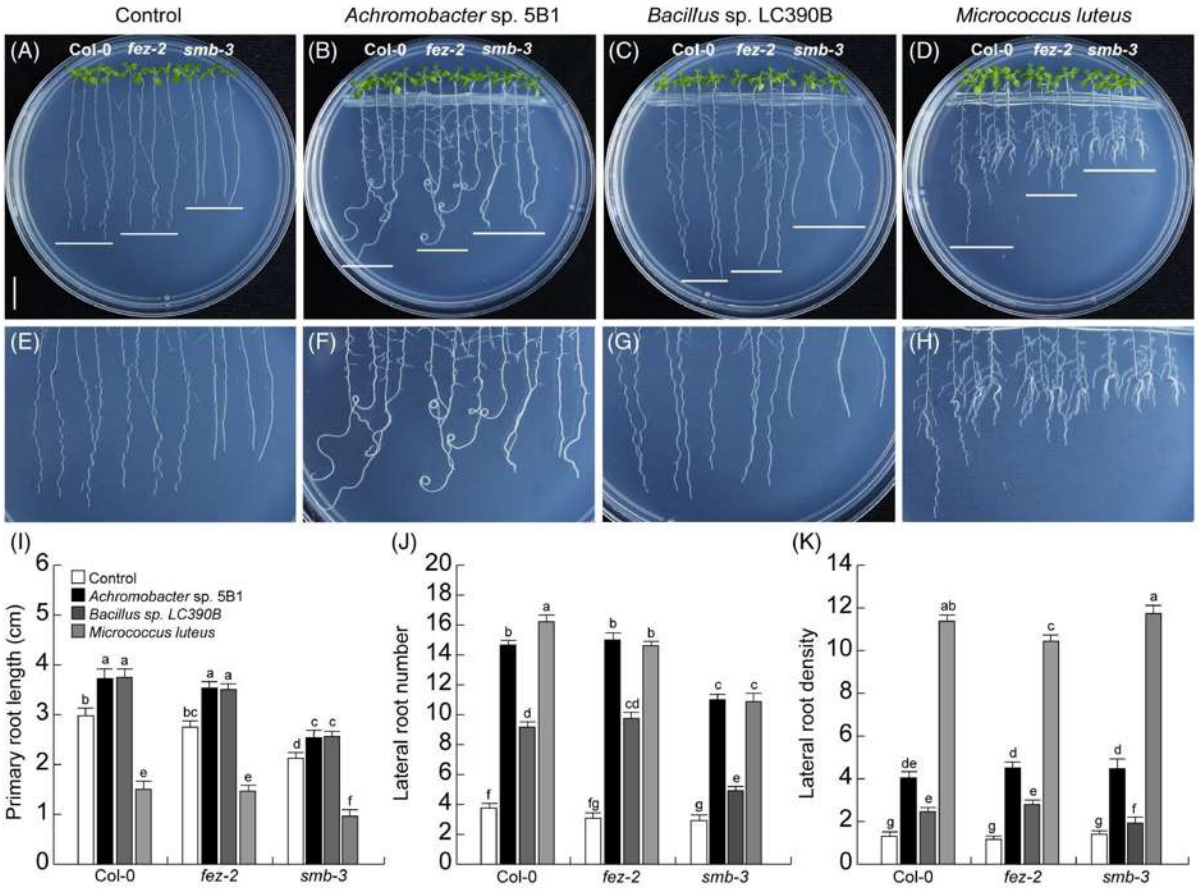


Figure 3. Root architectural response of Arabidopsis WT, *fez-2*, and *smb-3* mutants in response to *Achromobacter* sp. 5B1, *Bacillus* sp. LC390B and *Micrococcus luteus*.

Representative images of WT, *fez-2*, and *smb-3* mutants transferred 4 days after germination to 0.2× MS axenic media (A) or with bacterial streaks and grown for six additional days (B–D). Close-up of root tips treated with *Achromobacter* sp. 5B1, *Bacillus* sp. LC390B and *Micrococcus luteus* (E–H). (I) Primary root length, (J) lateral root number, and (K) lateral root density. Scale bar = 1 cm. Bars show the mean ± SD. Different letters indicate statistically significant differences ($P < 0.05$; $n = 18$). The experiment was repeated three times with comparable results.

seedlings without inoculum, whereas *smb-3* roots exhibited similar cell growth on both root flanks (Figure 4D–F). Noticeably, the bacterium boosted the growth of epidermal and cortex cells in the left, concave side (L) or right, convex (R) side of the root cylinder of *fez-2* mutants but not in *smb-3* (Figure 4G,H). Taken together, these results suggest that signals from the root cap cause agravitropic root behavior through differential cell elongation in inoculated seedlings.

Achromobacter sp. 5B1 increases the size of the root cap in WT seedlings and *smb-3* mutants

To test if the observed changes in root growth patterns in response to the bacterium are related to root cap size, we used Lugol staining to identify differentiated columella cells in WT, *fez-2*, and *smb-3* mutant seedlings. As expected, in axenic medium, we found that *fez-2* roots exhibited a root cap of reduced size compared to the WT, whereas *smb-3* mutants had huge caps that doubled the normal size (Figure 5A–C). WT, *fez-2*, and *smb-3* seedlings placed on bacterial streak significantly increased root cap area; even more in *smb-3* mutant in which the root cap size increased up to three times (Figure 5D–H). These data suggest that FEZ and SMB may act to configure the root cap in response to *Achromobacter* sp. 5B1 signals.

Achromobacter sp. 5B1 influences the expression of *FEZ::FEZ:GFP* and *SMB::SMB:GFP* in the root cap

To understand the effect of *Achromobacter* sp. 5B1 on root cap development, we evaluated the expression patterns of transcription factors FEZ and SMB. Four-day-old transgenic Arabidopsis seedlings expressing *FEZ::FEZ:GFP* and *SMB::SMB:GFP* were transferred to fresh media or co-cultivated with *Achromobacter* sp. 5B1. After 6 days, live-cell imaging of *FEZ::FEZ:GFP* decreased at the root tip periphery (Figure 6A,C,E). In contrast, bacterial colonization of the

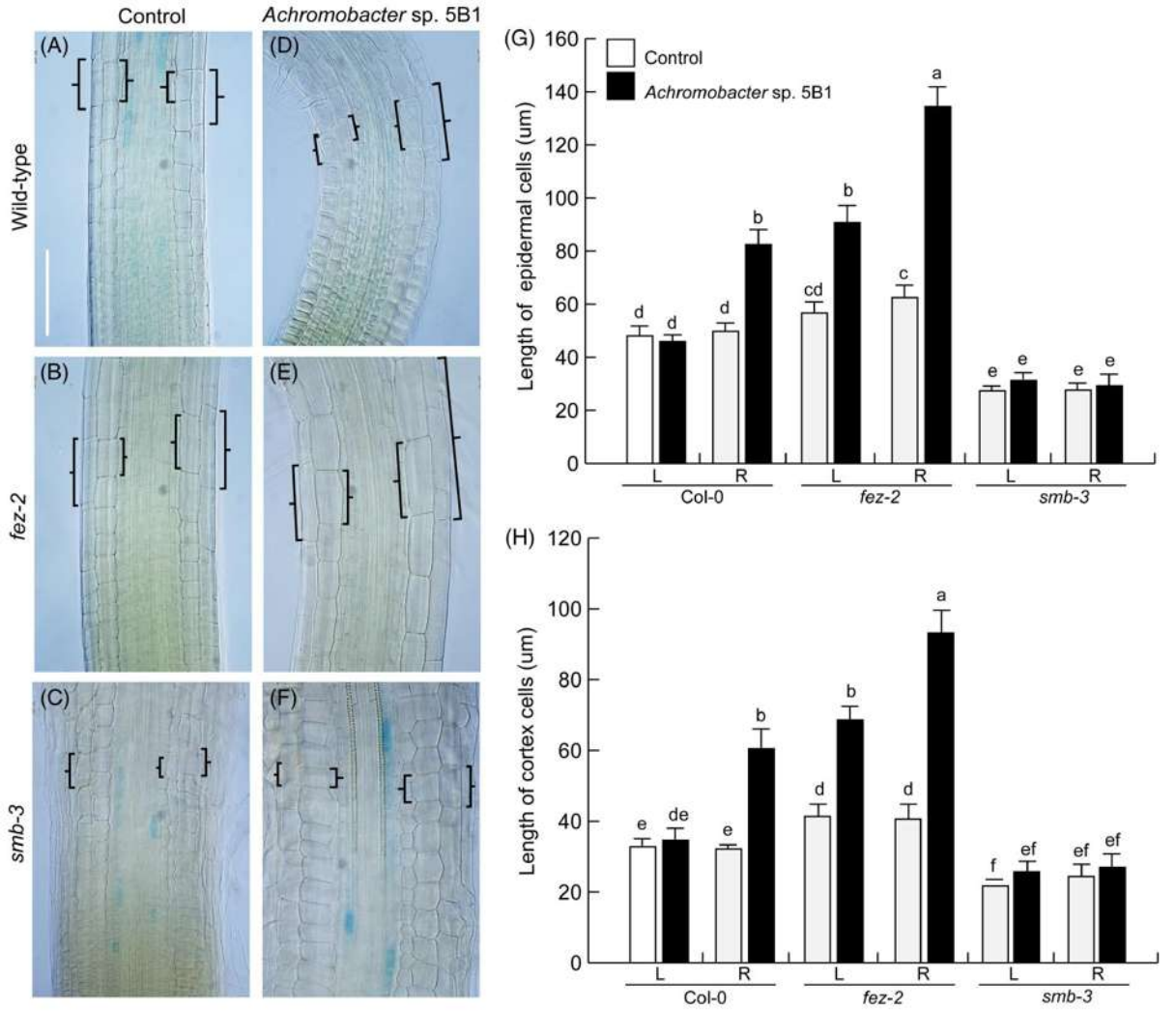


Figure 4. Effect of *Achromobacter* sp. 5B1 on the growth of root elongation zone cells in WT, *fez-2*, and *smb-3* Arabidopsis mutant seedlings. Representative images of WT, *fez-2*, and *smb-3* transferred 4 days after germination to axenic 0.2× MS medium (A–C) or placed over an *Achromobacter* sp. 5B1 inoculum streak (D–F) and co-cultivated for 6 days. Graphs show the effects of bacterial co-cultivation on the length of epidermal cells (G) and the length of cortex cells (H) in the left, concave side (L), or right, convex (R) side of the root cylinder. Bars show the mean ± SD. Different letters indicate statistically significant differences ($P < 0.05$; $n = 18$). Scale bar: 1 mm. These analyses were repeated three times with comparable results.

SMB::SMB:GFP reporter construct enhanced expression in more layers of the columella and LRC cells, which could be related to the greater number of cells in the root cap (Figure 6B,D,E). These results indicate that *Achromobacter* sp. 5B1 influences the expression of FEZ and SMB in the root cap.

The size of the root cap correlates with *DR5:GFP* auxin maximum within the Arabidopsis root tip and is regulated by FEZ and SOMBRERO

Achromobacter sp. 5B1 drives root growth through an asymmetric auxin distribution (Jiménez-Vázquez et al., 2020). However, whether root cap-associated elements, including FEZ and SMB, could be involved in the asymmetric auxin distribution that triggers directional root growth by the rhizobacteria is unknown. Therefore, we examined the expression patterns of *fez-2* and *smb-3* mutant seedlings carrying the *DR5:GFP* auxin-inducible reporter gene in primary root tips. As expected, cocultivation with *Achromobacter* sp. 5B1 enhanced auxin response in the root tip and the auxin redistribution toward the LRC and epidermal cells compared with root growth under axenic conditions, according to *DR5* fluorescence (Figure 7A,D). The *fez-2* mutant exhibited a

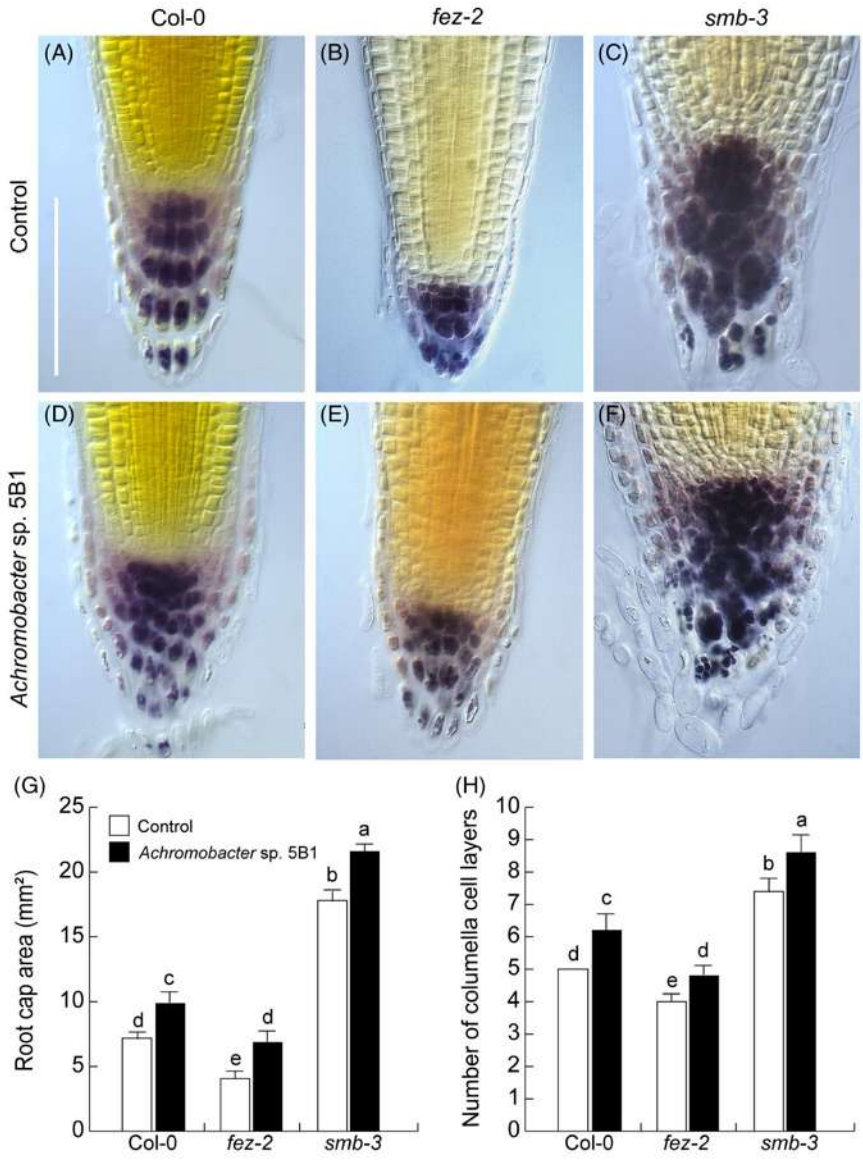


Figure 5. Root cap structure of Arabidopsis WT, *fez-2*, and *smb-3* mutants in response to *Achromobacter* sp. 5B1.
Representative images of root tips of WT, *fez-2*, and *smb-3* mutants stained with lugol to visualize starch grains in axenic conditions (A–C) and after 6 days in cocultivation with *Achromobacter* sp. 5B1 (D–F). (G) Root cap area and (H) Number of columella cell layers. Bars show the mean ± SD. Different letters indicate statistically significant differences ($P < 0.05$; $n = 18$). Scale bar: 100 μm. These analyses were repeated three times with comparable results.

lower level of *DR5:GFP* expression in the presence or absence of bacteria (Figure 7B,E), whereas *smb-3* seedlings had greater *DR5:GFP* expression domains irrespective of bacterization in columella cells (Figure 7C,F). These findings suggest that FEZ and SMB directly or indirectly, owing to the contrasting sizes of their root caps, influence auxin accumulation in the root tip.

Achromobacter sp. 5B1 regulates auxin transporters through the transcription factors FEZ and SMB

The directional auxin movement is controlled by auxin efflux transporters PIN to regulate root development (Blilou et al., 2005; Zhang et al., 2020). To further explore whether FEZ and SMB influence directional root growth through auxin transporters, we analyzed expression

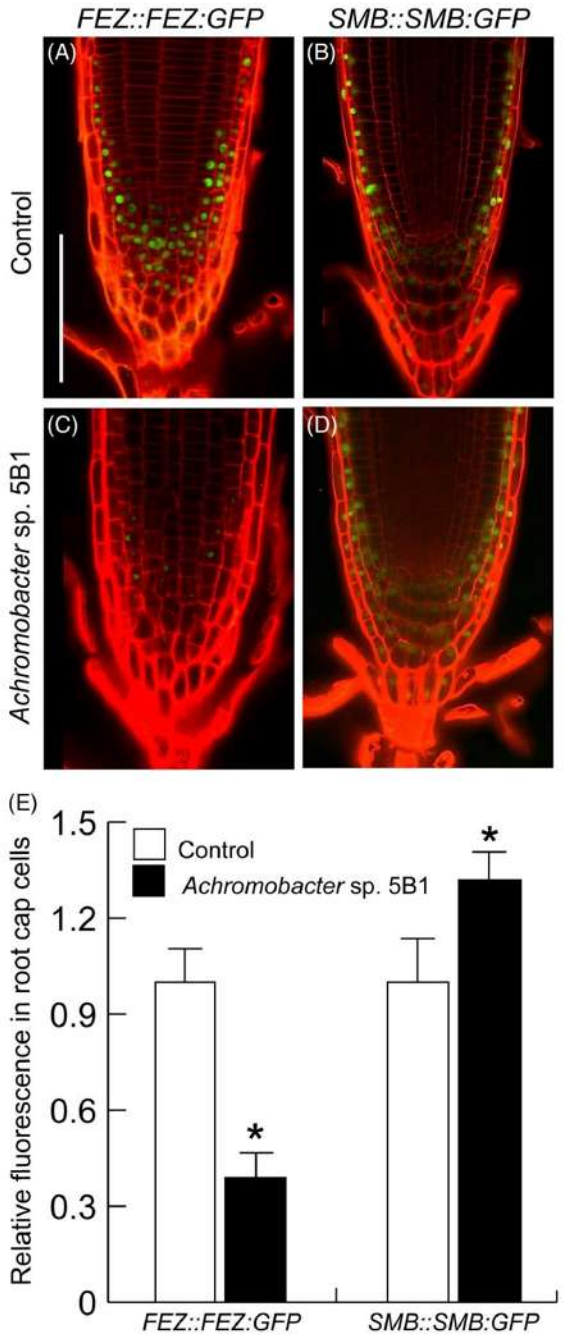


Figure 6. Effect of *Achromobacter* sp. 5B1 on expression of *FEZ::FEZ-GFP* and *SMB::SMB-GFP* genes in the root cap.
Transgenic Arabidopsis seedlings expressing *FEZ::FEZ-GFP* and *SMB::SMB-GFP* were germinated and grown on 0.2× MS media for 6 days and transferred to axenic MS 0.2× media or over a bacterial streak. Representative micrographs of primary roots from plants of *FEZ::FEZ-GFP* (A, C) and *SMB::SMB-FP* (B, D) were taken after six additional days. The graph shows the relative fluorescence intensity in the root cap (E). Bars show the mean ± SD. Asterisks indicate statistically significant differences ($P < 0.05$; $n = 10$). Scale bar: 100 μm. The experiment was repeated twice with comparable results.

patterns of auxin transporters PIN1, PIN2, PIN3, PIN4, and PIN7 outcrossing *PIN1::PIN1-GFP*, *PIN2::PIN2-GFP*, *PIN3::PIN3-GFP*, *PIN4::PIN4-GFP*, and *PIN7::PIN7-GFP* gene constructs into *fez-2* and *smb-3* mutants. Homozygous mutants harboring the construct that allows visualization of the corresponding proteins *in vivo* via confocal microscopy were grown side by side with their parental transgenics in Petri plates with axenic medium or over a streak of *Achromobacter* sp. 5B1 and were maintained in interaction for six additional days. The bacterium repressed PIN1, PIN2, and PIN7 while inducing PIN3 expression in columella cells (Figure 8A–J; Figure S7), as shown by the relative fluorescence intensity quantitation in the meristem and columella (Figure S7). In the *fez-2* mutant, PIN1, PIN3, and PIN4 were weakly expressed both in vascular tissue and root cap. However, PIN2 and PIN7 maintained their basal expression (Figure 8K–O). Interestingly, in this mutant inoculated with the bacterium, PIN expression on the provasculature and columella was severely reduced (Figure 8P–T).

On the other hand, *smb-3* seedlings exhibited lower levels of PIN1, PIN2, and PIN3 in the root tip, while PIN7 expression enhanced in root cap cells (Figure 9K–O). Bacterial root colonization repressed PINs expression in vascular tissue, except PIN7. These results were represented in graphs of the relative fluorescence intensity quantitation in the meristem and columella (Figure S8). Unlike the *fez-2* mutant, in columella cells, PIN3 and PIN4 levels were not reduced, although PIN7 displayed a broader expression pattern (Figure 9P–T). Collectively, these data revealed the dynamic changes of auxin transporters in two mutants with contrasting root cap development and their repression by *Achromobacter* sp. 5B1.

DISCUSSION

The root cap is a crucial innovation throughout plant evolution (Hetherington & Dolan, 2018). It protects the root meristem and functions as a sensory organ that receives and transmits environmental signals leading to changes in the direction of root growth (Kumpf & Nowack, 2015). Previously, we showed that the beneficial rhizobacterium *Achromobacter* sp. 5B1 disrupts gravitropic growth, a trait that enables roots to explore the substrate to find resources, resulting from the alteration in auxin transport and the establishment of asymmetric auxin distribution (Jiménez-Vázquez et al., 2020). In this study, we showed that this response is specific since plant growth-promoting bacteria *Bacillus* sp. LC390B (García-Cárdenas et al., 2023) and *Micrococcus luteus* LS570 (García-Cárdenas et al., 2022) did not deviate root growth from the gravity vector. Moreover, we uncovered the role of root cap-localized transcription factors FEZ and SMB in regulating agravitropic root behavior in response to *Achromobacter* sp. 5B1 through modulated expression of PIN auxin-transporters.

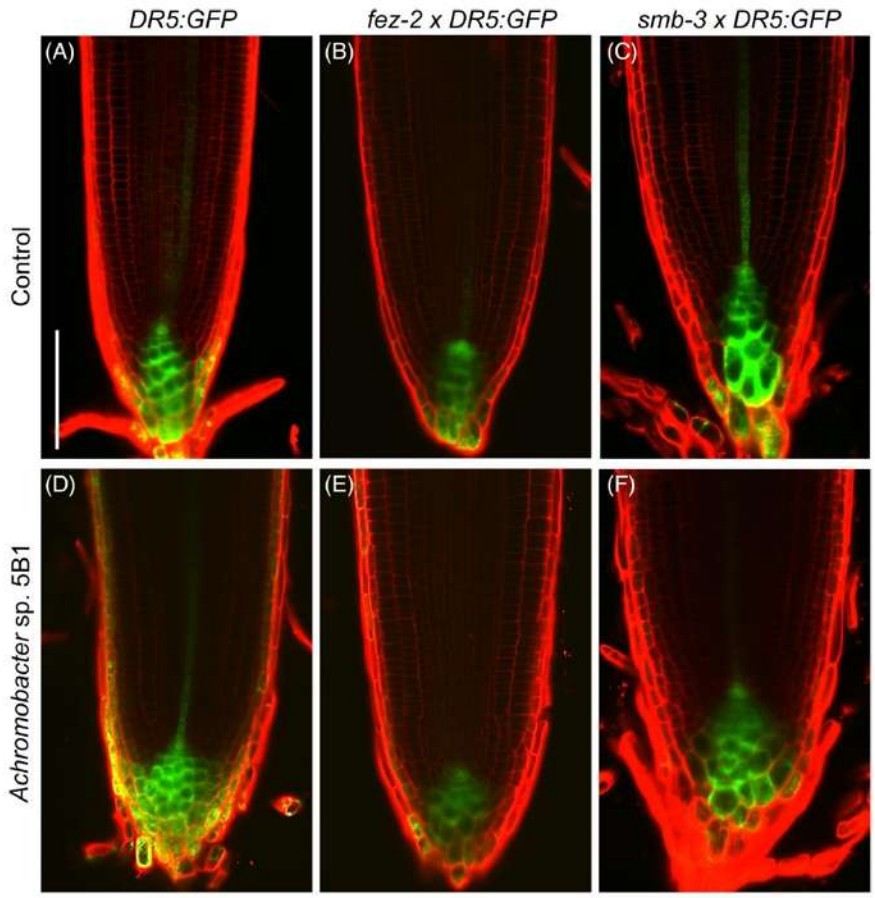


Figure 7. Effect of *Achromobacter* sp. 5B1 on *DR5:GFP* expression in root tips of WT, *fez-2*, and *smb-3* mutant seedlings.
Representative images of WT, *fez-2*, and *smb-3* seedlings harboring the *DR5:GFP* auxin-inducible reporter gene transferred 4 days after germination to 0.2× MS fresh media (A–C) or placed over *Achromobacter* sp. 5B1 bacterial streaks (D–F) for six additional days. Scale bar: 100 μm. The experiment was repeated twice with comparable results.

Root movements toward or away from environmental stimuli are known as tropisms, and detection of these signals usually occurs in the root cap. To investigate the possible role of the root cap as a sensing organ with the ability to respond to signals from *Achromobacter* sp. 5B1 and trigger the agravitropic root behavior, we evaluated the effect of bacterial colonization in Arabidopsis primary roots whose root caps were excised using a scalpel and in loss-of-function mutants *fez-2* and *smb-3*. Interestingly, WT roots without caps did not deviate in their growth upon physical contact with the bacteria, and the mutants displayed contrasting phenotypes; *fez-2* showed a supercoiling phenotype; in contrast, *smb-3* roots grew toward the gravity vector, indicating that directional root growth by *Achromobacter* sp. 5B1 is regulated by root cap-localized transcription factors. This is certainly comparable to a recent study in which SMB drives halotropic root curvature to escape from salt-containing areas, since *smb-3* roots are unable to activate halotropic bending to avoid salt stress (Zheng et al., 2024). These data suggest that the root cap may play a critical role in abiotic stress adaptation and to react to the plant-associated bacteria via discrete movements and changing their growth direction.

Waving, skewing, and coiling root growth patterns result from a combination of circumnutation, gravitropism, thigmotropism, and the intrinsic circular movements of root tips (Massa & Gilroy, 2003; Migliaccio & Piconese, 2001; Porat et al., 2024). Our results showed a higher frequency of waving in *fez-2* roots, whereas *smb-3* roots grew straightforwardly along the gravitational axis. WT and *fez-2* seedlings inoculated with the bacterium exhibited a remarkable waving and root coiling, possibly associated with an interplay between the agravitropic response by *Achromobacter* sp. 5B1, the gravitropic response, and contact with the medium. Differential cell elongation leads to the formation of curvatures to overcome obstacles in the ground and respond to environmental changes (Sato et al., 2015; Su et al., 2017). To further investigate whether

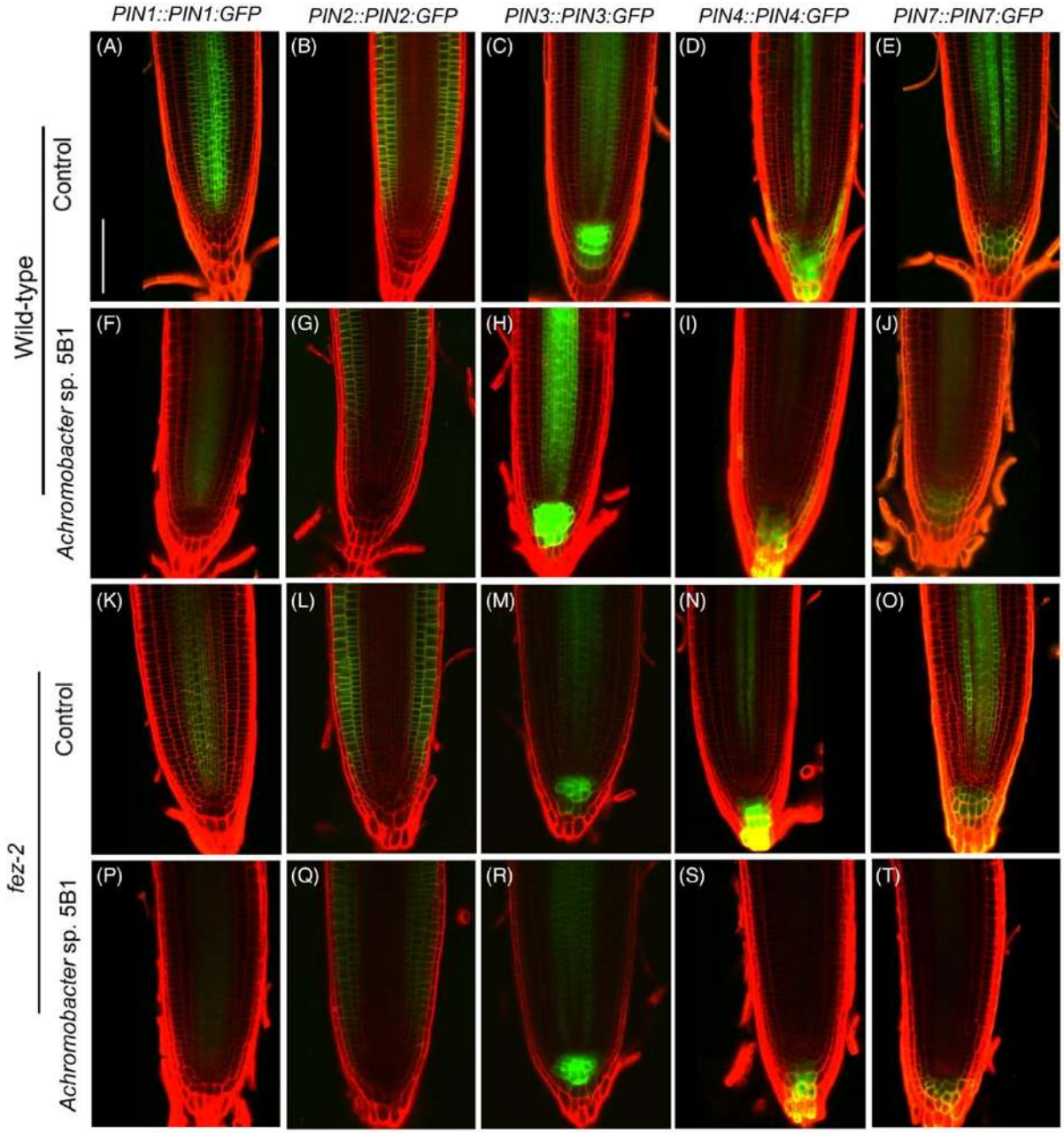


Figure 8. Root colonization by *Achromobacter* sp. 5B1 represses expression of auxin transporters in root tips of *fez-2* mutant seedlings.
Transgenic Arabidopsis seedlings expressing *PIN1::PIN1-GFP*, *PIN2::PIN2-GFP*, *PIN3::PIN3-GFP*, *PIN4::PIN4-GFP*, and *PIN7::PIN7-GFP*, and mutant Arabidopsis seedlings *fez-2* carrying these gene constructs were transferred to MS 0.2× media without and with bacterial inoculum and co-cultivated for 6 days. Representative confocal images of primary root tips show the expression of auxin transporters in axenic conditions (A–E), inoculated with *Achromobacter* sp. 5B1 (F–J), mutants *fez-2* harboring gene constructs mentioned above in the absence of bacterial streak (K–O) and the presence of *Achromobacter* sp. 5B1 (P–T). Scale bar: 100 μm. The experiment was repeated two times with comparable results.

agravitropic root behavior produced by *Achromobacter* sp. 5B1 involves differential cell elongation, we performed measurements of epidermis and cortex cells of the elongation zone of WT, *fez-2*, and *smb-3* roots. In *fez-2* roots, cells displayed a significant growth difference between the convex and concave sides of the root coil; conversely, in *smb-3* roots, the cell length remained similar on both sides of the root. This suggests that biotic stimuli such as

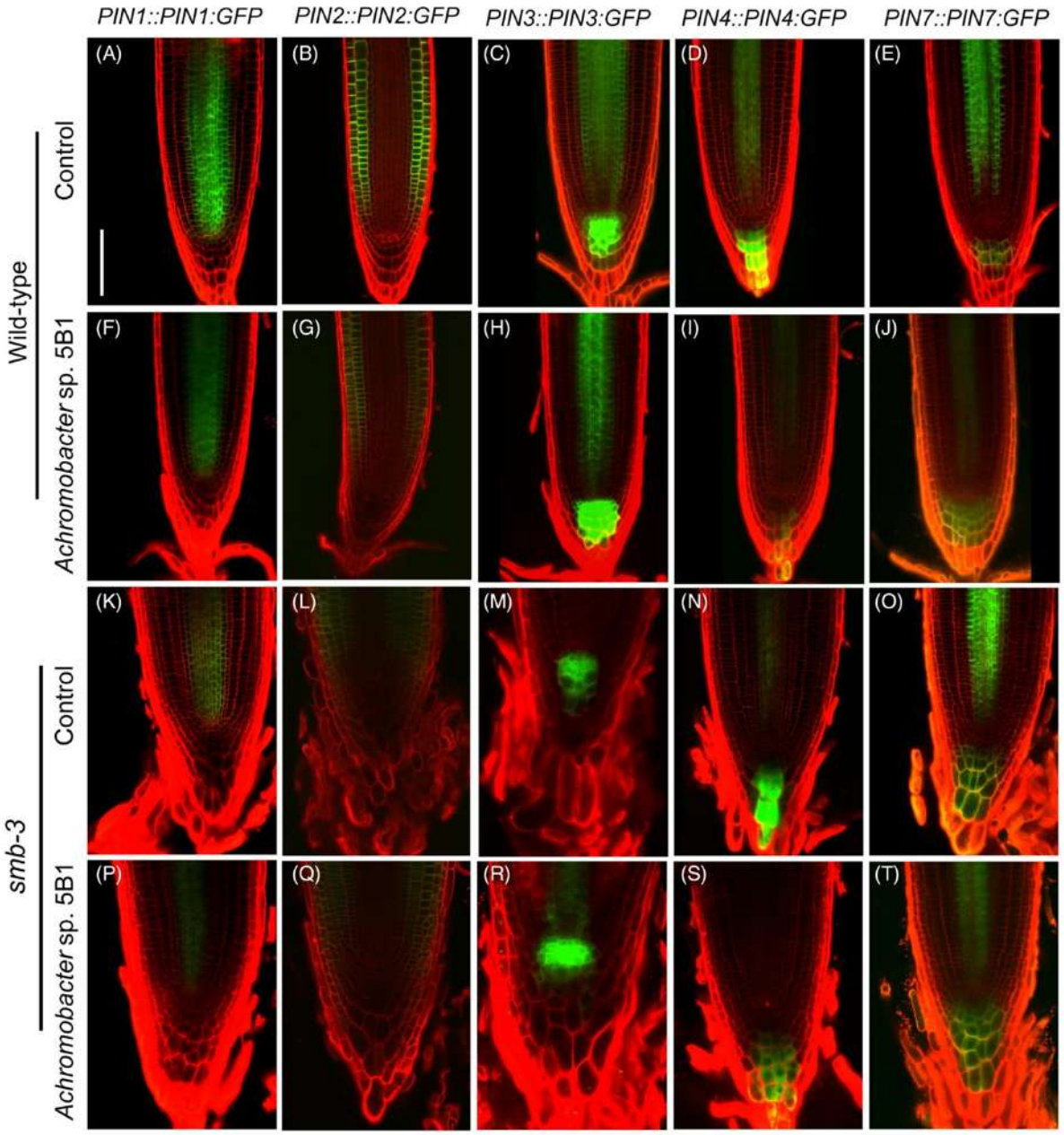


Figure 9. Effect of *Achromobacter* sp. 5B1 on the expression of auxin transporters in root tips of *smb-3* mutant seedlings.
Representative confocal micrographs of primary root tips show expression patterns of *PIN1::PIN1-GFP*, *PIN2::PIN2-GFP*, *PIN3::PIN3-GFP*, *PIN4::PIN4-GFP*, and *PIN7::PIN7-GFP* in the control condition (A–E) and co-cultivated with *Achromobacter* sp. 5B1 (F–J), *smb-3* mutants carrying gene constructs mentioned above un-inoculated (K–O) and interaction with rhizobacteria (P–T). Scale bar: 100 μm. Experiment was repeated twice with comparable results.

Achromobacter sp. 5B1 drive these root movements through differential cell growth and the involvement of root cap-localized elements.

In addition, we evaluated whether root cap structure could influence the root growth patterns by *Achromobacter* sp. 5B1, and found that the bacterium increased the root cap area in the three genotypes. Our data imply that although the bacterial inoculum induces root cap size, possibly the shape and root cap structure exhibited by the *fez-2* and *smb-3* mutants upon bacterial root colonization could trigger root coiling or guide growth toward gravity, respectively. Along with the apparent differences in root

cap size shown by the *fez-2* and *smb-3* mutants, a previous report noted that the shape of the root cap influences the root penetration ability through the medium; the *fez-2* mutant exhibits a pointed root cap, reducing its penetration ability, while *smb-3* mutants showed a rectangular-shaped root cap increasing the penetration ability of their root tips (Roue et al., 2020).

FEZ and SMB are strictly distributed at the root cap and interact with each other through a regulation loop for precise control of cell division and differentiation processes (Bennett et al., 2010; Willemsen et al., 2008). To test whether these transcription factors could play a role in response to *Achromobacter* sp. 5B1, we evaluated their expression in the root tip of seedlings inoculated with the bacterium. *FEZ-GFP* and *SMB-GFP* detection had an opposite trend; FEZ decreased, and SMB increased in root cap cells, indicating their participation in root behavior to *Achromobacter* sp. 5B1. These results suggest that the bacterium represses cell division and improves cell differentiation in the root cap, which could contribute to directional root growth.

We previously observed the formation of a lateral auxin gradient in root tips that accounted for directional root movements by *Achromobacter* sp. 5B1 (Jiménez-Vázquez et al., 2020). To confirm the involvement of the root cap in agravitropic root behavior to inoculation with the bacterium, we analyzed the auxin response in root tips of *fez-2* and *smb-3* mutants. Our data showed that *DR5:GFP* expression was reduced in the root cap of the *fez-2* mutant without bacterial inoculum compared with WT seedlings. In *fez-2* roots co-cultivated with *Achromobacter* sp. 5B1, although the *DR5* fluorescence signal is weakly visible, auxin was mobilized toward the lateral layer. Conversely, in *smb-3* roots, asymmetric auxin distribution was impaired. In this context, it was reported that in nutrient deficiency such as low phosphate conditions, SMB acts as a negative modulator of auxin signaling (Ravelo-Ortega et al., 2022). Recent evidence revealed the crucial role of SMB in the establishment of the lateral auxin gradient in the root cap for driving halotropic root curvature (Zheng et al., 2024). Thus, it is tempting to speculate that auxin loading to the LRC and epidermal cells could reduce auxin accumulation in the root cap and contribute to altering root gravitropism in response to *Achromobacter* sp. 5B1. According to Zhang et al. (2019), auxin mediates root gravitropism by regulating starch granule accumulation in columella cells to gravity perception and response. These findings indicate that transcription factors FEZ and SMB regulate auxin accumulation in the root tip. Essentially, SMB is required for the establishment of asymmetric auxin distribution across the root cap to trigger directional root growth by *Achromobacter* sp. 5B1.

Polar localization of PIN auxin-efflux carriers coordinates directional cell-to-cell transport of auxin to regulate developmental and tropic responses (Han et al., 2021). We provide evidence that FEZ and SMB regulate PIN proteins and interaction with *Achromobacter* sp. 5B1 showed the synergistic effect contributing to changes in the abundance and localization of auxin transporters in the root tip. Mutations in FEZ and SMB exhibited changes in the expression of PIN auxin transporters in a specific way. In the *fez-2* mutant, PIN1, PIN3, and PIN4 expression is reduced, whereas PIN2 and PIN7 expression did not change in non-inoculated seedlings; however, in co-culture with the bacterium, the expression of all PIN proteins evaluated was repressed. The mutation in SMB induced the repression of PIN auxin exporters except PIN7; concerning bacterial root colonization, PIN expression was decreased in the root tip except in the columella cells. Hence, SMB is required for specific modulation of root cap-localized PIN auxin transporters leading to the asymmetric auxin distribution. Zheng et al. (2024) demonstrated that SMB drives the expression of the auxin permease *AUX1* in the LRC for generating a lateral auxin gradient that triggers the root halotropic response. Another report evidenced that PIN-mediated polar auxin transport drives auxin accumulation on the concave side during root bending, leaving the growth away from the barrier during obstacle avoidance (Lee et al., 2020). This complex regulation of PIN proteins suggests that *Achromobacter* sp. 5B1 induces changes in auxin transporters to redirect auxin flow in the root tip through root cap-endogenous elements to promote tropic response by rhizobacterium.

Finally, from this complex plant–bacteria interaction, our results revealed that root cap-endogenous elements, including FEZ and SMB, play a regulatory role in root growth patterns by *Achromobacter* sp. 5B1, through modulation of PIN-mediated polar auxin transport promoting the redirection of auxin flow. Essentially, SMB is critical in establishing asymmetric auxin distribution across the root cap, triggering directional root growth (Figure 10). Our findings suggest that root tips sense and respond to *Achromobacter* sp. 5B1 through root cap-related transcription factors, which provide a framework to explore the rhizosphere heterogeneous environment through dynamic movements, to potentially reach areas where water and nutrients may be available. This possibility merits further research.

MATERIALS AND METHODS

Plant material and growth conditions

WT *A. thaliana* seeds (ecotype Columbia-0, Col-0), *smb-3* and *fez-2* mutants (Willemsen et al., 2008) and transgenic lines *DR5:GFP* (Ottenschlager et al., 2003), *DR5:GUS* (Ulmasov et al., 1997), *pPIN1::PIN1-GFP* (Benková et al., 2003), *pPIN2::PIN2-GFP* (Blilou et al., 2005), *pPIN3::PIN3-GFP* (Zádníková et al., 2010), *pPIN4::PIN4-GFP*, and *pPIN7::PIN7-GFP* (Blilou et al., 2005) were used in this study. Generation of *smb-3/pPIN1::PIN1-GFP*, *smb-3/pPIN2::PIN2-GFP*, *smb-3/pPIN3::PIN3-GFP*, *smb-3/pPIN4::PIN4-GFP*, *smb-3/pPIN::PIN7-GFP*, *fez-2/pPIN1::PIN1-GFP*, *fez-2/pPIN3::PIN3-GFP*,

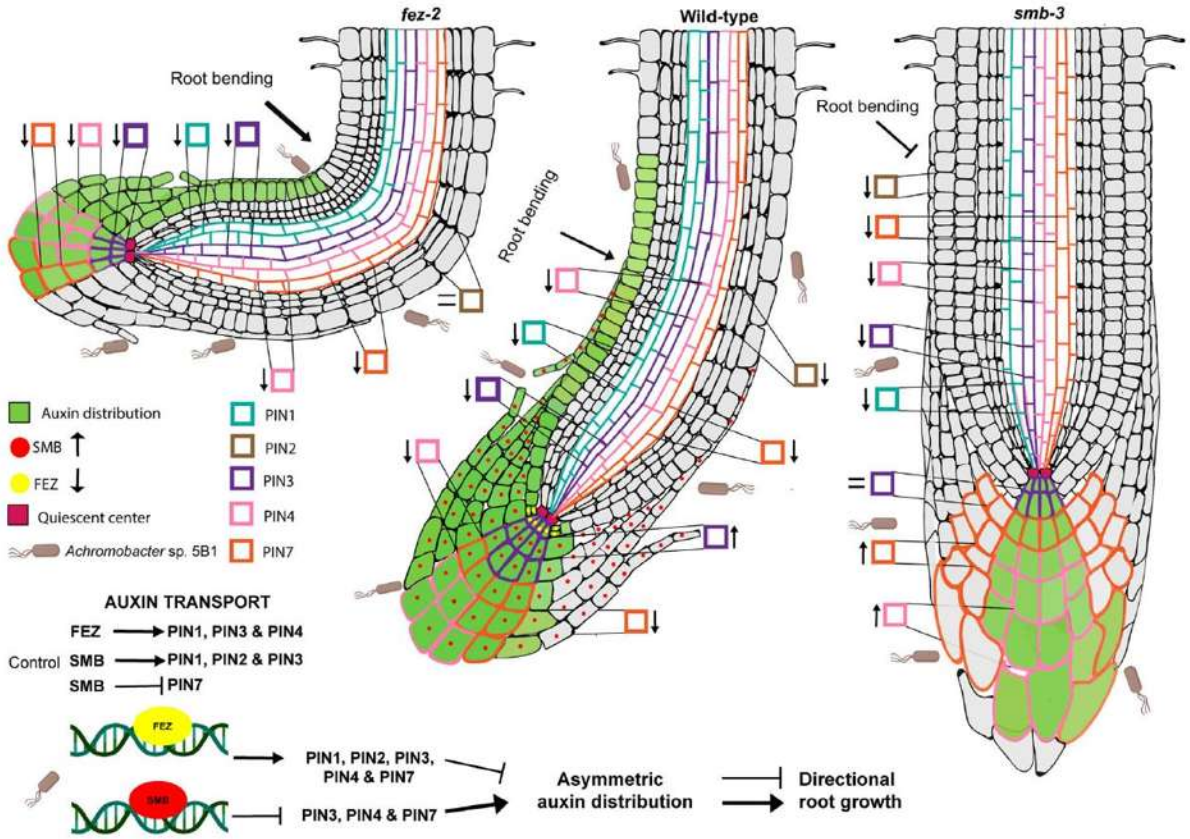


Figure 10. Schematic representation of directional root growth influenced by *Achromobacter* sp. 5B1 involving FEZ and SMB.
The rhizobacterium drives directional root growth through PIN auxin transporter modulation, leading to asymmetric auxin distribution in the root tip. In *fez-2* and *smb-3* loss-of-function mutants exhibit contrasting phenotypes exhibit, with *fez-2* roots showing reduced auxin levels; however, the lateral auxin gradient activating deviation root growth does occur. In *smb-3* roots, the asymmetric auxin distribution and directional root growth are impaired. Hence, the transcription factors FEZ and SMB regulatory play roles in agravitropic root behavior and are required for auxin accumulation in the root cap. In particular, SMB is crucial to the establishment of asymmetric auxin distribution to trigger directional root growth in response to *Achromobacter* sp. 5B1, indicating that root cap-localized elements participate in response to signals from soil microorganisms.

fez-2/IN4::PIN4-GFP, and *fez-2/pPIN::PIN7-GFP* were made by outcrossing individual *smb-3* and *fez-2* homozygous plants with pollen from individual homozygous plants from each transgenic line. The derived F1 siblings were grown in soil and allowed to self-pollinize to obtain F2 segregating populations, from which 20 independent seedlings with mutant phenotypes and harboring the transgenes were selected and self-pollinized for at least three generations. Seeds from each genotype were disinfected with 95% (v/v) ethanol and 20% (v/v) commercial bleach for 5 min each and then washed five times with sterilized deionized water. After 2-day cold stratification (4°C), the seeds were sown on Petri plates containing solidified MS 0.2× medium with 0.6% sucrose and 1% phytagar, pH 7.0. Plates were placed in a plant growth chamber (Percival Scientific AR-95L, Perry, IA, USA) at 22°C with a photoperiod of 16 h light/8 h darkness and light intensity of 200 μmol m^2 sec^{-1}.

Plant–bacteria co-cultivation

Four-day-old Arabidopsis seedlings were transferred to 0.2× MS media with or without a streak of *Achromobacter* sp. 5B1 (Jiménez-Vázquez et al., 2020, 2023), *Bacillus* sp. LC390B (García-Cárdenas et al., 2022) and *Micrococcus luteus* LS570 (García-Cárdenas et al., 2022). On the other hand, the seedlings treated with *Achromobacter* sp. 5B1 were removed from the plate with bacterial streak at 2, 4, and 6 days after co-inoculation, in order to assess its spread over roots. The bacterial inocula were prepared using bacteriological agar (BD Bioxon) and LB broth (Phytotechnology Laboratories, Lenexa, KS, USA). Six and 12 Arabidopsis seedlings, depending on each experiment, were transferred to fresh agar plates supplemented with 0.2× MS medium, placing their roots over or side the bacterial streaks, respectively, where the shoots were located approximately 1 cm above the bacterial streaks. Marks were placed on the bottom half of the Petri plates to indicate the location of the primary root tip at the moment of transfer and the growth was analyzed 6 days later. The plates were placed vertically and incubated in a plant growth chamber AR-95L (Percival Scientific) with a photoperiod of 16 h of light and 8 h of darkness, light intensity of 200 μmol m^2 sec^{-1}, and temperature of 22°C.

Analysis of green fluorescent protein

Transgenic Arabidopsis seedlings expressing the green fluorescent protein (GFP) were incubated in 10 mg ml^{-1} propidium

iodide for 1 min. Propidium iodide was purchased from Sigma-Aldrich (St. Louis, MO, USA). The seedlings were rinsed in water and mounted with 50% glycerol on glass slips and covered with coverslips. Each sample was analyzed separately for propidium iodide (with an excitation at 568 nm and an emission window at 585–610 nm) and GFP fluorescence (with an excitation at 488 nm and an emission window at 505–550 nm) using a confocal microscope (Olympus FV1200, Tokyo, Japan) after which the two micrographs were merged to produce a final image. For each treatment, 12 independent seedlings were analyzed.

GUS histochemical analysis

Transgenic seedlings expressing the β-glucuronidase reporter gene *uidA* (GUS) were incubated in 5-bromo-4-chloro-3-indolyl-β-D-glucuronide in sodium phosphate 100 mM buffer pH 7, 0.5 M, and incubated at 37°C overnight. The seedlings were cleared and fixed in 0.24 N HCl in 20% methanol (v/v) at 62°C solution, and 7% NaOH (w/v) in 60% ethanol (v/v) solution for 20 min at room temperature. Finally, the seedlings were dehydrated with 1 ml 40, 20, and 10% (v/v) ethanol for 20 min each and then fixed with 50% glycerol (v/v) on microscope slides. Images were taken using the Nomarski microscopy in 40× magnification.

Plant growth analysis

To evaluate *in vitro* growth, the length of the primary roots during the interaction with *Achromobacter* sp. 5B1 was measured with a ruler, and lateral roots present were recorded using a stereomicroscope (Leica MZ6) and the lateral root density was calculated by dividing the lateral root number by primary root length in 18 individuals of each growth condition. The total fresh weight, shoot fresh weight, and root fresh weight of six seedlings grown on the same plate were weighed with an analytical balance (Ohaus, Parsippany, NJ, USA), and three plates of each growth condition were evaluated. Ten micrographs (photographs acquired in the microscope) of each growth condition were analyzed in ImageJ software (http://rsbweb.nih.gov/ij/) to determine how cells expressed the reporter gene and to perform the measurements of the root cap. The dataset was statistically analyzed in all experiments with STATISTICA 10.0 program (Dell StatSoft, Austin, TX, USA). One-way ANOVA or two-way ANOVA were performed, and then data were analyzed with the Tukey's post hoc test. Different letters were used to indicate means that differ significantly ($P < 0.05$).

Root cap excision assay

The root caps of Arabidopsis seedlings were removed using a sterile scalpel under a stereoscope microscope (Leica MZ6) in a hood. The excisions were performed at the columella stem cells of the primary root tip. The seedlings with intact root caps or devoid of root caps were placed on fresh media and over a streak of *Achromobacter* sp. 5B, and analyzed 3 days post-cutting. Subsequently, plant roots were stained with propidium iodide (PI) and placed on slides to be observed with a confocal microscope (Olympus FV1200; Olympus Corp., Tokyo, Japan). The experiment included at least 40 seedlings for each treatment and was replicated at least three times.

AUTHOR CONTRIBUTIONS

KRJ-V and JL-B projected and designed the research. KRJ-V, JSL-B, and LFR-H performed the experiments and analyzed the data. KRJ-V and JL-B wrote the manuscript. All authors read and approved the manuscript.

ACKNOWLEDGMENTS

This work was supported by Secretaría de Educación Pública (SEP)—Consejo Nacional de Ciencia y Tecnología (CONACYT) grant number A1-S-34768.

CONFLICT OF INTEREST

The authors report no declarations of interest.

DATA AVAILABILITY STATEMENT

All relevant data can be found within the manuscript and its Supporting Information.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Effects of *Achromobacter* sp. 5B1 on root architecture of Arabidopsis seedlings under direct contact, in divided Petri plates and inoculated at a distance from the root tip.

Figure S2. Effect of *Achromobacter* sp. 5B1 on *DR5:GUS* expression in root tips of Arabidopsis seedlings under direct contact, exposed to bacterial volatile organic compounds or to a bacterial inoculum at a distance to react to diffusible compounds.

Figure S3. Arabidopsis root contact with *Achromobacter* sp. 5B1 determines root growth direction.

Figure S4. Root behavior of Arabidopsis seedlings expressing *DR5:GFP* in response to *Achromobacter* sp. 5B1.

Figure S5. Bacterial growth around Arabidopsis roots co-cultivated with *Achromobacter* sp. 5B1.

Figure S6. FEZ and SOMBRERO mediate root tip growth direction and coil formation in response to *Achromobacter* sp. 5B1.

Figure S7. Effect of *Achromobacter* sp. 5B1 and mutation of *FEZ* on the expression of the auxin transporters PIN1, PIN2, PIN3, PIN4 and PIN7.

Figure S8. Effect of *Achromobacter* sp. 5B1 and mutation of *SMB* on the expression of the auxin transporters PIN1, PIN2, PIN3, PIN4 and PIN7.

REFERENCES

Arnaud, C., Bonnot, C., Desnos, T. & Nussaume, L. (2010) The root cap at the forefront. *Comptes Rendus Biologies*, **333**, 335–343.

Badri, D.V. & Vivanco, J.M. (2009) Regulation and function of root exudates. *Plant, Cell & Environment*, **32**(6), 666–681.

Barlow, P.W. (2003) The root cap: cell dynamics, cell differentiation and cap function. *Journal of Plant Growth Regulation*, **21**(4), 261–286. Available from: https://doi.org/10.1007/s00344-002-0034-z

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G. *et al.* (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell*, **115**(5), 591–602.

Bennett, T., Sanchez-perez, G.F., Campilho, A., Willemsen, V., Snel, B. & Scheres, B. (2010) SOMBRERO, BEARSKIN1, and BEARSKIN2 regulate root cap maturation in Arabidopsis. *The Plant Cell*, **22**, 640–654.

Bennett, T., van den Toorn, A., Willemsen, V. & Scheres, B. (2014) Precise control of plant stem cell activity through parallel regulatory inputs. *Development*, **141**, 4055–4064.

Blancaflor, E.B. (2013) Regulation of plant gravity sensing and signaling by the actin cytoskeleton. *American Journal of Botany*, **100**(1), 143–152.

Blancaflor, E.B., Fasano, J. & Gilroy, S. (1999) Laser ablation of root cap cells: implications for models of graviperception. *Advances in Space Research*, **24**(6), 731–738.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J. *et al.* (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature*, **433**, 39–44.

Ding, Z. & Friml, J. (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12046–12051.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. *et al.* (1993) Cellular organisation of the *Arabidopsis thaliana* root. *Development*, **119**, 71–84.

Driouich, A., Gaudry, A., Pawlak, B. & Moore, J.P. (2021) Root cap-derived cells and mucilage: a protective network at the root tip. *Protoplasma*, **258**, 1179–1185.

Dubreuil, C., Jin, X., Grönlund, A. & Fischer, U. (2018) A local auxin gradient regulates root cap self-renewal and size homeostasis. *Current Biology*, **28**(16), 2581–2587.

Fendrych, M., Van Hautegem, T., Van Durme, M. *et al.* (2014) Programmed cell death controlled by ANAC033/SOMBRERO determines root cap organ size in Arabidopsis. *Current Biology*, **24**, 931–940.

Ganesh, A., Shukla, V., Mohapatra, A., George, A.P., Bhukya, D.P.N., Das, K.K. *et al.* (2022) Root cap to soil interface: a driving force toward plant adaptation and development. *Plant and Cell Physiology*, **63**(8), 1038–1051.

García-Cárdenas, E., Ortiz-Castro, R., Ruiz-Herrera, L.F., Valencia-Cantero, E. & López-Bucio, J. (2022) *Micrococcus luteus* LS570 promotes root branching in Arabidopsis via decreasing apical dominance of the primary root and an enhanced auxin response. *Protoplasma*, **259**(5), 1139–1155.

García-Cárdenas, E., Ortiz-Castro, R., Ruiz-Herrera, L.F., Valencia-Cantero, E. & López-Bucio, J. (2023) *Bacillus* sp. LC390B from the maize rhizosphere improves plant biomass, root elongation, and branching and requires the phytochromes PHYA and PHYB for phytostimulation. *Journal of Plant Growth Regulation*, **42**, 3056–3070.

Han, H., Adamowski, M., Qi, L., Alotaibi, S.S. & Friml, J. (2021) PIN-mediated polar auxin transport regulations in plant tropic responses. *New Phytologist*, **232**(2), 510–522.

Hetherington, A.J. & Dolan, L. (2018) Stepwise and independent origins of roots among land plants. *Nature*, **561**, 235–238.

Huysmans, M., Buono, R.A., Skorzinski, N., Radio, M.C., De Winter, F. *et al.* (2018) NAC transcription factors ANAC087 and ANAC046 control distinct aspects of programmed cell death in the Arabidopsis columella and lateral root cap. *The Plant Cell*, **30**, 2197–2213.

Jiménez-Vázquez, K.R., García-Cárdenas, E., Barrera-Ortiz, S., Ortiz-Castro, R., Ruiz-Herrera, L.F., Ramos-Acosta, B.P. *et al.* (2020) The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. *The Plant Journal*, **103**(5), 1639–1654. Available from: https://doi.org/10.1111/tpj.14853

Jiménez-Vázquez, K.R., López-Hernández, J., García-Cárdenas, E. *et al.* (2023) The plant growth promoting rhizobacterium *Achromobacter* sp. 5B1 rescues Arabidopsis seedlings from alkaline stress by enhancing root organogenesis and hormonal responses. *Microbiological Research*, **281**, 127594.

Kumar, N. & Iyer-Pascuzzi, A.S. (2020) Shedding the last layer: mechanisms of root cap cell release. *Plants*, **9**, 308.

Kumpf, R.P. & Nowack, M.K. (2015) The root cap: a short story of life and death. *Journal of Experimental Botany*, **66**(19), 5651–5662.

Kutschera, U. & Briggs, W.R. (2012) Root phototropism: from dogma to the mechanism of blue light perception. *Planta*, **235**, 443–452.

Lee, H.J., Kim, H.S., Park, J.M., Cho, H.S. & Jeon, J.H. (2020) PIN-mediated polar auxin transport facilitates root– obstacle avoidance. *New Phytologist*, **225**(3), 1285–1296.

Massa, G.D. & Gilroy, S. (2003) Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *The Plant Journal*, **33**, 435–445.

Méndez-Gómez, M., Barrera-Ortiz, S., Castro-Mercado, E., López-Bucio, J. & García-Pineda, E. (2020) The nature of the interaction *Azospirillum-Arabidopsis* determines the molecular and morphological changes in root and plant growth promotion. *Protoplasma*, **258**(1), 179–189. Available from: https://doi.org/10.1007/s00709-020-01552-7

Migliaccio, F. & Piconese, S. (2001) Spiralizations and tropisms in Arabidopsis roots. *Trends in Plant Science*, **6**(12), 561–565.

Nakamura, M., Nishimura, T. & Morita, M.T. (2019) Gravity sensing and signal conversion in plant gravitropism. *Journal of Experimental Botany*, **70**, 3495–3506.

Oliva, M. & Dunand, C. (2007) Waving and skewing: how gravity and the surface of growth media affect root development in Arabidopsis. *New Phytologist*, **176**(1), 37–43.

Ortiz-Castro, R., Campos-Garcia, J. & Lopez-Bucio, J. (2020) *Pseudomonas putida* and *Pseudomonas fluorescens* influence Arabidopsis root system architecture through an auxin response mediated by bioactive cyclodipeptides. *Journal of Plant Growth Regulation*, **39**, 254–265.

Ortiz-Castro, R., Diaz-Perez, C., Martinez-Trujillo, M., del Rio, R., Campos-Garcia, J. & Lopez-Bucio, J. (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 7253–7258.

Ortiz-Castro, R. & López-Bucio, J. (2019) Review: phytostimulation and root architectural responses to quorum-sensing signals and related molecules from rhizobacteria. *Plant Science*, **284**, 135–142.

Ottenschlager, I., Wolff, P., Wolverton, C., Bhalerao, R.P., Sandberg, G., Ishikawa, H. *et al.* (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(5), 2987–2991. Available from: https://doi.org/10.1073/pnas.0437936100

Porat, A., Tekinalp, A., Bhosale, Y., Gazzola, M. & Meroz, Y. (2024) On the mechanical origins of waving, coiling, and skewing in *Arabidopsis thaliana* roots. *Proceedings of the National Academy of Sciences of the United States of America*, **121**(11), e2312761121.

Ravelo-Ortega, G., Pelagio-Flores, R., López-Bucio, J., Campos-García, J., Reyes de la Cruz, H. & López-Bucio, J.S. (2022) Early sensing of phosphate deprivation triggers the formation of extra root cap cell layers via SOMBRERO through a process antagonized by auxin signaling. *Plant Molecular Biology*, **108**, 77–91.

Ravelo-Ortega, G., Raya-González, J. & López-Bucio, J. (2023) Compounds from rhizosphere microbes that promote plant growth. *Current Opinion in Plant Biology*, **73**, 102336.

Roue, J., Chauvet, H., Brunel-Michac, N., Bizet, F., Moulia, B., Badel, E. *et al.* (2020) Root cap size and shape influence responses to the physical strength of the growth medium in *Arabidopsis thaliana* primary roots. *Journal of Experimental Botany*, **71**(1), 126–137.

Rüger, L., Ganther, M., Freudenthal, J., Jansa, J., Heintz-Buschart, A., Tarkka, M.T. *et al.* (2023) Root cap is an important determinant of rhizosphere microbiome assembly. *New Phytologist*, **239**(4), 1434–1448.

Sato, E.M., Hijazi, H., Bennett, M.J., Vissenberg, K. & Swarup, R. (2015) New insights into root gravitropic signalling. *Journal of Experimental Botany*, **66**(8), 2155–2165.

Su, S.H., Gibbs, N.M., Jancewicz, A.L. & Masson, P.H. (2017) Molecular mechanisms of root gravitropism. *Current Biology*, **27**(17), R964–R972.

Tsugeki, R. & Fedoroff, N.V. (1999) Genetic ablation of root cap cells in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 12941–12946.

Ulmasov, T., Murfett, J., Hagen, G. & Guilfoyle, T.J. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *The Plant Cell*, **9**, 1963–1971.

Willemsen, V., Bauch, M., Bennett, T., Campilho, A., Wolkenfelt, H., Xu, J. *et al.* (2008) The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in Arabidopsis root stem cells. *Developmental Cell*, **15**, 913–922.

Zádníková, P., Petrásek, J., Marhavy, P., Raz, V., Vandenbussche, F., Ding, Z. *et al.* (2010) Role of PIN-mediated auxin efflux in apical hook development of *Arabidopsis thaliana*. *Development*, **137**, 607–617.

Zhang, Y., He, P., Ma, X., Yang, Z., Pang, C., Yu, J. *et al.* (2019) Auxin-mediated statolith production for root gravitropism. *New Phytologist*, **224**(2), 761–774.

Zhang, Y., Rodriguez, R., Li, L., Zhang, X. & Friml, J. (2020) Functional innovations of PIN auxin transporters mark crucial evolutionary transitions during rise of flowering plants. *Science Advances*, **6**(50), eabc8895.

Zheng, L., Hu, Y., Yang, T., Wang, Z., Wang, D., Jia, L. *et al.* (2024) A root cap-localized NAC transcription factor controls root halotropic response to salt stress in Arabidopsis. *Nature Communications*, **15**(1), 2061.

SUPPLEMENTARY INFORMATION

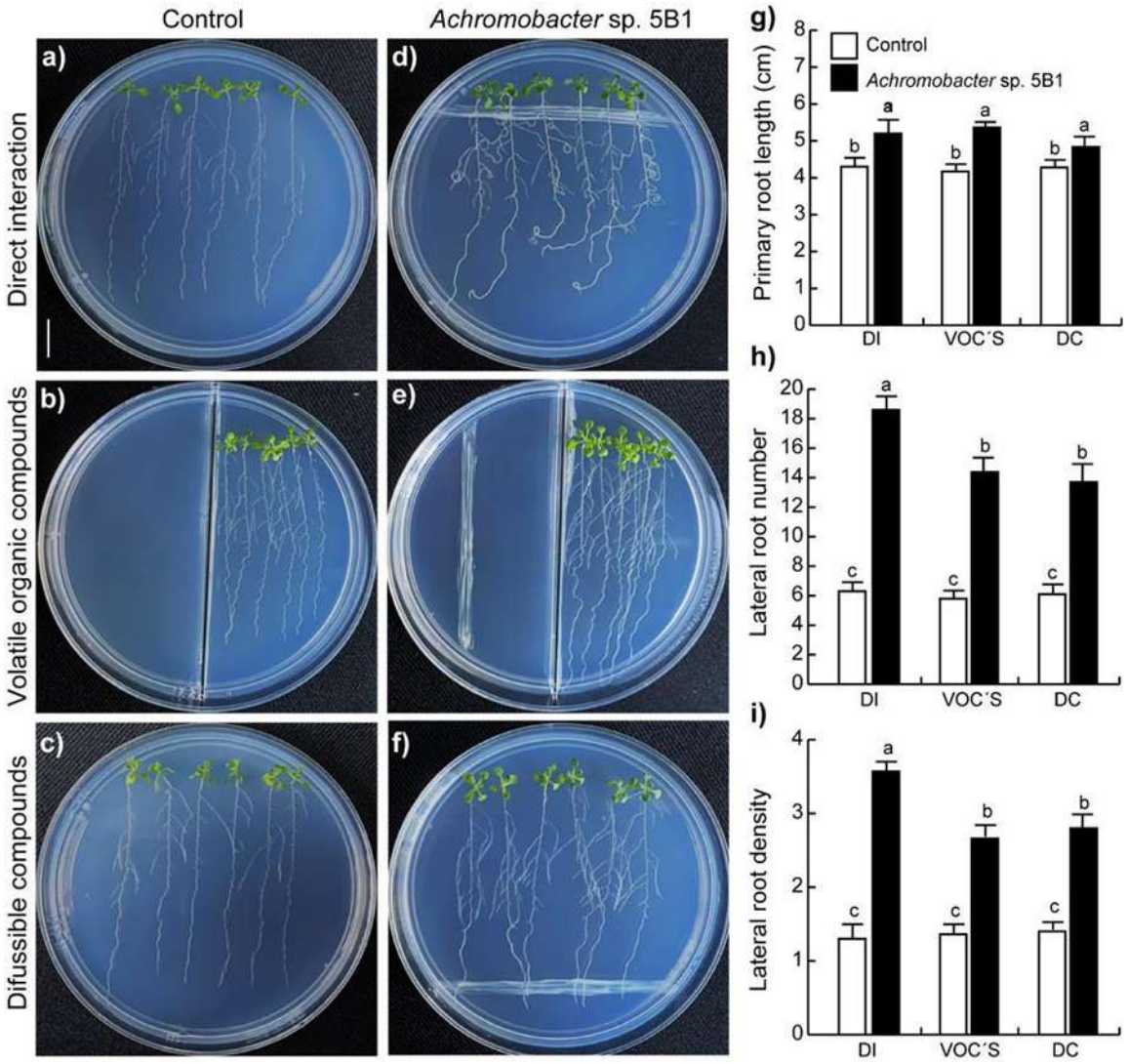


Figure S1. Effects of *Achromobacter* sp. 5B1 on root architecture of *Arabidopsis* seedlings under direct contact, in divided Petri plates and inoculated at a distance from the root tip. Representative images of WT seedlings transferred 4 days after germination to 0.2x MS axenic media (a-c) or co-cultivated with *Achromobacter* sp. 5B1 in different experimental conditions (d-f). Graphs show the effects of bacterial co-cultivation on primary root length, lateral root number and lateral root density (g-i). Different letters indicate statistically significant differences ($p < 0.05$; $n = 18$). Scale bar: 1 cm. These analyses were repeated three times with comparable results.

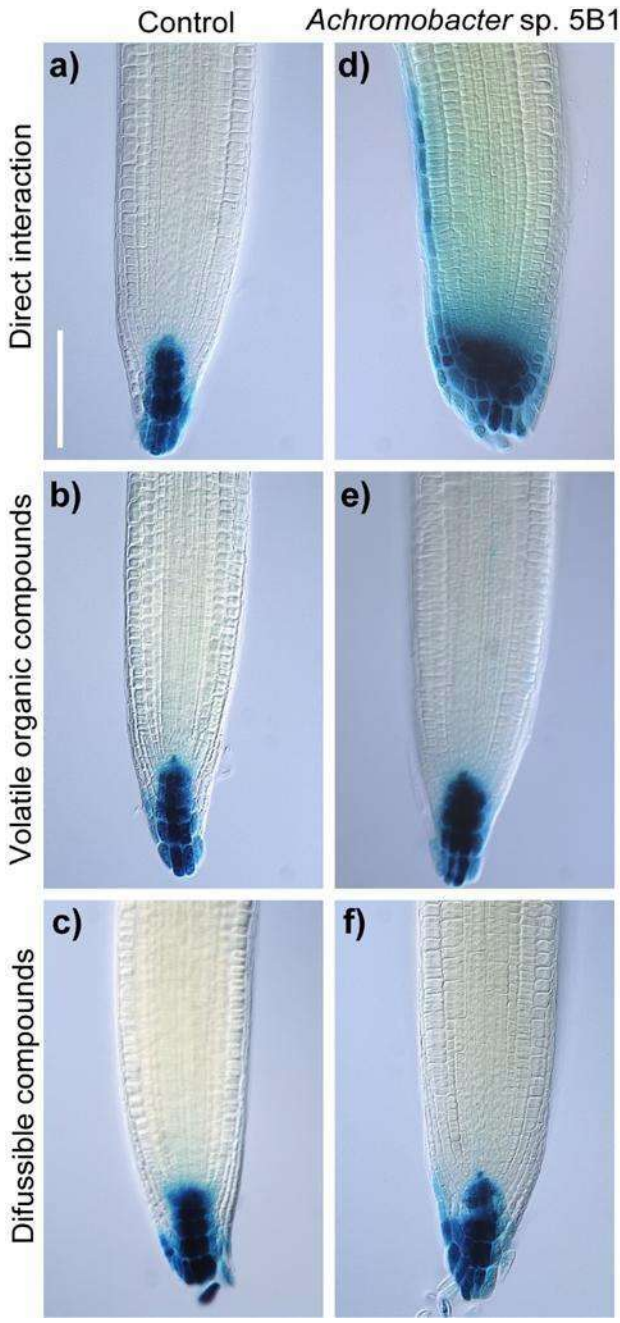


Figure S2. Effect of *Achromobacter* sp. 5B1 on *DR5:GUS* expression in root tips of *Arabidopsis* seedlings under direct contact, exposed to bacterial volatile organic compounds or to a bacterial inoculum at distance to react to diffusible compounds. Representative images of transgenic seedlings *DR5:GUS* transferred 4 days after germination to 0.2x MS fresh media (a-c) or co-cultivated with *Achromobacter* sp. 5B1 (d-f) for 6 additional days. Scale bar: 100 µm. The experiment was repeated twice with comparable results.

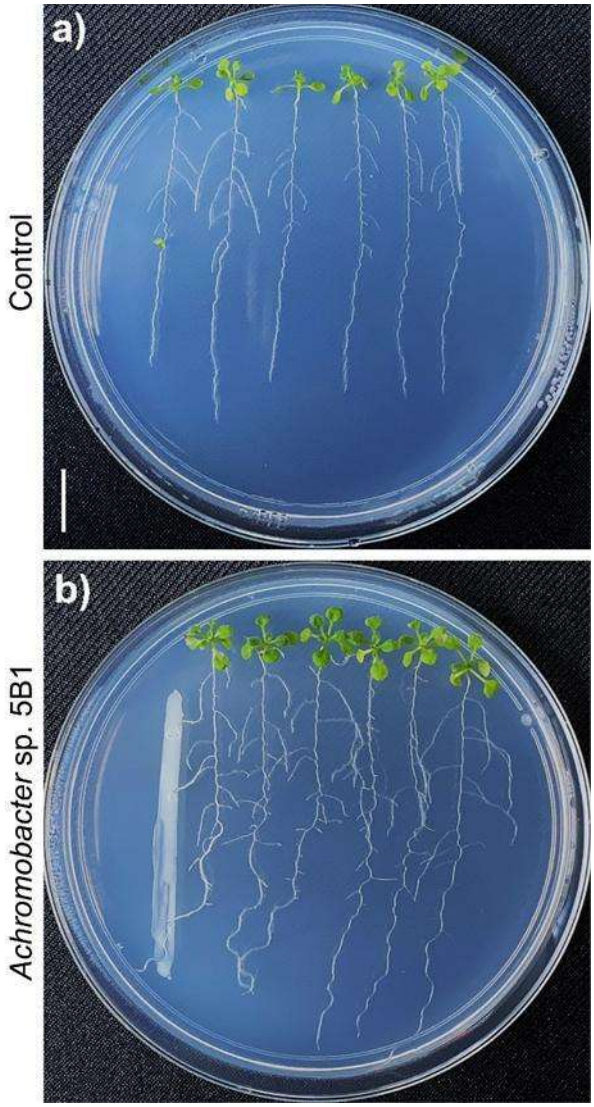


Figure S3. *Arabidopsis* root contact with *Achromobacter* sp. 5B1 determines root growth direction. *Arabidopsis* WT seedlings were germinated and grown on agar-solidified 0.2x MS medium, and 4 days after germination, transferred to fresh media (a) or placed side by side of *Achromobacter* sp. 5B1 streak (b), and grown for 6 additional days. Note that only the root in direct contact with the streak deviates its growth. Scale bar: 1 cm. The experiment was repeated three times with comparable results.

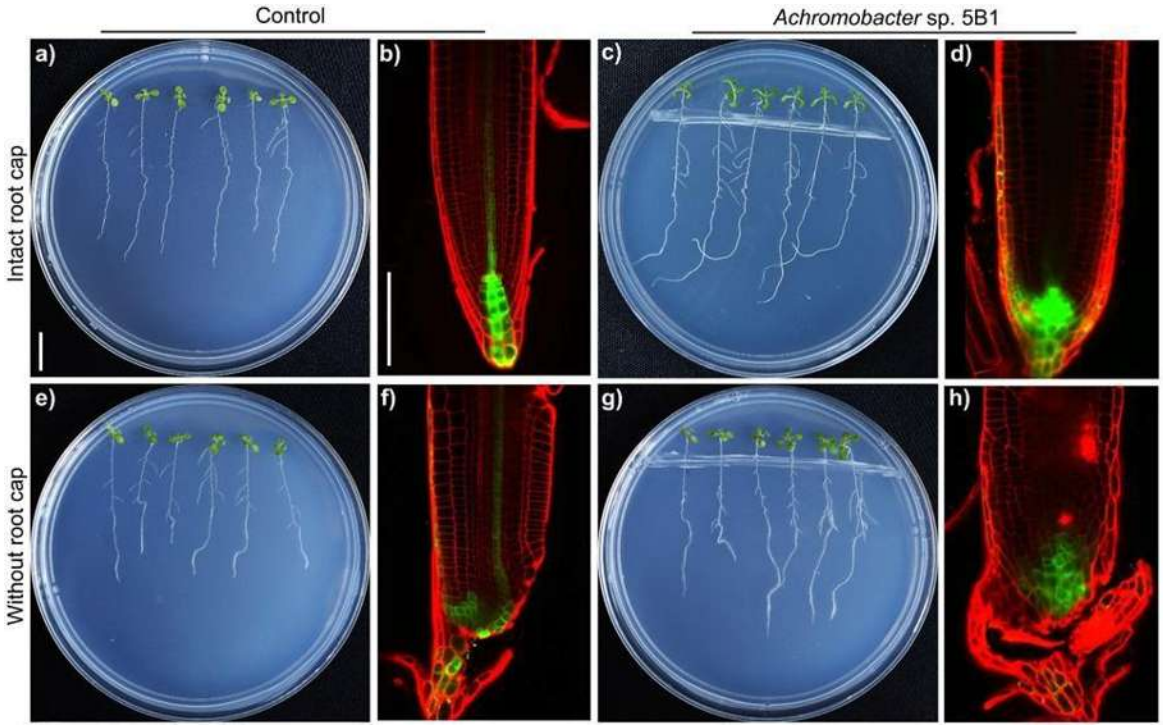


Figure S4. Root behavior of *Arabidopsis DR5:GFP* seedlings with or without root caps in response to *Achromobacter* sp. 5B1. Transgenic *Arabidopsis* seedlings expressing *DR5:GFP* were germinated and grown on 0.2x MS media for 6 days. Subsequently, plants with root caps (a-c) or removed root caps (e-h) were transferred to axenic MS 0.2x media (a,b,e,f) or over a bacterial streak (c,d,g,h). Representative images were taken after 3 additional days. Scale bars: 1 cm and 100 µm, respectively. The experiment was repeated twice with comparable results.

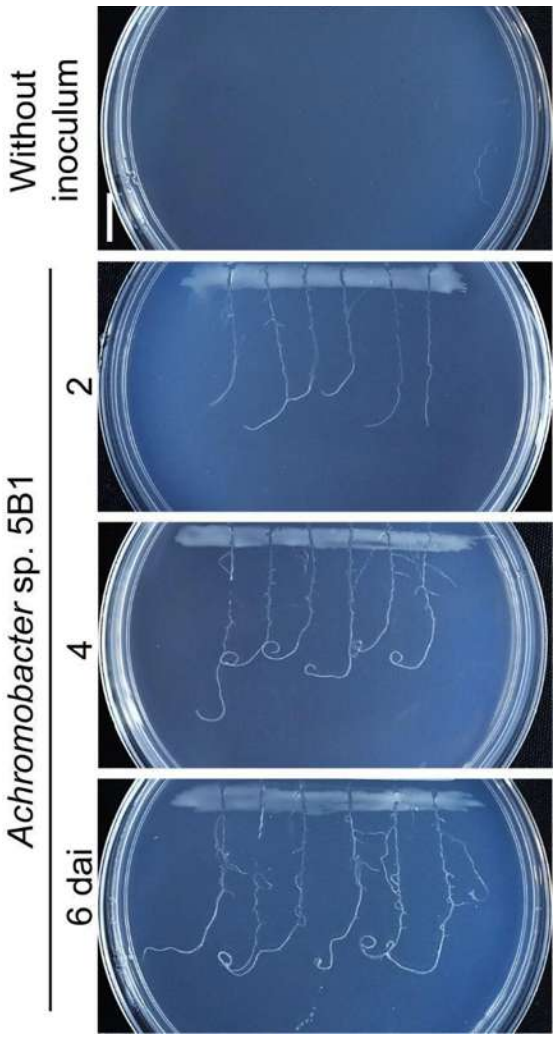
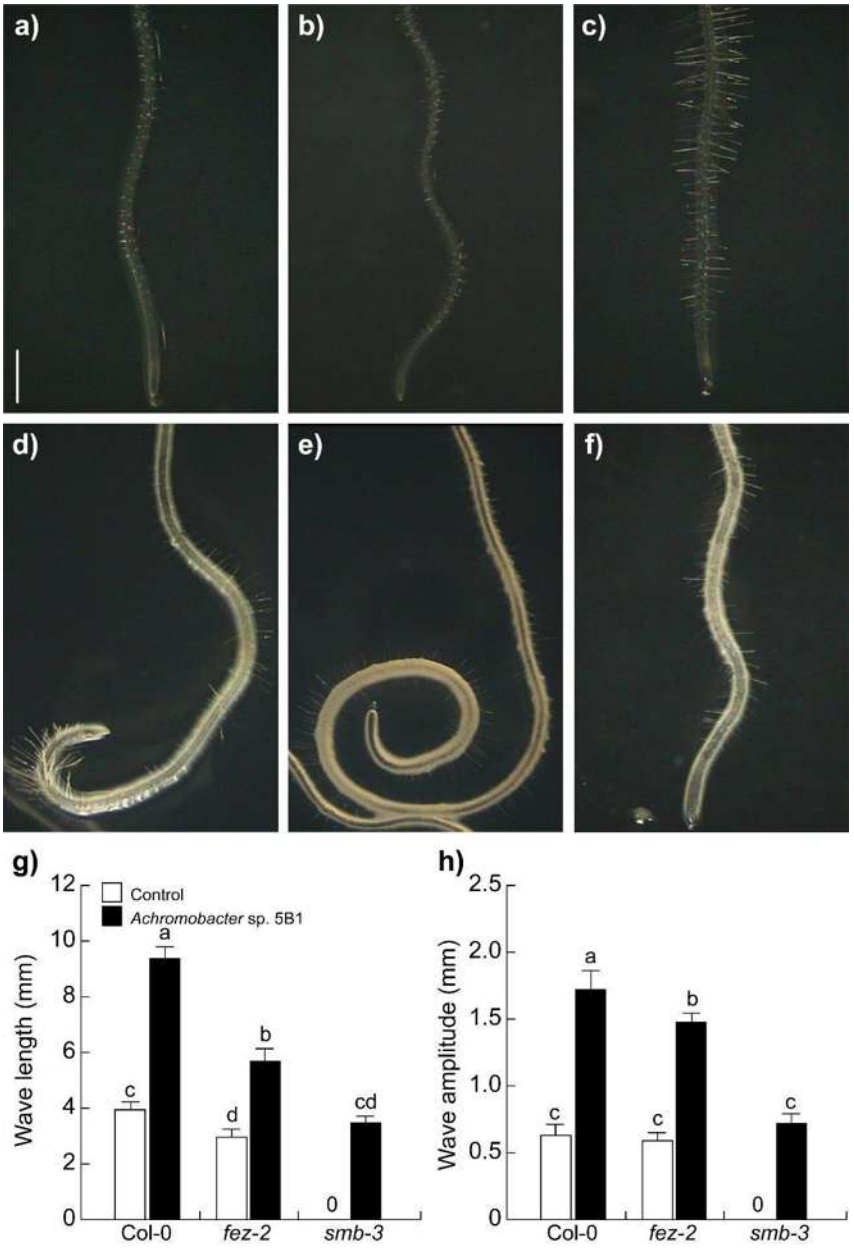


Figure S5. Bacterial growth around *Arabidopsis* roots co-cultivated with *Achromobacter* sp. 5B1. Representative images of Petri dishes where the plants were co-cultivated with *Achromobacter* sp. 5B1 in direct contact with roots. The seedlings were removed from the plates and the remaining bacteria developed a fully visible colony around roots that mimics of the root, indicating its spread over the root system. Scale bar: 1 cm.



S6. FEZ and SOMBRERO mediate root tip growth direction and coil formation in response to *Achromobacter* sp. 5B1. Representative images of *Arabidopsis* WT and *fez-2* and *smb-3* root tips of six-day-old seedlings transferred to 0.2x MS fresh media (controls) (a-c) or placed over *Achromobacter* sp. 5B1 bacterial streaks (d-f) and co-cultivated for 6 days. Graphs show the effects of bacterial co-cultivation on wave length (g) and wave amplitude (h). Bars show the mean ± SD. Different letters indicate statistically significant differences ($p < 0.05$; $n = 18$). Scale bar: 1 mm. These analyses were repeated three times with comparable results.

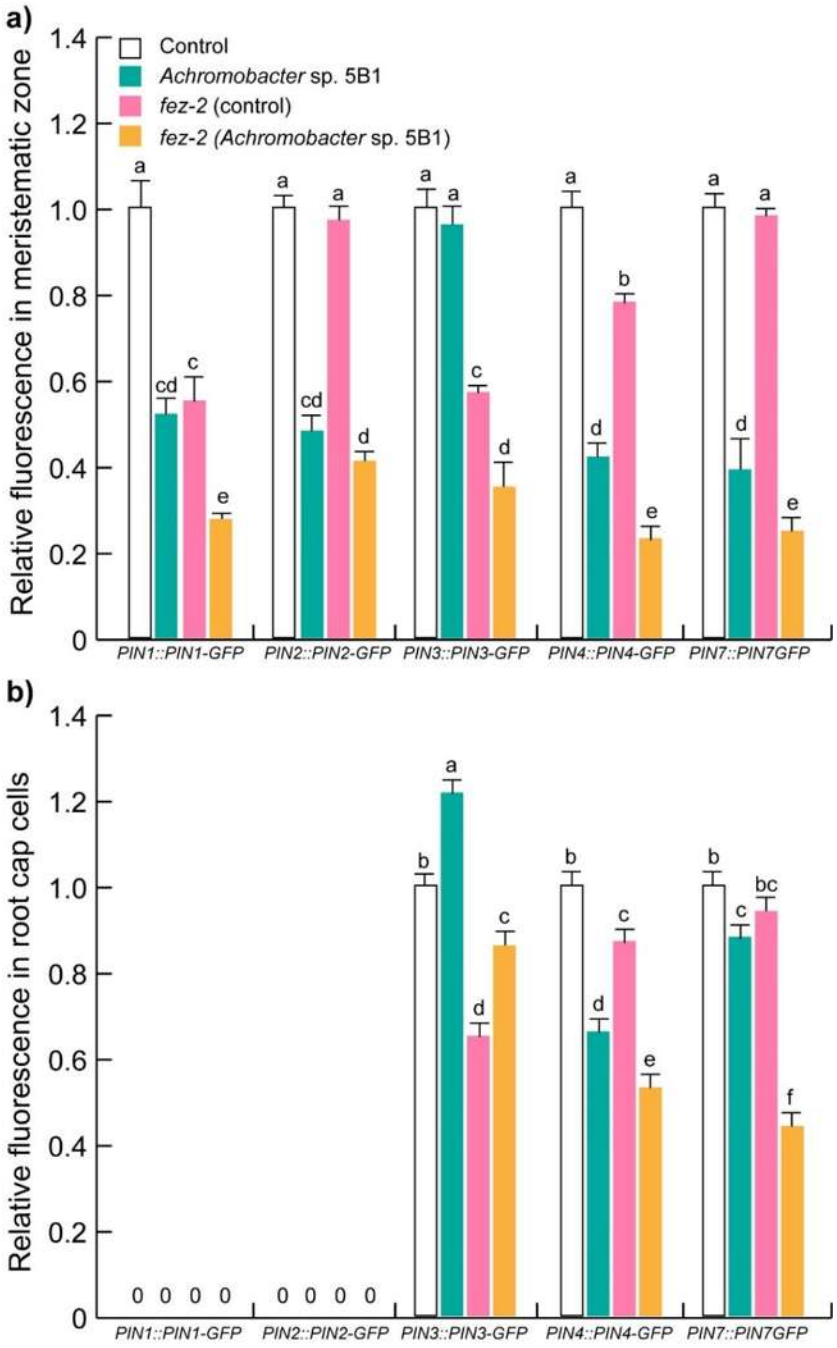


Figure S7. Effect of *Achromobacter* sp. 5B1 and mutation of *FEZ* on the expression of the auxin transporters PIN1, PIN2, PIN3, PIN4 and PIN7. Graphs show the relative fluorescence intensity at (a) the meristem and (b) columella cells. Bars show the mean ± SD. Different letters indicate statistically significant differences ($p < 0.05$; $n = 10$). These analyses were repeated three times with comparable results.

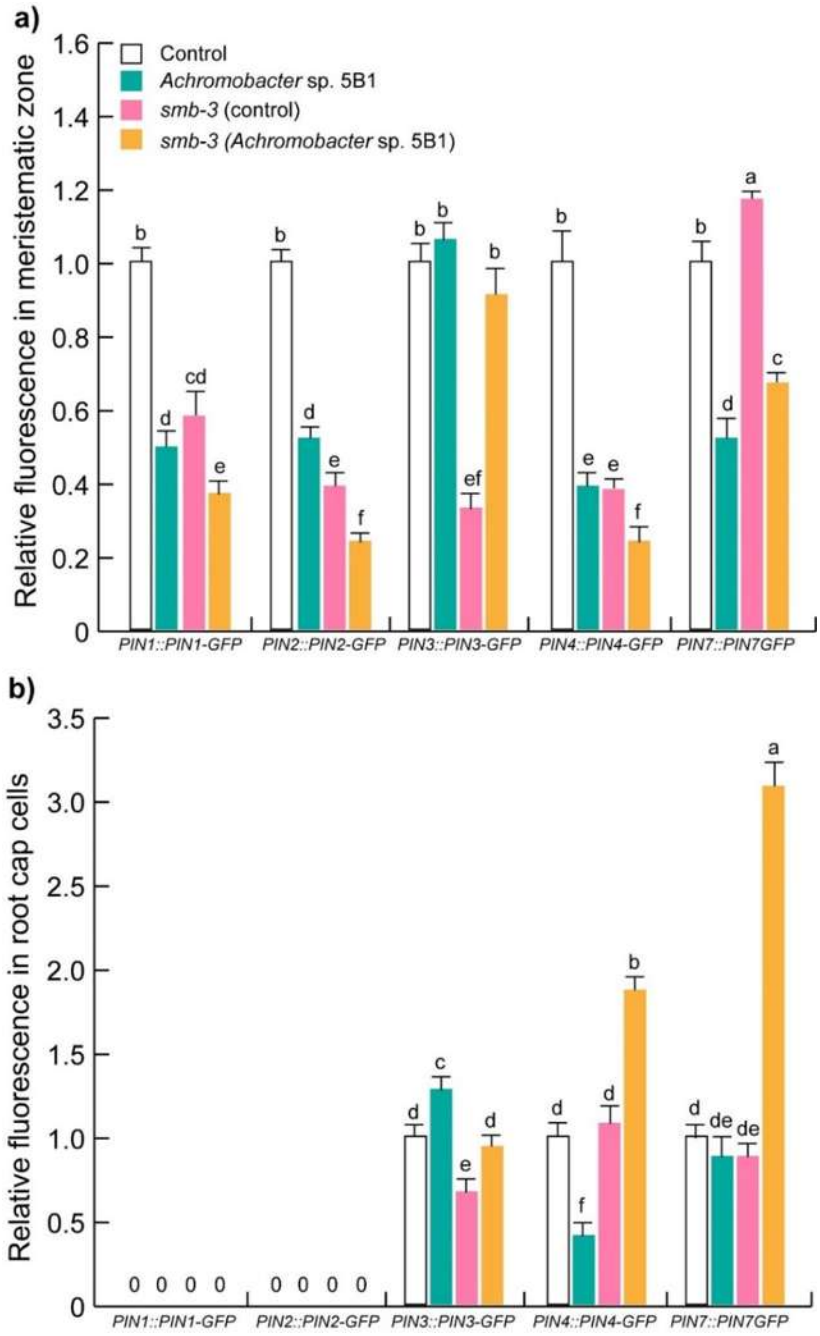


Figure S8. Effect of *Achromobacter* sp. 5B1 and mutation of *SMB* on the expression of the auxin transporters PIN1, PIN2, PIN3, PIN4 and PIN7. Graphs show the relative fluorescence intensity at (a) the meristem and (b) columella cells. Bars show the mean ± SD. Different letters indicate statistically significant differences ($p < 0.05$; $n = 10$). These analyses were repeated three times with comparable results.

8.2. Capítulo II.

Microbiological Research 281 (2024) 127594

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

The plant growth promoting rhizobacterium *Achromobacter* sp. 5B1, rescues *Arabidopsis* seedlings from alkaline stress by enhancing root organogenesis and hormonal responses

Kirán Rubí Jiménez Vázquez [a,1], José López-Hernández [a,1], Elizabeth García-Cárdenas [a], Ramón Pelagio-Flores [b], Jesús Salvador López-Bucio [c], Anahí Canedo Téxon [d,e,3], Enrique Ibarra-Laclette [d], José López-Bucio [a,2,*]

[a] *Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C. P., 58030 Morelia, Michoacán, Mexico*

[b] *Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Tzintzuntzan 173; Col. Matamoros, 58240 Morelia, Michoacán, Mexico*

[c] *Catedrático CONACYT-Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C. P., 58030 Morelia, Michoacán, Mexico*

[d] *Red de Estudios Moleculares Avanzados, Instituto de Ecología A.C., Carretera Antigua a Coatepec 351, El Haya, C.P. 91070, Xalapa, Ver, Mexico*

[e] *Departamento de la Conservación de la Biodiversidad, El Colegio de la Frontera Sur., Carretera Villahermosa-Reforma Km 15.5, Ranchería el Guineo, Sección II C.P., 86280 Villahermosa, Tabasco, Mexico*

ARTICLE INFO

Keywords:
Alkaline soils
Abiotic stress
Arabidopsis thaliana
Achromobacter sp. 5B1
Root architecture
Auxin

ABSTRACT

Soil alkalinity is a critical environmental factor for plant growth and distribution in ecosystems. An alkaline condition (pH > 7) is imposed by the rising concentration of hydroxides and cations, and prevails in semiarid and arid environments, which represent more than 25% of the total arable land of the world. Despite the great pressure exerted by alkalinity for root viability and plant survival, scarce information is available to understand how root microbes contribute to alkaline pH adaptation. Here, we assessed the effects of alkalinity on shoot and root biomass production, chlorophyll content, root growth and branching, lateral root primordia formation, and the expression of CYCB1, TOR kinase, and auxin and cytokinin-inducible trangenes in shoots and roots of *Arabidopsis* seedlings grown in Petri plates with agar-nutrient medium at pH values of 7.0, 7.5, 8.0, 8.5, and 9.0. The results showed an inverse correlation between the rise of pH and most growth, hormonal and genetic traits analyzed. Noteworthy, root inoculation with *Achromobacter* sp. 5B1, a beneficial rhizospheric bacterium, with plant growth promoting and salt tolerance features, increased biomass production, restored root growth and branching and enhanced auxin responses in WT seedlings and auxin-related mutants *aux1–7* and *eir1*, indicating that stress adaptation operates independently of canonical auxin transporter proteins. Sequencing of the *Achromobacter* sp. 5B1 genome unveiled 5244 protein-coding genes, including genes possibly involved in auxin biosynthesis, quorum-sensing regulation and stress adaptation, which may account for its plant growth promotion attributes. These data highlight the critical role of rhizobacteria to increase plant resilience under high soil pH conditions potentially through genes for adaptation to an extreme environment and bacteria-plant communication.

* Corresponding author.
E-mail address: jbucio@umich.mx (J. López-Bucio).
[1] These authors contributed equally to this research
[2] ORCID: 0000–0002-4849–5212
[3] Current address: Departamento de la Conservación de la Biodiversidad, El Colegio de la Frontera Sur. Carretera Villahermosa-Reforma Km 15.5, Ranchería el Guineo, Sección II C.P

https://doi.org/10.1016/j.micres.2023.127594
Received 12 September 2023; Received in revised form 21 December 2023; Accepted 23 December 2023
Available online 29 December 2023

1. Introduction

Soil pH refers to the level of acidity or alkalinity, usually modified when water dissolves substrate components such as organic matter, ash or minerals. It can also be dynamically modulated by plant activities such as root exudation of protons, organic acids, sugars, aminoacids and allelopathics (Esparza-Reynoso et al., 2021; Hinsinger et al., 2003; Korenblum et al., 2020). The pH goes from 0 to 14, where the optimal values for plant growth and productivity are believed to occur between pH 5.5 to pH 7.5 and most macro, and micronutrients may be available for root uptake (Rengel, 2015). Alkaline soils are thoroughly distributed across the world, particularly in arid and semiarid climates and are typically rich in calcium (Ca) and poor in phosphate and micronutrients, including iron (Fe), zinc (Zn), and manganese (Mn). These soils are inhabited by plant communities with species of diverse ecological affinities, but most crops and horticultural species fail to perform properly under these conditions (Gentili et al., 2018; Laliberté, 2012; Lee, 1998; Wang et al., 2017).

The plants that fail to thrive in alkaline soils are referred to as calcifuges (Lee et al., 1998). These high pH-sensitive plants may suffer from nutrient imbalances that compromise growth and survival, and include cereals, citrics and horticultural crops (Hayes et al., 2019; Kotula et al., 2021; Pereira et al., 2021). As a response to alkalinity, roots may adjust growth, waving and branching patterns to help soil exploration and scape from the stress (Liu et al., 2022). Research to understand molecular targets of alkalinity has been performed in *Arabidopsis*, in which the inhibition of root elongation through reduction of cell division in the meristem mediated by local changes in auxin transport via PIN2/EIR1, PIN7 and AUX1 and ethylene responses is a hallmark (X. Li et al., 2017; J. Li et al., 2017; Liu et al., 2022; Xu et al., 2012). In apple trees, an alkaline hydroponic medium imposed severe stress symptoms on both shoots and roots leading to growth inhibition. Noteworthy, overexpression of tyrosine decarboxylase, an enzyme involved in dopamine biosynthesis, rescued growth by supporting photosynthetic capacity and improving nitrogen absorption capacity (Liu et al., 2021). The growth response of *Arabidopsis* seedlings in acidic medium (pH 5.8) and mild alkaline conditions (pH 7.5) in vitro was recently reported by Duan and associates (2023), and the authors found the stimulation of primary root growth and lateral root formation at pH 7.5 that correlated with enhanced mitosis in root meristems and required auxin signaling and transport components (Duan et al., 2023). Understanding the influence of pH higher than 7.5 on root morphogenesis, growth and differentiation as well as hormonal responses associated with the activity of root and shoot meristems may provide tools to manage the adaptive mechanisms to make agriculture more resilient in alkaline soils.

The contribution of the root microbiome to abiotic stress adaptation has just begun to be unveiled (Kandhol et al., 2023; Otto, 2021). The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 was isolated from the rhizosphere of a mezquite tree growing around a salty pool in the Chihuahua desert, and whose banks were composed of alkaline-calcareous soil. It helped *Arabidopsis* plants to grow and produce more biomass in vitro and *in soil*, and to resist salt stress through inducing discrete root movements that primed pericycle cells to form lateral roots via auxin signaling (Jiménez-Vázquez et al., 2020). To the best of our knowledge, the contribution of root-associated microbes to plant resilience under alkaline stress remains unknown.

Here, we investigated the growth, developmental and physiological processes that are compromised in *Arabidopsis thaliana* seedlings grown under a wide range of alkaline pH through comparing the growth of the WT ecotype Col-0, transgenic lines expressing reporter genes on selected cellular and hormonal processes and auxin transport mutants. Moreover, we report that *Achromobacter* sp. 5B1 root inoculation rescues plants from alkaline stress and show that the genome of this bacterium harbors genes potentially involved in plant-bacteria communication and stress resistance that may account to grow under soil limiting conditions and improve biomass production.

2. Materials and methods

2.1. Plant material and growth conditions

Homozigous seedlings of the *Arabidopsis thaliana* ecotype Columbia-0 (Col-0), the transgenic lines *CycB1:GUS* (Colón-Carmona et al., 1999), *AtTOR:GUS* (Menand et al., 2002), *ARR5:GUS* (D'Agostino et al., 2000), *DR5:GUS* (Ulmasov et al., 1997) and the auxin-related mutants *eir1–1* (Luschnig et al., 1998), and *aux1–7* (Swarup et al., 2004) were employed for the experiments.

Seeds from WT, trangenic and mutant lines were disinfected with 95% (v/v) ethanol and 20% (v/v) commercial bleach for five minutes each, and then washed five times with sterilized deionized water. After 2 day cold stratification (4 °C), the seeds were sown on Petri plates containing solidified MS 0.2x medium with 0.6% sucrose and 1% phytagar, pH 7.0. Plates were placed in a plant growth chamber at 21 °C with a photoperiod of 16 h light/8 h darkness and light intensity of 200 $\mu mol/m^{-2}/s$. After five days, the seedlings were transferred to MS 0.2x medium, pH adjusted to 7.0, 7.5, 8.0, 8.5 and 9.0 with drops of potassium hydroxide and grown for seven more days.

2.2. Growth analysis and measurements

The root growth and branching were analyzed in *Arabidopsis* seedlings seven days after transfer to media with changing pH conditions. The roots and shoots were photographed using a Leica DFC450C microscope or Leica DM5000B with Nomarski optics, depending upon the magnification of the image. The stages of lateral root primordia were registered according to Malamy and Benfey (1997), which include seven developmental stages (Stages I to VII) and those primordia already emerging from the parent root were considered as stage emerged (E).

2.3. Total chlorophyll content analysis

For chlorophyll determination, *Arabidopsis* seedlings were recovered from the media and weighted to register the shoot fresh weight parameter. The shoot was submerged in 1 ml ethanol (96% v/v) for 30 min in microplates. All treatments were placed separately and covered with aluminum foil. Spectrum absorbance was recorded at 649 and 664 nm wavelengths for chlorophyll "b" and "a", respectively, using ethanol as a blank. The values of total chlorophyll were calculated according to Lichtenthaler (1987), following the equation: [(5.24 x Absorbance 664) + (22.24 x Absorbance 649)/(1000 x (shoot fresh weight)]. The units were reported in mg/g of fresh weight.

2.4. Histochemical analysis

Histochemical analysis of β-glucuronidase (GUS) activity was performed in the transgenic lines *CycB1:GUS*, *AtTOR:GUS*, *ARR5:GUS* and *DR5:GUS*. The transgenic seedlings from each genotype were transferred to varying pH media for seven days, taken up from the media and incubated at 37 °C in 1 ml of X-Gluc reaction buffer (0.5 mg ml-1 5-bromo-4-chloro-3-indolyl-D-glucuronide in 100 mM sodium phosphate, pH 7). The seedlings were cleared and fixed according to Malamy and Benfey (1997), in 0.24 N HCl in 20% methanol (v/v), at 62 °C solution, and 7% NaOH (w/v) in 60% ethanol (v/v) solution for 20 min at room temperature. Finally, the seedlings were dehydrated with 1 ml 40%, 20% and 10% (v/v) ethanol for 20 min each and then fixed with 50% glycerol (v/v) on microscope slides. 8 transgenic seedlings per treatment were analyzed using the Nomarski optics on a Leica DM5000B microscope.

2.5. Statistical analysis

All experiments were repeated at least three times with varied sampled seedlings per treatment. Each treatment included at least six

Petri plates with eight Arabidopsis seedlings each. The analyses were repeated five times with comparable results.The data were analyzed using the STATISTICA software. The graphs show the mean value ± standard error subjected to a one-way ANOVA test and a Tukey significance test. Letters above bars indicate means that differ significantly ($P \leq 0.05$).

2.6. Genome sequencing, assembly, and annotation of Achromobacter sp. 5B1

A single DNA library for Illumina MiSeq sequencing was constructed using the Nextera XT DNA kit (Illumina). The library was quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific) and quality was evaluated using a 7500 DNA kit on a 2100 Bioanalyzer instrument (Agilent Technologies). The library was sequenced on two independent runs using a MiSeq sequencer (Illumina) and the high output v2.0 MiSeq Reagent Kits from 300- and 600-cycles (2 ×150 bp and 2 ×300 bp paired-end (PE) reads, respectively). Prior to assembly, paired-end reads were filtered (https://github.com/Czh3/NGSTools/blob/master/qualityControl.py) to obtain only high quality (HQ) reads. SeqPrep software (https://github.com/jstjohn/SeqPrep) with default parameters was used to remove adapters and merge in a longer read those paired sequences (R1 and R2), which contained overlapping ends. Genome de novo assembly was performed using Newbler v3.0 with default settings and including adapters-free HQ paired-end reads and those longer sequences resulting from merging reads. Also, a reference-based scaffolding process was done using the MeDuSa software (Bosi et al., 2015), and a total of eight *Achromobacter* genomes as a guide for alignment, including those from *A. denitrificans*, *A. arsenitoxydans*, *A. xylosoxidans*, *A. spanius*, *A. ruhlandii*, *A. piechaudii*, *A. marplatensis*, and *A. insuavis* downloaded from the latest version available on GenBank. The *Achromobacter* sp. 5B1 genome was error-corrected by a mapping of Illumina reads onto the finished assembly. The Pilon software (Walker et al., 2014), which significantly improves draft genome assemblies, was used for this purpose. Finally, an annotation (including ORF finding and gene function annotation) was generated using Proksee server (https://proksee.ca/; Seemann, 2014; Grant et al., 2023). The raw PE reads can be found in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number PRJNA1041825. The *Achromobacter* sp. 5B1 genome assembly and gene models are available on Comparative Genomics (CoGe) database (http://genomevolution.org/CoGe/).

2.7. Ortholog groups identification

To identify ortholog genes shared across the *Achromobacter* species, all annotated proteins into the different species used in the scaffolding process (see section above) and in the genome of *Achromobacter* sp. 5B1 were compared. OrthoMCL (Li et al., 2003), a pipeline that uses a reciprocal BLAST and the Markov CLuster (MCL) algorithm to infer and group orthologs (and paralogs), were used for this purpose. Default parameters were used to define the orthologs cluster structure, including an inflation value of 1.5 and a threshold *e*-value of 10^{-10} in the BLAST step.

It is worth mentioning that some reference proteins were also included in the OrthoMCL analysis, which was added to the list of 51,432 *Achromobacter* proteins. These reference proteins belong to different bacterial species and are well-known enzymes involved in indole-3-acetic acid (IAA) biosynthesis pathways and were identified using the MetaCyc database (https://metacyc.org/; Caspi et al., 2018, Karp et al., 2019). The UniProt entry accession from reference enzymes is listed below: Tryptophan 2-monooxygenase (P0A3V3 and P06617 from *Rhizobium radiobacter* and *Pseudomonas savastanoi*, respectively); Indoleacetamide hydrolase (P0A2X0 and P06618 from *Rhizobium radiobacter* and *Pseudomonas savastanoi*, respectively); Nitrile hydratase subunit alpha and beta (Q70X88, Q70X87, P97051, and P97052 from *Rhizobium radiobacter* and *Pseudomonas putida*); Nitrilase (P20960 and Q500U1 from *Alcaligenes faecalis* and *Pseudomonas syringae* pv. *syringae*); Tryptophanase (A0A0M3F2I7 from *Enterobacter cloacae*); Indolepyruvate decarboxylase (P71323 and P23234 from *Enterobacter agglomerans* and *E. cloacae*); Indolepyruvate oxidoreductase subunit a and b (F9VNL4 and F9VNL6 from *Sulfurisphaera tokodaii*).

3. Results

3.1. Alkaline pH disrupts Arabidopsis seedling growth and development

Alkaline stress is deleterious for plant growth and development (Lee et al., 1998). To analyze the effects of basic pH during early plant growth, *Arabidopsis* seedlings were germinated and grown for 5 d in Petri plates with agar-solidified Murashige and Skoog (MS) 0.2x medium pH 7.0, and then transferred to the same medium adjusting the pH to 7.0, 7.5, 8.0, 8.5 and 9.0 with a potassium hydroxide solution. Seedlings from the wild-type ecotype Columbia-0 (Col-0) were grown for seven additional days over the surface of the plates to allow easy visualization of both the root and shoot systems without any interference of the substrate. Overall inspection of the seedlings transferred to medium with pH 7.0 (Fig. 1a), pH 8.5 (Fig. 1b), or pH 9.0 (Fig. 1c), showed that the rise of pH repressed shoot and root growth, while close up from a group of seedlings at neutral pH or high alkaline pH clearly showed the repression of leaf formation and chlorosis as a consequence of alkaline stress (Fig. 1d-g). Along with the drastic reduction of plant size at pH 9.0, the primary roots manifested an enhaced wavy pattern compared to plants grown in medium with pH 7.0 (Fig. 1h, i).

3.2. Alkaline pH represses root growth, plant biomass and chlorophyll content

To obtain quantitative data into how mild or strong alkaline stress impairs plant growth, we analyzed the following parameters: I) Primary root growth, II) Total lateral root primordia (LRP), III) Chlorophyll content, IV) Total fresh weight, V) Shoot fresh weight, and VI) Root fresh weight. Along with the drastic reduction of plant size by rising pH, the roots were shorter (Fig. 2a), had a reduced number of lateral root primordia (Fig. 2b), and the levels of chlorophyll in leaves decreased (Fig. 2c), reaching the maximum inhibition al pH 9.0.

The phenotype of shoot and root systems in plants grown in alkaline media indicates that biomass production and energetic metabolism may be compromised. Biomass measurements were consistent with this notion, since total fresh weight, shoot fresh weight and root fresh weight strongly decreased at pH 9.0 (Fig. 2d, e, f). Thus, alkaline stress is a major determinant for plant health and productivity.

3.3. The initiation of lateral roots is highly sensitive to alkaline stress

Lateral roots have the fundamental functions of plant support and nutrient and water acquisition (Yokawa and Baluška, 2018). Thus, the next aim of the research was to understand the impact of alkaline pH on a very important trait that accounts for the root branching process, namely lateral root primordia formation. The effects of alkaline pH in the developmental stages of lateral root primordia were assessed by observing each lateral root primordium within a given primary root from seedlings that were cleared to visualize the corresponding developmental stages. The primordia stages (I-VII) and emerged lateral roots (E) were counted from seedlings transferred for 7 days to media with pH 7.0, 8.0, and 9.0. Both the number and developmental stage of the lateral root primordia decreased with the rise of pH (Fig. 3a-c). However, the number of emerged branches increased at pH 8.0 and then decreased at pH 9.0 (Fig. 3b, c), which indicates that mild alkalinity may promote lateral root maturation, and that the development of lateral roots is highly sensitive to alkaline pH.

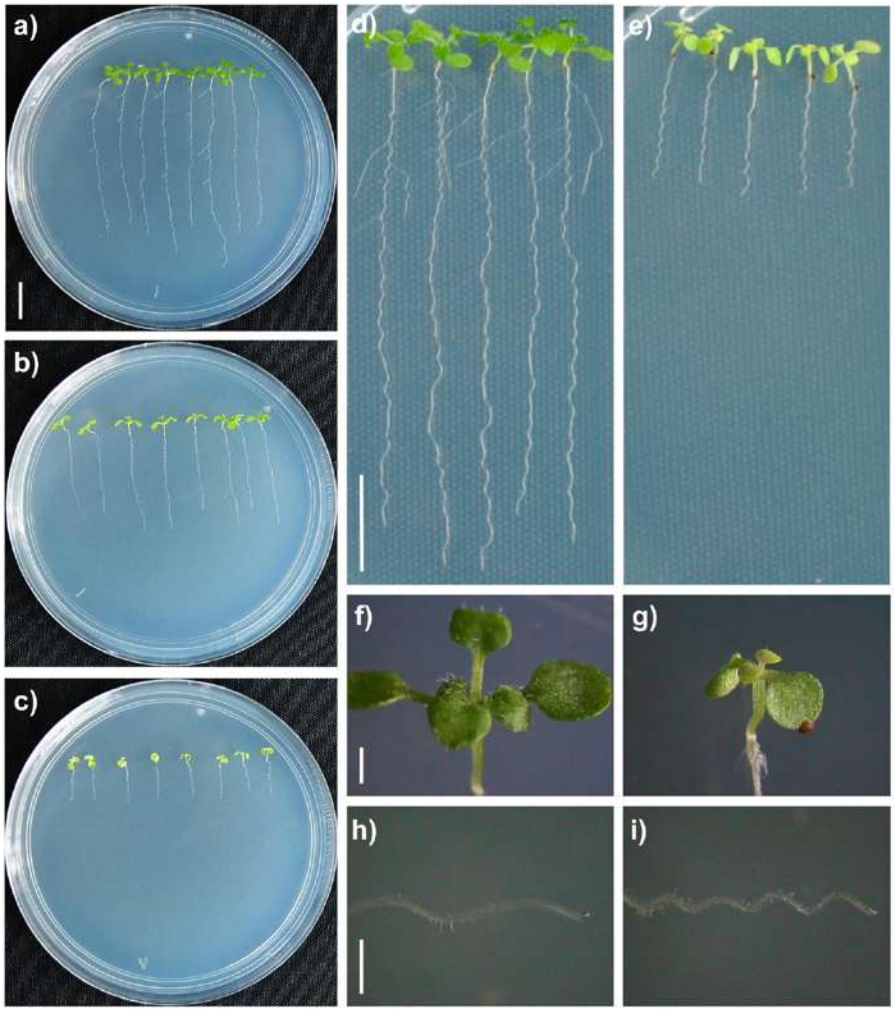


Fig. 1. Phenotypes of *Arabidopsis thaliana* seedlings exposed to alkaline pH. The plants were germinated and grown in MS 0.2x agar-solidified medium pH 7.0 and transferred 5 days after germination to fresh media adjusting the pH of the medium to high pH. The images show photos of the plates taken 7 days after the transfer to medium with pH 7.0 (**a**), pH 8.5 (**b**) or pH 9.0 (**c**). Scale bar: 1 cm. Close up of seedlings transferred to pH 7.0 (**d**, **f**, **h**) or pH 9.0 (**e**, **g**, **i**). The experiment included three independent plates for each treatment and the experiment was repeated 5 times with comparable results.

3.4. Decreased shoot and root growth under alkaline pH correlates with dramatically reduced hormonal signatures

Plant biomass relies on cell production at the shoot meristem, leaf primordia formation and organ extension (Huerta-Venegas et al., 2022), and strong alkalinity (pH 9.0) halts the progression of leaf primordia to mature leaves. To understand the basis of such growth repression, we analyzed the growth of transgenic *Arabidopsis* seedlings that express the *CycB1:GUS*, *TOR:GUS*, *ARR5:GUS* and *DR5:GUS* gene constructs at pH 7.0 (neutral) and pH 9.0 (strong alkaline). As expected, the shoots of seedlings from all transgenic lines grown at pH 9.0 showed delayed development of leaf primordia when compared to seedlings at pH 7.0 (Fig. 4 a-h). Histochemical analysis of GUS expression did not reveal clear expression of CYCB1 at the shoot system irrespective of the pH condition (Fig. 4a, e), instead GUS transcript directed by the TOR promoter could be clearly detected in developing leaf primordia of plants at pH 7.0 and 9.0 by the blue staining (Fig. 4b, f), whereas the cytokinin-inducible and auxin-inducible reporters *ARR5* and *DR5*, respectively, were drastically inhibited at pH 9.0, which correlated with the delay of leaf primordia development (Fig. 4c-h).

The root tip includes the meristem and cell elongation zones that are responsible of producing the cell biomass for organogenesis, cell signaling and establishment of hormonal gradients (Salvi et al., 2020). An assessment of the gene expression of *CycB1:GUS*, *TOR:GUS*, *ARR5:GUS* and *DR5:GUS* at pH 7.0 unveiled the domains covered by the promoters, for which *CYCB1* was specifically expressed in the root meristem (Fig. 4i), *TOR* was located in the meristem and cell elongation regions (Fig. 4j), and *ARR5* and *DR5* had their maximum expression within the columella, at the root tip (Fig. 4k, l). Noteworthy, in seedlings of all four marker lines grown in medium with pH 9.0, their corresponding expression domains decreased drastically (Fig. 4m, n, o, p), which correlated with the halted root growth imposed by the alkaline stress.

3.5. Achromobacter sp. 5B1 promotes growth at neutral pH and rescues Arabidopsis seedlings from strong alkaline stress

Root interactions with a select group of beneficial bacteria promote plant growth and productivity (Lugtenberg and Kamilova, 2009). Our work led to the identification of the bacterium *Achromobacter* sp. 5B1, coming from a saline/alkaline environment (Jiménez-Vázquez et al., 2020), so we hypothesized that the interaction of *Arabidopsis* with this

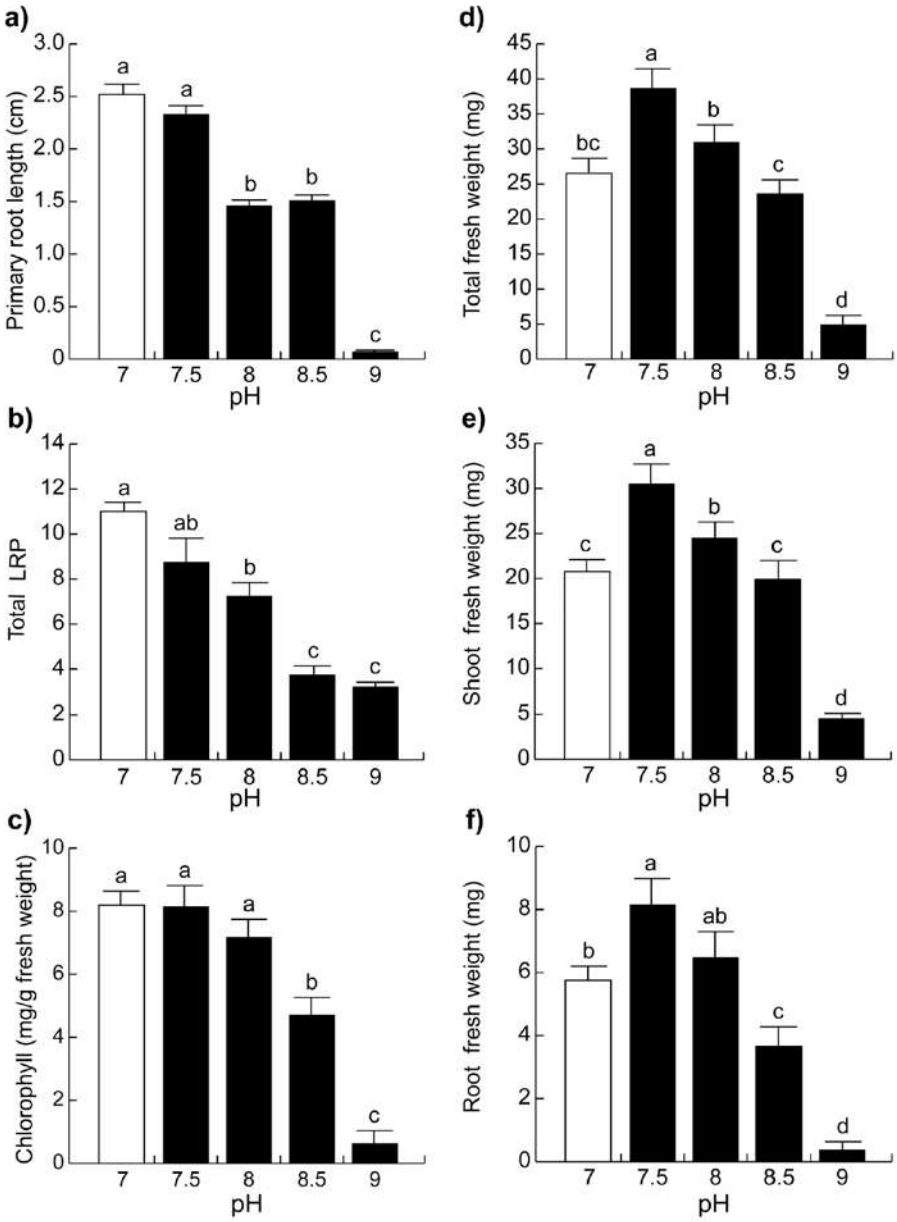


Fig. 2. Effects of alkaline pH on plant growth and developmental traits. *Arabidopsis* seedlings were germinated and grown in MS 0.2x agar-solidified medium pH 7.0 and transferred 7 days after germination to fresh media with pH medium of 7.0, 7.5, 8.0, 8.5, and 9.0 for five subsequent days. **a**). Primary root length, **b**). Total lateral root primordia (LRP), **c**). Chlorophyll content, **d**). Total fresh weight, **e**). Shoot fresh weight, **f**). Root fresh weight. Bars represent the mean ± SE (n = 24). Different letters indicate means that statistically differ at $p < 0.05$ with a Tukey post-hoc test. The analyses were repeated five times with comparable results.

bacterium could help plants to resist alkaline stress. To test this, the effect of plant cocultivation by placing the roots over a streak of *Achromobacter* sp. 5B1 on *Arabidopsis* biomass production at neutral (pH 7.0) and strong alkaline pH (9.0) on total fresh weight, shoot fresh weight, and root fresh weight was determined. The cultivation of plants whose root was in contact with the inoculum, promoted about twice the total fresh weight, fresh weight of the foliage and fresh weight of the root in medium with neutral pH (Fig. 5a-c). As expected, the alkaline environment repressed the production of biomass, considering the three variables analyzed, while the incorporation of the bacterium to the alkaline medium reversed the negative effect of alkalinity and resulted in a much higher biomass of plants in this adverse condition (Fig. 5a-c). Representative images of axenically grown seedlings are shown at pH 7.0 (Fig. 5d), pH 9.0 (Fig. 5e), or inoculated with a streak of *Achromobacter* sp. 5B1 and grown at pH 7.0 (Figs. 5f) or 9.0 (Fig. 5g). Considering the representative images of the Petri dishes containing the plants in the different treatments, it is noteworthy that the root growth deviation caused by the bacteria in the medium with pH 7.0 (Fig. 5f), is reversed, and now the primary roots of the plants inoculated in the medium with pH 9.0 restored their growth, recovering the straightness of its root towards the normal gravity vector (Fig. 5g). Under both pH conditions there was a clear increase in the formation of lateral roots and the growth of these structures as a consequence of bacterial inoculation (Fig. 5d-g).

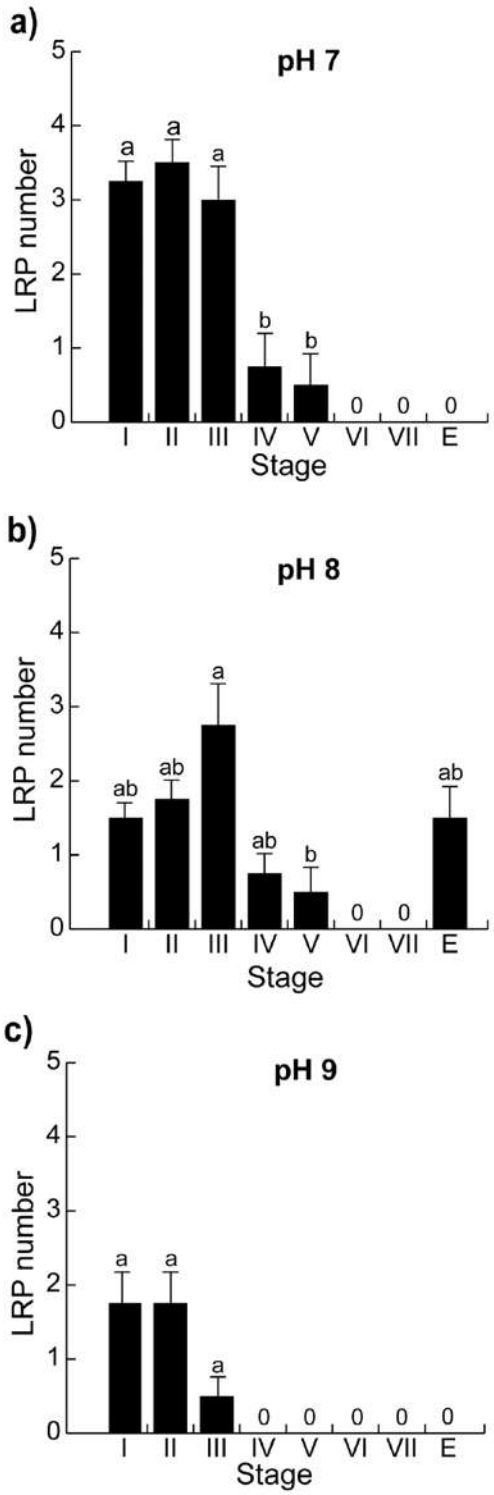


Fig. 3. Effects of alkaline pH in the developmental stages of lateral root primordia. Developmental stages of the primordia (I-VII) and emerged lateral roots (E) were counted in Arabidopsis seedlings transferred for 7 days to media with pH 7.0 (**a**), 8.0 (**b**), and 9.0 (**c**). The seedlings were cleared to visualize the primordia. Bars represent the mean ± SE (n = 8). Different letters mean statistically significant differences at $p < 0.05$ with a Tukey post-hoc. The analysis was repeated twice with comparable results.

3.6. The plant growth recovery under alkaline stress promoted by Achromobacter sp. 5B1 is independent of EIR1 and AUX1 genes

The deviation of root growth promoted by *Achromobacter* sp. 5B1 in *Arabidopsis* was postulated to prime pericycle cells to produce more lateral roots and these changes were related to an auxin response (Jiménez-Vázquez et al., 2020). Moreover, since the adaptation of roots to alkaline stress involved auxin transport components PIN2/EIR1 (Xu et al., 2012) and AUX1 (X. Li et al., 2017; J. Li et al., 2017), we hypothesized that mutation of the corresponding genes would compromise the bacterially-mediated resilience of plants to grow at high pH. To test this possibility, *Arabidopsis* WT (Col-0), *eir1–1* and *aux1–7* mutants, were inoculated or not with *Achromobacter* sp. 5B1 at pH 7.0 or 9.0, and primary root length, lateral root number, lateral root density, and lateral root length were analyzed as selected traits. All these traits, which are strongly affected by alkalinity, could be normalized by the inoculation at pH 9.0 (Fig. 6a-d), and interestingly, the bacterium reverted the agravitropic roots of both *eir1–1* and *aux1–7* mutants at high pH (Fig. 6e-l), which suggests that combinatorial mechanisms, and not a single mechanism, accounts for plant resistance to high pH, relieving not only the adverse effects of alkalinity on WT plants but also the agravitropic root behavior of auxin transport mutants.

3.7. Achromobacter sp. 5B1 restores the auxin response of WT seedlings and auxin transport-related mutants eir1-1 and aux1-7 in primary root tips under strong alkaline stress

Since *Achromobacter* sp. 5B1 helped plants to resist better alkaline stress in WT and auxin transport mutants *eir1–1* and *aux1–7*, it was of interest to determine the changes in auxin response in root meristems of the mutants grown at neutral pH (7.0) or strong alkaline pH (9.0). In WT seedlings, *DR5:GUS* had their maximum expression within the columella at the root tip and the expression strongly decreased at pH 9.0. Alkaline pH also inhibited *DR5:GUS* expression within primary root tips in *eir1–1* and *aux1–7* mutants (Fig. 7). In contrast, bacterial inoculation strongly enhanced *GUS* expression at both pH 7.0 and pH 9.0 in the WT and mutant lines (Fig. 7), which accounts for adequate meristem structure and primary root growth.

3.8. Assembly, annotation, and comparative analysis of the Achromobacter sp. 5B1 genome

Several bacterial traits account for plant growth promotion and may contribute to plant growth under adverse conditions. To gain insight into the possible mechanisms by which *Achromobacter* sp. 5B1 rescues plants from alkaline stress, we proceeded to sequence its genome. A total of 4,125,285 paired-end reads (~1.2 Gbp) were generated from the genomic DNA sequenced library [1,629,485 (2 ×150 bp) paired-end reads and 2,495,800 (2 ×250 bp) paired-end reads). Before de novo assembly, raw reads were filtered to only include those with acceptable quality (see Methods for more details). Longer reads were generated by merging paired-end reads with overlapping regions and both data sets (long reads and no merged paired-end reads) were used to assemble the genome, which resulted in a total of seven scaffolds (with a minimum size of 2828 bp). The scaffold N50 was 2,082,648 bp, and the maximum scaffold length was 2,297,750 bp. The genome assembly comprises a total of 5,835,596 bp (~200x coverage), slightly smaller in comparison with other published genomes of *Achromobacter* species (*A. denitrificans*, ≈ 6.54 Mbp (Reis et al., 2017); *A. arsenitoxydans*, ≈ 6.15 Mbp (Li et al., 2012); *A. xylosoxidans*, ≈ 6.52 Mbp (Strnad et al., 2011; Jakobsen et al., 2013; Badalamenti and Hunter, 2015; Jeukens et al., 2015); *A. spanius*, ≈ 6.42 Mbp (Li et al., 2018); *A. ruhlandii*, ≈ 6.44 Mbp (Rodrigues et al., 2016); *A. piechaudii*, ≈ 6.25 Mbp (Trimble et al., 2012); *A. marplatensis*, ≈ 6.88 Mbp (Li et al., 2023); *A. insuavis*, ≈ 6.87 Mbp (Veschetti et al., 2021). The assembly statistics of the *Achromobacter* sp. 5B1 are presented in Supplementary table S1.

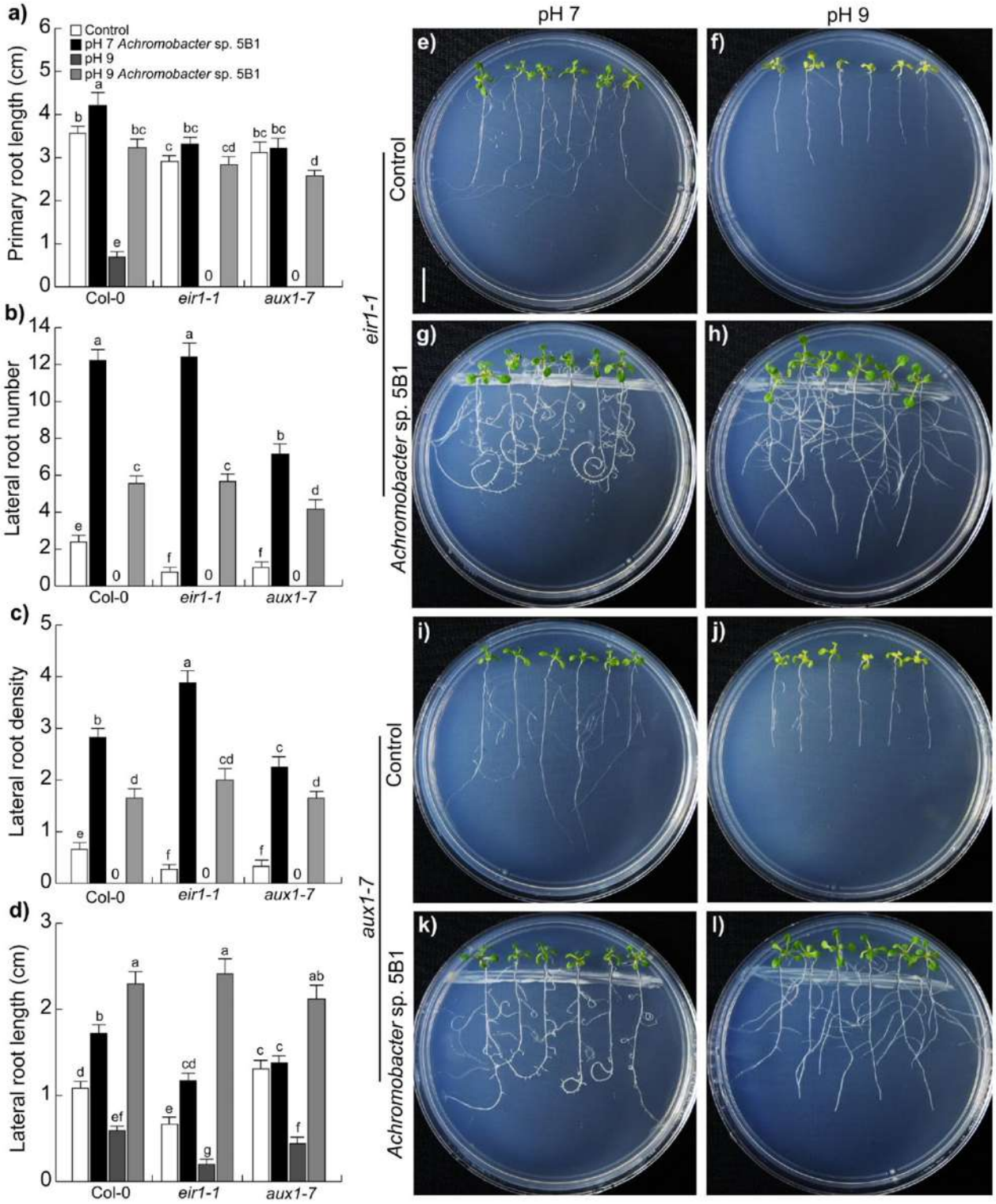


Fig. 4. *GUS*, T pH 7.0 seedlin repeat

Ge annot et al., *Achro* S2). A *A. ruh* 5556, with (6204

Fig. 6. Effect of root cocultivation with *Achromobacter* sp. 5B1 on growth and development of *Arabidopsis* auxin-related mutants at neutral and strong alkaline pH. *Arabidopsis* WT, *eir1–1* and *aux1–7* mutant seedlings were germinated and grown in MS 0.2x agar-solidified medium pH 7.0 and transferred 5 days after germination to fresh media with pH 7.0 or 9.0 for 7 subsequent days. **a**). Primary root length, **b**). Lateral root number, **c**). Lateral root density, **d**). Lateral root length. Representative images of *eir1–1* mutants axenically grown at pH 7.0 (**e**), or pH 9.0 (**f**), or inoculated with a streak of *Achromobacter* sp. 5B1 and grown at pH 7.0 (**g**) or 9.0 (**h**). Representative images of *aux1–7* mutants axenically grown at pH 7.0 (**i**), or pH 9.0 (**j**), or inoculated with a streak of *Achromobacter* sp. 5B1 and grown at pH 7.0 (**k**) or 9.0 (**l**). Bars represent the mean ± SE (n = 24). Different letters indicate means that statistically differ at $p < 0.05$ with a Tukey post-hoc test. Each treatment included six plates. The analyses were repeated three times with comparable results.

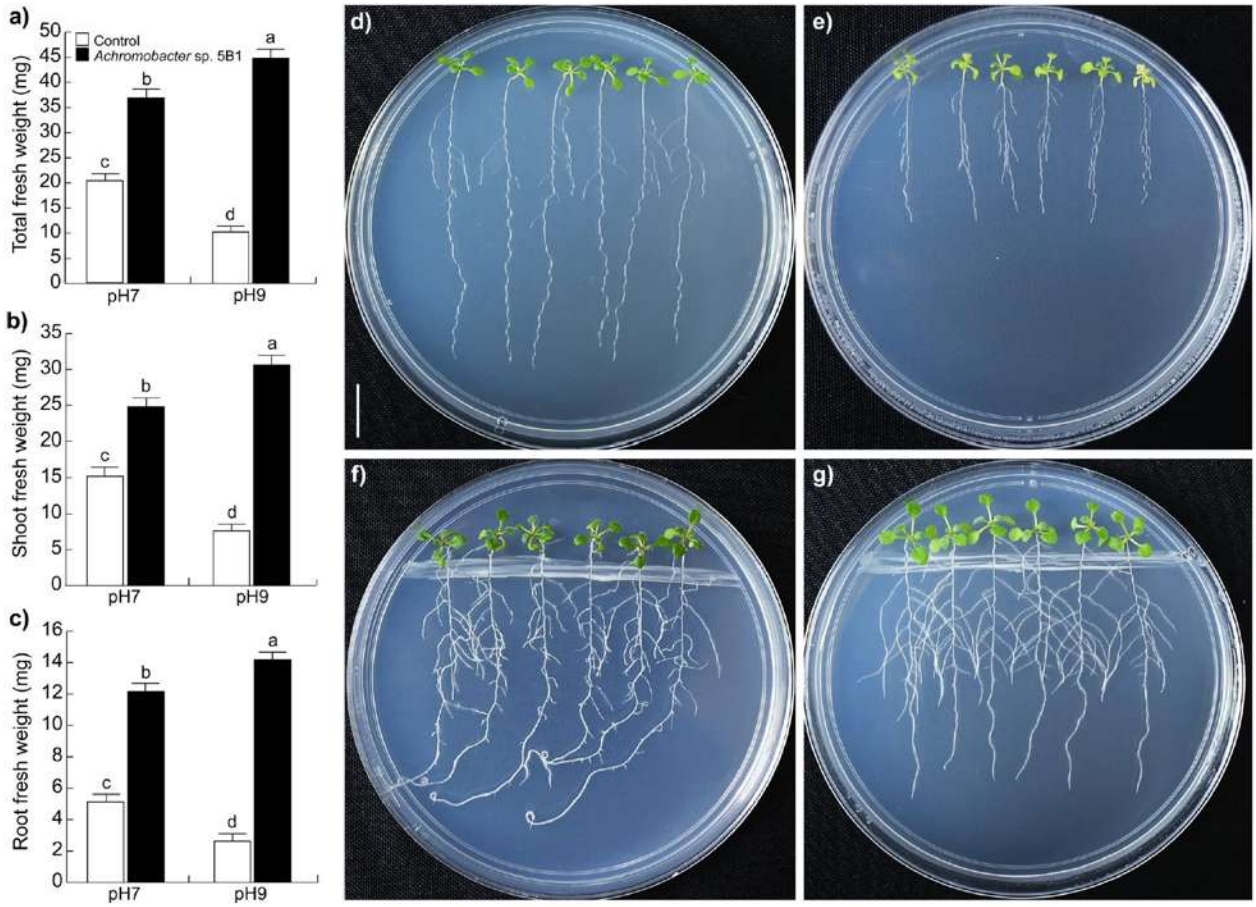


Fig. 5. Effect of root cocultivation with *Achromobacter* sp. 5B1 on *Arabidopsis* biomass production at neutral and strong alkaline pH. **a**). Total fresh weight, **b**). Shoot fresh weight, **c**). Root fresh weight. Representative images of axenically grown seedlings at pH 7.0 (**d**), or pH 9.0 (**e**), or inoculated with a streak of *Achromobacter* sp. 5B1 and grown at pH 7.0 (**f**) or 9.0 (**g**). Bars represent the mean ± SE (n = 24). Different letters indicate means that statistically differ at $p < 0.05$ with a Tukey post-hoc test. Each treatment included six plates. The analyses were repeated five times with comparable results.

were assigned to at least one these groups while 3281 proteins (grouped in a total of 3201 families) resulted common among all *Achromobacter* species (Fig. 9 and Supplementary table S3).

3.10. *Achromobacter sp. 5B1 genes related to plant growth promotion*

3.10.1. *Indole-3-acetic acid biosynthesis*

Considering the reference enzymes included in the OrthoMCL analysis (see methods from details), *Achromobacter* sp. 5B1 genes likely involved in indole-3-acetic acid (IAA) biosynthesis were identified. The gene LOCUS_22090 encodes the indoleacetamide hydrolase (iaaH), which hydrolyzes indole-3-acetamide (IAM) into indole-3-acetic acid (IAA). This enzyme was 50% identical to homologs identified in some other betaproteobacteria from the order Burkholderiales. It also shared 37.5% of identity to indoleacetamide hydrolase (UniProt entry accession P0A2X0) encoded by the *tms2* gene (Klee et al., 1984; Sciaky and Thomashow, 1984) of *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*). This enzyme has been characterized by its expression in prokaryotic and eukaryotic systems (Schröder et al., 1984). Similarly, the gene LOCUS_22670, which was annotated as an aliphatic nitrilase, is 34.5% identical and was grouped in the same orthogroup that the nitrilase enzymes used as reference (P20960 from *Alcaligenes faecalis*, and Q500U1 from *Pseudomonas syringae* pv. *syringae*). Enzymes encoded by the genes LOCUS_26670, LOCUS_26240, and LOCUS_45210 were grouped, respectively, in same orthogroups that the reference enzymes tryptophane aminotransferase (A0A0M3F2I7 from *Enterobacter cloacae*), indole pyruvate decarboxylase (P71323 and P71324 from *Enterobacter agglomerans* and *E. cloacae*), and indole pyruvate dehydrogenase (F9VNL4 from *Sulfurisphaera tokodaii*). However, for all these enzymes the identity percentage with *Achromobacter* proteins is only around 23.5–25.9%. Based on these results, *Achromobacter* sp. 5B1 might synthesize IAA at least by two pathways, but a third route may also occur (Fig. 10), which is consistent with the induction of the auxin-inducible reporter gene *DR5* in *Arabidopsis* seedlings.

3.10.2. *Genes for cross-kingdom bacteria-plant communication*

Gram negative bacteria employ quorum-sensing signals from the *N*-acyl-*L* homoserine lactone (AHL) class, and these molecules can be perceived by plants to orchestrate root system architecture (Ortiz-Castro et al., 2008; Ortiz-Castro and López-Bucio, 2019). Our assessment of the *Achromobacter* sp. 5B1 genome uncovered genes for AHL synthesis such as LOCUS_02070 encoding an *N*-acyltransferase YncA, while LOCUS_11520, LOCUS_11540, LOCUS_12350, LOCUS_25260, LOCUS_25890, and LOCUS_31160 encode homoserine lactone efflux proteins. Termination of AHL signaling may involve LOCUS_17880 encoding *N*-acyl homoserine lactonase, an enzyme that degrades the lactone ring of AHLs, whereas LOCUS_18310, LOCUS_33450, LOCUS_45350, and LOCUS_45830 encode acylamidase, which disrupts QS signaling, also termed quorum quenching (Supplementary table S2).

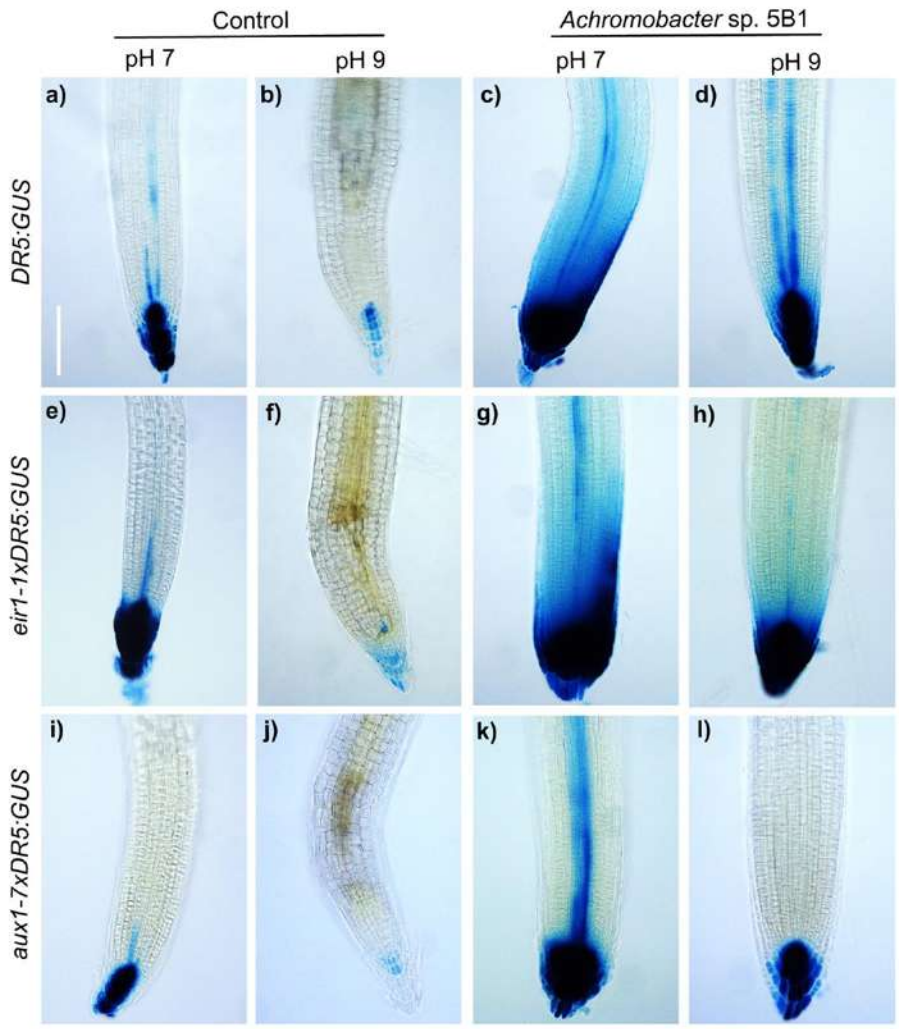


Fig. 7. Expression of auxin-response reporter gene *DR5:GUS* in root tips of WT, *eir1–1*, and *aux1–7* mutant seedlings grown in media with neutral or strong alkaline pH inoculated or not with *Achromobacter* sp. 5B1. The seedlings were transferred at 5 days after germination to media with pH 7.0 or 9.0 and grown for 7 additional days. GUS expression in the WT *DR5:GUS* line at pH 7.0 (**a**) or 9.0 (**b**) and with bacteria at pH 7.0 (**c**) or 9.0 (**d**). GUS expression in the *eir1–1* mutant background at pH 7.0 (**e**) or 9.0 (**f**) and with bacteria at pH 7.0 (**g**) or 9.0 (**h**). GUS expression in the *aux1–7* mutant background at pH 7.0 (**i**) or 9.0 (**j**) and with bacteria at pH 7.0 (**k**) or 9.0 (**l**). The images are representative from 8 independent seedlings that were cleared and processed for histochemical GUS detection and taken using a Leica DM 5000B microscope with the Nomarsky optics. The analysis was repeated three times with comparable results.

3.10.3. Genes related to abiotic stress responses

The *Achromobacter* sp. 5B1 strain was isolated from a high salinity rhizosphere (Jiménez-Vázquez et al., 2020), and despite its survival mechanisms are not yet understood, identification of a sequence encoding 1-aminocyclopropane-1-carboxylate deaminase (ACCD) codified by LOCUS_05640 gene, strongly draw our attention (Supplementary table S2). This protein can be found in *Achromobacter* sp. FB-14 a probiotic isolate that augmented rice growth by upregulating the expression of stress-responsive CIPK genes under salinity stress (Shahid et al., 2020). Regarding alkaline pH, we suggest that like other plant growth-promoting microbes, *Achromobacter* sp. 5B1 can use sodium-dependent ATP synthases as an alkali tolerance mechanism or improve Fe nutrition and root architecture that account for better plant growth (Zhou et al., 2016). The genes encoding the eighth polypeptides of the ATP synthase from *Achromobacter* sp. 5B1 are LOCUS_23310, LOCUS_23300, LOCUS_23290, LOCUS_23280, LOCUS_23270, LOCUS_23260, LOCUS_23250, LOCUS_23240 (atpε, atpβ, atpγ, atpα, atpΔ, atpB, atpC, and atpA, respectively). While it is not clear how some bacteria can use this mechanism, it is known that they modify their surrounding medium to near neutrality to survive high pH. Sodium is crucial in intracellular pH maintenance because it allows the exchange of H^+/Na^+ antiporters (Satyanarayana et al., 2005). Furthermore, the H^+ concentration gradients across the membranes play an essential role in producing ATP during cellular respiration, and thus, it is possibly that through proton motive force and the ATP synthase activity, some microbes modulate the tolerance to alkalinity (Celiker and Gore, 2013).

4. Discussion

4.1. Alkaline pH disrupts the activity of plant meristems, hormonal homeostasis and lateral root primordia development

Understanding the impact of soil pH on plant growth and development and the mechanisms that enable adaptation to alkalinity is critical to develop efficient agronomic management and support productivity under environmental stress. Alkaline and acid soils both share the problem of phosphorus deficiency (Agegnehu et al., 2021), thus understanding how alkaline stress affects critical plant traits such as

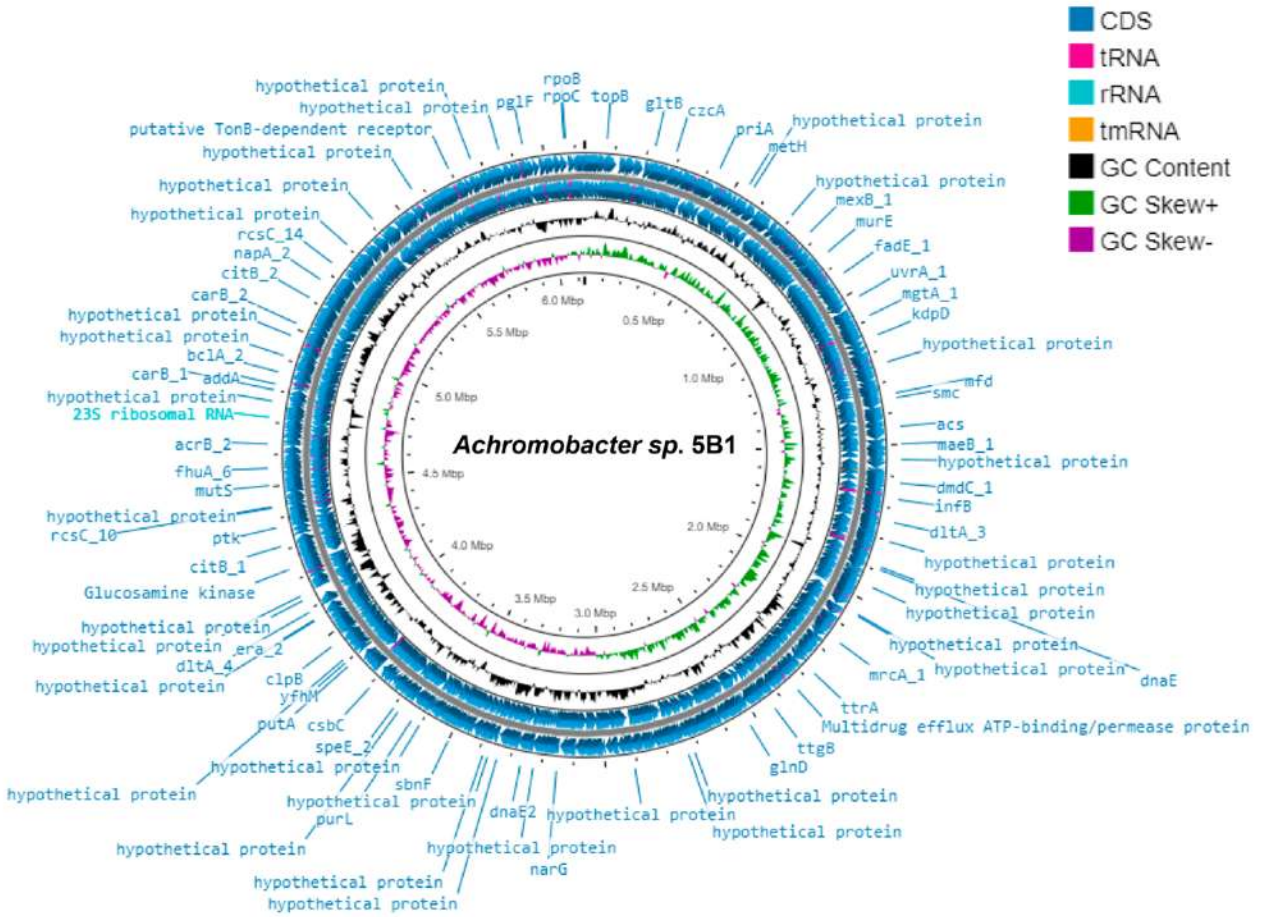


Fig. 8. Circular view of the genome of *Achromobacter* sp. strain 5B1. The contents of the rings (starting with the outermost ring) depict the CDS and RNAs from the forward and reverse strands, respectively, followed by content and skew of GC. The figure was created using Proksee Server (https://proksee.ca/).

biomass production and the activity of shoot and root meristems may help support plant growth in about 25% of global land used by farmers, mainly in arid and semiarid regions (Rengel, 2015).

The current study focused on the analysis of the *Arabidopsis* response to alkaline stress using an agar-plate system with MS 0.2x nutrient medium at pH values of 7.0, 7.5, 8.0, 8.5, and 9.0. The effects of alkaline stress on the seedlings was the reduction of growth and concomitantly, both shoot and root biomass strongly decreased at high pH. This could be correlated with the yellowing of leaves probably caused by iron deficiency and the lack of chlorophyll. We could corroborate the dose-dependent reduction in leaf chlorophyll content as a response to the rise of pH, which also reduced leaf primordia development, growth and seedling vigor. These effects of alkaline stress have been documented in many crop species, particularly in cereals and citrus plants and is thought to be caused by the formation of insoluble ferric hydroxide (Li et al., 2021).

Plants may adapt to alkaline/calcareous soils by several strategies, including lowering of the pH via exudation of organic acids such as citrate, malate, and oxalate, or protons, or through the metabolic effects of microbial communities (López-Bucio et al., 2000). In a recent study, the microbial populations associated with plants grown in neutral and strongly alkaline soil from a sand dune complex was characterized via 16S rRNA amplicon sequencing (Lopes et al., 2021). The most noticeable effect of plants on their belowground microbiomes was in alkaline soils, suggesting that host physiological factors shape the microbial population to resist alkaline stress. Indeed, fungal plant symbionts of the *Trichoderma* genus manifest strong soil acidification capacity via proton extrusion (Pelagio-Flores et al., 2017), which may be part of the adaptive behavior that improves plant survival.

Although some recent advances have been made toward deciphering the molecular targets of alkaline stress in plants (X. Li et al., 2017; J. Li et al., 2017; Liu et al., 2022; Xu et al., 2012), to the best of our knowledge, no previous studies had been conducted to analyze the influence of alkaline stress in critical targets for root architecture configuration such as lateral root initiation. The use of semi-transparent, agar-solidified medium to adjust the pH enabled detailed characterization of the growth and root branching capacity of *Arabidopsis* seedlings under a wide variety of pH values ranging from 7.0 to 9.0. Our data clearly showed the reduction of root growth and formation of lateral root primordia, which indicates that alkalinity may block the overall capacity of plants to take up nutrients and water through an impaired root foraging. Root dysfunction may also explain why the shoot system grows slowly and does not produce fully developed leaves at pH 9.0. The importance of the roots in the search of a new "green revolution" has been noted (Den Herder et al., 2010), because an adequate root branching may optimize fertilizer usage leading to an increase in shoot biomass and seed yield, something urgently needed not only in problem soils but also in fertile soils.

An assessment of the gene expression patterns of *CycB1:GUS*, *TOR:GUS*, *DR5:GUS* and *ARR5:GUS* in shoots and roots, using transgenic seedlings that express these reporters, indicated major changes in their corresponding expression domains. CycB1 is a mitotic cyclin acting at

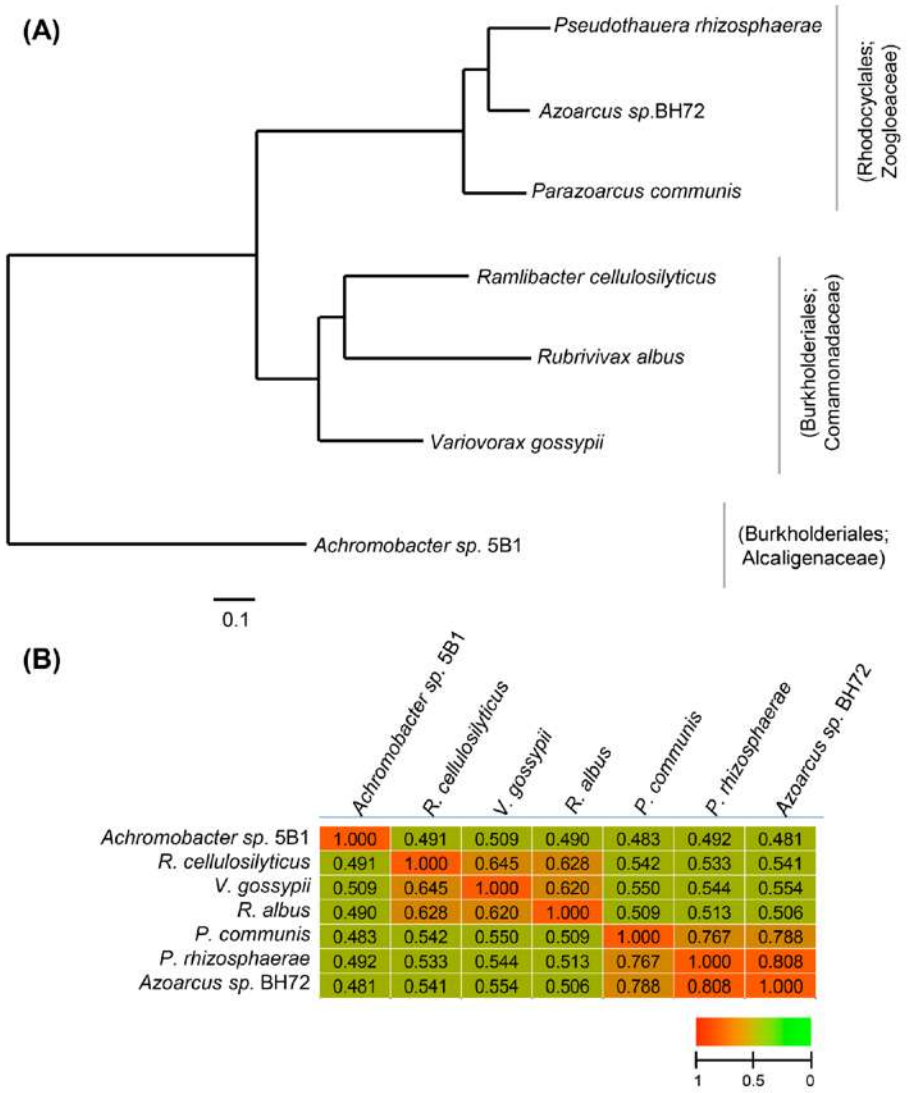


Fig. 9. Maximum likelihood phylogenetic tree of the indoleacetamide hydrolase (iaaH). (A) The tree includes the enzyme annotated/identified into the *Achromobacter* sp. 5B1 genome and some homologs from the betaproteobacteria class, which were downloaded from UniProt database. Numbers on branches indicate the percentage of 1000 bootstrap replicates that support the adjacent node. The scale bar corresponds to 0.1 estimated amino acid substitutions per site. In parenthesis (next to the names of the species), the order and family of compared species are shown. Besides, **(B)** a percentage identity matrix of the protein alignments is shown.

the G2-M transition of the cell cycle being expressed specifically in root meristems, whereas Target of Rapamycin (TOR) is a kinase responsive to sugars and auxins required to drive mitosis in root and shoot meristems (X. Li et al., 2017; J. Li et al., 2017). The fact that both CycB1 and TOR expression disappeared in *Arabidopsis* primary roots grown in medium with pH 9.0 indicated that major targets of alkaline stress are the progression of the cell cycle and TOR-dependent signaling at meristems. The reduction of expression of auxin (DR5) and cytokinin (ARR5) transgenes further indicates that plants under alkaline stress are unable to support the synthesis of these phytohormones, perhaps due to the halted biomass production, and impaired leaf development and photosynthesis. Additional factors may account for such growth repression, perhaps phosphate or iron availability may be critical to sustain growth under alkaline stress.

4.2. Achromobacter sp. 5B1 increases plant resilience to survive in alkaline medium

In their natural environment plants are not alone, but colonized by a myriad of bacterial, protozoa and fungal species that require carbon resources exuded by the roots as nutritional cues (Lugtenberg and Kamilova, 2009). These microbes may contribute to plant resilience under adverse growth conditions such as alkalinity by releasing a number of plant growth promoting compounds (Ravelo Ortega et al., 2023), but no information is currently available into the influence of specific isolates on plants at high pH. Here, we selected *Achromobacter* sp. 5B1 as a promising bacterium to help plants to resist alkaline stress, since it was formerly isolated from the rhizosphere of a mesquite tree growing around a salty pool in the Chihuahua desert (Jiménez-Vázquez et al., 2020). In our previous report, we could demonstrate that the interactions of *Arabidopsis* roots with a streak of *Achromobacter* sp. 5B1 in Petri plates, dynamically changed the root growth direction, and promoted lateral root formation through an auxinic mechanism

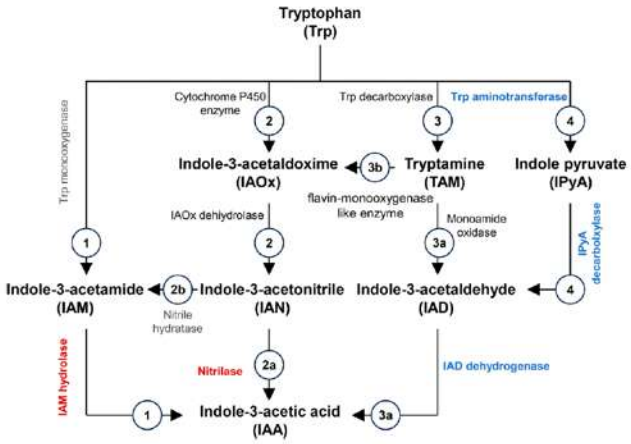


Fig. 10. Indole-3-acetic acid (IAA) biosynthesis pathways in bacteria whose enzymes are reported and described in MetaCyc database (https://metacyc.org/). IAA can be synthesized through indole-3-acetamide (IAM) (1), the indole-3-acetaldoxime/indole-3-acetonitrile (IAOx-IAN) (2, 2a and 2b), the tryptamine (TAM) (3a and 3b), and the indole-3-pyruvic acid (IPyA) pathway (4). Enzymes highlighted in colors are candidate proteins encoded in *Acrhomobacter* sp. 5b1 genome that were grouped in the same orthogroups with functional-characterized enzymes used as a reference and belonging to bacteria from beta- or gamma proteobacteria class. Red represents enzymes with at least 35% identity regarding their counterparts. In blue are those enzymes with 23–25% of identity.

(Jiménez-Vázquez et al., 2020). The findings that this bacterium rescues *Arabidopsis* seedlings from alkaline stress and improves total plant biomass, shoot biomass and root biomass not only at neutral pH (7.0), but also at high pH (9.0) are very promising. Noteworthy, the bacterium improved the growth of auxin related mutant *eir1*, which encodes the auxin-efflux permease PIN2, as well as the auxin intake transporter AUX1. The analysis of expression of the auxin inducible reporter gene *DR5:GUS* in root tips of WT, *eir1–1*, and *aux1–7* mutant roots clearly showed an increase in the auxin response of all these plant lines upon bacterial cocultivation, which safeguards the meristem under alkaline pH and certainly account for the recovery of root growth. It would be of interest to test the performance of plants to alkaline pH in the presence of other probiotic microorganisms that release volatiles and secondary metabolites that reduce oxidative stress, enhance root branching and promote growth of plants via auxin and TOR signaling such as *Azospirillum* (Méndez-Gómez et al., 2020; Mora et al., 2023).

The fact that alkaline medium normalizes root gravitropism in *eir1–1* and *aux1–7* that manifest agravitropic root behavior at pH 7.0 indicates that environmental cues may contribute to auxin transport independently of the canonical auxin transporters PIN2/EIR1 and AUX1. In this regard, a subset of ATP-BINDING CASSETTE subfamily B (ABCB) transporters has been identified in *Arabidopsis*, which play an important role in auxin export and re-uptake at the plasma membrane. Loss of ABCB21 reduces auxin transport in roots and delays lateral root emergence (Jenness et al., 2019), perhaps any/few ABCB transporters could be acting as mediators of plant resistance to alkaline conditions, but this possibility merits further research.

4.3. Achromobacter sp. 5B1 genome highlights potential mechanisms to assist plants to resist alkaline stress

Several bacterial traits may account to plant resistance to alkaline stress. Highlights from the *Achromobacter* sp. 5B1 genome enabled correlation of plant growth with three major bacterial procesess, namely auxin biosynthesis, quorum-sensing modulation and stress tolerance. Bacteria synthesize indole-3-acetic auxin as a major auxin via tryptophan (Trp)-dependent and/or -independent pathways (Costacurta and Vanderleyden, 1995). In the Trp-independent pathways, indole is most likely used as an IAA precursor while the Trp-dependent pathways involve different biosynthetic routes that may be interconnected and are named according to the primary intermediate: indole-3-pyruvic acid (IPyA), indole-3-acetamide (IAM), tryptamine (TAM), and indole-3-acetaldoxime/indole-3-acetonitrile (IAOx–IAN). However, genes and enzymes involved in the IAA biosynthesis steps are not entirely resolved, and this is important considering the many bacterial species that influence root development through auxinic mechanisms (Tzipilevich et al., 2021; García Cárdenas et al., 2022; Xu et al., 2023). In bacteria, a few enzymes have been characterized mainly in some alpha and gammaproteobacteria. The analysis of the *Achromobacter* sp. 5B1 genome led to the identification of genes that seem to be orthologs to those previously characterized and which encode enzymes involved in the IAA biosynthesis (e.g. indoleacetamide hydrolase and nitrilase), suggesting that this strain can synthesize IAA at least by the IAM and IAOx–IAN routes.

Treatment of *Arabidopsis* seedlings with AHLs, the major QS signals from Gram negative bacteria configure root architecture via primary root growth and lateral root formation (Ortiz-Castro et al., 2008). In the *Achromobacter* sp. 5B1 genome, several genes encoding proteins potentially involved in AHL synthesis, transport and degradation could be identified. Noteworthy, six genes putatively encoded homoserine lactone efflux proteins and four genes encoded acylamidase enzymes, indicating the importance of both AHL transport and degradation not only for bacteria-bacteria communication, but also crooskindom recognition with their eukaryotic hosts. AHLs belong into the major class of quorum sensing signals in Gram negative species, comprising of an acyl chain of variable length and an homoserine lactone group. Cellular release of AHLs happens at high population density and help bacteria to synchronize behavioral traits such as virulence or symbiosis. The fact the *Achromobacter* sp. 5B1 harbors genes for both lactonases and acylases, shows its wide repertoire of enzymes for quorum-quenching, thus modulating bacterial interactions. Treatment with AHLs decreased protease activity in *Serratia* and *Aeromonas* species, and differentially modulated biofilm formation in these bacterial genera. In *Shewanella*, AHLs decreased the abundance of proteins involving in growth and metabolism such as citrate synthase, succinate semialdehyde dehydrogenase, and environment adaptation such as polysaccharide deacetylases and transaldolase, while inducing the abundance of stress response proteins and DNA ligase (Zhang et al., 2016, 2017). Moreover, since plants possess bioactive metabolites that mimic AHLs such as alkamides and *N*-acyl-ethanolamines (Ramírez-Chávez et al., 2004; Blancaflor et al., 2014), the bacterial acylases may be part of a mechanism for bacteria-plant transkingdom signaling important for root organogenesis.

Based on the potential of some protein-coding genes such as ACC deaminase, *Achromobacter* sp. 5B1 can cope with extreme environmental conditions (i.e., high salinity or alkaline pH). In alkaline (basic) pH conditions, this enzyme may be helpful to decrease the growth repressing effects of the stress hormone ethylene and to resume growth of plants (Santoyo et al., 2016; Shahid et al., 2023). This strain probably adjusts its surrounding medium via secreting protons and/or organic acids, decreasing in this manner the pH of the rhizosphere and making it favorable for plant growth. IAA secretion itself may help reducing the pH to less suboptimal levels and at the same time boost root branching that contributes to more efficient soil exploration and nutrient mining, particularly phosphate and iron, which are strongly limiting as the soil pH rises.

Ethical approval

Not applicable.

Funding

This work was supported by Secretaría de Educación Pública (SEP)-

Consejo Nacional de Ciencia y Tecnología (CONACYT) grant number A1-S-34768.

CRediT authorship contribution statement

López-Bucio Jesús Salvador: Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **García-Cárdenas Elizabeth:** Writing – review & editing, Methodology, Investigation. **Pelagio-Flores Ramón:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Jiménez-Vázquez Kirán Rubí :** Writing – original draft, Methodology, Investigation, Conceptualization. **López-Hernández José :** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **López-Bucio José :** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Canedo-Texón Anahí :** Writing – review & editing, Software, Methodology, Investigation. **Ibarra-Laclette Enrique:** Writing – review & editing, Software, Methodology, Investigation.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgments

We acknowledge Tom Guilfoyle, Peter Doerner, Benoit Menand, and Joseph Kieber, for sharing seed materials. This project was financially supported by SEP-CONACYT grant A1-S-34768. The authors wish to thank the Institute of Ecology A.C. (INECOL) for the access to the high-performance computing system (HUTZILIN) required to carry out this research, and we also thank Emanuel Villafán for his assistance as the administrator of this resource.

Consent to participate

All authors manifest their consent to participate as co-authors.

Consent to publish

All authors manifest their consent to publish.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2023.127594.

References

Agegnehu, G., Amede, T., Erkossa, T., Yirga, C., Henry, C., Tyler, R., Nosworthy, M.G., Beyene, S., Sileshi, G.W., 2021. Extent and management of acid soils for sustainable crop production system in the tropical agroecosystems: a review. Acta Agric. Scand. Sect. B- Soil Plant Sci. 71, 852–869. https://doi.org/10.1080/09064710.2021.1954239.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Badalamenti, J.P., Hunter, R.C., 2015. Complete genome sequence of *Achromobacter xylosoxidans* MN001, a cystic fibrosis airway isolate. Genome Ann. 3 (4).

Blancaflor, E.B., Kilaru, A., Keereetaweep, J., Khan, B.R., Faure, L., Chapman, K.D., 2014. *N*-Acylethanolamines: lipid metabolites with functions in plant growth and development. Plant J. 79, 568–583. https://doi.org/10.1111/tpj.12427.

Bosi, E., Donati, B., Galardini, M., Brunetti, S., Sagot, M.F., Lió, P., Crescenzi, P., Fani, R., Fondi, M., 2015. MeDuSa: a multi-draft-based scaffolder. Bioinformatics 31, 2443–2451. https://doi.org/10.1093/bioinformatics/btv171.

Caspi, R., Billington, R., Fulcher, C.A., Keseler, I.M., Kothari, A., Krummenacker, M., Latendresse, M., Midford, P.E., Ong, Q., Ong, W.K., et al., 2018. The MetaCyc database of metabolic pathways and enzymes. Nucleic Acids Res. 46 (D1), D633–D639. https://doi.org/10.1093/nar/gkx935.

Celiker, H., Gore, J., 2013. Cellular cooperation: insights from microbes. Trends Cell Biol. 23, 9–15.

Colón-Carmona, A., You, R., Haimovitch-Gal, T., Doerner, P., 1999. Spatio temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J. 20, 503–508. https://doi.org/10.1046/j.1365-313x.1999.00620.x.

Costacurta, A., Vanderleyden, J., 1995. Synthesis of phytohormones by plant-associated bacteria. Crit. Rev. Microbiol. 21, 1–18. https://doi.org/10.3109/10408419509113531.

D'Agostino, I.B., Deruère, J., Kieber, J.J., 2000. Characterization of the response of the *Arabidopsis* response regulator gene family to cytokinin. Plant Physiol. 124, 1706–1717. https://doi.org/10.1104/pp.124.4.1706.

Den Herder, G., Van Isterdael, G., Beeckman, T., De Smet, I., 2010. The roots of a new green revolution. Trends Plant Sci. 15, 600–607. https://doi.org/10.1016/j.tplants.2010.08.009.

Duan, X., Luo, L., Wang, Z., Wang, W., Ye, C., Jia, L., Xie, Y., Chen, J., Han, Y., Yuanda, L., QI, W., Xuan, W., 2023. Promotion of root development by slightly alkaline pH involves an auxin mediated adaption mechanism. Soil Sci. Environ. 2, 6.

Esparza-Reynoso, S., Ruíz-Herrera, L.F., Pelagio-Flores, R., Macías-Rodríguez, L.I., Martínez-Trujillo, M., López-Coria, M., Sánchez-Nieto, S., Herrera-Estrella, A., López-Bucio, J., 2021. *Trichoderma atroviride*-emitted volatiles improve growth of *Arabidopsis* seedlings through modulation of sucrose transport and metabolism. Plant Cell Environ. 44, 1961–1976. https://doi.org/10.1111/pce.14014.

García-Cárdenas, E., Ortiz-Castro, R., Ruiz-Herrera, L.F., Valencia-Cantero, E., López-Bucio, J., 2022. *Micrococcus luteus* LS570 promotes root branching in Arabidopsis via decreasing apical dominance of the primary root and an enhanced auxin response. Protoplasma 259, 1139–1155. https://doi.org/10.1007/s00709-021-01724-z.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., Citterio, S., 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of Ambrosia artemisiifolia L. Front. Plant Sci. 9, 1335 https://doi.org/10.3389/fpls.2018.01335.

Grant, J.R., Enns, E., Marinier, E., Mandal, A., Herman, E.K., Chen, C.Y., Graham, M., Van Domselaar, G., Stothard, P., 2023. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res. 51 (W1), W484–W492. https://doi.org/10.1093/nar/gkad326.

Hayes, P.E., Pereira, C., Clode, P.L., Lambers, H., 2019. Calcium-enhanced phosphorus toxicity in calcifuge and soil-indifferent Proteaceae along the Jurien Bay chronosequence. N. Phytol. 221, 764–777. https://doi.org/10.1111/nph.15447.

Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248, 43–59. https://doi.org/10.1023/A:1022371130939.

Huerta-Venegas, P.I., Raya-González, J., López-García, C.M., Barrera-Ortiz, S., Ruiz-Herrera, L.F., López-Bucio, J., 2022. Mutation of MEDIATOR16 promotes plant biomass accumulation and root growth by modulating auxin signaling. Plant Sci. 314, 111117 https://doi.org/10.1016/j.plantsci.2021.111117.

Jakobsen, T.H., Hansen, M.A., Jensen, P.O., Hansen, L., Riber, L., Cockburn, A., Kolpen, M., Ronne Hansen, C., Ridderberg, W., Eickhardt, S., et al., 2013. Complete genome sequence of the cystic fibrosis pathogen *Achromobacter xylosoxidans* NH44784-1996 complies with important pathogenic phenotypes. PLOS One 8, e68484.

Jenness, M.K., Carraro, N., Pritchard, C.C., Murphy, A.S., 2019. The Arabidopsis ATP-BINDING CASSETTE transporter ABCB21 regulates auxin levels in cotyledons, the root pericycle, and leaves. Front. Plant Sci. 10, 806 https://doi.org/10.3389/fpls.2019.00806.

Jeukens, J., Freschi, L., Kukavica-Ibrulj, I., Nguyen, D., Levesque, R.C., 2015. Draft genome sequence of triclosan-resistant cystic fibrosis isolate *Achromobacter xylosoxidans* CF304. Genome Ann. 3 (4), e00865-15 https://doi.org/10.1128/genomeA.00865-15.

Jiménez-Vázquez, K.R., García-Cárdenas, E., Barrera-Ortiz, S., Ortiz-Castro, R., Ruiz-Herrera, L.F., Ramos-Acosta, B.P., Coria-Arellano, J.L., Sáenz-Mata, J., López-Bucio, J., 2020. The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. Plant J. 103, 1639–1654. https://doi.org/10.1111/tpj.14853.

Kandhol, N., Pandey, S., Singh, V.P., Herrera-Estrella, L., López-Bucio, J., Tran, L.P., Tripathi, D.K., 2023. Bacterial community and root endodermis: a complementary relationship. Trends Plant Sci. 28, 749–751. https://doi.org/10.1016/j.tplants.2023.03.021.

Klee, H., Montoya, A., Horodyski, F., Lichtenstein, C., Garfinkel, D., Fuller, S., Flores, C., Peschon, J., Nester, E., Gordon, M., 1984. Nucleotide sequence of the tms genes of the pTiA6NC octopine Ti plasmid: two gene products involved in plant tumorigenesis. Proc. Natl. Acad. Sci. USA 81, 1728–1732.

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., Meir, S., Rogachev, I., Aharoni, A., 2020. Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. Proc. Natl. Acad. Sci. USA 117, 3874–3883. https://doi.org/10.1073/pnas.1912130117.

Kotula, L., Clode, P.L., Ranathunge, K., Lambers, H., 2021. Role of roots in adaptation of soil-indifferent Proteaceae to calcareous soils in south-western Australia. J. Exp. Bot. 72, 1490–1505. https://doi.org/10.1093/jxb/eraa515.

Laliberté, E., Turner, B.L., Costes, T., Pearse, S.J., Wyrwoll, K.H., Zemunik, G., Lambers, H., 2012. Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. J. Ecol. 100, 631–642. https://doi.org/10.1111/j.1365-2745.2012.01962.x.

Lee, J.A., 1998. The calcicole-calcifuge problem revisited. In: Advances in Botanical Research, 29. Academic Press,, pp. 1–30.

Li, D., Han, S., Zhang, K., Xu, G., Zhang, H., Chen, F., Wang, L., Liu, Q., Guo, Z., Zhang, J., Li, J., 2023. Genome analysis and safety assessment of *Achromobacter*

marplatensis strain YKS2 isolated from the rumen of yaks in China. Probiot. Antimicrob. Proteins. https://doi.org/10.1007/s12602-023-10124-z.
Li, G., Yang, L., Zhang, T., Guo, X., Qin, J., Cao, Y., Yang, Q., You, S., Yuan, G., Wan, X., et al., 2018. Complete genome sequence of *Achromobacter spanius* type strain DSM 23806(T), a pathogen isolated from human blood. J. Glob. Antimicrob. Res. 14, 1–3. https://doi.org/10.1016/j.jgar.2018.05.003.
Li, J., Xu, H.H., Liu, W.C., Zhang, X.W., Lu, Y.T., 2017. Ethylene inhibits root elongation during alkaline stress through AUXIN1 and associated changes in auxin accumulation. Plant Physiol. 168, 1777–1791. https://doi.org/10.1104/pp.15.00523.
Li, J., Cao, X., Jia, X., Liu, L., Cao, H., Qin, W., Li, M., 2021. Iron deficiency leads to chlorosis through impacting chlorophyll synthesis and nitrogen metabolism in *Areca catechu* L. Front. Plant Sci. 12, 710093 https://doi.org/10.3389/fpls.2021.710093.
Li, L., Stoeckert Jr., C.J., Roos, D.S., 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. 13, 2178–2189. https://doi.org/10.1101/gr.1224503.
Li, X., Hu, Y., Gong, J., Lin, Y., Johnstone, L., Rensing, C., Wang, G., 2012. Genome sequence of the highly efficient arsenite-oxidizing bacterium *Achromobacter arsenitoxydans* SY8. J. Bacteriol. 194, 1243–1244. https://doi.org/10.1128/JB.06667-11.
Li, X., Cai, W., Liu, Y., Li, H., Fu, L., Liu, Z., Xu, L., Liu, H., Xu, T., Xiong, Y., 2017. Differential TOR activation and cell proliferation in *Arabidopsis* root and shoot apexes. Proc. Natl. Acad. Sci. USA 114, 2765–2770. https://doi.org/10.1073/pnas.1618782114.
Lichtenthaler, H.K., 1987. Chlorophyll and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.
Liu, X., Jin, Y., Tan, K., Zheng, J., Gao, T., Zhang, Z., Zhao, Y., Ma, F., Li, C., 2021. MdTyDc overexpression improves alkalinity tolerance in Malus domestica. Front. Plant Sci. 12, 625890 https://doi.org/10.3389/fpls.2021.625890.
Liu, Y., Mu, C., Du, D., Yang, Y., Li, L., Xuan, W., Kircher, S., Palme, K., Li, X., Li, R., 2022. Alkaline stress reduces root waving by regulating PIN7 vacuolar transport. Front. Plant Sci. 13, 1049144 https://doi.org/10.3389/fpls.2022.1049144.
Lopes, L.D., Hao, J., Schachtman, D.P., 2021. Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. FEMS Microbiol. Ecol. 97, fiab028 https://doi.org/10.1093/femsec/fiab028.
López-Bucio, J., Nieto-Jacobo, M.F., Ramírez-Rodríguez, V., Herrera-Estrella, L., 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Sci. 160, 1–13. https://doi.org/10.1016/S0168-9452(00)00347-2.
Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Annu Rev. Microbiol. 63, 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918.
Luschnig, C., Gaxiola, R.A., Grisafi, P., Fink, G.R., 1998. EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. Genes Dev. 12, 2175–2187 https://doi.10.1101/gad.12.14.2175.
Malamy, J.E., Benfey, P.N., 1997. Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124, 33–44. https://doi.org/10.1242/dev.124.1.33.
Menand, B., Desnos, T., Nussaume, L., Berger, F., Bouchez, D., Meyer, C., Robaglia, C., 2002. Expression and disruption of the *Arabidopsis* TOR (*target of rapamycin*) gene. Proc. Natl. Acad. Sci. USA 99, 6422–6427. https://doi.org/10.1073/pnas.09214189.
Méndez-Gómez, M., Castro-Mercado, E., Peña-Uribe, C.A., Reyes-de la Cruz, H., López-Bucio, J., García-Pineda, E., 2020. TARGET OF RAPAMYCIN signaling plays a role in Arabidopsis growth promotion by *Azospirillum brasilense* Sp245. Plant Sci. 293, 110416 https://doi.org/10.1016/j.plantsci.2020.110416.
Mora, V., López, G., Molina, R., Coniglio, A., Nievas, F., De Diego, N., Ćavar Zeljković, S., Salcedo Sarmiento, S., Spíchal, L., Robertson, S., Wilkins, O., Elías, J., Pedraza, R., Estevez, J.M., Belmonte, M.F., Cassán, F., 2023. *Azospirillum argentinense* modifies Arabidopsis root architecture through auxin-dependent pathway and flagellin. J. Soil Sci. Plant Nutr. 23, 4543–4557. https://doi.org/10.1007/s42729-023-01371-8.
Ortiz-Castro, R., López-Bucio, J., 2019. Review: phytostimulation and root architectural responses to quorum-sensing signals and related molecules from rhizobacteria. Plant Sci. 284, 135–142. https://doi.org/10.1016/j.plantsci.2019.04.010.
Ortiz-Castro, R., Martínez-Trujillo, M., López-Bucio, J., 2008. *N*-acyl-*L*-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ. 31, 1497–1509. https://doi.org/10.1111/j.1365-3040.2008.01863.x.
Otto, G., 2021. The root of nutrient stress resistance. Nat. Rev. Microbiol. 19, 139. https://doi.org/10.1038/s41579-021-00521-y.
Pelagio-Flores, R., Esparza-Reynoso, S., Garnica-Vergara, A., López-Bucio, J., Herrera-Estrella, A., 2017. *Trichoderma*-induced acidification is an early trigger for changes in Arabidopsis root growth and determines fungal phytostimulation. Front. Plant Sci. 17, 822 https://doi.org/10.3389/fpls.2017.00822.
Pereira, C., Hayes, P.E., Clode, P.L., Lambers, H., 2021. Phosphorus toxicity, not deficiency, explains the calcifuge habit of phosphorus-efficient Proteaceae. Physiol. Plant. 172, 1724–1738. https://doi.org/10.1111/ppl.13384.
Ramírez-Chávez, E., López-Bucio, J., Herrera-Estrella, L., Molina-Torres, J., 2004. Alkamides isolated from plants promote growth and alter root development in Arabidopsis. Plant Physiol. 134, 1058–1068. https://doi.org/10.1104/pp.103.034553.
Ravelo-Ortega, G., Raya-González, J., López-Bucio, J., 2023. Compounds from rhizosphere microbes that promote plant growth. Curr. Opin. Plant Biol. 73, 102336 https://doi.org/10.1016/j.pbi.2023.102336.
Reis, A.C., Kroll, K., Gomila, M., 2017. Complete genome sequence of *Achromobacter denitrificans* PR1. Genome Announc 5, e00762-17. https://doi.org/10.1128/genomeA.00762-17.
Rengel, Z., 2015. Availability of Mn, Zn and Fe in the rhizosphere. J. Soil Sci. Plant Nutr. 15, 397–409.
Rodrigues, E.R.A., Rocha, G.A., Ferreira, A.G., Leão, R.S., Albano, R.M., Marques, E.A., 2016. Draft genome sequences of four *Achromobacter ruhlandii* strains isolated from cystic fibrosis patients. Mem. do Inst. Oswaldo Cruz 111, 777–780.
Salvi, E., Rutten, J.P., Di Mambro, R., Polverari, L., Licursi, V., Negri, R., Dello Ioio, R., Sabatini, S., Ten Tusscher, K.A., 2020. Self-organized PLT/auxin/ARR-B network controls the dynamics of root zonation development in *Arabidopsis thaliana*. Dev. Cell 53, 431–443. https://doi.org/10.1016/j.devcel.2020.04.004.
Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda, M.C., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol. Res. 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008.
Satyanarayana, T., Raghukumar, C., Shivaji, S., 2005. Extremophilic microbes: diversity and perspectives. Curr. Sci. 89, 78–90.
Schröder, G., Waffenschmidt, S., Weiler, E.W., Schröder, J., 1984. The T-region of Ti plasmids codes for an enzyme synthesizing indole-3-acetic acid. Eur. J. Biochem. 138, 387–391.
Sciaky, D., Thomashow, M.F., 1984. The sequence of the tms transcript 2 locus of the *A. tumefaciens* plasmid pTiA6 and characterization of the mutation in pTiA66 that is responsible for auxin attenuation. Nucleic Acids Res. 12, 1447–1461.
Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
Shahid, M., Shah, A.A., Basit, F., Noman, M., Zubair, M., Ahmed, T., Naqqash, T., Manzoor, I., Maqsood, A., 2020. *Achromobacter* sp. FB-14 harboring ACC deaminase activity augmented rice growth by upregulating the expression of stress-responsive CIPK genes under salinity stress. Braz. J. Microbiol. 51, 719–728.
Shahid, M., Singh, U.B., Khan, M.S., Singh, P., Kumar, R., Singh, R.N., Kumar, A., Singh, H.V., 2023. Bacterial ACC deaminase: insights into enzymology, biochemistry, genetics, and potential role in amelioration of environmental stress in crop plants. Front. Microbiol. 14, 1132770 https://doi.org/10.3389/fmicb.2023.1132770.
Strnad, H., Ridl, J., Paces, J., Kolar, M., Vlcek, C., Paces, V., 2011. Complete genome sequence of the haloaromatic acid-degrading bacterium *Achromobacter xylosoxidans* A8. J. Bacteriol. 193, 791–792.
Swarup, R.J., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, S., Millner, P., Tsurumi, S., Moore, I., Napier, R., Kerr, I.D., Bennett, M.J., 2004. Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. Plant Cell 16, 3069–3083. https://doi.org/10.1105/tpc.104.024737.
Trimble, W.L., Phung le, T., Meyer, F., Silver, S., Gilbert, J.A., 2012. Draft genome sequence of *Achromobacter piechaudii* strain HLE. J. Bacteriol. 194, 6355.
Tzipilevich, E., Russ, D., Dangl, J.L., Benfey, P.N., 2021. Plant immune system activation is necessary for efficient root colonization by auxin-secreting beneficial bacteria. Cell Host Microbe 29, 1507–1520.e4. https://doi.org/10.1016/j.chom.2021.09.005.
Ulmasov, T., Murfett, J., Hagen, G., Guilfoyle, T.J., 1997. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9, 1963–1971. https://doi.org/10.1105/tpc.9.11.1963.
Veschetti, L., Sandri, A., Patuzzo, C., Melotti, P., Malerba, G., Lleo, M.M., 2021. Genomic characterization of *Achromobacter* species isolates from chronic and occasional lung infection in cystic fibrosis patients. Micro Genom. 7, 000606 https://doi.org/10.1099/mgen.0.000606.
Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K., et al., 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLOS One 9, e112963. https://doi.org/10.1371/journal.pone.0112963.
Wang, S.J., Chen, Q., Li, Y., Zhuo, Y.Q., Xu, L.Z., 2017. Research on saline-alkali soil amelioration with FGD gypsum. Resour. Conserv. Recycl. 121, 82–92. https://doi.org/10.1016/j.resconrec.2016.04.005.
Xu, F., Liao, H., Yang, J., Zhang, Y., Yu, P., Cao, Y., Fang, J., Chen, S., Li, L., Sun, L., Du, C., Wang, K., Dang, X., Feng, Z., Cao, Y., Li, Y., Zhang, J., Xu, W., 2023. Auxin-producing bacteria promote barley rhizosheath formation. Nat. Commun. 14, 5800 https://doi.org/10.1038/s41467-023-40916-4.
Xu, W., Jia, L., Baluška, F., Ding, G., Shi, W., Ye, N., Zhang, J., 2012. PIN2 is required for the adaptation of Arabidopsis roots to alkaline stress by modulating proton secretion. J. Exp. Bot. 63, 6105–6114. https://doi.org/10.1093/jxb/ers259.
Yokawa, K., Baluška, F., 2018. Sense of space: Tactile sense for exploratory behavior of roots. Commun. Integr. Biol. 11, 1–5. https://doi.org/10.1080/19420889.2018.1440881.
Zhang, C., Zhu, S., Jatt, A.N., Zeng, M., 2016. Characterization of *N*-acyl homoserine lactones (AHLs) producing bacteria isolated from vacuum-packaged refrigerated turbot (*Scophthalmus maximus*) and possible influence of exogenous AHLs on bacterial phenotype. J. Gen. Appl. Microbiol. 62, 60–67. https://doi.org/10.2323/jgam.62.60.
Zhang, C., Zhu, S., Jatt, A.N., Pan, Y., Zeng, M., 2017. Proteomic assessment of the role of *N*-acyl homoserine lactone in *Shewanella putrefaciens* spoilage. Lett. Appl. Microbiol. 65, 388–394. https://doi.org/10.1111/lam.12795.
Zhou, C., Guo, J., Zhu, L., Xiao, X., Xie, Y., Zhu, J., Ma, Z., Wang, J., 2016. *Paenibacillus polymyxa* BFKC01 enhances plant iron absorption via improved root systems and activated iron acquisition mechanisms. Plant Physiol. Biochem. 105, 162–173. https://doi.org/10.1016/j.plaphy.2016.04.025.

8.3. Capítulo III

Planta (2023) 258:80
https://doi.org/10.1007/s00425-023-04236-7

ORIGINAL ARTICLE

Pseudomonas aeruginosa LasI-dependent plant growth promotion requires the host nitrate transceptor AtNRT1.1/CHL1 and the nitrate reductases NIA1 and NIA2

José López-Bucio[1] · Randy Ortiz-Castro[2] · Viridiana Magaña-Dueñas[1] · Elizabeth García-Cárdenas[1] · Kirán Rubí Jiménez-Vázquez[1] · Javier Raya-González[3] · Ramón Pelagio-Flores[3] · Enrique Ibarra-Laclette[2] · Luis Herrera-Estrella[4]

Received: 18 January 2023 / Accepted: 4 September 2023

Abstract

Main conclusion **In *P. aeruginosa*, mutation of the gene encoding *N*-acyl-*L*-homoserine lactone synthase LasI drives defense and plant growth promotion, and this latter trait requires adequate nitrate nutrition.**

Abstract Cross-kingdom communication with bacteria is crucial for plant growth and productivity. Here, we show a strong induction of genes for nitrate uptake and assimilation in *Arabidopsis* seedlings co-cultivated with *P. aeruginosa* WT (PAO1) or *ΔlasI* mutants defective on the synthesis of the quorum-sensing signaling molecule *N*-(3-oxododecanoyl)-*L*-homoserine lactone. Along with differential induction of defense-related genes, the change from plant growth repression to growth promotion upon bacterial QS disruption, correlated with upregulation of the dual-affinity nitrate transceptor CHL1/AtNRT1/NPF6.3 and the nitrate reductases NIA1 and NIA2. *CHL1-GUS* was induced in *Arabidopsis* primary root tips after transfer onto *P. aeruginosa ΔlasI* streaks at low and high N availability, whereas this bacterium required high concentrations of nitrogen to potentiate root and shoot biomass production and to improve root branching. *Arabidopsis chl1-5* and *chl1-12* mutants and double mutants in NIA1 and NIA2 nitrate reductases showed compromised growth under low nitrogen availability and failed to mount an effective growth promotion and root branching response even at high NH_4NO_3. WT *P. aeruginosa* PAO1 and *P. aeruginosa ΔlasI* mutant promoted the accumulation of nitric oxide (NO) in roots of both the WT and *nia1nia2* double mutants, whereas NO donors SNP or SNAP did not improve growth or root branching in *nia1nia2* double mutants with or without bacterial cocultivation. Thus, inoculation of *Arabidopsis* roots with *P. aeruginosa* drives gene expression for improved nitrogen acquisition and this macronutrient is critical for the plant growth-promoting effects upon disruption of the *LasI* quorum-sensing system.

Keywords Quorum-sensing · Root development · Gene expression · Nitrate · Ammonium · Nitric oxide

Communicated by Anastasios Melis.

✉ José López-Bucio
jbucio@umich.mx

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, 58030 Morelia, Michoacán, Mexico

[2] Red de estudios moleculares avanzados, Instituto de Ecología A. C., Carretera Antigua a Coatepec 351, El Haya, 91070 Xalapa, Veracruz, Mexico

[3] Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Tzintzunzan 173, Col. Matamoros, 58240 Morelia, Michoacán, Mexico

[4] Centro de Investigación y de Estudios Avanzados del IPN, Unidad de Genómica Avanzada, Laboratorio Nacional de Genómica para la Biodiversidad, Campus Irapuato, Km. 9.6 Libramiento Norte Carretera Irapuato-León, 36821 Irapuato, Guanajuato, Mexico

Published online: 16 September 2023

Introduction

The microbial population (microbiome) residing in the rhizosphere includes ecologically diverse bacteria, which may establish symbiotic or pathogenic relationships with their plant hosts. Bacteria rely on root exudates for nutrition and can remodel plant root architecture via the production of phytohormones such as auxins (Jiménez-Vázquez et al. 2020; García-Cárdenas et al. 2021), releasing *N*-acyl-*L*-homoserine lactones (Ortiz-Castro et al. 2008; 2020) and/or cyclodipeptides (González-López et al. 2021). Some bacteria may protect their host plants against pathogens and improve abiotic stress tolerance (Zamioudis et al. 2013; Carlson et al. 2020; Nishad et al. 2020), but the genetic and molecular mechanisms orchestrating these responses remain mostly unknown.

Pseudomonas aeruginosa PAO1, is a facultative plant pathogen. The virulence of PAO1 is controlled by the *N*-acyl-*L*-homoserine lactone (acyl-HSL)-dependent quorum-sensing (QS) systems, in which the enzymes LasI and RhlI play a major role by synthesizing *N*-3-oxo-dodecanoyl-*L*-homoserine lactone (3-oxo-C12-HSL) and *N*-butanoyl-*L*-homoserine lactone (C4-HSL), respectively (Subramoni et al. 2021). These metabolites interact with the transcription factors LasR or RhlR to regulate the expression of downstream target genes encoding virulence factors, siderophore biosynthesis, and those related to the establishment of multispecies biofilm communities (Miller and Bassler 2001; Papenfort and Bassler 2016; Subramoni et al. 2021).

A single variant of *Pseudomonas aeruginosa* PAO1, caused by mutation of the gene encoding 3-oxo-C12-HSL synthase (*ΔlasI*) impaired in 3-oxo-C12-HSL biosynthesis did not cause disease and instead improved growth and root branching of *Arabidopsis thaliana* and tomato in vitro and in an artificial substrate under greenhouse conditions (Ortiz-Castro et al. 2011, 2017). Besides the quorum-sensing-deficient phenotype of *P. aeruginosa ΔlasI,* this mutant has an increased production of cyclodipeptides (CDPs), which activate the canonical auxin-response pathway via the receptors TIR1, AFB2, and AFB3 and transcriptional regulators ARF7 and ARF19 (Ortiz-Castro et al. 2011, 2014; Díaz-Pérez et al. 2022). These findings indicate that CDPs biosynthesis changes the bacterial cell behavior and at the same time activates hormone signaling in roots, but how the plant nutritional status modifies the plant-bacteria interaction is still unclear.

Mineral nutrition mediates signaling between bacteria and plants (Castrillo et al. 2017; López-Hernández et al. 2022). Nitrate (NO_3^-) is an essential nutrient for plants to complete their life cycle and to boost productivity in crops, particularly cereals. Although N levels in the atmosphere and biological N-fixation warrant reliable sources of NO_3^-, specific molecular mechanisms must be activated to perceive the local and systemic N status in roots and shoots (Kant 2018; Wang et al. 2020). Plants improve nitrate nutrition and access organic or inorganic pools through symbiosis with soil fungi that degrade decaying materials, and through root architectural configurations that enhance nitrate import from the soil (Wang et al. 2012; Feng et al. 2020; López-Bucio et al. 2022; Ye et al. 2022).

Nitrate enters into root epidermal cells through NPF, NRT2, CLC, and SLAC/SLAH protein transporters (Li et al. 2007a, b; O'Brien et al. 2016; Hachiya and Sakakibara 2017; Sakuraba et al. 2021). One of their best-characterized nitrate transporters in *Arabidopsis* is AtCHL1 (AtNRT1.1/NPF6.3), which takes nitrate from soil and acts as a sensor of N availability (Wang et al. 1998; Liu et al. 1999; Ho et al. 2009; Bouguyon et al. 2015). Moreover, AtCHL1 can also be an auxin transporter, playing in this manner its multilevel roles in nutrition, metabolism, growth, and development (Bouguyon et al. 2015; Wang et al. 2020). Prior to its conversion into amino acids, nitrate is reduced by the subsequent activity of the enzymes nitrate reductase and nitrite reductase, respectively, to produce ammonium. In *Arabidopsis*, the NIA1 and NIA2 genes encode nitrate reductases, which play redundant functions in nitrate to nitrite reduction (Li et al. 2007a, b; Park et al. 2011). This conversion releases nitric oxide (NO), a diffusible, reactive gas that acts as a reactive nitrogen species and as a cellular messenger important for the regulation of root growth and branching (Méndez-Bravo et al. 2010; Fernandez-Marcos et al. 2011; Dolch et al. 2017; Oláh et al. 2020). Although nitrate sensing, transport, and reduction are critical for plant productivity, the impact of root-associated bacteria in these processes has been scarcely investigated.

In this report, detailed experimentation was performed to analyze the molecular mechanisms underlying the responses of *Arabidopsis* seedlings to the root interaction with *P. aeruginosa* PAO1 and its derived growth-promoting, quorum-sensing mutant *ΔlasI*. Global changes in gene expression of *Arabidopsis* seedlings during the early stages of root colonization with *P. aeruginosa* (PAO1) and *ΔlasI* mutant identified genes for both defense and nutrition clearly induced by these bacteria, and we took advantage of pharmacological and genetic analyses to identify nitrate-regulated genes directly involved in the plant growth promoting traits. Increased plant biomass accumulation and root branching occurred as major responses to the *ΔlasI* mutant, required high amounts of nitrogen, and were disrupted in *chl1-5* and *chl12* single mutants, and *nia1nia2* double mutants. Root bacterized plants accumulated high levels of nitric oxide, indicating its possible role in root architecture configuration, which is consistent with the fact that NO donors SNP and SNAP could not promote growth or branching in *nia1nia2*

double mutants. These findings have implications for understanding the principles of cross-kingdom plant-bacteria communication, plant growth, adaptation to the environment, and productivity.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana ecotype Col-0, the transgenic lines *CHL1-GUS* (AT1G12110) (Guo et al. 2002), *JAZ1/TIFY10A-GUS-GFP* (AT1G19180) (Grunewald et al. 2009), *pLOX2:GUS* (AT3G45140) (Schommer et al. 2008) and *pPR1a:GUS* (AT2G14610) (Contreras-Cornejo et al. 2011), the mutants *chl1-5*, *chl1-12* (Tsay et al. 1993), *nia1*, *nia2* and *nia1nia2* (Méndez-Bravo et al. 2010) were used. Seeds were surface disinfected with 95% (v/v) ethanol for 5 min and 20% (v/v) bleach for 7 min. After five washes with sterile distilled water, seeds were germinated and grown on agar plates containing 0.2 × Murashige and Skoog medium (MS basal salts mixture, M5524; Sigma). The suggested formulation is 4.3 g L^{-1} of salts for 1 × medium; we used 0.9 g L^{-1}, which we consider and refer to as 0.2 × MS and lacks amino acids and vitamins. Phytagar (micropropagation grade) was purchased from Phytotechnology. Plants were placed in a plant growth chamber (Percival Scientific AR-95L) with a photoperiod of 16 h of light, 8 h of darkness, a light intensity of 100 µmol m^2 s^{-1}, and a temperature of 22 °C.

GUS histochemical analysis

Transgenic plants expressing *CHL1-GUS* (AT1G12110) from the different treatments were incubated in a reaction buffer containing 0.5 mg mL 5-bromo-4-chloro-3-indolyl-β-d-glucuronide in 100 mM sodium phosphate, pH 7, for 6 h at 37 °C covering the reaction with aluminum foil. The seedlings were processed according to Malamy and Benfey (1997) to eliminate carbohydrates and pigments and then included overnight into 50% glycerol solution (v/v). The seedlings were mounted onto glass slides and the blue color indicative of *GUS* expression was recorded using the Nomarski differential interference contrast (DIC) on a Leica DMR microscope. Eight plants per treatment were analyzed and the expression patterns were further confirmed in two subsequent repetitions of the experiment.

In vitro plant/bacteria inoculation assays

Bacteria used in this work were *P. aeruginosa* PAO1, and the quorum-sensing mutant PAO-JP1 (*ΔlasI*) (Li et al. 2007a, b). Bacterial densities of 2.5×10^8 cfu were streaked on agar plates containing 0.2 × MS medium. *Arabidopsis* seedlings were germinated and grown for 6d on agar-solidified 0.2 × MS medium and then transferred over the bacterial streak. The seedlings were kept for 3, 6, 12, and 24 h in axenic growth or in co-cultivation with PAO1 or *ΔlasI Pseudomonas* strains and then RNA from the shoot and root system was obtained to analyze the differential gene expression. In an independent experiment, we monitored root system architecture and whole plant growth during 8-day placing the plates into the growth chamber in a completely randomized design. All experiments were replicated at least three times.

Analysis of growth and statistical analysis

The growth of primary roots was registered using a ruler. The lateral root number was determined by counting the lateral roots present in the primary root from the tip to the root/stem transition. The fresh weight of plants was determined with an analytical balance (Ohaus Corp.) with a 0.0001 g precision value. For all experiments, the overall data were statistically analyzed in the SPSS software version 10 (Statistical Package for the Social Sciences). Univariate and multivariate analyses with Tukey's post-hoc tests were used for testing differences in growth and root development responses in wild-type and mutant seedlings. Different letters are used to indicate means that differ significantly ($P \leq 0.05$).

RNA extraction

Total RNA was isolated from shoots and roots using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. To remove polysaccharide contamination, 0.25 mL of isopropanol was added to the aqueous phase, followed by 0.25 mL of a high-salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 mL of TRIzol reagent to precipitate RNA. Total RNA samples were treated with DNase I (NEB) and re-purified by an RNeasy kit (Qiagen) to remove DNA contamination and salts. RNA was quantified by using a Nanodrop 1000 spectrophotometer (Labtech) and assessed for purity via UV absorbance measurements at 260 and 280 nm. Total RNA integrity was confirmed by using an Agilent 2100 Bioanalyzer (Agilent Technologies).

RNA-Seq analysis: cDNA library preparation and sequencing

RNA-Seq libraries were prepared using the TruSeq RNA sample preparation kit (Illumina) following the manufacturer's protocol. Each library was independently labeled with a specific multiplexing index and then, all of them were pooled together and run on two lanes of a single flow cell of an Illumina HiSeq 2000 (one line for root samples and one for shoot samples). The samples were sequenced using TruSeq SBS

kit v3-HS (200 cycles; 2 × 100 pb) (Illumina) following the manufacturer's instructions. The RNA-seq data were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) with accession number PRJNA885452.

Data processing

The sequence libraries for each sample were processed by using CASAVA version 1.8.2 to produce 100-bp paired-end sequence data in fastq format. To ensure the good quality of the reads, the fastq files were processed using a python script (https://github.com/Czh3/NGSTools/blob/master/qualityControl.py) with the parameters −q 20 (Minimum quality score to keep), −p 85 (Minimum percent of bases that must have [−q] quality) and −a 25 (the average quality of paired-end). The high-quality R1–R2 read pairs, for each sample, were aligned to the annotated *Arabidopsis* genome (http://www.arabidopsis.org/) by using the RSEM (RNA-Seq by Expectation–Maximization) software (Li et al. 2011). RSEM, which directly executes Bowtie (Langmead et al. 2009) during the mapping process, calculates maximum likelihood abundance estimates as well as posterior mean estimates and 95% credibility intervals for genes (the expected read counts). Normalized expression values as Fragments Per Kilobase per Million (FPKM) and Transcripts Per Million (TPM) were also computed by RSEM. To compare expression levels of the different *Arabidopsis* genes across samples, perl scripts included in the Trinity software package (Grabherr et al. 2011) were used to perform a normalization process using the TMM (weighted Trimmed Mean of *M* values) method (Robinson and Oshlack 2010; Robinson et al. 2013) and the subsequent identification of differentially expressed genes by using the EdgeR bioconductor package (Robinson et al. 2010). The differential gene expression analysis was focused on *Arabidopsis* seedlings whose roots are grown in physical contact with PAO1 or *ΔlasI* *P. aeruginosa* bacterial streaks (the evaluated times were 3, 6, 12, and 24 h in the interaction). The tagwise dispersions were estimated and then used for logFC ($\log_2$ fold change) estimating and testing. Differentially expressed genes were extracted by applying the threshold false discovery rate (FDR) of less than 0.05 to adjusted *P* values, which were generated by using the Benjamini and Hochberg approach (Benjamini and Hochberg 1995). FPKM values estimated for each gene were used for comparing expression levels among samples.

Results

P. aeruginosa induces changes in root growth and gene expression

Previous work showed that unlike *P. aeruginosa* PAO1, which inhibits *Arabidopsis* growth, root co-cultivation with *ΔlasI* mutant enhances root branching and biomass accumulation (Ortiz-Castro et al. 2011, 2014). The contrasting phenotypes caused by these two near-isogenic bacteria are clearly distinguishable in seedlings after a few hours of interaction. The earliest visible *Arabidopsis* response to *P. aeruginosa* PAO1 is the curvature of the root tip, followed by primary root growth inhibition. In contrast, *ΔlasI* mutant neither causes root tip bending nor inhibits primary root growth and instead promotes root hair development (Supplementary Fig. S1). 3 days after contact with bacterial streaks, *ΔlasI* mutant induced both lateral root and root hair development (Supplementary Fig. S2).

RNA Seq analysis of *Arabidopsis* roots and shoots was performed at 3, 6, 12 and 24 h after root inoculation in experiments that included 2000 plants from 40 independent Petri plates co-cultivated with *P. aeruginosa* PAO1 or *ΔlasI* strains; seedlings grown without bacteria served as control samples (Supplementary Fig. S3; Supplementary Table S1). Differentially expressed genes in response to PAO1 and *ΔlasI* strains are shown in Supplementary Tables S2 and S3, respectively, each divided into two blocks, the upper part showing root genes, and the lower shoot genes. A total of 2079 genes were identified as differentially expressed overtime during the *Arabidopsis-P. aeruginosa* PAO1 interaction compared to the non-inoculated controls (1972 from roots and 202 from shoots). In contrast, in the *Arabidopsis-ΔlasI* interaction, according to the stringency levels on the statistical test (FDR ≤ 0.05), almost six times fewer genes (348 from roots and 67 from shoots) were differentially expressed and most of them were also represented in the interaction with the WT PAO1 strain (Supplementary Tables S2 and S3; Fig. 1). These data suggest that *Arabidopsis* seedlings differentially react to the root inoculation of these two bacteria, which correlates with the strength of the transcriptional response established.

P. aeruginosa induces defense-related genes in *Arabidopsis*

The virulence of *P. aeruginosa* PAO1 depends on the production of 3-oxo-C12-HSL and C4-HSL. The *ΔlasI* strain does not synthesize 3-oxo-C12-HSL (Ortíz-Castro et al.

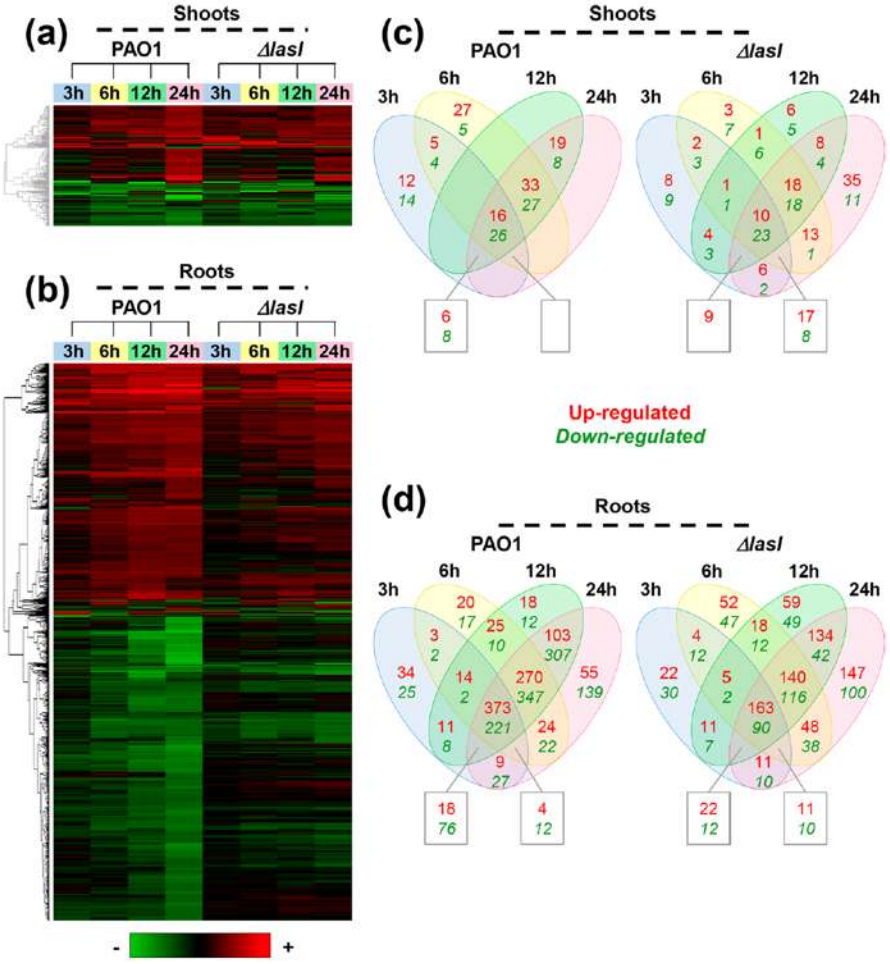


Fig. 1 Heat map of hierarchical clustering analysis of differentially expressed genes in shoots (**a**) or in roots (**b**) from *Arabidopsis* seedlings. The heat map was constructed with the logFC values (log_2 fold change) using an untethered correlation as a distance metric and the complete linkage method. Horizontal rows represent individual genes and vertical columns represent each time sampled during the interaction with PAO1 or *ΔlasI P. aeruginosa* strains. Green indicates downregulated (−), red upregulated (+) and black unchanged values, as shown on the color scale at the top of the figure. Venn diagrams showing common or distinct regulated genes over the sampled time-points in shoots (**c**) and roots (**d**). The number of genes up- or down-regulated each time is shown in red or green bold font, respectively

2011). Regarding the transcriptome, the expression patterns of defense-related genes were contrasting between *P. aeruginosa* PAO1 and *ΔlasI* strains, while PAO1 elicited a strong defense reaction, enhancing the expression of genes not induced by *ΔlasI* such as those encoding WRKY33, 53, and 70, PHYTOALEXIN DEFICIENT 3, and PLANT DEFENSIN 2.1 (Table1). However, both of these bacteria had a common set of genes up-regulated in the plant, including two peroxidases and a chitinase, *WRKY 46* and *51*, *cysteine-rich RLKs 36* and *40* as well as proteins involved in the plant reaction to pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs), which suggest that *ΔlasI* can still activate plant defense (Table 2). To validate this observation, we evaluated the expression of jasmonate and salicylic acid-related gene markers *pJAZ1:GUS*, *pLOX2:GUS*, and *pPR-1:GUS* in transgenic *Arabidopsis* seedlings that were grown for 6d in MS 0.2 × medium and then transferred over PAO1 or *ΔlasI* streaks for 6 h, 12 h, and 24 h to concur with the transcriptomic analysis. Under axenic conditions, expression driven by the *JAZ1* promoter was not observable in roots (Fig. 2), whereas in shoots, it shows a weak expression (Supplementary Fig. S4). Interestingly, since 6 h of exposure, PAO1 induced strong *JAZ1* expression in a time-dependent manner both in root and shoot regions, whereas *ΔlasI* showed a mild induction compared to PAO1 (Fig. 2 and Supplementary Fig. S4).

The expression of *LOX2* and *PR-1* promoters, using the GUS reporter gene, was analyzed in shoots 24 h after transfer to PAO1 and *ΔlasI* strains. PAO1 triggered a strong activation of these gene markers, whereas the effect of *ΔlasI* strain was indistinguishable when compared to non-inoculated seedlings (Supplementary Fig. S5). These data indicate that 3-oxo-C12-HSL production in *P. aeruginosa* controls the strength of the defense reaction elicited in plants.

P. aeruginosa induces genes for nitrate assimilation

Information from the transcriptome indicates that the interaction of *Arabidopsis* seedlings with *P. aeruginosa*

Table 1 Plant defense-related genes differentially regulated by PAO1, but not by *ΔlasI* mutant in Arabidopsis seedlings

Genes	Gene ID	Symbol	Description	*Arabidopsis*-PAO1 interaction (logFC)			
				3 h	6 h	12 h	24 h
Up-regulated	AT1G15520	PDR12	Pleiotropic drug resistance 12	1.41	3.85	5.50	4.95
	AT3G26830	PAD3	Phytoalexin deficient 3	1.06	4.57	5.69	4.69
	AT2G02120	PDF2.1	Plant defensin 2.1	0.35	1.23	2.19	3.63
	AT3G50930	BCS1	Cytochrome BC1 synthesis	2.25	2.41	4.10	3.01
	AT3G56710	SIB1	Sigma factor binding protein 1	2.98	3.42	3.06	2.92
	AT2G38470	WRKY33	WRKY DNA binding protein 33	1.28	1.74	2.46	2.26
	AT4G23810	WRKY53	WRKY DNA binding protein 53	1.41	1.42	2.46	2.21
	AT3G56400	WRKY70	WRKY DNA binding protein 70	1.16	1.02	1.88	2.61
	AT5G47220	ERF2	Ethylene-responsive element binding factor 2	0.53	1.16	1.44	2.25
	AT4G02380	SAG21	Senescence-associated gene 21	1.83	1.73	2.35	0.35
	AT1G72520	LOX4	Lipoxygenase 4	1.27	0.99	3.54	4.27
	AT3G12500	PR-3	Pathogenesis-related protein 3	−0.55	0.49	1.92	2.66
	AT5G47910	RBOHD	Respiratory burst oxidase homologue D	0.35	1.28	2.56	2.30
	AT1G74310	HSP101	Heat shock protein 101	−0.28	−0.53	0.43	2.28
Down-regulated	AT1G09090	RBOHB	Respiratory burst oxidase homologue D	−1.47	−0.54	−0.59	−2.35
	AT1G66950	PDR11	Pleiotropic drug resistance 11	−1.03	0.16	−0.83	−6.73
	AT1G34540	CYP94D1	Cytochrome P450, family 94, subfamily D, polypeptide 1	−1.98	−1.39	−8.02	−6.53
	AT2G46660	CYP78A6	Cytochrome P450, family 78, subfamily A, polypeptide 6	0.18	−2.54	−4.16	−3.18
	AT4G01830	PGP5	P-glycoprotein 5	−1.05	−0.04	−4.50	−6.83

Table 2 Plant defense-related genes with differential expression in Arabidopsis seedlings in response to inoculation with *P. aeruginosa* PAO1 and *ΔlasI* strains

Genes	Gene ID	Symbol	Description	*Arabidopsis*-PAO1 interaction (logFC)				*Arabidopsis*-*ΔlasI* interaction (logFC)			
				3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h
Up-regulated	AT5G19880	PER58	Peroxidase 58	3.29	8.81	8.32	8.49	3.25	6.89	6.53	7.70
	AT5G39580	PER62	Peroxidase 62	3.11	3.85	5.90	6.28	2.60	3.64	5.30	7.01
	AT2G43570	CHI	Chitinase	1.07	2.20	2.83	4.30	1.15	1.56	2.70	3.86
	AT2G46400	WRKY46	WRKY DNA binding protein 46	0.65	0.91	1.01	3.61	−0.13	−0.05	1.49	2.88
	AT5G64810	WRKY51	WRKY DNA binding protein 51	2.28	2.02	3.09	3.86	0.67	0.37	1.69	3.13
	AT4G11480	CRK32	Cysteine-rich RLK 36	1.84	8.45	6.68	6.10	2.15	7.93	4.27	1.12
	AT4G04570	CRK40	Cysteine-rich RLK 40	1.40	3.25	3.53	8.91	1.54	2.40	3.41	9.34
	AT4G28460	PIP1	PAMP-induced secreted peptide 1	3.80	5.37	6.14	6.81	2.60	4.08	4.96	5.52
	AT1G79680	WAKL10	Wall-associated receptor kinase 1	1.68	6.90	6.38	10.14	1.25	4.77	3.79	7.93
	AT2G19190	FRK1	FLG22-induced receptor-like kinase 1	−0.09	1.61	4.48	4.36	0.19	1.36	4.35	2.11
	AT5G57220	CYP81F2	Cytochrome P450, family 81, subfamily F, polypeptide 2	2.17	3.67	5.33	4.35	2.21	2.85	3.85	3.88
	AT5G41750	MUF8.3	Disease resistance protein family	1.84	2.80	3.45	4.09	1.18	1.90	2.57	2.81
	AT2G29350	SAG13	Senescence-associated gene 13	1.75	4.11	10.67	7.86	1.78	2.25	8.52	5.26

PAO1 or *ΔlasI* strains increased transcript levels of some nitrate transporters in roots (Table 3), including AtNRT1.1 (At1g12110), AtNRT1.8 (At4g21680) and AtNRT2.1 (At1g08090). Most interesting was the identification of genes encoding the nitrate reductases NIA1 (At1g77760) and NIA2 (At1g37130) as *P. aeruginosa*-inducible genes, whose expression increased in response to both PAO1 and *ΔlasI* strains (Table 3). Considering the result that PAO1 causes disease and consequently induces a much higher number of genes, it was surprising that the *ΔlasI* mutant

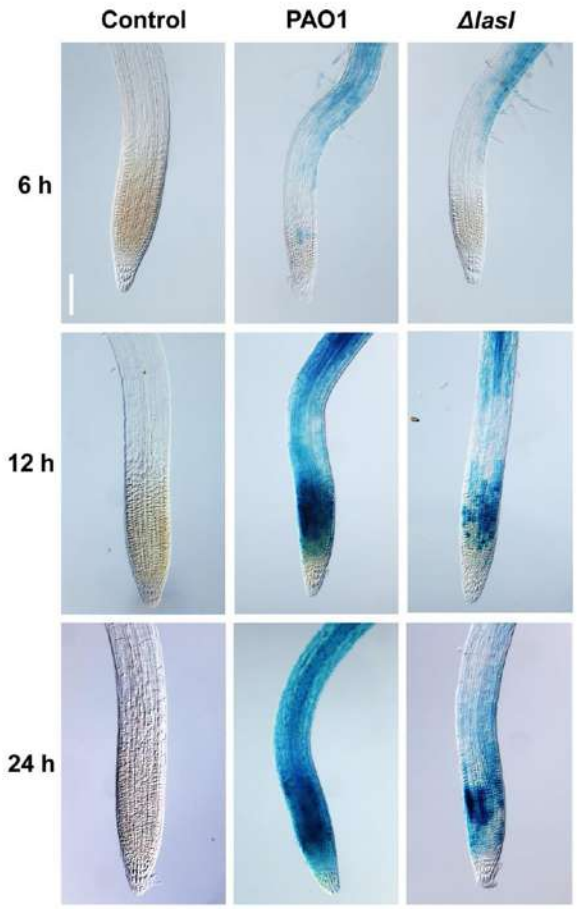


Fig. 2 Expression of *JAZ1:GUS* in *Arabidopsis* primary roots in contact with PAO1 or *ΔlasI* streaks. Six-day-old transgenic *A. thaliana* seedlings expressing *JAZ1:GUS* were transferred to fresh media (control) or placed over *P. aeruginosa* PAO1 or *ΔlasI* bacterial streaks and co-cultivated for 6 h, 12 h, or 24 h. Representative images of roots in the different treatments ($n = 20$) are provided. These analyses were repeated three times with comparable results. Scale bar = 100 µm

still manifested induction of AtNRT1.1 and NIA1 and NIA2 genes (Table 3), thus opening the possibility that the growth-promoting effects reported for the *P. aeruginosa ΔlasI* (Ortiz-Castro et al. 2011) could rely on an improved nitrate nutrition. The root transcriptome revealed that the nitrate transporter NRT1.1 could be induced by PAO1 or *ΔlasI* mutant 2.0 and 2.4-fold, respectively, at 24 h of root co-cultivation (Table 3). Thus, we aimed to confirm these data by testing the inducibility of the *CHL1-GUS* reporter construct in transgenic seedlings expressing this construct transferred to axenic media with low (10 µM) or high (1 mM) NH_4NO_3 or over *ΔlasI* bacterial streaks. Our data showed the induction of expression of *CHL1* in primary root tips by both nitrogen concentrations by the bacterium at an early time (24 h) or at a later time (6 d) of interaction (Fig. 3). These data show that AtNRT1 is induced upon bacterial inoculation.

The dual affinity nitrate transceptor CHL1 (AtNRT1.1) and the nitrate reductases NIA1 and NIA2 are required for plant growth promotion induced by *P. aeruginosa ΔlasI* under high nitrogen availability

The nitrate assimilation pathway starts with nitrate uptake by roots followed by nitrate reduction to ammonium, which is required for glutamine and glutamate biosynthesis (Kant 2018). The nitrate transceptor CHL1 (AtNRT1), and the nitrate reductases NIA1 and NIA2 play critical roles in the nitrate signaling, acquisition, and reduction pathways, respectively. To test the roles of genes involved in nitrate uptake and reduction on the beneficial effects of *P. aeruginosa ΔlasI*, seedlings of *Arabidopsis* WT and mutants defective in two independent alleles of *CHL1*, namely *chl1-5* and *chl1-12* (Tsay et al. 1993) were co-cultivated with *P. aeruginosa ΔlasI*. CHL1 loss-of-function drastically compromised the phytostimulation elicited by *P. aeruginosa ΔlasI*, since co-cultivated *Arabidopsis chl1-5* and *chl1-12* were no longer responsive to *ΔlasI* and, in fact, seedlings had a shoot, root, and total biomass significantly decreased when inoculated with the bacterial strain (Fig. 4a–c). Slight differences in root biomass could be observed between *chl1-5* and *chl1-12* in response to bacterization, being *chl1-5* more inhibited than *chl1-12* (Fig. 4b). Primary root growth was inhibited in WT, *chl1-5,* and *chl1-12* seedlings following inoculation (Fig. 4d), and in contrast to the WT, the number of first and second order lateral roots decreased in the *chl1-5 and chl1-12* mutants when compared to Col-0 plants inoculated with *ΔlasI* (Fig. 4e, f).

To further correlate the described phenotypical responses of WT and *chl1* mutants with nitrate reduction, the effect of *ΔlasI* on biomass production and root development of *nia1* and *nia2* single mutants and the *nia1nia2* double mutant was investigated. In these experiments *P. aeruginosa ΔlasI* increased shoot and root biomass production, and first- and second-order lateral root formation of WT, *nia1,* and *nia2* single mutants (Fig. 5a–f), but this effect was severely compromised in *nia1nia2* seedlings, which failed to support a strong root branching response and instead showed decreased biomass production following inoculation with the bacteria (Fig. 5a–f). Taken together, these data show the critical role of CHL1, and NIA1, and NIA2 in *Arabidopsis* to support a beneficial bacterial interaction under high nitrogen conditions.

Table 3 Nitrogen-related genes with differential expression in roots in response to inoculation with *P. aeruginosa* PAO1 and *ΔlasI* strains

Genes	Gene ID	Symbol	Description	*Arabidopsis*-PAO1 interaction (logFC)				*Arabidopsis*-*ΔlasI* interaction (logFC)			
				3 h	6 h	12 h	24 h	3 h	6 h	12 h	24 h
Up-regulated	AT4G21680	NRT1.8	Nitrate transporter 1.8	1.44	4.97	6.24	5.06	−0.61	1.01	0.11	0.46
	AT1G08090	NRT2.1	Nitrate transporter 2.1	−0.37	1.59	3.52	2.81	1.44	1.38	2.45	2.62
	AT1G12110	NRT1.1	Nitrate transporter 1.1	−0.04	1.57	1.96	2.09	0.79	1.19	1.96	2.45
	AT2G38290	AMT2	Ammonium transporter 2	0.51	1.56	2.22	2.36	0.02	0.86	0.83	1.49
	AT1G77760	NIA1	Nitrate reductase 1	1.89	3.32	4.79	4.94	1.91	2.50	4.51	4.49
	AT1G37130	NIA2	Nitrate reductase 2	0.87	1.64	2.53	1.96	0.79	0.94	1.56	1.45
	AT5G22300	NIT4	Nitrilase 4	1.54	3.05	3.40	2.47	0.50	1.08	1.43	0.17
	AT3G21720	ICL	Isocitrate lyase	−0.89	2.70	3.56	4.05	0.05	−0.12	−0.36	0.76
	AT1G73010	PS2	Phosphate starvation-induced gene 2	0.73	1.53	0.36	2.99	0.75	0.89	0.36	1.86
	AT5G06860	PGIP1	Polygalacturonase inhibiting protein 1	0.56	1.71	2.69	2.84	0.50	1.07	2.51	2.90
	AT3G09350	Fes1A	Fes1A	−0.42	−0.24	1.58	2.74	−0.05	−1.01	0.07	0.17
	AT1G02850	BGLU11	Beta glucosidase	0.72	1.75	2.84	2.29	0.07	0.27	0.44	0.61
	AT4G11280	ACS6	1-Aminocyclopropane-1-carboxylic acid synthase 6	1.06	2.18	2.86	2.12	0.42	1.12	0.58	0.41
	AT2G19190	FRK1	Fructokinase 1	−0.09	1.61	4.48	4.36	0.19	1.36	4.35	2.11
	AT2G16060	GLB1	Hemoglobin 1	1.59	0.93	2.33	0.25	0.71	1.00	2.54	1.75
	AT4G04610	APR1	APS reductase 1	0.10	1.98	2.24	0.23	−0.01	1.76	1.80	1.81
Down-regulated	AT2G02780	LRR1	Leucine-rich repeat protein 1	−0.73	−0.75	−1.70	−2.50	−0.32	−0.98	−1.75	−3.15
	AT1G34940	AXR3	AUX/IAA transcriptional regulator	0.01	−0.01	−1.01	−2.76	0.40	0.11	−0.42	−0.21
	AT5G34940	GUS3	Glucuronidase 3	−0.73	−0.65	−1.10	−2.91	−0.15	−0.68	−0.55	−1.12
	AT1G01170	DUF1138	Ozone-responsive stress like protein	−0.10	−1.30	−2.63	−3.52	−0.34	−1.20	−1.50	-2.12
	AT5G40890	CLC-A	Chloride channel A	−0.94	−1.04	−1.36	−2.17	−0.94	−1.15	−1.05	−1.55

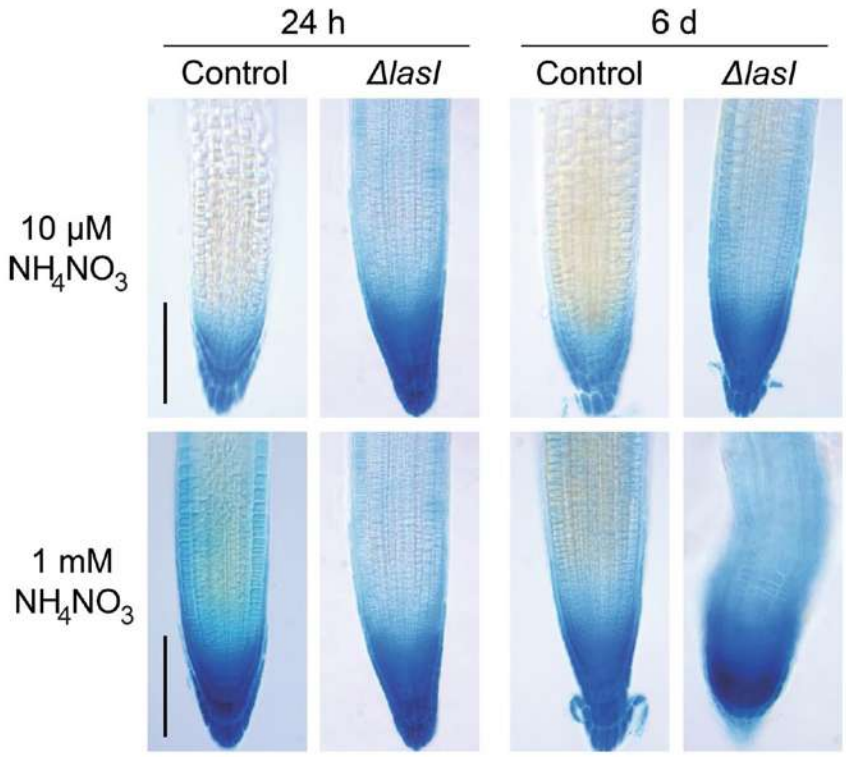


Fig. 3 Expression of *CHL1-GUS* in transgenic *Arabidopsis* seedlings exposed to contrasting NH_4NO_3 availability with or without bacteria. Six-day-old transgenic *A. thaliana* seedlings expressing *CHL1-GUS* were transferred to fresh media (controls) or placed over *P. aeruginosa ΔlasI* bacterial streaks and co-cultivated for 24 h or 6 days in medium supplemented with a low (10 µM NH_4NO_3) or high (1 mM NH_4NO_3) supplements. Representative images of root tips in the different treatments ($n=8$). These analyses were repeated three times with similar results. Scale bar = 100 µm

P. aeruginosa ΔlasI did not promote growth of *Arabidopsis* WT seedlings under low nitrogen availability and requires CHL1 (AtNRT1.1) and the nitrate reductases NIA1 and NIA2 for its phytostimulating effects at high nitrogen

The plant response to the bacteria may change according to N availability. To assess if the beneficial effects of bacterization could be seen at low and high N levels, the effect of co-cultivation with *P. aeruginosa ΔlasI* in *Arabidopsis* WT seedlings and *chl1-5* mutants was assessed at 10 µM NH_4NO_3 and 1 mM NH_4NO_3. We found that 1 mM NH_4NO_3 improved shoot biomass and total biomass in the WT and to a lesser extent in *chl1-5* mutant seedlings compared to the 10 µM NH_4NO_3 treatment (Fig. 6a–c). *P. aeruginosa ΔlasI* enhanced total plant biomass in WT seedlings at 1 mM NH_4NO_3, which correlated with a 2.5-fold increase in lateral root number and over sixfold enhancement of lateral root density (Fig. 6e–g). Although the bacterium also promoted lateral root density in *chl1-5* mutants, the impact of bacterization in the shoot, root, and total biomass was negligible. In 10 µM NH_4NO_3 medium, no signs of *P. aeruginosa ΔlasI* phytostimulation or root branching promotion could be observed in WT or *chl1-5* seedlings, suggesting the essentiality of N to improve plant growth (Fig. 6a–g).

Comparison of the growth of WT seedlings and *nia1nia2* mutants evidenced that *P. aeruginosa ΔlasI* neither improves shoot nor root biomass in the WT in 10 µM NH_4NO_3 and although root branching could be somewhat induced *P. aeruginosa ΔlasI,* it failed to promote growth under N scarcity (Fig. 7a–g). A slow growth phenotype of the *nia1nia2* double mutant seedlings was observed in 10 µM NH_4NO_3 medium and although 1 mM NH_4NO_3 could resume their primary root growth, the mean length was still shorter than in the WT (Fig. 7d). These data not only show the dependence of nitrogen for plant growth promotion by *P. aeruginosa ΔlasI*, but also indicate the involvement of CHL1 and the NIA1 and NIA2 proteins for growth and developmental adjustments.

Role of nitric oxide in root architectural and growth response to bacteria

Nitric oxide (NO) mediates both primary root growth and lateral root development and its cellular levels rise during nitrate reduction (Méndez-Bravo et al. 2010; Fernandez-Marcos et al. 2011). The increase of NO in roots as a possible response to *P. aeruginosa* PAO1 and *ΔlasI* was tested in *Arabidopsis* WT and *nia1nia2* mutants using the fluorescent probe 4,5-diaminofluorescein diacetate (DAF-2DA) and confocal microscopy 5 days after root interaction with bacterial streaks. Roots from WT (Col-0) and *nia1nia2 Arabidopsis* seedlings grown with PAO1 showed an increase of NO, which results more evident in

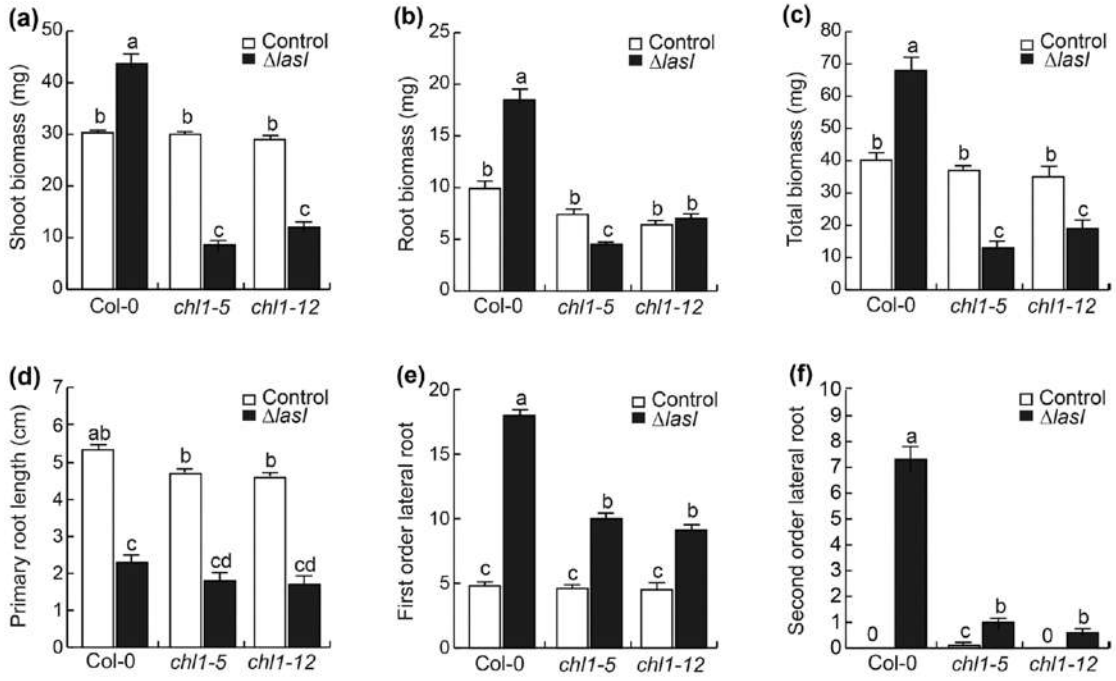


Fig. 4 Effect of co-cultivation with Δ*lasI P. aeruginosa* in *Arabidopsis* WT seedlings and CHL1 mutants grown in high nitrogen medium. Six-day-old *A. thaliana* WT, *chl1-5*, and *chl1-12* mutants were transferred to MS 0.2X fresh media (controls) or placed over *P. aeruginosa* Δ*lasI* bacterial streaks and co-cultivated for 8 days. Effects of bacterial co-cultivation on shoot biomass (**a**), root biomass (**b**), total biomass (**c**), primary root length (**d**), first-order lateral roots per plant (**e**), and second order lateral roots per plant (**f**). Data show the mean ± SD ($n = 30$). These analyses were repeated three times with similar results. Different letters indicate means statistically different at $P < 0.05$

the primary root meristems (Fig. 8). Interestingly, NO in Col-0 and *nia1nia2* mutants inoculated with *ΔlasI* did not increase in root meristems, but instead spread over the dense root hairs that covered the primary roots (Fig. 8). These data indicate that NO accumulation changes differentially in roots depending upon the type of bacteria being inoculated and is likely independent of NIA1 and NIA2.

A widely used strategy to investigate NO signaling in plant development involves the pharmacological application of NO donors such as SNP or SNAP to emulate NO production. To determine if bacterial growth promotion is related to NO accumulation, we analyzed the effect of two NO donors in *Arabidopsis* WT (Col-0) and *nia1nia2* mutants inoculated with *ΔlasI* applying 10 µM or 75 µM SNP or SNAP, respectively, and shoot, root and total biomass and root architectural traits were analyzed 8 days after root contact with *ΔlasI* streaks. In these experiments, NO donors did not further increase growth promotion parameters elicited by the bacterium in the WT, and failed to induce a strong root branching response in *nia1nia2* mutants (Fig. 9). These data indicate that *ΔlasI* mediated phytostimulation cannot be mimicked by application of NO donors.

Discussion

In this report, we investigated the early growth and developmental consequences of root co-cultivation with *P. aeruginosa* PAO1 and *ΔlasI* strains, and the underlying changes in global gene expression in *Arabidopsis* seedlings. The shoot and root transcriptomes allowed the identification of several classes of differentially expressed genes related to growth, carbon transport, and metabolism, defense, and nutrition. We wondered if the *P. aeruginosa* interaction with roots could contribute to carbon assimilation in host plants since N and C ratios are important and correlated for plant development processes. In fact, plant sugars can induce nitrogen reductase and can improve plant biomass (Schofield et al. 2009). Surprisingly, several genes related to sugar synthesis and transport were repressed, including *SWEET3*, *SWEET11*, *SWEET12*, *SWEET16*, *SWEET17*, and *STP1*. However, over-induced genes could be also found, including *SPS4F*, *SPP1*, *SUS3*, *SUS4*, and *SWEET14*, indicating that *Pseudomonas aeruginosa ΔlasI* affects sugar metabolism and transport responses.

As expected, *P. aeruginosa* PAO1 a well-known plant pathogen (Walker et al. 2004; Ortiz-Castro et al. 2014;

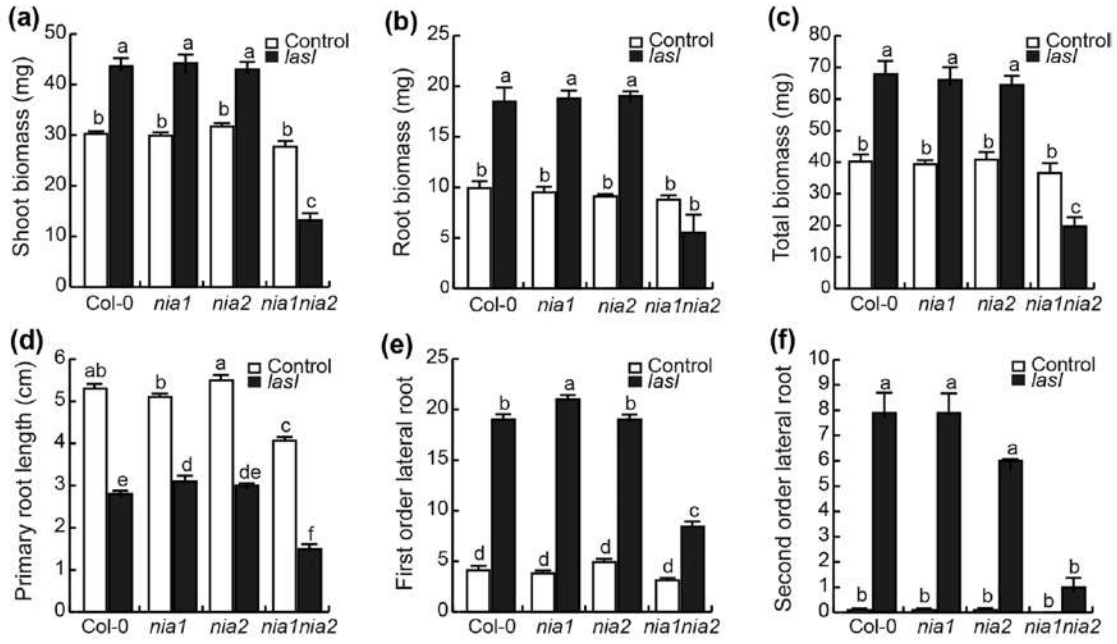


Fig. 5 Effect of co-cultivation with Δ*lasI P. aeruginosa* in *Arabidopsis* WT seedlings and nitrate reductase mutants grown in high nitrogen medium. Six-day-old *A. thaliana* WT, *nia1*, *nia2*, and *nia1nia2* mutants were transferred to MS0.2X fresh media (controls) or placed over *P. aeruginosa* Δ*lasI* bacterial streaks and co-cultivated for 8 d. Effects of bacterial co-cultivation on shoot biomass (**a**), root biomass (**b**), total biomass (**c**), primary root length (**d**), first-order lateral roots per plant (**e**), and second order lateral roots per plant (**f**). Data represent the mean ± SD ($n = 30$). These analyses were repeated three times with similar results. Different letters indicate means statistically different at $P < 0.05$

Solis et al. 2022), strongly induced the expression of defense-related genes, many of which increased with time, including *PDR12* (At1g15520), *SIB1* (AT3G56710), *WRKY33* (AT2G38470), *WRKY28* (AT4G18170), *WRKY70* (AT3G56400), *BCS1* (AT3G50930), *LOX4* (AT1G72520), and *PAD3* (AT3G26830), to mention a few. *PDR12* encodes an ABC transporter, a member of the pleiotropic drug resistance (PDR) family, probably involved in the export of toxic secondary metabolites and/or in the detoxification of pathogen toxins (Stein et al. 2006). *SIB1* encodes for one of the two sigma factor binding proteins present in the *Arabidopsis* genome, induced by infection with *Pseudomonas syringae* and treatments with salicylic acid (SA) and jasmonic acid (JA) and it is an activator of WRKY33 (Xie et al. 2010). *BCS1* is an *Arabidopsis* stress-responsive gene encoding a mitochondrial protein, which positively regulates SA accumulation, cell death, and tolerance to the biotrophic pathogen *Pseudomonas syringae*, but renders plants more sensitive to the necrotrophic fungus *Botrytis cinerea* (Zhang et al. 2014). WRKY70 has a well-known role in defense and appears to integrate SA and JA responses (Li et al. 2006). Meanwhile, LOX4 is expressed in phloem-associated cells, contributes to JA synthesis and defense against the generalist herbivore *Spodoptera littoralis* (Chauvin et al. 2013; 2016), and along with LOX3 is responsible for early JA biosynthesis in the *Arabidopsis* response to *Botrytis cinerea* (Windram et al. 2012). Noteworthy, a common set of defense-regulated genes by both PAO1 and Δ*lasI* strains, included two peroxidases, potentially involved in reactive oxygen species detoxification and adaptation to stress, a chitinase and regulatory proteins transducing plant responses to microbial components such as WRKY46 and WRKY51 transcription factors and cysteine-rich receptor-like kinases CRK3 and CRK40 (Idänheimo et al. 2014; Minibayeva et al. 2015). This suggests that *LasI* mutation in *P. aeruginosa* affects the strength of the defense-related transcriptional response mounted by *Arabidopsis* seedlings. Confirmation in vivo of such a hypothesis could be done by direct interaction of *Arabidopsis* transgenic seedlings expressing *JAZ1*, *LOX2*, and *PR-1* promoters fused to the GUS reporter gene. *JAZ1* encodes for a JA-signaling repressor, *LOX2* for an enzyme involved in JA biosynthesis, and *PR-1* for a pathogenesis-related protein induced by SA. In our experiments, all three gene constructs were strongly induced in a time-dependent manner by PAO1, but only JAZ1 could be upregulated by Δ*lasI*.

The transcriptomic analysis also evidenced critical points for the regulation of nitrate nutrition in the *Arabidopsis-P. aeruginosa* interaction, for instance three nitrate

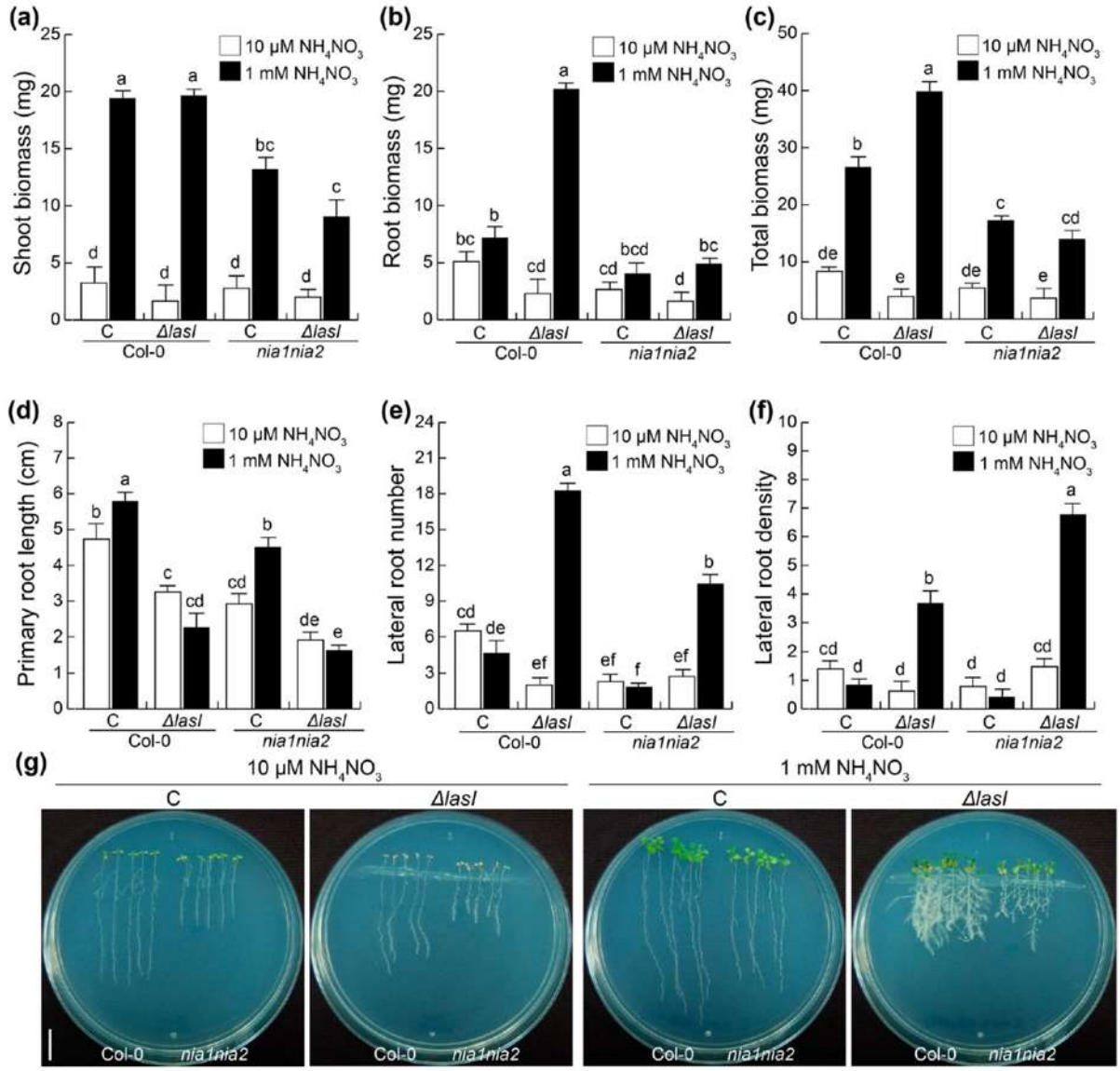


Fig. 7 Effect of co-cultivation with *P. aeruginosa ΔlasI* in *Arabidopsis* WT seedlings and *nia1nia2* double mutants at contrasting NH_4NO_3 availability. Six-day-old *A. thaliana* WT and *nia1nia2* mutants were transferred to fresh media (controls) or placed over *P. aeruginosa ΔlasI* bacterial streaks and co-cultivated for 8 days in medium supplemented with a low (10 µM NH_4NO_3) or high (1 mM NH_4NO_3) media. The graphs show the effects of bacterial co-cultivation on shoot biomass (**a**), root biomass (**b**), total biomass (**c**), primary root length (**d**), lateral root number per plant (**e**), and lateral root density (**f**). Data from (**a**) to (**f**) represent the mean ± SD ($n = 30$). (**g**) Representative images of WT and *nia1nia2* seedlings grown side by side for 8 days in high or low NH_4NO_3 media inoculated or not with *P. aeruginosa ΔlasI*. These analyses were repeated three times with similar results. Different letters indicate means statistically different at $P < 0.05$. Scale bar = 1 cm

bacteria (Castrillo et al. 2017). Nutrient demand of phosphate and nitrate could be of adaptive importance since hundreds of transcripts and proteins are de novo synthesized in response to biotic interactions, especially those eliciting development or defense adjustments (Weir et al. 2008; Castrillo et al. 2017; Wang et al. 2020).

Among the nitrate-related genes induced by root contact with PAO1, *NRT1.1*, *NRT2.1* and both nitrate reductases *NIA1* and *NIA2* were also induced in *Arabidopsis* roots co-cultivated with the *P. aeruginosa ΔlasI* mutant. However, *NRT1.8* that was strongly induced by PAO1 was not induced by the *P. aeruginosa ΔlasI*. These data showed that although

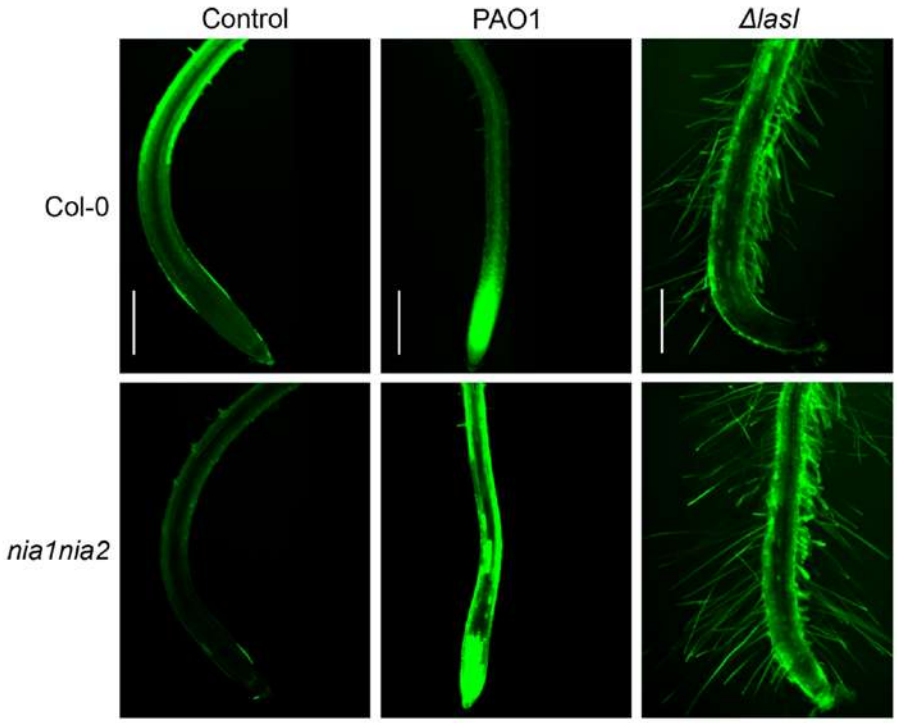


Fig. 8 Effect of co-cultivation with *P. aeruginosa* WT and *P. aeruginosa* PAO1 or Δ*lasI* on nitric oxide (NO) accumulation in primary root tips. Six-day-old *A. thaliana* seedlings and *nia1nia2* mutants were germinated and grown on 0.2X MS medium and transferred to fresh medium or co-cultivated with *P. aeruginosa* WT and Δ*lasI* strains by 2 and 5 days. Roots were uploaded with DAF-2DA and green fluorescence indicative of nitric oxide accumulation was recorded by confocal microscopy. Photographs are representative of individuals of at least 10 seedlings analyzed (Scale bar = 500 µm)

somewhat comparable, the gene expression patterns elicited by PAO and *ΔlasI* are not identical and particular signatures might exist that enable plants to specifically recognize each strain. Previous studies located CHL1 expression in the tips of primary and lateral roots and it was induced by auxin in a similar manner to the *DR5::GUS* auxin-responsive promoter (Guo et al. 2002). Our previous research failed to detect indole-3-acetic acid in cell-free extracts from *P. aeruginosa* PAO1 or *ΔlasI* cultures (Ortiz-Castro et al. 2011). However, the production of cyclodipeptides by *ΔlasI* mutant, which possesses weak auxin activity may explain the induction of CHL1 expression in root tips by *P. aeruginosa* PAO1 or *ΔlasI*, particularly when N is available. In a recent report, AtNRT1 was induced in roots of *Arabidopsis* seedlings inoculated with the plant-beneficial fungus *Trichoderma atroviride* and its phytostimulation indeed required the function of the corresponding transporter (López-Bucio et al. 2022). These findings can be explained because *T. atroviride* secretes indole-3-acetic acid and auxin precursors, already been reported to induce AtNRT1 (Guo et al. 2002).

Roots sense structural components of bacterial cells, such as flagellin, lipopolysaccharides, and diffusible substances including auxins and AHLs (Ortiz-Castro et al. 2008; von Rad et al. 2008), for which root exudation drives bacterial growth and synchronizes cell behavior (Fan et al. 2012; Kierul et al. 2015). These reports showed that nitrogen-deprived maize plants triggered a *Bacillus amyloliquefaciens* FZB42 response, modulating its protein synthesis, chemotaxis, and motility, that according to our findings link the plant nutritional status with photosynthesis and root colonizing bacteria, perhaps by releasing rich carbon resources into the rhizosphere.

Nitrate availability triggers root foraging, a highly sophisticated behavior, which involves changes in root growth and branching patterns. Our data suggest that *P. aeruginosa ΔlasI* root colonization may influence the plant nutrient status by releasing auxin-mimetic cyclodipeptides that enhance root branching and soil exploration, thus helping the acquisition of organic resources from detritus that accumulate in the upper layers of soil. In addition, through activating the molecular mechanisms of nitrate intake and reduction, roots may grow more properly, which coincides with the proliferation of root hairs and lateral roots after bacterial colonization. Regarding the impact of microbes or microbial signals on plant nutrient uptake systems, *Phyllobacterium brassicacearum* STM196 improved nitrate influx in *Arabidopsis* 24 h post-inoculation via inducing the putative nitrate transporters *NRT2.5* and *NRT2.6*. Comparison of root development and phytostimulation in WT, *nrt2.5*, *nrt2.6,* and *nia2* mutants, indicated the importance of nitrate assimilation for the plant growth promotion capacity of this bacterium, since all three mutations abolished plant growth and root architecture responses to STM196 (Mantelin et al. 2006; Kechid et al. 2013). A role for *NRT2.6* in the resistance against *Erwinia amylovora* further revealed the direct connection between N nutrition and tolerance to a plant

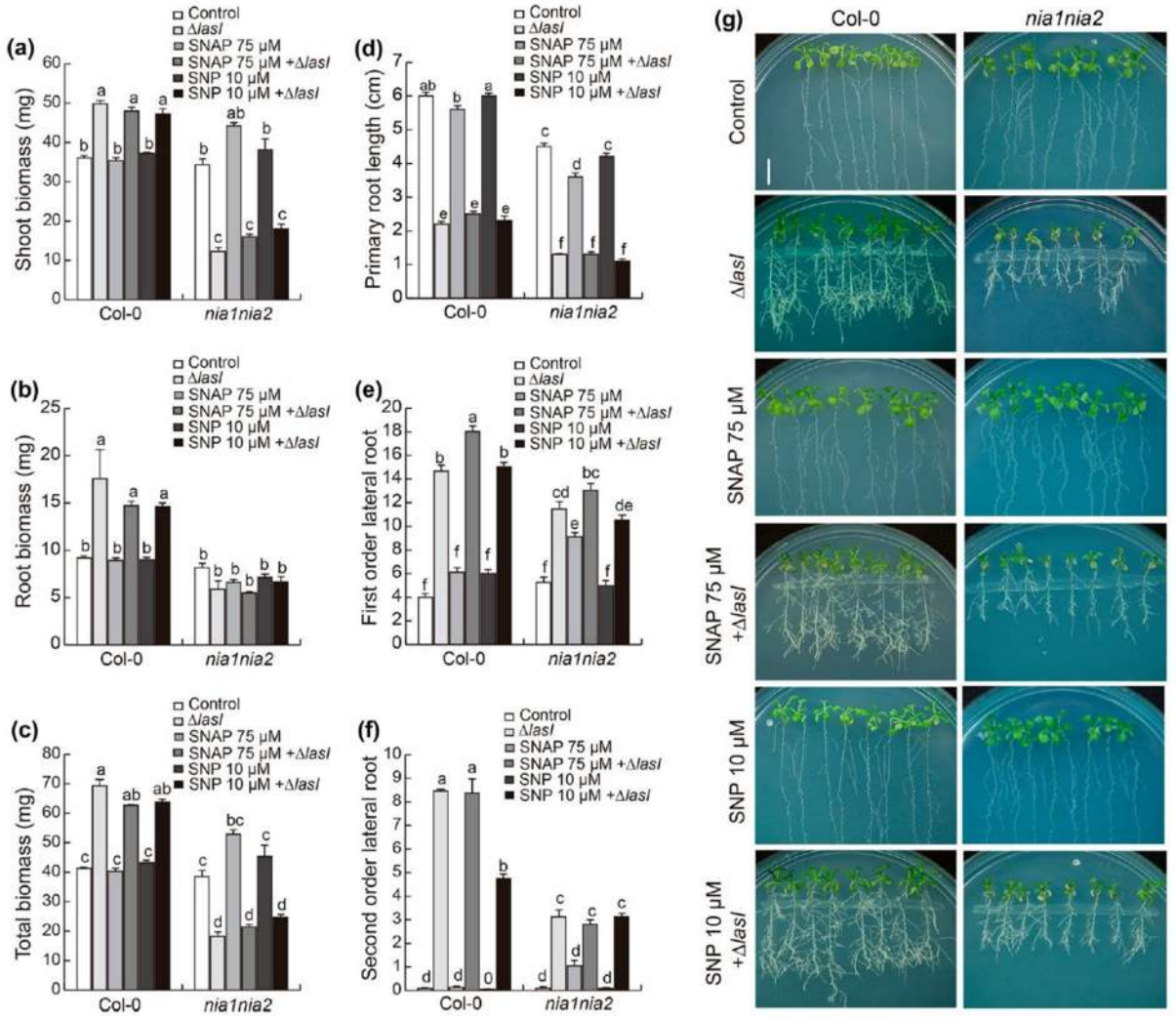


Fig. 9 Effect of nitric oxide donors SNP and SNAP on growth and root architecture of *Arabidopsis* seedlings and the response to *P. aeruginosa ΔlasI*. *Arabidopsis* WT (Col-0) seedlings were grown for 6 days on agar plates supplied with the solvent or with the NO donors SNP (10 µM) or SNAP (75 µM) and transferred to fresh medium or placed over *P. aeruginosa ΔlasI* bacterial streak in the medium with NO donors. After 8 days of growth effects on shoot biomass (**a**), root biomass (**b**), total biomass (**c**), primary root length (**d**), first-order lateral roots per plant (**e**), and second-order lateral roots per plant (**f**) were quantified. (**g**) Representative photographs of plant development in the interaction. Data from (**a**) to (**f**) represent the mean ± SD ($n = 30$). These analyses were repeated three times with similar results. Different letters indicate means statistically different at $P < 0.05$. Scale bar in (**g**) = 1 cm

pathogen (Dechorgnat et al. 2012). Here, we identified three N transporter-encoding genes that are induced in *Arabidopsis* in response to the phytopathogen *P. aeruginosa* PAO1. One of these, CHL1 (AtNRT1.1) was induced in the plants in interaction with *ΔlasI* despite this bacterium did not cause disease. This motivated us to analyze the phenotype of plants defective in two CHL1 independent alleles in response to interaction with *ΔlasI* regarding plant biomass accumulation and root architecture configuration. The diminished phytostimulation driven by the bacterium in the single *chl1-5* and *chl1-12* mutants demonstrates the critical role of nitrate acquisition in the beneficial relationship established with the plant growth-promoting *P. aeruginosa ΔlasI* mutant.

The following lines of evidence are provided to probe that nitrate reduction via the activity of NIA1 and NIA2 enzymes mediate the plant response to *P. aeruginosa*: (1) the NR genes, *NIA1* and *NIA2*, are up-regulated in the interactions with PAO1 and *ΔlasI* strains; (2) *Arabidopsis nia1nia2* double mutants are severely compromised during the interaction with the plant growth promoting *P. aeruginosa ΔlasI*;

and (3) root co-cultivation with *P. aeruginosa* PAO1 and *ΔlasI* mutant induced NO accumulation in the root system, albeit at different sites, PAO1 in meristems and *ΔlasI* in root hairs. Since NR activity is a major source of NO in plants, it explains the drastic increase in NO production in root meristems of seedlings interacting with PAO1 and the exacerbated NO accumulation in root hairs in the region of the root in contact with *ΔlasI* streaks.

Nitric oxide increase (using DAF-2DA) was shown in *nia1nia2* double mutant roots when inoculated with PAO1 or *ΔlasI* strains. NO production may occur from oxidative and reductive pathways and from different sources. Oxidative pathways (oxygen-dependent) involve L-arginine, polyamine, and hydroxylamine (Gupta et al. 2011), whereas reductive pathways occur during low O_2 and are dependent on NO_3^-, NO_2^-, and the nitrate reductase activity driven in *Arabidopsis* by the NIA1 and NIA2 enzymes, plasma membrane-bound nitrite reductase (PM NiNOR), xanthine oxidoreductase in plant peroxisomes, photosynthetic-electron-transport-chain-dependent NO_2^- reduction in chloroplasts, and mitochondrial electron transporter chains (Gupta et al. 2011; Kumari et al. 2019). On the other hand, plants produce NO via a NITRIC OXIDE SYNTHASE1 (NOS1), which has been reported as a key component for NO biosynthesis (since *nos1* mutant is severely affected in NO biosynthesis; Guo et al. 2002), and it has been shown to be needed for NO production and signaling during defense responses (Zeidler et al. 2004). We think that besides NIA1NIA2 activity, *ΔlasI* strains could activate any other, still unknown pathway(s) for NO production.

The fact that supplementation of *nia1nia2* double mutants with the NO donors SNP and SNAP did not improve root growth or biomass production in the mutants, indicates that NO alone is not sufficient to drive organogenesis and biomass production in these mutants and suggests the essential role of adequate nitrate availability/reduction to mount an effective response to bacterization. To the best of our knowledge, the possible role of nitric oxide in root responses to rhizosphere bacteria has been assessed only in the interaction of *Azospirillum brasilense* Sp245 with tomato, where this gaseous molecule triggered adventitious and lateral root development regardless of the bacterial capacity to produce auxin (Molina-Favero et al. 2008), an effect likely explained because NO promotes the formation of lateral and adventitious roots in many plant species from different families (Raya-González et al. 2019).

Considering the reported overproduction of cyclodipeptides by *P. aeruginosa ΔlasI* mutants, which induce the canonical auxin response in *Arabidopsis* roots (Ortiz-Castro et al. 2011), we cannot discard that a combined hormonal and nitrate-dependent nutritional effect underlies the strong growth promoting and root branching effect observed in the interaction of plants with *ΔlasI* mutant, particularly regarding the proposed role of AtNRT1.1 in transporting both nitrate and auxin (Wang et al. 2020).

AHLs produced by Gram-negative bacteria share the core homoserine lactone moiety but differ in the length of the fatty acid incorporated by their respective synthase enzymes. C-10 to C14-chained molecules strongly repress root growth and promote lateral root formation (Ortiz-Castro et al. 2008), a follow-up of the current research is determining whether any of the AHL-depending signaling pathways in *P. aeruginosa* may relate to the nutritional-mediated bacterial traits and if the length of the fatty acid chain could influence nitrate-dependent nutritional responses in the plant. Overall, the findings reported here, contribute to a better understanding of the adaptive and nutritional roles of rhizobacteria to plants, with great promise for the development of bacterial strains for best crop performance and adaptability and open a new avenue towards optimizing N acquisition efficiency highly needed in the current conditions of declining environmental resources.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00425-023-04236-7.

Acknowledgements We thank the staff of 'Laboratorio de Servicios Genómicos' at LANGEBIO for their technical support and sequencing services. The authors wish to thank the Institute of Ecology A.C. (INECOL), for the access to the high-performance computing system (HUTZILIN) required to carry out this research and we also thank Emanuel Villafán for his assistance as the administrator of this resource. We appreciate the kind support of Drs. Barbara Iglewski and Gabriel Krouk for donation of WT *P. aeruginosa* and mutant strains, and *Arabidopsis* mutants, respectively.

Author contributions JLB, ROC, and LHE conceived and designed the experiments; JRG, RPF, KRJV, VMD and EGC performed experiments; JLB, EIL, RPF, ROC, and JRG analysed the data; JLB, ROC, LHE, and EIL provided reagents/materials/analytical tools; JLB wrote the paper; All authors reviewed and edited the manuscript. LHE and JLB applied for funding.

Funding This work was financed by grants from the Consejo Nacional de Ciencia y Tecnología (CONACYT, México, Grants A1-S-34768, PDCPN-2015-882 and FORDECYT-PRONACES 292399) and UMSNH (CIC 2.26).

Declarations

Conflict of interest The authors declare that there are no competing interests.

Availability of data and materials All data generated or analyzed during this study are included in this article and are available upon reasonable request to the corresponding author.

References

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat

Soc Ser B (methodol) 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bouguyon E, Brun F, Meynard D, Kubeš M, Pervent M, Leran S, Lacombe B, Krouk G, Guiderdoni E, Zažímalová E, Hoyerová K, Nacry P, Gojon A (2015) Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1.1. Nat Plants 2(1):15015. https://doi.org/10.1038/nplants.2015.15

Carlson R, Tugizimana F, Steenkamp PA, Dubery IA, Hassen AI, Labuschagne N (2020) Rhizobacteria-induced systemic tolerance against drought stress in *Sorghum bicolor* (L.) Moench. Microbiol Res 232:126388. https://doi.org/10.1016/j.micres.2019.126388

Castrillo G, Teixeira PJ, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl JL (2017) Root microbiota drive direct integration of phosphate stress and immunity. Nature 543:513–518. https://doi.org/10.1038/nature21417

Chauvin A, Caldelari D, Wolfender J-L, Farmer E-E (2013) Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded Arabidopsis thaliana leaves: a role for lipoxygenase 6 in responses to long-distance wound signals. New Phytol 197(2):566–575. https://doi.org/10.1111/nph.12029

Chauvin A, Lenglet A, Wolfender J-L, Farmer E (2016) Paired hierarchical organization of 13-lipoxygenases in Arabidopsis. Plants 5(2):16. https://doi.org/10.3390/plants5020016

Contreras-Cornejo HA, Macías-Rodríguez L, Beltrán-Peña E, Herrera-Estrella A, López-Bucio J (2011) *Trichoderma*-induced plant immunity likely involves both hormonal- and camalexin-dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. Plant Signal Behav 6(10):1554–1563. https://doi.org/10.4161/psb.6.10.17443

Dechorgnat J, Patrit O, Krapp A, Fagard M, Daniel-Vedele F (2012) Characterization of the Nrt2.6 gene in Arabidopsis thaliana: a link with plant response to biotic and abiotic stress. PLoS ONE 7(8):e42491. https://doi.org/10.1371/journal.pone.004249

Díaz-Pérez SP, Solis CS, López-Bucio JS, Valdez Alarcón JJ, Villegas J, la Cruz R-D, Campos-García J (2022) Pathogenesis in *Pseudomonas aeruginosa* PAO1 biofilm-associated is dependent on the pyoverdine and pyocyanin siderophores by quorum sensing modulation. Microb Ecol. https://doi.org/10.1007/s00248-022-02095-5

Dolch LJ, Lupette J, Tourcier G, Bedhomme M, Collin S, Magneschi L, Conte M, Seddiki K, Richard C, Corre E, Fourage L, Laeuffer F, Richards R, Reith M, Rébeillé F, Jouhet J, McGinn P, Maréchal E (2017) Nitric oxide mediates nitrite-sensing and acclimation and triggers a remodeling of lipids. Plant Physiol 175:1407–1423. https://doi.org/10.1104/pp.17.01042

Fan B, Carvalhais L-C, Becker A, Fedoseyenko D, von Wirén N, Borriss R (2012) Transcriptomic profiling of *Bacillus amyloliquefaciens* FZB42 in response to maize root exudates. BMC Microbiol 12:116. https://doi.org/10.1186/1471-2180-12-116

Feng H, Fan X, Miller AJ, Xu G (2020) Plant nitrogen uptake and assimilation: regulation of cellular pH homeostasis. J Exp Bot 71:4380–4392. https://doi.org/10.1093/jxb/eraa150

Fernandez-Marcos M, Sanz L, Lewis D-R, Muday G-K, Lorenzo O (2011) Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN-FORMED 1 (PIN1)-dependent acropetal auxin transport. Proc Natl Acad Sci USA 108:18506–18511. https://doi.org/10.1073/pnas.1108644108

García-Cárdenas E, Ortiz-Castro R, Ruiz-Herrera LF, Valencia-Cantero E, López-Bucio J (2021) *Micrococcus luteus* LS570 promotes root branching in *Arabidopsis* via decreasing apical dominance of the primary root and an enhanced auxin response. Protoplasma 259:1139–1155. https://doi.org/10.1007/s00709-021-01724-z

González-López O, Palacios-Nava B, Peña-Uribe C, Campos-García J, López-Bucio J, García-Pineda E, Reyes de la Cruz H (2021) Growth promotion in *Arabidopsis thaliana* by bacterial cyclodipeptides involves the TOR/S6K pathway activation. J Plant Physiol 257:153343. https://doi.org/10.1016/j.jplph.2020.153343

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29:644–652. https://doi.org/10.1038/nbt.1883

Grunewald W, Vanholme B, Pauwels L, Plovie E, Inzé D, Gheysen G, Goossens A (2009) Expression of the Arabidopsis jasmonate signalling repressor JAZ1/TIFY10A is stimulated by auxin. EMBO Rep 10(8):923–928. https://doi.org/10.1038/embor.2009.103

Guo FQ, Wang R, Crawford NM (2002) The Arabidopsis dual-affinity nitrate transporter gene AtNRT1.1 (CHL1) is regulated by auxin in both shoots and roots. J Exp Bot 53(370):835–844. https://doi.org/10.1093/jexbot/53.370.835

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011) On the origins of nitric oxide. Trends Plant Sci 16:160–168. https://doi.org/10.1016/j.tplants.2010.11.007

Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J Exp Bot 68(10):2501–2512. https://doi.org/10.1093/jxb/erw449

Ho C-H, Lin S-H, Hu H-C, Tsay Y-F (2009) CHL1 functions as a nitrate sensor in plants. Cell 138:1184–1194. https://doi.org/10.1016/j.cell.2009.07.004

Idänheimo N, Gauthier A, Salojärvi J, Siligato R, Brosché M, Kollist H, Mähönen AP, Kangasjärvi J, Wrzaczek M (2014) The *Arabidopsis thaliana* cysteine-rich receptor-like kinases CRK6 and CRK7 protect against apoplastic oxidative stress. Biochem Biophys Res Commun 445(2):457–462. https://doi.org/10.1016/j.bbrc.2014.02.013

Jiménez-Vázquez KR, García-Cárdenas E, Barrera-Ortiz S, Ortiz-Castro R, Ruiz-Herrera LF, Blanca P, Ramos-Acosta BP, Coria-Arellano JL, Saenz-Mata J, López-Bucio J (2020) The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. Plant J 103:1639–1654. https://doi.org/10.1111/tpj.14853

Kant S (2018) Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. Semin Cell Dev Biol 74:89–96. https://doi.org/10.1016/j.semcdb.2017.08.034

Kechid M, Desbrosses G, Rokhsi W, Varoquaux F, Djekoun A, Touraine B (2013) The NRT2.5 and NRT2.6 genes are involved in growth promotion of *Arabidopsis* by the plant growth-promoting rhizobacterium (PGPR) strain *Phyllobacterium brassicacearum* STM196. New Phytol 198:514–515. https://doi.org/10.1111/nph.12158

Kierul K, Voigt B, Albrecht D, Chen X-H, Carvalhais L-C, Borriss R (2015) Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Microbiology 161:131–147. https://doi.org/10.1099/mic.0.083576-0

Kumari A, Pathak PK, Bulle M, Igamberdiev AU, Gupta KJ (2019) Alternative oxidase is an important player in the regulation of nitric oxide levels under normoxic and hypoxic conditions in plants. J Exp Bot 70:4345–4354. https://doi.org/10.1093/jxb/erz160

Langmead B, Trapnell C, Pop M, Salzberg S-L (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10:R25. https://doi.org/10.1186/gb-2009-10-3-r25

Li B, Dewey C (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinform 12:323. https://doi.org/10.1186/1471-2105-12-323

Li J, Brader G, Kariola T, Palva E-T (2006) WRKY70 modulates the selection of signaling pathways in plant defense. Plant J 46(3):477–491. https://doi.org/10.1111/j.1365-313X.2006.02712.x

Li LL, Malone JE, Iglewski BH (2007a) Regulation of the *Pseudomonas aeruginosa* quorum-sensing regulator VqsR. J Bacteriol 189:4367–4374. https://doi.org/10.1128/JB.00007-07

Li W, Wang Y, Okamoto M, Crawford NM, Yaeesh-Siddiqi M, M-Glass AD (2007b) Dissection of the AtNRT2.1:AtNRT2.2 inducible high-affinity nitrate transporter gene cluster. Plant Physiol 143:425–433. https://doi.org/10.1104/pp.106.091223

Liu K-H, Huang C-Y, Tsay Y-F (1999) CHL1 is a dual-affinity nitrate transporter of *Arabidopsis* involved in multiple phases of nitrate uptake. Plant Cell 11:865–874. https://doi.org/10.1105/tpc.11.5.865

López-Bucio J, Esparza-Reynoso S, Pelagio-Flores R (2022) Nitrogen availability determines plant growth promotion and the induction of root branching by the probiotic fungus *Trichoderma atroviride* in *Arabidopsis* seedlings. Arch Microbiol 204:380. https://doi.org/10.1007/s00203-022-03004-7

López-Hernández J, García-Cárdenas E, López-Bucio JS, Jiménez-Vázquez KR, De la Cruz HR, Ferrera-Rodríguez O, Santos-Rodríguez DL, Ortiz-Castro R, López-Bucio J (2022) Screening of phosphate solubilization identifies six *Pseudomonas* species with contrasting phytostimulation properties in *Arabidopsis* seedlings. Microb Ecol. https://doi.org/10.1007/s00248-022-02080-y

Malamy JE, Benfey PN (1997) Organization and cell diferentiation in lateral roots of *Arabidopsis thaliana*. Development 124:33–44. https://doi.org/10.1242/dev.124.1.33

Mantelin S, Desbrosses G, Larcher M, Tranbarger TJ, Cleyet-Marel JC, Touraine B (2006) Nitrate-dependent control of root architecture and N nutrition are altered by a plant growth-promoting *Phyllobacterium* sp. Planta 223:591–603. https://doi.org/10.1007/s00425-005-0106-y

Méndez-Bravo A, Raya-González J, Herrera-Estrella L, López-Bucio J (2010) Nitric oxide is involved in alkamide-induced lateral root development. Plant Cell Physiol 51:1612–1626. https://doi.org/10.1093/pcp/pcq117

Miller MB, Bassler B-L (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Minibayeva F, Beckett RP, Kranner I (2015) Roles of apoplastic peroxidases in plant response to wounding. Phytochemistry 112:122–129. https://doi.org/10.1016/j.phytochem.2014.06.008

Molina-Favero C, Creus CM, Simontacchi M, Puntarulo S, Lamattina L (2008) Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. Mol Plant-Microbe Interact 21(7):1001–1009. https://doi.org/10.1094/MPMI-21-7-1001

Nishad R, Ahmed T, Rahman VJ, Kareem A (2020) Modulation of plant defense system in response to microbial interactions. Front Microbiol 11:1298. https://doi.org/10.3389/fmicb.2020.01298

O'Brien JA, Vega A, Bouguyon E, Krouk G, Gojon A, Coruzzi G, Gutiérrez RA (2016) Nitrate transport, sensing, and responses in plants. Mol Plant 9(6):837–856. https://doi.org/10.1016/j.molp.2016.05.00

Oláh D, Feigl G, Molnár Á, Ördög A, Kolbert Z (2020) Strigolactones interact with nitric oxide in regulating root system architecture of *Arabidopsis thaliana*. Front Plant Sci 11:1019. https://doi.org/10.3389/fpls.2020.01019

Ortiz-Castro R, Martinez-Trujillo M, Lopez-Bucio J (2008) *N*-acyl-*L*-homoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. Plant Cell Environ 31(10):1497–1509. https://doi.org/10.1111/j.1365-3040.2008.01863.x

Ortiz-Castro R, Díaz-Pérez C, Martinez-Trujillo M, Del Rio RE, Campos-García J, López-Bucio J (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc Natl Acad Sci USA 108:7253–7258. https://doi.org/10.1073/pnas.1006740108

Ortiz-Castro R, Pelagio-Flores M-B, Ruiz-Herrera LF, Campos-García J, López-Bucio J (2014) Pyocyanin, a virulence factor produced by *Pseudomonas aeruginosa*, alters root development through reactive oxygen species and ethylene signaling in *Arabidopsis*. Mol Plant-Microbe Interact 27:364–378. https://doi.org/10.1094/MPMI-08-13-0219-R

Ortiz-Castro R, Campos-García J, López-Bucio J (2020) *Pseudomonas putida* and *Pseudomonas fluorescens* influence *Arabidopsis* root system architecture through an auxin response mediated by bioactive cyclodipeptides. J Plant Growth Regul 39:254–265. https://doi.org/10.1007/s00344-019-09979-w

Ortiz-Castro R, López-Bucio JS, López-Bucio J (2017) Physiological and molecular mechanisms of bacterial phytostimulation. In: Singh HB, Sarma BK, Keswani C (eds) Advances in PGPR research. CABI, ISBN-13: 978 1 78639 032 5

Papenfort K, Bassler B-L (2016) Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol 14:576–588. https://doi.org/10.1038/nrmicro.2016.89

Park BS, Song JT, Seo HS (2011) *Arabidopsis* nitrate reductase activity is stimulated by the E3 SUMO ligase AtSIZ1. Nat Commun 2:400. https://doi.org/10.1038/ncomms1408

Raya-González J, López-Bucio JS, López-Bucio J (2019) Nitric oxide and hydrogen peroxide in root organogenesis. Springer, Cham. https://doi.org/10.1007/978-3-030-11129-8_8

Robinson MD, Oshlack A (2010) A scaling normalization method for differential expression analysis of RNA-Seq data. Genome Biol 11:R25. https://doi.org/10.1186/gb-2010-11-3-r25

Robinson MD, McCarthy D-J, Smyth G-K (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616

Robinson MA, Rau A, Aubert J, Hennequet-Antier C, Jeanmougin M, Servant N, Keime C, Marot G, Castel D, Estelle J, Guernec G, Jagla B, Jouneau L, Laloë D, Le Gall C, Schaëffer B, Le Crom S, Guedj M, Jaffrézic F (2013) A comprehensive evaluation of normalization methods for Illumina high-throughput RNA sequencing data analysis. Brief Bioinform 14:671–683. https://doi.org/10.1093/bib/bbs046

Sakuraba Y, Chaganzhana A, Mabuchi A, Iba K, Yanagisawa S (2021) Enhanced NRT1.1/NPF6.3 expression in shoots improves growth under nitrogen deficiency stress in Arabidopsis. Commun Biol 4(1):256. https://doi.org/10.1038/s42003-021-01775-1

Schofield RA, Bi YM, Kant S, Rothstein SJ (2009) Over-expression of STP13, a hexose transporter, improves plant growth and nitrogen use in *Arabidopsis thaliana* seedlings. Plant Cell Environ 32(3):271–285. https://doi.org/10.1111/j.1365-3040.2008.01919.x

Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Nath U, Weigel D (2008) Control of jasmonate biosynthesis and senescence by miR319 targets. PLoS Biol 6(9):e230. https://doi.org/10.1371/journal.pbio.0060230

Solis-Ortiz CS, Gonzalez-Bernal J, Kido-Díaz HA, Peña-Uribe CA, López-Bucio JS, López-Bucio J, Guevara-García ÁA, García-Pineda E, Villegas J, Campos-García J, Reyes de La Cruz H (2022) Bacterial cyclodipeptides elicit *Arabidopsis thaliana* immune responses reducing the pathogenic effects of *Pseudomonas aeruginosa* PAO1 strains on plant development. J Plant Physiol 275:153738. https://doi.org/10.1016/j.jplph.2022.153738

Stein M, Dittgen J, Sánchez-Rodríguez C, Hou BH, Molina A, Schulze-Lefert P, Lipka V, Somerville S (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 18(3):731–746. https://doi.org/10.1105/tpc.105.038372

Subramoni S, Muzaki MZBM, Booth SCM, Kjelleberg S, Rice SA (2021) *N*-acyl homoserine lactone-mediated quorum sensing regulates species interactions in multispecies biofilm communities. Front Cell Infect Microbiol 11:646991. https://doi.org/10.3389/fcimb.2021.646991

Tsay Y-F, Schroeder J-I, Feldmann K-A, Crawford N-M (1993) The herbicide sensitivity gene CHL1 of *Arabidopsis* encodes a nitrate-inducible nitrate transporter. Cell 72:705–713

von Rad U, Klein I, Dobrev PI, Kottova J, Zazimalova E, Fekete A, Hartmann A, Schmitt-Kopplin P, Durner J (2008) Response of *Arabidopsis thaliana* to *N*-hexanoyl-DL-homoserine-lactone, a bacterial quorum sensing molecule produced in the rhizosphere. Planta 229:73–85. https://doi.org/10.1007/s00425-008-0811-4

Walker TS, Bais HP, Déziel E, Schweizer HP, Rahme LG, Fall R, Vivanco JM (2004) Pseudomonas aeruginosa-plant root interactions. Pathogenicity, biofilm formation, and root exudation. Plant Physiol 134(1):320–331. https://doi.org/10.1104/pp.103.027888

Wang R, Liu D, Crawford N-M (1998) The *Arabidopsis* CHL1 protein plays a major role in high-affinity nitrate uptake. Proc Natl Acad Sci USA 95:15134–15139. https://doi.org/10.1073/pnas.95.25.15134

Wang Y-Y, Hsu P-K, Tsay Y-F (2012) Uptake, allocation and signaling of nitrate. Trends Plant Sci 17:458–467. https://doi.org/10.1016/j.tplants.2012.04.006

Wang W, Hu B, Li A, Chu C (2020) NRT1.1s in plants: functions beyond nitrate transport. J Exp Bot 71(15):4373–4379. https://doi.org/10.1093/jxb/erz554

Weir TL, Stull VJ, Badri D, Trunck LA, Schweizer HP, Vivanco J (2008) Global gene expression profiles suggest an important role for nutrient acquisition in early pathogenesis in a plant model of *Pseudomonas aeruginosa* infection. Appl Environ Microbiol 74:5784–5791. https://doi.org/10.1128/AEM.00860-08

Windram O, Madhou P, McHattie S et al (2012) *Arabidopsis* defense against *Botrytis cinerea*: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell 24(9):3530–3557. https://doi.org/10.1105/tpc.112.102046

Xie YD, Li W, Guo D, Dong J, Zhang Q, Fu Y, Ren D, Peng M, Xia Y (2010) The Arabidopsis gene SIGMA FACTOR-BINDING PROTEIN 1 plays a role in the salicylate- and jasmonate-mediated defence responses. Plant Cell Environ 33(5):828–839. https://doi.org/10.1111/j.1365-3040.2009.02109.x

Ye JY, Tian WH, Jin CW (2022) Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation. Stress Biol 2:4. https://doi.org/10.1007/s44154-021-00030-1

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CM (2013) Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. Plant Physiol 162(1):304–318. https://doi.org/10.1104/pp.112.212597

Zeidler D, Zähringer U, Gerber I, Dubery I, Hartung T et al (2004) Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci 101(44):15811–15816. https://doi.org/10.1073/pnas.0404536101

Zhang B, Van Aken O, Thatcher L, De Clercq I, Duncan O, Law SR, Murcha MW, van der Merwe M, Seifi HS, Carrie C, Cazzonelli C, Radomiljac J, Höfte M, Singh KB, Van Breusegem F, Whelan J (2014) The mitochondrial outer membrane AAA ATPase AtOM66 affects cell death and pathogen resistance in *Arabidopsis thaliana*. Plant J 80(4):709–727. https://doi.org/10.1111/tpj.12665

8.4. Capítulo IV

Microbial Ecology
https://doi.org/10.1007/s00248-022-02080-y

PLANT MICROBE INTERACTIONS

Screening of Phosphate Solubilization Identifies Six *Pseudomonas* Species with Contrasting Phytostimulation Properties in *Arabidopsis* Seedlings

José López-Hernández[1] · Elizabeth García-Cárdenas[1] · Jesús Salvador López-Bucio[2] · Kirán Rubí Jiménez-Vázquez[1] · Homero Reyes de la Cruz[1] · Ofelia Ferrera-Rodríguez[3] · Dulce Lizbeth Santos-Rodríguez[3] · Randy Ortiz-Castro[4] · José López-Bucio[1]

Received: 21 March 2022 / Accepted: 15 July 2022

Abstract
The interaction of plants with bacteria and the long-term success of their adaptation to challenging environments depend upon critical traits that include nutrient solubilization, remodeling of root architecture, and modulation of host hormonal status. To examine whether bacterial promotion of phosphate solubilization, root branching and the host auxin response may account for plant growth, we isolated and characterized ten bacterial strains based on their high capability to solubilize calcium phosphate. All strains could be grouped into six *Pseudomonas* species, namely *P. brassicae*, *P. baetica*, *P. laurylsulfatiphila*, *P. chlororaphis*, *P. lurida*, and *P. extremorientalis* via 16S rRNA molecular analyses. A *Solibacillus isronensis* strain was also identified, which remained neutral when interacting with *Arabidopsis* roots, and thus could be used as inoculation control. The interaction of *Arabidopsis* seedlings with bacterial streaks from pure cultures in vitro indicated that their phytostimulation properties largely differ, since *P. brassicae* and *P. laurylsulfatiphila* strongly increased shoot and root biomass, whereas the other species did not. Most bacterial isolates, except *P. chlororaphis* promoted lateral root formation, and *P. lurida* and *P. chlororaphis* strongly enhanced expression of the auxin-inducible gene construct *DR5:GUS* in roots, but the most bioactive probiotic bacterium *P. brassicae* could not enhance the auxin response. Inoculation with *P. brassicae* and *P. lurida* improved shoot and root growth in medium supplemented with calcium phosphate as the sole Pi source. Collectively, our data indicate the differential responses of *Arabidopsis* seedlings to inoculation with several *Pseudomonas* species and highlight the potential of *P. brassicae* to manage phosphate nutrition and plant growth in a more eco-friendly manner.

Keywords Phosphate-Solubilizing Bacteria · *Pseudomonas* spp · Plant Growth Promotion · Root Branching · *Arabidopsis thaliana*

✉ José López-Bucio
jbucio@umich.mx

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C. P. 58030 Morelia, Michoacán, México

[2] Catedrático CONACYT-Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C. P. 58030 Morelia, Michoacán, México

[3] Instituto de Ecología, Red de Estudios Moleculares Avanzados, Clúster BioMimic®, Carretera Antigua a Coatepec 351, El Haya, A.C 91073 Veracruz, México

[4] Catedrático CONACYT-Instituto de Ecología, Red de Estudios Moleculares Avanzados, Clúster BioMimic®, Carretera Antigua a Coatepec 351, El Haya, A.C 91073 Veracruz, México

Introduction

Plants interact with bacteria in many ways and depend on their microbial partners to grow and resist environmental challenges. Among the several bacterial properties that account for an efficient phytostimulation, nutrient solubilization, modification of root growth and branching, and modulation of the hormonal status through production of metabolites and endogenous plant hormones may lead to an improved biomass production [1–4].

Among the essential macronutrients, phosphate (P_i) availability critically determines plant success in natural and agricultural ecosystems. In acid soils, P_i combines with aluminum and iron; whereas in alkaline-calcareous soils, P_i binds to calcium; in both cases, the corresponding cations precipitate P_i,

Published online: 22 July 2022

making it unavailable for uptake by roots [5]. The potential of the bacterial microbiota to assist plants to more efficiently solubilize phosphate may be a highly desirable trait to improve not only plant nutrition, but also to optimize fertilizer usage and plant production in the long term [6, 7]. However, how phosphate-solubilizing bacteria may influence plant processes such as root growth and morphogenesis and their possible effects on plant hormonal status remain largely unknown.

Bacteria may produce quorum-sensing signals, secondary metabolites, and endogenous plant hormones, all of which contribute to root growth and branching [2, 8]. The production of auxin or molecules that mimic auxin action impairs primary root growth while improving root branching, and it appears to be the major mechanism by which *P. aeruginosa*, *P. putida*, and *P. fluorescens* affect plant growth and development [9, 10]. Comparable results have also been obtained in interactions with *Azospirillum brasilense* [11, 12] and *Micrococcus luteus* [13], which repress cell division and elongation of the primary root while triggering more pericycle cells to produce lateral roots that further extend the root absorptive potential.

In a recent study of the Gram negative bacteria *Achromobacter* sp. 5B1 that inhabits the rhizosphere of mesquite plants in a saline soil, root colonization could promote primary root growth, deviated the growth direction making waves and coils, and enhanced the initiation of lateral roots. These effects were attributed to auxin redistribution at the root meristem [14]. The slow root movements driven by the bacteria not only helped plants to better resist salt stress, but also contributed to an enhanced biomass production [14]. These results suggest that auxin transport and signaling are influenced by bacterial traits, which change root coiling and waving.

In this report, we regarded the P_i-solubilizing trait as a major bacterial property potentially responsible for plant growth promotion. We employed plant-bacteria co-cultivation systems to assess its relevance to improved *Arabidopsis* biomass production and altered auxin-directed root morphogenesis. In media designed to support optimal seedling growth or using calcium phosphate as the only P_i source, six bacterial species belonging to the *Pseudomonas* genus were discovered, which differ in their phytostimulation properties and their regulation of root branching irrespective of their common P_i-solubilizing attributes. Moreover, we identified *P. brassicae* as a plant growth-promoting species that boosts plant biomass production and growth in medium with sparingly available phosphate.

Materials and Methods

Plant Growth and Development

Arabidopsis thaliana wild-type Columbia-0 (Col-0) and transgenic line *DR5:uidA* [15] were used for experiments. *Arabidopsis* seeds were superficially disinfected with ethanol 96% (v/v) for 7 min, followed by chlorine 20% (v/v) for 7 min and five rinses with sterilized distilled water. Seeds were stored at 4 °C for 48 h. Then, disinfected seeds were germinated and grown over the surface of 0.2 × Murashige and Skoog (MS) agar medium [16] in Petri plates, which were incubated vertically in a Percival growth chamber (Percival Scientific AR95L) at 22 °C, with 80% humidity, 16-/8-h light/darkness photoperiod, and light intensity of 100 µmol m^{-2} s^{-1}. Four days after germination, the Petri plates were opened and the seedlings were transferred onto fresh 0.2 × MS agar medium, previously streaked with pure cultures of each bacterial isolate under axenic conditions. The primary root was placed over the streak and biomass and root architecture analyzed 6 days after transfer. To assess growth of plants with sparingly soluble phosphate, the potassium phosphate from the original recipe was replaced by 1 mM calcium phosphate. Plant growth and developmental parameters were recorded 15 days after transfer over the bacterial streak and included measurements of primary and lateral root length using a ruler, whereas the lateral root number was counted using a Leica L2 stereomicroscope. The fresh weight of root and shoot systems was quantified using an OHAUS Adventurer^{tm} analytical scale.

Bacterial Isolation

To obtain and identify the pure bacterial cultures, 100 g of soil was collected from 4 different sites showing different degrees of perturbation, near the community of Ajuno, Michoacán state, México (19°30′46″N, 101°43′20″´W). The sampled zones included an avocado farmland, a preserved pine forest, a preserved oak forest, and a soil patch affected by forest fires. We also sampled earthworm humus enriched with disposed domestic residual fruit peels and vegetables, this latter as an organic, nutrient rich substrate that in principle would increase bacterial performance. All samples were stored at 4 °C. Serial dilutions were performed with 1 g of soil or substrate in 9 ml of distilled, sterilized water. A sample of 100 µl was taken from each dilution and streaked into Petri dishes containing Luria Bertani (LB) solid medium at 30 °C. The isolated colonies were collected and streaked into LB media at 30 °C for 72 h. Finally, pure cultures of the isolated bacteria were conserved in glycerol 20% (v/v) and liquid LB medium at − 80 °C.

Phosphate-Solubilizing Assay

The phosphate-solubilizing capacity of the bacterial isolates was assayed in pure bacterial cultures pre-grown overnight in Luria–Bertani (LB) medium and then cultured on Pikovskaya-bromophenol blue (PKV-BMP) medium at 0.025 OD600 ($n = 3$) according to Tzec-Interian

et al. (2020) [17]. The cultures were incubated at 30 °C, 180 rpm for 48 h, harvested by centrifugation at 8000 rpm for 5 min, and the supernatant analyzed at 590 nm by using an Epoch2 (Biotek) spectrophotometer. The experiment was repeated twice and included six biological replicates each.

Molecular Characterization and Phylogenetic Analysis

Extraction of genomic DNA from pure bacterial cultures was done following a standardized protocol [17]. The 16S rRNA gene was amplified with forward and reverse primers 27F (3′-AGAGTTTGATCCTGGCTCAG-5′) and 1492R (3′-TAC CTTGTTACGACTT-5′). The amplicons were sequenced in the LABSERGEN capillary sequencing service unit of LANGEBIO, CINVESTAV, México. Chimeric sequences, as identified by DECIPHER v2.0 [18], were discarded. The closest homologues of the remaining sequences were identified using the NCBI BLAST algorithm [19] and SILVA rRNA database [20]. The phylogenetic relationships were further confirmed by using the Ribosomal Database Project (RDP) tools [21]. We used the Classifier [22] and the Sequence Match [23, 24] tools. Phylogenetic reconstructions were performed using MEGA7 [25] and the free trial of Geneious, Bioinformatics Software for Sequence Data Analysis (https://www.geneious.com). 16S rRNA sequences obtained from the isolates were deposited in GenBank (Accession numbers: OK031074-OK031083).

Histochemical Determination of GUS Activity

Arabidopsis seedlings expressing the synthetic auxin-responsive promoter *DR5:uidA* were used to study the auxin response in direct root-bacteria interactions. The transgenic seedlings were immersed in a solution of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc) in sodium phosphate 100 mM buffer, pH 7, supplemented with 2 mM $K_4Fe(CN)_6$ and 2 mM $K_3Fe(CN)_6$ at 37 °C and incubated overnight. Tissue clearing was done by placing the seedlings in 2 ml solution with methanol 20% (v/v) and HCl 0.24 N final concentrations, incubating at 60 °C for 45 min; after careful removal of the solution using 1 ml micropipette tip, NaOH 7% was added to cover the seedlings and maintained at room temperature for 30 min, and then the solution was discarded and the seedlings were washed with ethanol 40, 20, and 10% (v/v) for 30 min each, and finally stored with glycerol 50% (v/v) at 4 °C [26]. Plant tissues were fixed on clean glass slides with glycerol 50% (v/v), and representative images were taken using the Nomarsky optics in a Leica DM5000B microscope at 20 × magnifications.

Statistical Analysis

All the treatments were performed in triplicate, and yielded comparable results. Data processing was made through STATISTICA 10 StatSoft program with factorial ANOVA, and Tukey's HSD method. Different letters are used to indicate means that statistically differ ($P < 0.05$).

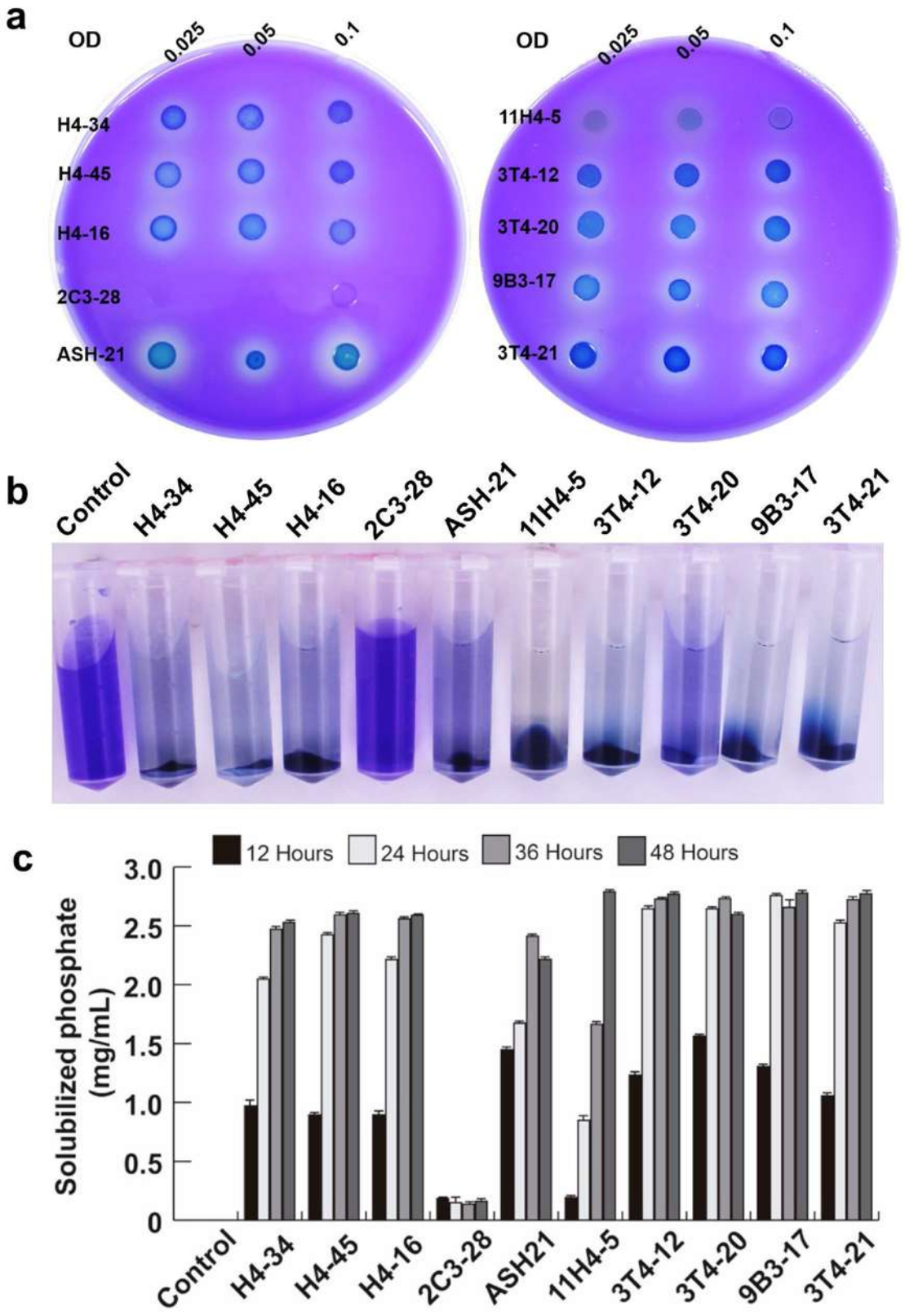
Results

Screening for Phosphate-Solubilizing Bacteria from Different Environments

Phosphate-solubilizing attribute may be desirable for agriculture. To understand whether this property may be found in soil bacteria from different natural environments, we screened 266 bacterial isolates from pure cultures coming from five different environments: an agricultural soil with avocado trees, an undisturbed soil from an oak grove, a soil from a well-conserved pine forest, as well as sampling earthworm humus, and ash from a 2-year-old fire in a forest patch (Table 1). The idea was to collect as much as possible of the bacterial diversity inhabiting all these different environments. From the pure cultures, individual isolates were further selected using Pikovskaya-bromophenol blue (PKB-BMP) medium for screening P_i-solubilizing microorganisms [27, 28]. Nine strains, H4-34, H4-45, H4-16, ASH21, 11H4-5, 3T4-12, 3T4-20, 9B3-17, and 3T4-21, manifested strong phosphate solubilization when cultured on PKV-BMP agar medium at 30 °C for 48 h, as revealed by clear halos surrounding the cultures (Fig. 1a). The isolate 2C3-28 did not show clear halos when compared to the other strains and represents a good control for P_i-solubilizing properties (Fig. 1a). P_i-solubilizing trait was further confirmed in PKV-BMP liquid medium, where all strains except 2C3-28 manifested strong P_i solubilization (Fig. 1b). In time-course

Table 1 Description of bacterial isolates, their predicted species based on 16S rRNA analysis and site of origin

Isolate	Predicted species	Origin
H4-34	*P. brassicae*	Earthworm humus
H4-45	*P. brassicae*	Earthworm humus
H4-16	*P. brassicae*	Earthworm humus
2C3-28	*S. isronensis*	Avocado farmland
ASH-21	*P. lurida*	Soil patch affected by forest fires
11H4-5	*P. extremorientalis*	Earthworm humus
3T4-12	*P. chlororaphis*	Well-preserved oak forest
3T4-20	*P. chlororaphis*	Well-preserved oak forest
9B3-17	*P. laurylsulfatiphila*	Well-preserved pine forest
3T4-21	*P. baetica*	Well-preserved oak forest


a
OD
0.025
0.05
0.1
H4-34
H4-45
H4-16
2C3-28
ASH-21
OD
0.025
0.05
0.1
11H4-5
3T4-12
3T4-20
9B3-17
3T4-21
b
Control
H4-34
H4-45
H4-16
2C3-28
ASH-21
11H4-5
3T4-12
3T4-20
9B3-17
3T4-21
c
12 Hours
24 Hours
36 Hours
48 Hours
Solubilized phosphate (mg/mL)
3.0
2.5
2.0
1.5
1.0
0.5
0
Control
H4-34
H4-45
H4-16
2C3-28
ASH21
11H4-5
3T4-12
3T4-20
9B3-17
3T4-21

◂**Fig. 1** The effects of bacterial isolates on phosphate solubilization. **a** Bacterial isolates were cultured on PKV-BMP medium supplemented with agar at 30 °C for 48 h. **b** Bacterial isolates were cultured on PKV-BMP liquid medium starting at 0.025 OD_{600} at 30 °C, 180 rpm for 48 h. **c** Solubilized phosphate. The data represent the mean ± SD ($n = 3$). The experiment was repeated twice with similar results

analyses, P_i solubilization started at 12 h and was maximum after 36 h (Fig. 1c). These data evidence the P_i-solubilizing properties of the bacteria isolated from the above-mentioned environments.

Molecular Characterization of Highly Efficient Phosphate-Solubilizing Bacteria

In order to identify the different isolates, including the poorly P_i-solubilizing strain 2C3-28, their 16S rRNA genes were sequenced and compared with public database sequences to establish phylogenetic relationships. The closest homologues for Pi-solubilizing strains were from *Pseudomonas brassicae* (H4-34, H4-45, and H4-16 isolates), *Pseudomonas lurida* (ASH21), *Pseudomonas extremorientalis* (11H4-5), *Pseudomonas chlororaphis* (3T4-12 and 3T4-20), *Pseudomonas laurylsulfatiphila* (9B3-17), and *Pseudomonas baetica* (3T4-21) (Fig. 2a), whereas 2C3-28 was close to *Solibacillus isronensis* (Fig. 2b). From the tree illustration, there is an *S. kalamii* and an *S. silvestris* sequence equally close to *S. isronensis*. It was of interest that all Pi-solubilizing strains belong to the genus *Pseudomonas* (Fig. 2a), suggesting the ubiquity of this trait amongst this group.

Arabidopsis Biomass Production upon Root Contact with Bacterial Streaks

To investigate if the already isolated bacteria could promote plant growth along with their high Pi-solubilizing capability [29], *Arabidopsis* seedlings were germinated and grown for 5 days on agar plates supplemented with 0.2 × MS agar medium and then transferred over single bacterial streaks from each bacterial species and incubated for six additional days. Notably, seedlings in contact with *P. brassicae* and *P. laurylsulfatiphila* affiliates had 2-to 3.5-fold greater shoot biomass than seedlings not in contact with bacterial streaks, whereas the isolates related to *P. chlororaphis*, *P. lurida*, *P. extremorientalis*, *P. baetica*, and *Solibacillus isronensis* had no significant effect (Fig. 3a). For root biomass production, *P. brassicae*, *P. extremorientalis*, and *P. laurylsulfatiphila* affiliates were inducers, whereas the other isolates were not (Fig. 3b). Thus, an enhanced capability to solubilize Pi did not correlate with plant growth promotion in a medium where nutrient supplements are adequate for plant growth.

Regulation of Root Architecture upon Root Contact with Bacterial Streaks

The spread of bacteria over the root system could explain the different results among strains, and possibly may configure root growth and branching [13, 14]. We assessed root system traits including primary root length, lateral root number, lateral root density, and lateral root length in bacterized seedlings six days after contact with streaks. Six *Pseudomonas* isolates repressed primary root growth by 50–60% (Fig. 4a), while increasing root branching (Fig. 4a,b). *P. baetica*, *P. laurylsulfatiphila*, and *P. brassicae* were the most bioactive species; plants incubated with these reached fourfold to sixfold higher lateral root number and density than uninoculated seedlings (Fig. 4b,c). *S. isronensis* did not affect any of the above-mentioned plant traits and *P. chlororaphis* strongly repressed overall root system architecture development. Figure 5 shows representative images of *Arabidopsis* seedlings grown axenically in the 0.2 × MS agar medium, and transferred or not over bacterial streaks of *P. brassicae*, *P. lurida*, and *P. chlororaphis* after 6 days of interaction, which highlights the highly contrasting effects of bacterial inoculation.

Induction of Auxin-Responsive Gene Expression by Selected Phosphate-Solubilizing Bacteria

Suppression of primary root growth and enhancement of lateral root formation is a common effect of inoculation with auxin-producing bacteria, for instance *Azospirillum brasilense* and *Micrococcus luteus* [11, 13]. To analyze if the changes in root architecture driven by Pi-solubilizing *Pseudomonas* species could be explained by an enhanced auxin response; *Arabidopsis* seedlings expressing the auxin-inducible reporter gene *DR5:GUS* were inoculated with *P. brassicae*, *P. lurida*, and *P. chlororaphis*, which manifested the most contrasting effects in the plant. Unexpectedly, the strong growth promotion elicited by *P. brassicae* did not correlate with an enhanced histochemical detection of GUS activity (Fig. 6a-d), while seedlings co-cultivated with *P. lurida* and *P. chlororaphis* had a strong induction of the marker over the entire root system (Fig. 6e-h). These results show that although the different bacterial isolates share the property to solubilize phosphate, they clearly differ in modulating auxin-inducible gene expression in the plant host.

Plant Growth Promotion by Selected Phosphate-Solubilizing Bacteria in Medium with Calcium Phosphate

Most agricultural soils are characterized by poor phosphate availability, which can be explained by the strong fixation of this macronutrient with cations. In alkaline-calcareous

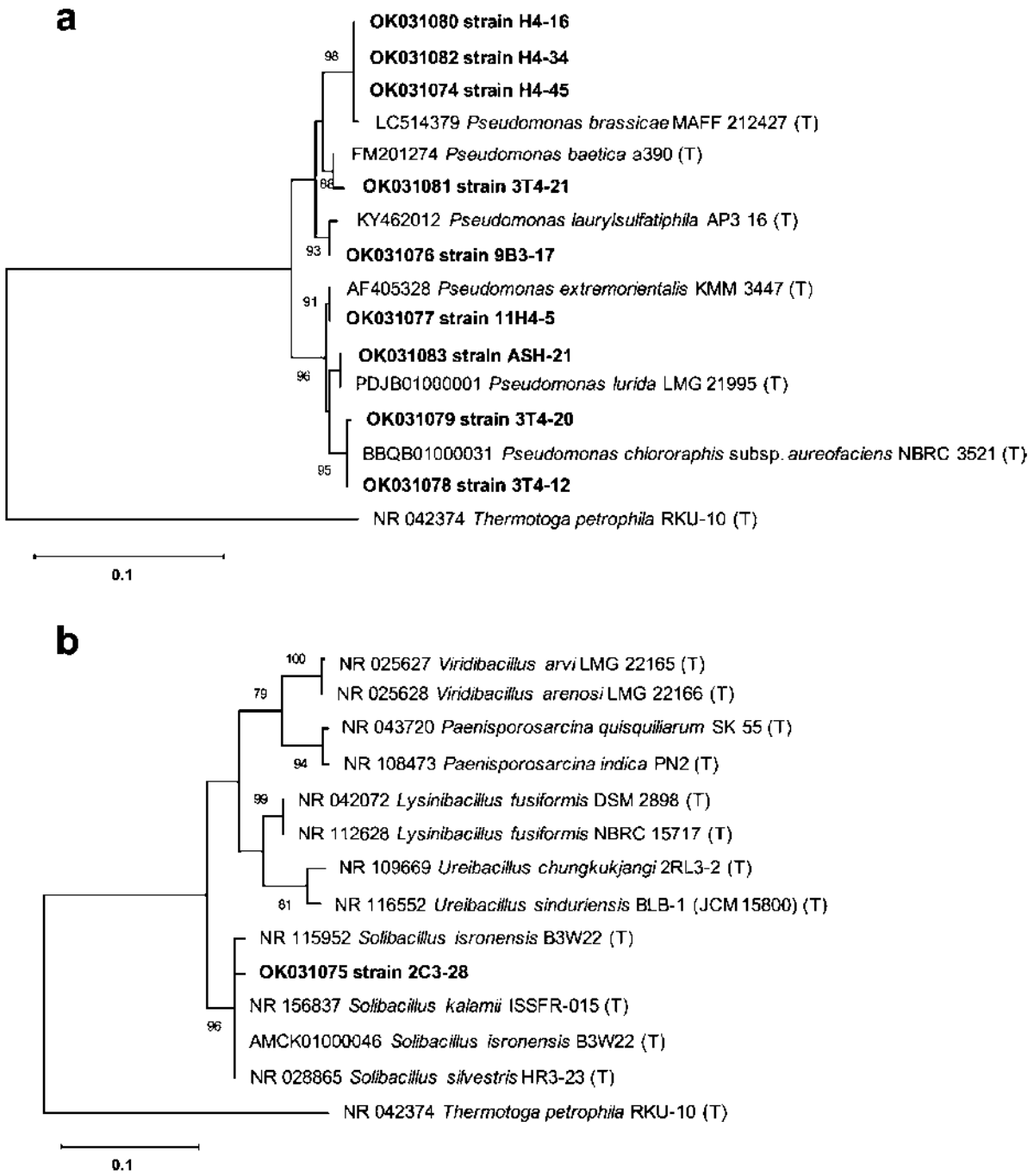


Fig. 2 Phylogenetic tree based on 16S rRNA gene sequences of bacterial strains and additional references were generated using the maximum likelihood method. **a** *Pseudomonas* cluster. **b** *Solibacillus isronensis*. Bootstrap values from 1000 replications are shown as percentages at branch points. 16S rRNA sequence of NR 042,374 *Thermotoga petrophila* was used as the outgroup. Scale bar 0.1 substitutions per site

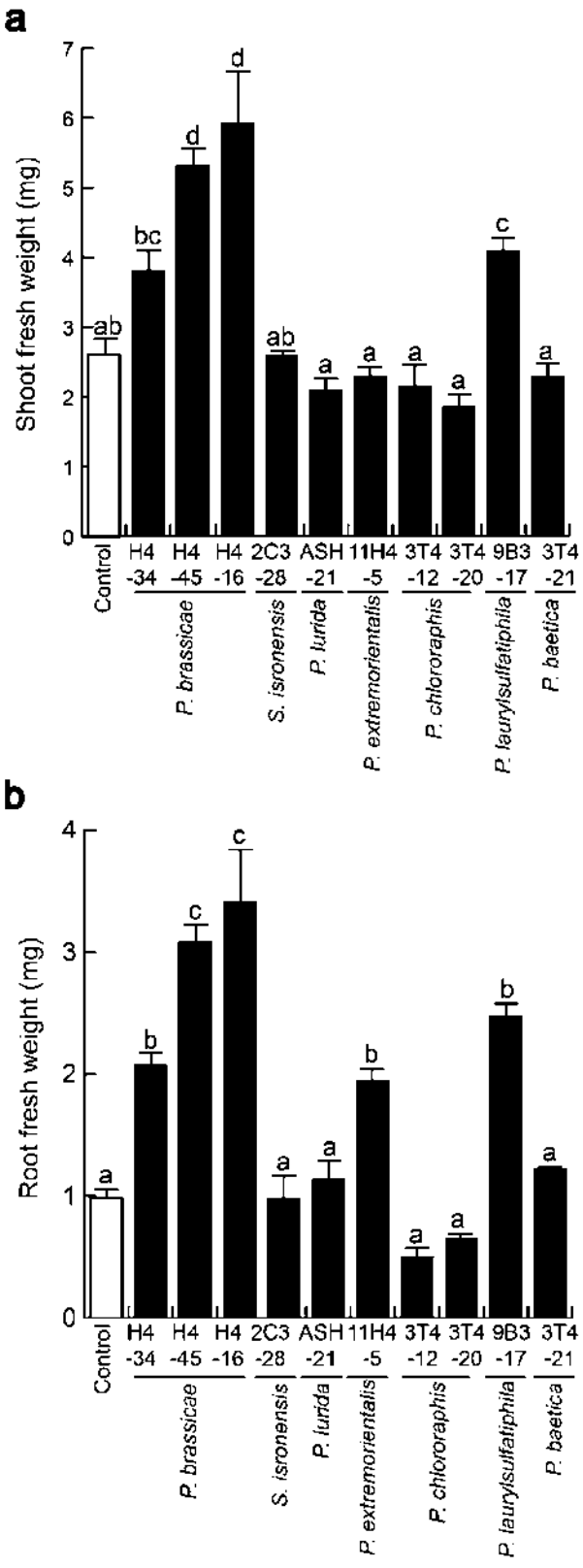
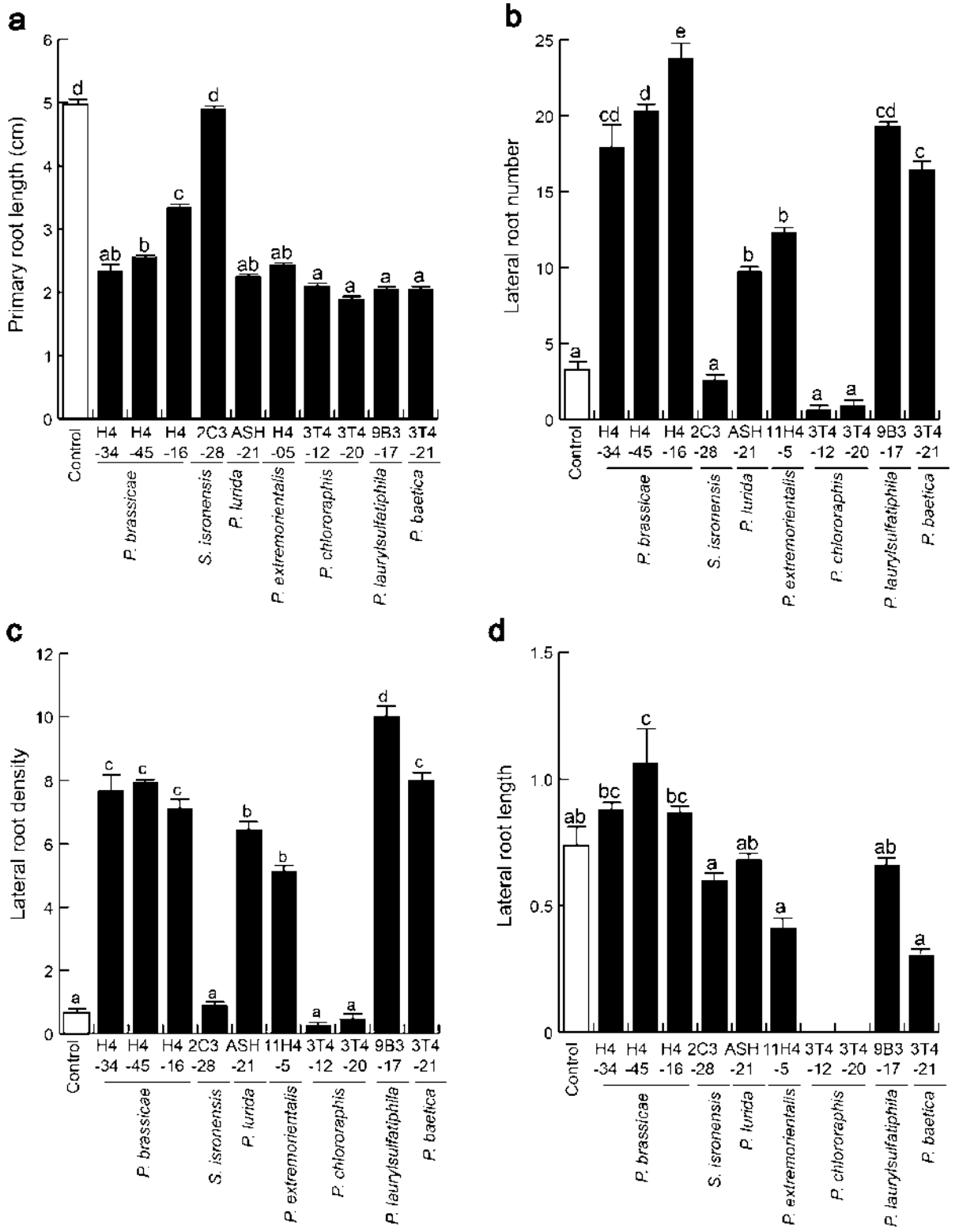


Fig. 3 The effect of phosphate-solubilizing bacteria on *Arabidopsis* seedling biomass production. **a** Shoot fresh weight. **b** Root fresh weight. Data points indicate mean ± SD ($n = 18$). Different letters mean statistically significant differences at $p < 0.05$. Tukey post hoc $p < 0.05$


a
Primary root length (cm)
b
Lateral root number
c
Lateral root density
d
Lateral root length
Control
H4 -34
H4 -45
H4 -16
2C3 -28
ASH -21
H4 -05
11H4 -5
3T4 -12
3T4 -20
9B3 -17
3T4 -21
P. brassicae
S. isronensis
P. lurida
P. extremorientalis
P. chlororaphis
P. laurylsulfatiphila
P. baetica

◂**Fig. 4** Root system architecture of bacterized seedlings. Changes in root architecture were registered 6 days after bacterial exposure. **a** Primary root length. **b** Lateral root number. **c** Lateral root density. **d** Lateral root length. Data points indicate mean ± SD ($n = 18$). Different letters mean statistical differences. Tukey post hoc with $p < 0.05$

soils, P_i binds calcium to form sparingly soluble minerals [30]. To analyze if the root inoculation with P_i-solubilizing *Pseudomonas* or non Pi-solubilizing *Solibacillus isronensis* could provide an advantage to plants grown in medium supplemented with poorly soluble calcium phosphate as the sole P_i source, the basal MS 0.2 × medium was modified via substitution of highly soluble potassium phosphate by low soluble calcium phosphate and growth and development of *Arabidopsis* seedlings was examined. The growth of the un-inoculated seedlings or seedlings inoculated with *S. isronensis* in the calcium phosphate medium was limited and the shoot and root growth was drastically compromised (Fig. 7a,b). By contrast, inoculation with *P. brassicae* or *P. lurida*, but not *P. chlororaphis*, restored shoot and root growth, indicating their potential to help plants to grow and utilize a sparingly available P_i source (Fig. 7c-e). Comparison of primary root growth suggested that both *P. brassicae* and *P. lurida* were equally efficient in supporting P_i usage (Fig. 7f). These data unveil the contrasting potential of P_i-solubilizing *Pseudomonas* as plant growth promoters under stressful conditions.

Discussion

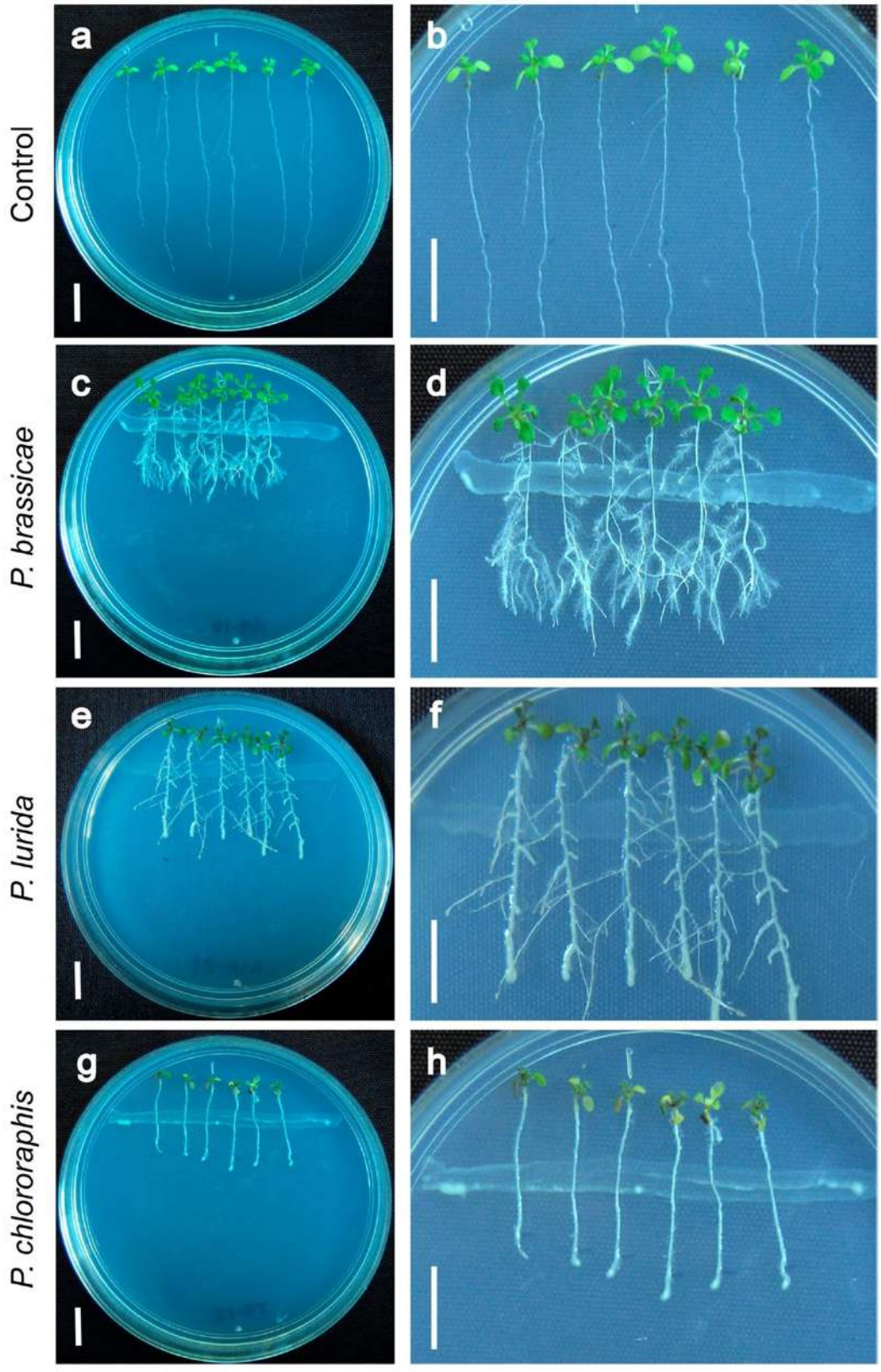
To gain further insights into the correlation of P_i solubilization with other important traits that support plant growth, such as root architecture remodeling, endogenous auxin response and ultimately, growth of plants under sparingly soluble phosphate, we screened a collection of 266 bacterial isolates from diverse environments to select strains with high P_i-solubilizing properties. Our analysis identified nine isolates that were grouped into six different species all belonging to the *Pseudomonas* genus. We also identified a strain close to *Solibacillus isronensis*, a bacterial species with poor P_i-solubilizing capacity, which was used as a control to compare with seedlings grown aseptically.

It was not really surprising that the isolates H4-34, H4-45, H4-16, ASH21, 11H4-5, 3T4-12, 3T4-20, 9B3-17, and 3T4-21, which manifested strong phosphate-solubilizing property when cultured on PKV-BMP media, all corresponded to *Pseudomonas* species, such as *P. brassicae* (H4-34, H4-45, H4-16), *P. lurida* (ASH21), *P. extremorientalis* (11H4-5), *P. chlororaphis* (3T4-12, 3T4-20), *P. laurylsulfatiphila* (9B3-17), and *P. baetica* (3T4-21). Consistent with our screening and 16S rRNA identification, previous reports demonstrated the Pi-solubilizing capability of *Pseudomonas* species and their probiotic effects when assayed in interactions with plants under varied growth conditions, ranging from growth chambers to the field, using model plants and crops. For instance, *P. putida* (SP21 and SP22) promoted shoot and root development in soybean [31]. *P. corrugata* improved growth of wheat under greenhouse conditions [32], and *P. proteolytica* and *P. azotoformans* increased rosette diameter, leaf area, and biomass of *Arabidopsis* grown in a growth chamber [33]. *Pseudomonas* spp. improved plant growth in the field, under moderate and high Pi levels, thus augmenting the use of fertilizers [34–36]. For endophytic *Pseudomonas*, the Pi-solubilizing properties rely at least in part on gluconic acid (GA) [37].

To gain further insight into the plant probiotic properties of P_i-solubilizing *Pseudomonas* species, the roots of *Arabidopsis* seedlings were placed over bacterial streaks in MS 0.2 × medium, which provides readily available nutrients. *P. brassicae* and *P. laurylsulfatiphila* strongly promoted shoot and root growth whereas inoculation with *P. lurida*, *P. extremorientalis*, *P. baetica, P. chlororaphis*, or *Solibacillus isronensis* did not enhance shoot or root biomass relative to un-inoculated controls. Moreover, detailed analysis of primary root growth and branching indicated that amongst the newly identified Pi-solubilizing *Pseudomonas* species, all of them repressed root growth by 50–60% while increasing lateral root number and density fourfold to sixfold, with *P. baetica*, *P. laurylsulfatiphila*, and *P. brassicae* being the most bioactive species. Inoculation with *P. brassicae* H4-45 also led to the production of longer lateral roots than in un-inoculated seedlings, suggesting that its beneficial effects promoting root branching may directly improve plant biomass through better exploration of the substrate. Interestingly, *P. chlororaphis* inhibited root system development despite its good P_i-solubilizing properties.

In our plant-bacteria interaction system, the "bacterial streaks" methodology used enabled co-cultivation of many plants with the inoculum at the same time. However, it is not easy to explain how the morphological changes of roots in areas where there is no direct contact with the bacterial streak occur. In this regard, motility systems have been reported for *Pseudomonas* species. In the case of *P. aeruginosa*, a polar flagellum and type 4 pili allow initial attachment of the bacteria, with additional steps required for colonization [38]. Alternatively, spread of diffusible metabolites and/or volatiles from the bacterial streak may induce the development of lateral roots and root hairs above and below the bacterial streaks. To the best of our knowledge, how bacterial motility influences root behaviors has not been investigated and remains as an important aspect that deserves attention.

Several species specific factors may contribute to plant biomass accumulation. *Pseudomonas* is one of the most common and thoroughly investigated bacterial genera, which


Control
P. brassicae
P. lurida
P. chlororaphis
a
b
c
d
e
f
g
h

◂**Fig. 5** Phenotypes of *Arabidopsis* seedlings inoculated with selected phosphate-solubilizing *Pseudomonas* species. Representative images demonstrate different biostimulation categories of seedlings 6 days after transfer over bacterial streaks (**a**, **b**). Non-inoculated seedlings (**c**, **d**). *P. brassicae* (**e**, **f**). *P. lurida* (**g**, **h**). *P. chlororaphis*. Scale bar = 1 cm, $n = 18$. Different letters mean statistical differences. Tukey post hoc with $p < 0.05$

includes both plant growth-promoting (e.g. *P. putida* and *P. fluorescens*) and -pathogenic (e.g., *P. aeruginosa*) species. From our results, *P. baetica*, *P. laurylsulfatiphila*, and *P. brassicae* can be regarded as plant growth promoters, whereas *P. chlororaphis* behaved as a growth repressing bacterium, probably due to the production of as yet unidentified metabolites, antibiotics, and/or virulence factors. Very recently, Raio and Puopolo (2021) noted that the *P. fluorescens* complex contains at least eight phylogenetic groups and each of these includes several species sharing ecological and physiological traits [39]. According to the authors, *P. chlororaphis* belongs to a separate group, and these bacteria produce metabolites that are toxic to other organisms, including

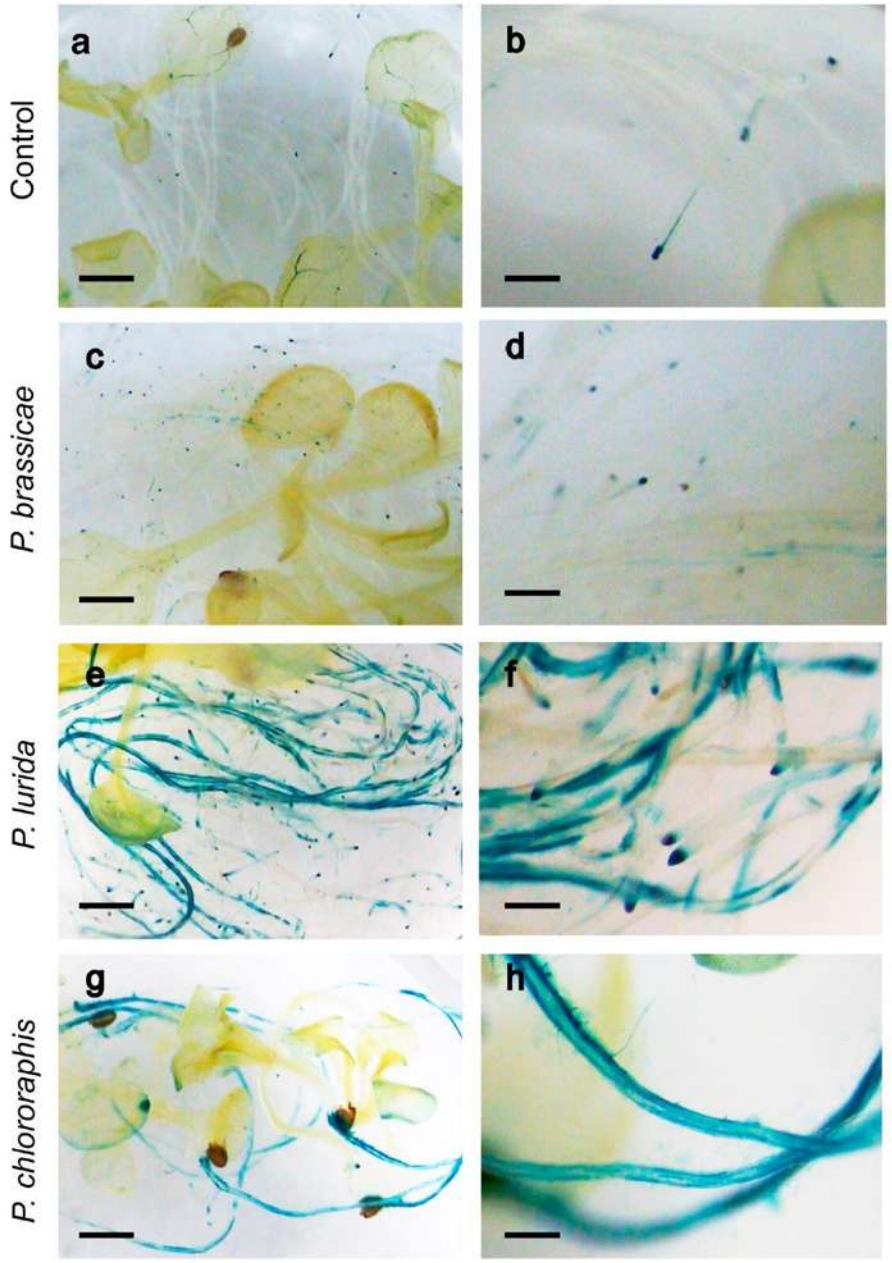


Fig. 6 Auxin-responsive *DR5:uidA* expression pattern in shoot and root of *Arabidopsis* seedlings. Seedlings expressing the *DR5:uidA* marker were placed over streaks of selected *Pseudomonas* species (**a**, **b**). Basal GUS expression in non-inoculated seedlings (**c**, **d**). The promoting effects by *P. brassicae* showed a similar expression with the control (**e**–**h**). *P. lurida* and *P. chlororaphis* strongly increased the auxin-inducible expression in the root system. Scale bar = 1 mm, $n = 18$. Different letters mean statistically significant differences. Tukey post hoc with $p < 0.05$

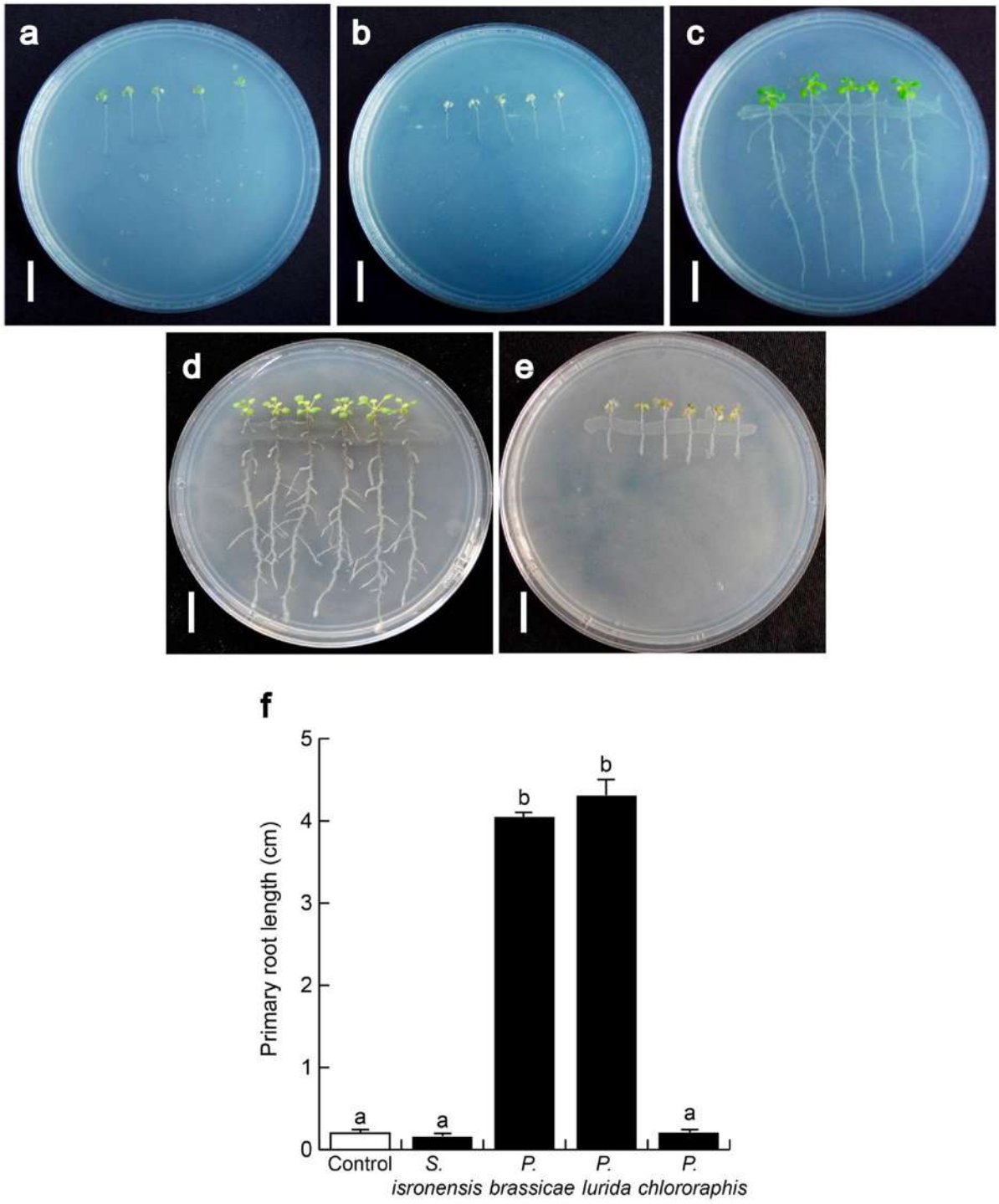


Fig. 7 *P. brassicae* and *P. lurida* improve plant growth in medium supplemented with sparingly soluble phosphate source. The MS 0.2× solid media was modified replacing potassium phosphate with calcium phosphate. **a** Un-inoculated seedlings **b** *S. isronensis*, **c** *P. brassicae*, **d** *P. lurida*, **e** *P. chlororaphis*, **f** Primary root length. Scale bar = 1 cm. Data points indicate mean ± SD ($n = 18$). Different letters mean statistical differences. Tukey post hoc with $p < 0.05$

antibiotics such as phenazines, pyrrolnitrine, 2-hexyl, 5-propyl resorcinol, and hydrogen cyanide; siderophores such as pyoverdine and achromobactine; and a complex blend of volatile organic compounds (VOCs) that effectively contribute to the control of several plant pathogens, nematodes, and insects. Although in a field situation the number of possible interactions is much higher, for plants grown in vitro the release of siderophores and VOCs may directly account for repression of plant growth or may behave as auxin signal mimics. Our data, certainly, contribute to the understanding of *P. chlororaphis*-plant interactions.

Alterations in auxin homeostasis have been considered a critical factor in the phytostimulation driven by *Azospirllum brasilense* and *Micrococcus luteus,* which interact with roots and increase root expansion by means of root branching [11, 13]. Comparable effects have been reported for *Pseudomonas putida*, *Pseudomonas fluorescens*, and the plant pathogen *Pseudomonas aeruginosa*, which strongly repress primary root growth through the production of cyclodipeptides that mimic auxin action [9, 10]. This was not the case for *P. chlororaphis*, which despite strongly inducing the auxin-responsive reporter gene *DR5:GUS* failed to enhance the production of more lateral roots. In contrast, *P. brassicae* strongly increased root branching and length of the lateral roots, but could not induce the auxin-responsive reporter gene. The seedlings co-cultivated with *P. lurida* or *P. chlororaphis* had a strong induction of the marker over the entire root system, indicating its participation in the molecular communication established with *Arabidopsis* through an auxinic mechanism. These results show that although different *Pseudomonas* species share the property to solubilize phosphate, the suppression of primary root growth can apparently occur by more than one mechanism.

Phosphate is a critical macronutrient that influences both auxin synthesis or auxin sensitivity with an important role in root architecture responses of plants, drives plant-bacteria interaction, and modulates the composition of root exudates as well as the diversity of the microbiome [40–43]. The root-inhabiting bacteria may contribute to plant mineral nutrient homeostasis by releasing organic acids, protons, and chelating molecules that release P_i from poorly soluble minerals and allow its translocation to photosynthetic and reproductive tissues. To determine if root inoculation with P_i-solubilizing bacteria could provide an advantage to plants grown in medium supplemented with calcium phosphate, the impact of the inoculation of *Arabidopsis* seedlings with *P. brassicae*, *P. lurida*, and *P. chlororaphis* on overall seedling growth and primary root elongation was assessed after 15 days of interaction. *P. brassicae* and *P. lurida* but not *P. chlororaphis* enhanced shoot and root growth, indicating their potential to help plants to grow and utilize a sparingly soluble Pi source. Thus, Pi-solubilizing bacteria may be interesting candidates as plant growth promoters, from which the three newly identified isolates of *P. brassicae* (H4-34, H4-45, H4-16) represent true plant beneficial bacteria that can assist Pi nutrition and plant growth under different conditions.

Author Contribution J.L.H., R.O.C., and J.L.B. conceived and designed the experiments; J.L.H., E.G.C., K.R.J.V., D.L.S.R., and O.F.R. performed experiments; J.L.H., J.S.L.B., O.F.R., R.O.C., and J.L.B analyzed the data; R.O.C., J.S.L.B, H.R.C, and J.L.-B. provided reagents/materials/analytical tools; J.L.H. and J.L.B. wrote the paper; all authors reviewed and edited the manuscript. R.O.C. and J.L.B. applied for funding.

Funding This work was financed by grants from the Consejo Nacional de Ciencia y Tecnología (CONACYT, México, grants A1-S-34768, PDCPN-2015–882, and FORDECYT-PRONACES 292399) and UMSNH (CIC 2.26).

Data Availability All data generated or analysed during this study are included in this article and are available upon reasonable request to the corresponding author.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Backer R, Rokem SJ, Ilangumaran G et al (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front Plant Sci 9:1473. https://doi.org/10.3389/fpls.2018.01473
2. Finkel OM, Castrillo G, Paredes SH et al (2017) Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol 38:155–163. https://doi.org/10.1016/j.pbi.2017.04.018
3. Finkel OM, Salas-González I, Castrillo G et al (2020) A single bacterial genus maintains root growth in a complex microbiome. Nature 587:103–108. https://doi.org/10.1038/s41586-020-2778-7
4. Wang S, Na X, Yang L et al (2021) *Bacillus megaterium* strain WW1211 promotes plant growth and lateral root initiation via regulation of auxin biosynthesis and redistribution. Plant Soil 466:491–504. https://doi.org/10.1007/s11104-021-05055-z
5. López-Arredondo D, Leyva-González MA, González-Morales S et al (2014) Phosphate nutrition: improving low phosphate tolerance in crops. Annu Rev Plant Biol 65:95–123. https://doi.org/10.1146/annurev-arplant-050213-035949
6. Shahid M, Hameed S, Tariq M et al (2015) Characterization of mineral phosphate-solubilizing bacteria for enhanced sunflower growth and yield-attributing traits. Ann Microbiol 65:1525–1536. https://doi.org/10.1007/s13213-014-0991-z
7. Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8:971. https://doi.org/10.3389/fmicb.2017.00971
8. Ortiz-Castro R, López-Bucio J (2019) Review: Phytostimulation and root architectural responses to quorum-sensing signals and related molecules from rhizobacteria. Plant Sci 284:135–142. https://doi.org/10.1016/j.plantsci.2019.04.010

9. Ortiz-Castro R, Díaz-Pérez C, Martínez-Trujillo M et al (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc Natl Acad Sci USA 108:7253–7258. https://doi.org/10.1073/pnas.1006740108
10. Ortiz-Castro R, Campos-García J, López-Bucio J (2020) *Pseudomonas putida* and *Pseudomonas fluorescens* influence *Arabidopsis* root system architecture through an auxin response mediated by bioactive cyclodipeptides. J Plant Growth Regul 39:254–265. https://doi.org/10.1007/s00344-019-09979-w
11. Spaepen S, Bossuyt S, Engelen K et al (2014) Phenotypical and molecular responses of *Arabidopsis thaliana* roots as a result of inoculation with the auxin-producing bacterium *Azospirillum brasilense*. New Phytol 201(3):850–861. https://doi.org/10.1111/nph.12590
12. Méndez-Gómez M, Barrera-Ortíz S, Castro-Mercado E et al (2020) The nature of the interaction *Azospirillum-Arabidopsis* determines the molecular and morphological changes in root and plant growth promotion. Protoplasma 258:179–189. https://doi.org/10.1007/s00709-020-01552-7
13. García-Cárdenas E, Ortiz-Castro R, Ruiz-Herrera LF, et al (2021) *Micrococcus luteus* LS570 promotes root branching in *Arabidopsis* via decreasing apical dominance of the primary root and an enhanced auxin response. Protoplasma https://doi.org/10.1007/s00709-021-01724-z
14. Jiménez-Vázquez K, García-Cárdenas E, Barrera-Ortíz S et al (2020) The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. Plant J 103:1639–1654. https://doi.org/10.1111/tpj.14853
15. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971. https://doi.org/10.1105/tpc.9.11.1963
16. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
17. Tzec-Interian JA, Desgarennes D, Carrión G et al (2020) Characterization of plant growth-promoting bacteria associated with avocado trees(*Perseaamericana*Miller) and their potential use in the biocontrol of*Scirtothripsperseae*(avocado thrips). PloS One 15(4):e0231215.https://doi.org/10.1371/journal.pone.0231215
18. Wright ES (2012) DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl Environ Microbiol 78(3):717–725. https://doi.org/10.1128/AEM.06516-11
19. Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
20. Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219
21. Cole JR, Wang Q, Fish JA et al (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucl Acids Res 42((Database issue)):D633-42. https://doi.org/10.1093/nar/gkt1244
22. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07
23. Larsen N, Olsen GJ, Maidak BL et al (1993) The ribosomal database project. Nucl Acids Res 21:3021–3023. https://doi.org/10.1093/nar/21.13.3021
24. Cole JR, Chai B, Farris RJ et al (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucl Acids Res 33:D294–D296. https://doi.org/10.1093/nar/gki038
25. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054
26. Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124:33–44. https://doi.org/10.1242/dev.124.1.33
27. Gupta RR, Singal A, Sankar RC et al (1994) Amodified plate assay for screening phosphate-solubilizing microorganisms. J Gen Appl Microbiol 40:255–260. https://doi.org/10.2323/jgam.40.255
28. Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x
29. Ortiz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. Plant SignalBehav 4(8):701–712. https://doi.org/10.4161/psb.4.8.9047
30. López-Bucio J, De la Vega OM, Guevara-García A, Herrera-Estrella L (2000) Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nat Biotechnol 18(4):450–453. https://doi.org/10.1038/74531
31. Rosas SB, Andrés JA, Rovera M, Correa NS (2006) Phosphate-solubilizing*Pseudomonasputida*can influence the rhizobia–legume symbiosis. Soil Biol Biochem 38:3502–3505.https://doi.org/10.1016/j.soilbio.2006.05.008
32. Trivedi P, Sa T (2008) *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. Curr Microbiol 56(2):140–144. https://doi.org/10.1007/s00284-007-9058-8
33. Adhikari P, Jain R, Sharma A, Pandey A (2021) Plant growth promotion at low temperature by phosphate-solubilizing Pseudomonas spp isolated from high-altitude Himalayan soil. Microbial Ecol 82(3):677–687. https://doi.org/10.1007/s00248-021-01702-1
34. Majeed A, KaleemAbbasi M, Hameed S et al (2018) *Pseudomonas* sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of *Helianthus annuus* L. under reduced fertilizer input. Microbiol Res 216:56–69. https://doi.org/10.1016/j.micres.2018.08.006
35. Bautista-Cruz A, Antonio-Revuelta B, Martínez-Gallegos VC, Báez-Pérez A (2019) Phosphate-solubilizing bacteria improve Agave angustifolia Haw. growth under field conditions. J Sci Food Agric 99:6601–6607. https://doi.org/10.1002/jsfa.9946
36. Tahir M, Naeem MA, Shahid M et al (2020) Inoculation of *pqqE* gene inhabiting *Pantoea* and *Pseudomonas* strains improves the growth and grain yield of wheat with a reduced amount of chemical fertilizer. J Appl Microbiol 129(3):575–589. https://doi.org/10.1111/jam.14630
37. Oteino N, Lally RD, Kiwanuka S et al (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745. https://doi.org/10.3389/fmicb.2015.00745
38. Kazmierczak BI, Schniederberend M, Jain R (2015) Cross-regulation of *Pseudomonas* motility systems: the intimate relationship between flagella, pili and virulence. Curr Opin Microbiol 28:78–82. https://doi.org/10.1016/j.mib.2015.07.017
39. Raio A, Puopolo G (2021) *Pseudomonas chlororaphis* metabolites as biocontrol promoters of plant health and improved crop yield. World J Microbiol Biotechnol 37(6):99. https://doi.org/10.1007/s11274-021-03063-w
40. López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in

hormone sensitivity in the *Arabidopsis* root system. Plant Physiol 129(1):244–256. https://doi.org/10.1104/pp.010934

41. Castrillo G, Teixeira P, Paredes S et al (2017) Root microbiota drive direct integration of phosphate stress and immunity. Nature 543:513–518. https://doi.org/10.1038/nature21417
42. Brisson VL, Richardy J, Kosina SM et al (2022) Phosphate availability modulates root exudate composition and rhizosphere microbial community in a Teosintle and a modern maize cultivar. Phytobiomes J 6(1):83–94. https://doi.org/10.1094/PBIOMES-06-21-0041-R
43. Kawasaki A, Dennis PG, Forstner C et al (2021) Manipulating exudate composition from root apices shapes the microbiome throughout the root system. Plant Physiol 184:2279–2285. https://doi.org/10.1093/plphys/kiab337

8.5. Capítulo V

Biológicas (2022) 21: 1–8

Influencia de las rizobacterias promotoras del crecimiento vegetal en la respuesta hormonal y la arquitectura de la raíz

Kirán Rubí Jiménez Vázquez, José López Bucio y Elda Beltrán Peña

Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B1, C. P. 58030 Morelia, Michoacán, México.

Plant growth promoting rhizobacteria influence on hormonal response and root architecture

Abstract

A wide variety of mutualistic relations are established between plants and microorganisms in the different ecosystems. Plant growth promoting rhizobacteria (PGPR) influence many aspects in the development, physiology and productivity of plants because they colonize roots and therefore, improve soil resource acquisition. In this review article, we discuss how the modifications in the root organogenesis program that are influenced by rhizobacteria are related with the response to auxins, a group of growth regulators widely distributed in the plant kingdom. It is also discussed the chemical nature and bioactivity of diverse molecules, which are synthesized and secreted by beneficial bacteria and affect plant growth via an auxin hormonal response or through changes in gene expression, and consequently modify the plant phenotype leading to better adaptive properties such as better fertilizer use and to a higher and more sustainable productivity.

Keywords: auxin, rhizobacteria, root architecture.

Resumen

En los diferentes ecosistemas se establece una amplia variedad de relaciones mutualistas entre las plantas y los microorganismos. Las rizobacterias promotoras del crecimiento vegetal (Plant Growth Promoting Rhizobacteria, PGPR, por sus siglas en inglés) influyen sobre diversos aspectos del desarrollo, fisiología y la productividad vegetal, ya que colonizan la raíz y, por lo tanto, posibilitan un mejor aprovechamiento de los recursos del suelo. En este artículo de revisión se analiza cómo es que las modificaciones en los programas de organogénesis de la raíz que están influenciadas por las rizobacterias se relacionan con la respuesta a las auxinas, un grupo de reguladores del crecimiento distribuidos ampliamente en el reino vegetal. También se discute sobre la naturaleza química y la bioactividad de diversas moléculas de bacterias benéficas que influyen en el desarrollo vegetal a través de una respuesta hormonal auxínica, o porque alteran la expresión genética y el fenotipo de las plantas, alcanzando éstas mejores propiedades adaptativas, incluyendo el uso más eficiente de los fertilizantes que se suministran como fuente de nutrientes y consecuentemente una productividad más sostenible y redituable.

Palabras clave: auxinas, rizobacteria, arquitectura radicular.

Introducción

Las raíces son órganos dinámicos y fascinantes de las plantas que les permiten una mejor adaptación al agobio ambiental, ya que extraen del suelo el agua y los nutrientes minerales requeridos para su desarrollo. Las interacciones de la raíz con los microorganismos contribuyen al aprovechamiento de los recursos disponibles y conllevan a la formación de relaciones simbióticas duraderas y efectivas. Algunos grupos de bacterias estimulan el desarrollo radicular a través de cambios en los niveles endógenos de reguladores del crecimiento, también llamados fitohormonas, incluyendo a las auxinas, por lo que se les considera probióticos con amplio potencial para estimular la producción agrícola (Salwan *et al.*, 2019).

Las auxinas influyen en la embriogénesis, la dominancia apical, la regeneración tisular y los tropismos (Saini *et al.*, 2013: Motte *et al.*, 2019). Cerca del 80% de las bacterias del suelo tienen la capacidad de producir auxinas, las cuales pueden ser percibidas por receptores de naturaleza proteínica presentes en las membranas de las células de la raíz. En contraparte, las raíces producen y secretan ácidos orgánicos, fitosideróforos, azúcares, vitaminas y aminoácidos que representan una fuente importante de energía para las rizobacterias y las atraen hacia su zona de influencia en el suelo, también conocida como rizósfera (Patten y Glick, 1996; Dakora y Phillips, 2002; Bulgarelli *et al.*, 2013).

✉ **Kirán Rubí Jiménez Vázquez, kiran.jimenez@umich.mx**
Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B1, CP 58030 Morelia, Michoacán, México.

Las auxinas y el desarrollo de la raíz

La raíz es el órgano de las plantas que crece típicamente bajo el suelo y responde a las condiciones fluctuantes del ambiente. Algunas de sus funciones son:

1. Proporciona soporte estructural al tallo y follaje
2. La adquisición de agua y nutrientes
3. La exploración del substrato

Este órgano puede ser muy complejo, y sus características están sujetas a un control genético que es dependiente de la especie vegetal, la edad de la

planta, y los factores bióticos y abióticos del ambiente.

Desde hace dos décadas, se ha utilizado a la especie *Arabidopsis thaliana* como una planta modelo para el estudio de la organogénesis de la raíz, con tres dominios celulares bien definidos. En el meristemo, las células entran en un programa repetitivo de mitosis, y luego se expanden en la zona de elongación, antes de adquirir sus funciones y características especializadas (**Fig. 1a**; Motte *et al.*, 2019). El desarrollo del sistema radicular ocurre a partir del embrión, desde donde surge una raíz primaria; las ramificaciones posteriores son derivadas del periciclo, un tejido interno que rodea al cilindro vascular, y se permite que se aumente la superficie de exploración (Atkinson *et al.*, 2014).

En las plantas dicotiledóneas, de las que *A. thaliana* forma parte, los distintos grados de ramificación dependen de las raíces laterales, en tanto que en las plantas monocotiledóneas como el maíz (*Zea mays* L.) es más frecuente la formación de raíces adventicias (Motte *et al.*, 2019). El proceso de formación de las raíces laterales consta de cuatro etapas: 1) La activación de las células del periciclo, 2) La iniciación de un primordio, 3) El desarrollo del primordio, que conduce a la formación de una estructura en forma de domo, que

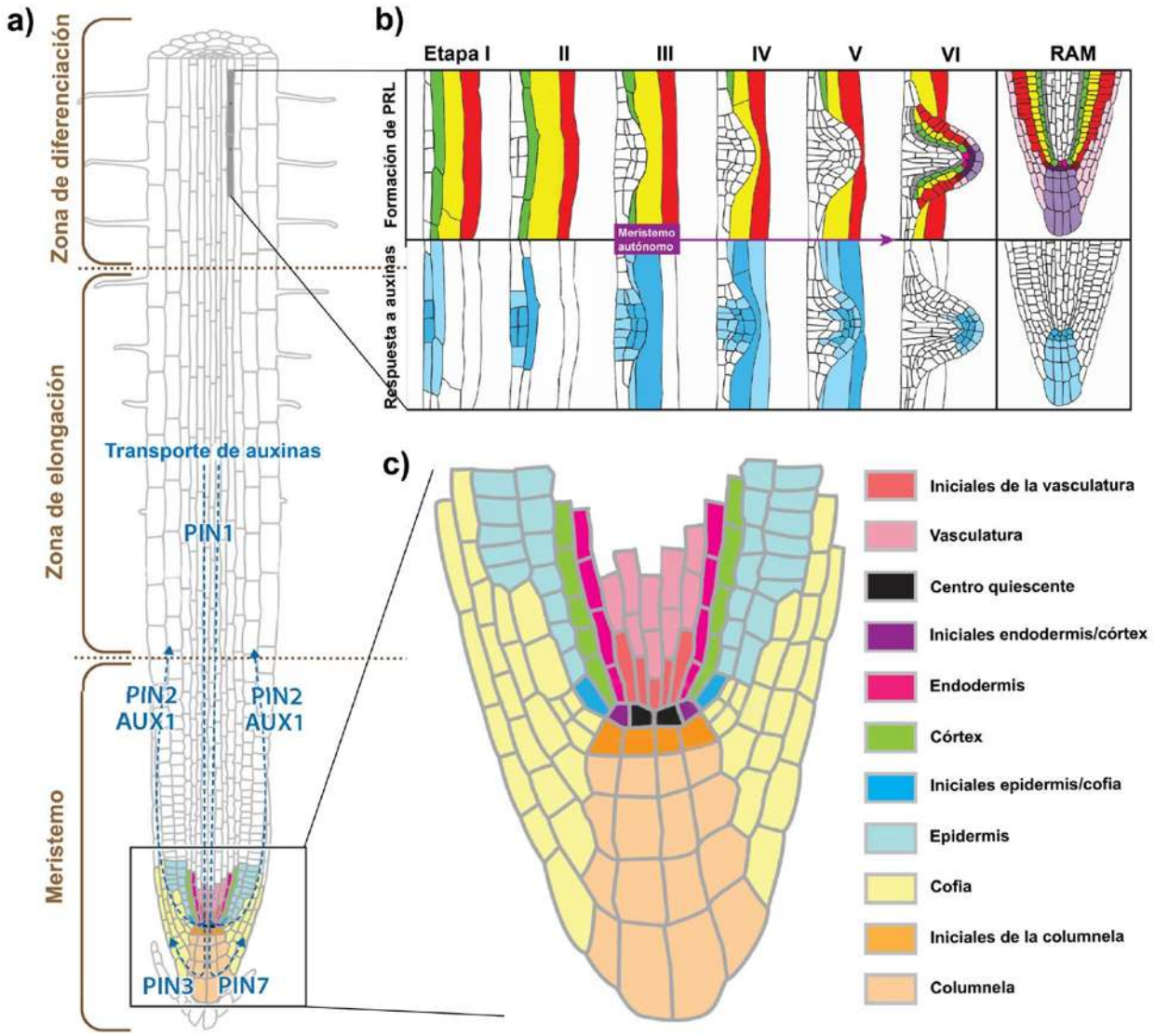


Figura 1. Estructura de la raíz primaria y las etapas de formación del primordio de la raíz lateral. a) La raíz consta de tres zonas de desarrollo: la meristemática, de elongación y diferenciación, en las cuales el transporte de auxinas desempeña una función fundamental. b) La formación del primordio de la raíz lateral ocurre en la zona de diferenciación, en donde a través de una división celular asimétrica y a través de varias etapas se forma un domo con crecimiento autónomo, culminando con la emergencia de una nueva ramificación. c) El nicho de células iniciales y los tejidos que conforman la raíz dependen del centro quiescente, que comprende un grupo de células con escaso potencial mitótico (Adaptado de Motte *et al.*, 2019; Du y Scheres, 2018).

crece a través de las capas celulares superpuestas en el interior de la raíz primaria, 4) La emergencia de las raíces laterales como estructuras con crecimiento autónomo e independiente de la raíz primaria (**Fig. 1b**; Du y Scheres, 2018). A partir del cilindro vascular, es factible visualizar los tejidos concéntricos del periciclo, endodermis, córtex y epidermis, desde el interior hacia el exterior. Por otra parte, en la zona más distal de la raíz se localiza la cofia, estructura de protección del meristemo (**Fig. 1c**).

En la formación de la raíz participan múltiples fitohormonas (Chaiwanon *et al.*, 2016; Motte *et al.*, 2019). La síntesis de auxinas ocurre en los brotes jóvenes del follaje, sitio desde el cual se distribuyen local y sistémicamente a todos los tejidos (Olatunji *et al.*, 2017; Brumos *et al.*, 2018). Se han descrito dos tipos de transporte, uno pasivo, rápido y directo a través del floema, y otro con gasto de energía, célula a célula a través de transportadores de membrana conocido como transporte polar. En este último, participan dos clases generales de permeasas; las que permiten el paso al interior de la célula e incluyen a las proteínas AUXIN RESISTANT 1/LIKE-AUX1 (AUX1/LAX) y las de eflujo, agrupadas en las familias PIN-FORMED (PIN) y ATP-Binding Cassette B/MultiDrugs Resistent/P-GlicoProteínas (ABCB/MDR/PGP) que bombean las auxinas al exterior de la célula (Petrásek y Friml, 2009; Marhavý *et al.*, 2013; Porco *et al.*, 2016).

Las auxinas ejercen su efecto celular a través del complejo receptor SCFTIR1/AFB-Aux/IAA (SCF [(SKP, CULLIN, F-BOX), TIR1/AFB (TRANSPORT INHIBITOR RESPONSE 1/AUXIN-RELATED F-BOX PROTEINS), AUX/IAA (AUXIN/INDOLE-3- ACETIC ACID)]. Su interacción con el receptor promueve la unión de los represores Aux/IAA con el complejo ubiquitina E3-ligasa SCFTIR1/AFB provocando su degradación, lo que resulta en la activación de los factores de trascripción de respuesta a auxinas y el inicio subsecuente de la síntesis de RNA mensajero (Strader y Zhao, 2016; Leyser, 2018). Los genes de respuesta a auxinas se agrupan en tres familias: 1) *AUX/IAA*, 2) *Gretchen Hagen-3* (*GH3*) y 3) *Small Auxin-up Regulated RNA* (*SAURs*). Las proteínas codificadas por los genes *AUX/IAA* bloquean a los factores de respuesta a auxinas y por lo tanto actúan como inhibidores transcripcionales. Un ejemplo de la función de dichas proteínas en la organogénesis de la raíz, es el módulo SOLITARY ROOT (SLR)/IAA14-ARF7-ARF19, en el cual una mutación que cambia a un aminoácido en la secuencia de la proteína bloquea la degradación de SLR1, lo que causa la inhibición de la formación de las raíces laterales (Kim y Lee, 2013; Lee y Kim, 2013). La familia de genes *GH3* participa en la homeostasis hormonal mediante la conjugación de aminoácidos con las formas libres del ácido indol-3-acético (AIA), ácido jasmónico y ácido salicílico (Park *et al.*, 2007b). Los genes *SAUR* codifican proteínas involucradas en la expansión de las células, como *SAUR41*, que se expresa en el centro quiescente y la célula inicial del córtex/endodermis en el meristemo de la raíz (Kong *et al.*, 2013; Qiu *et al.*, 2013).

Influencia de las rizobacterias promotoras del crecimiento vegetal (PGPR) en el desarrollo del sistema radicular

La necesidad de optimizar el rendimiento de los cultivos ha intensificado el estudio de las interacciones planta-microorganismo, enfatizándose en la identificación de bacterias con función probiótica y en las diversas facetas de los microbiomas sobre la productividad (Barrett *et al.*, 2011; Bulgarelli *et al.*, 2013). Diversas bacterias tienen la capacidad de colonizar la raíz, ya que cuentan con flagelos móviles, y los exudados vegetales ejercen un efecto atrayente (**Fig. 2;** Barea *et al.*, 2005; Vacheron *et al.*, 2013). Hasta ahora, la mayoría de los aislados estudiados afectan el crecimiento de la raíz primaria, incrementan el número y la longitud de las raíces laterales, y estimulan la elongación de los pelos radiculares (Chamam *et al.*, 2013; Sukumar *et al.*, 2013). Estas modificaciones mejoran la absorción de agua y nutrientes, por lo que el uso de las rizobacterias contribuye en prácticas agrícolas más sostenibles.

Entre las rizobacterias con actividad sobre el crecimiento vegetal se encuentran diversos géneros de bacterias Gram-negativas como *Azospirillum*, *Pseudomonas*, *Gluconacetobacter* y *Rhizobium*, además de algunos géneros de bacterias Gram-positivas, como *Bacillus* y *Paenibacillus* (Bulgarrelli *et al.*, 2013). Las raíces ejercen una presión de selección sobre los microorganismos y éstos les proveen ventajas competitivas. Por ejemplo, uno de los factores clave para la colonización exitosa es la motilidad flagelar, característica fundamental para el movimiento hacia la raíz y la fase de adhesión inicial; como es de esperarse, ésta se modifica por moléculas presentes en los exudados radicales. Consecuentemente, bacterias mutantes de *Pseudomonas fluorescens*, *Pseudomona putida* y *Azospirillum brasilense* que carecen de flagelos, se ven afectadas o incluso pierden la capacidad de colonizar las raíces de *A. thaliana* (De Weger *et al.*, 1987; Vande-Broek, 1988).

Las rizobacterias promueven el crecimiento a través de la fijación biológica del nitrógeno, proceso por el cual se reduce el nitrógeno gaseoso a nitrato y amonio, iones precursores de aminoácidos y otras moléculas orgánicas (Urquiaga *et al.*, 2012). Durante la interacción también se producen auxinas, propiedad fisiológica aparentemente presente en diversos microorganismos **(Fig. 2;** Patten y Glick, 1996). Los experimentos realizados con bacterias mutantes afectadas en la producción de ácido indol-3-acético (AIA) han demostrado su participación en la fitoestimulación (Spaepen *et al.*, 2007a; Vokou *et al.*, 2012), como lo es en la adaptación de las plantas para sobrevivir en suelos carentes de hierro, un micronutriente importante para la

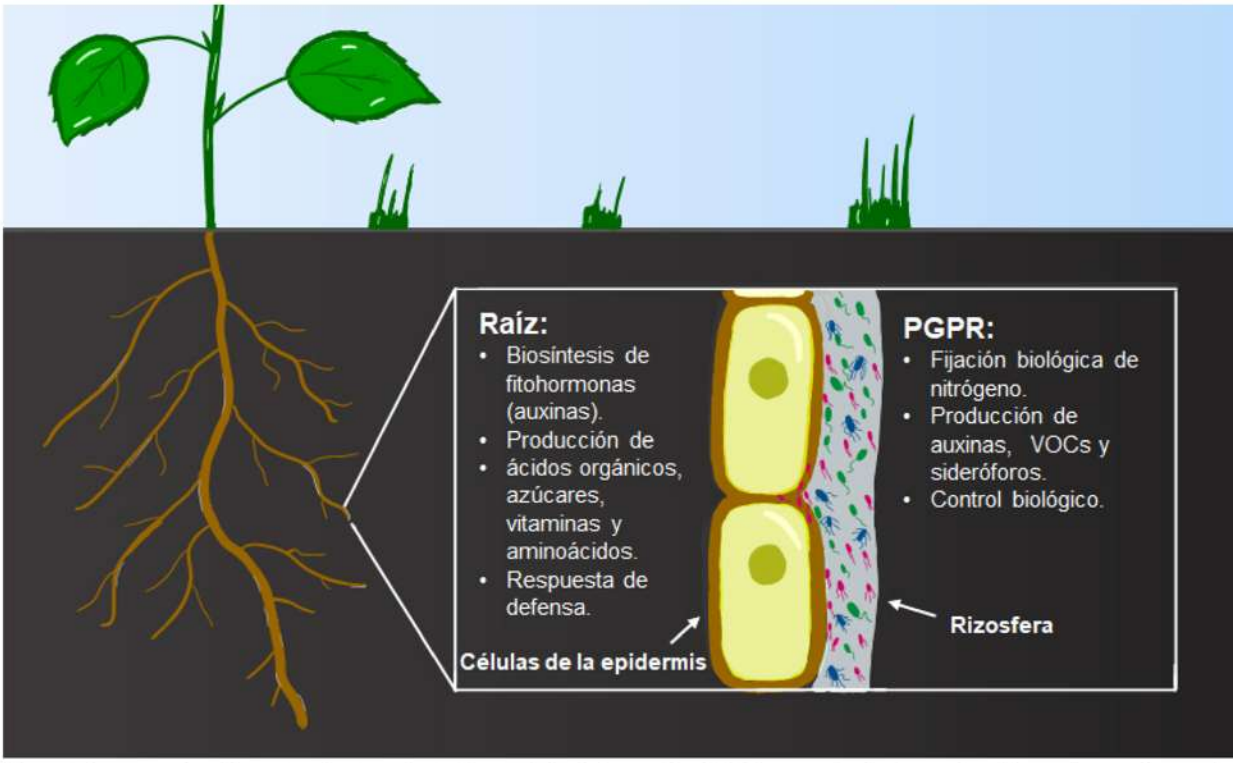


Figura 2. La comunicación entre plantas y bacterias modula el desarrollo del sistema radicular. Las raíces liberan diversas fuentes de carbono y moléculas con funciones en la señalización para el reconocimiento de las especies microbianas con las que interactúan, ejerciendo un efecto atrayente para las bacterias, estas últimas, mediante diferentes mecanismos influyen sobre el crecimiento, la nutrición y la salud vegetal. La producción de auxinas, tanto en la raíz como por las rizobacterias (PGPR) contribuye en el establecimiento de la interacción y las modificaciones en la arquitectura radicular.

fotosíntesis (Lemanceau *et al.,* 2009; Marschner *et al.,* 2011). En estos procesos, ocurre una comunicación química entre los simbiontes en la que participan compuestos orgánicos volátiles (Ryu *et al.,* 2004; Zhang *et al.,* 2007).

Las auxinas y la señalización entre plantas y bacterias

Las bacterias utilizan una amplia variedad de moléculas para adaptarse y sobrevivir en diversos nichos ambientales (Waters y Bassler, 2005; Ortiz-Castro y López Bucio, 2019). Entre ellas, la producción de compuestos como los sideróforos, antibióticos, péptidos, y lípidos, que se relacionan con la virulencia, la formación de biopelículas y por lo tanto llegan a afectar sus relaciones con las plantas (Waters y Bassler, 2005; Vega *et al.*, 2013). El indol modula la virulencia, confiere resistencia a ácidos y fármacos, además de participar en la formación de biopelículas en las bacterias Gram-negativas (Lee y Lee, 2010). En las células epiteliales de humanos, contribuye en el funcionamiento del sistema inmune (Bansal *et al.*, 2010) y derivados del indol, como el ácido indol-3-acético y el indol-3-acetonitrilo desempeñan una función relevante en las interacciones planta-microorganismo (Lee *et al.*, 2011; Spaepen y Vanderleyden, 2011). *Spaepen* y col. (2007), cuantificaron las fitohormonas secretadas por diversas especies de *Azospirillum* y encontraron que las auxinas eran las más abundantes, lo que se correlacionaba con un mejor desarrollo del sistema radicular y una mayor producción de biomasa. Un posible blanco fisiológico de las auxinas es la respuesta de defensa, que protege a las plantas o prepara sus tejidos para reaccionar eficientemente ante una posible infección, en cuyo caso podría ocurrir un reajuste del crecimiento (Bais *et al.*, 2004; Gourion *et al.,* 2015; Lebeis *et al.,* 2012).

Una vez que una planta reconoce propiedades bioquímicas de las bacterias, se activan cascadas de señalización que afectan el fenotipo. Cada interacción es diferente, debido a que no todas las bacterias producen la misma cantidad y tipo de auxinas y la planta responde de manera distinta a cada microorganismo, sin olvidar que factores como la luz, pH, temperatura, humedad, entre otros, también influyen en el establecimiento de una posible simbiosis (Bulgarelli *et al.,* 2013).

Influencia de las bacterias sobre la respuesta auxínica

Algunas rizobacterias alteran el desarrollo de la raíz, cambiando su estructura, su longitud y el grado de ramificación (**Fig. 3**; Zhang *et al.,* 2007; López-Bucio

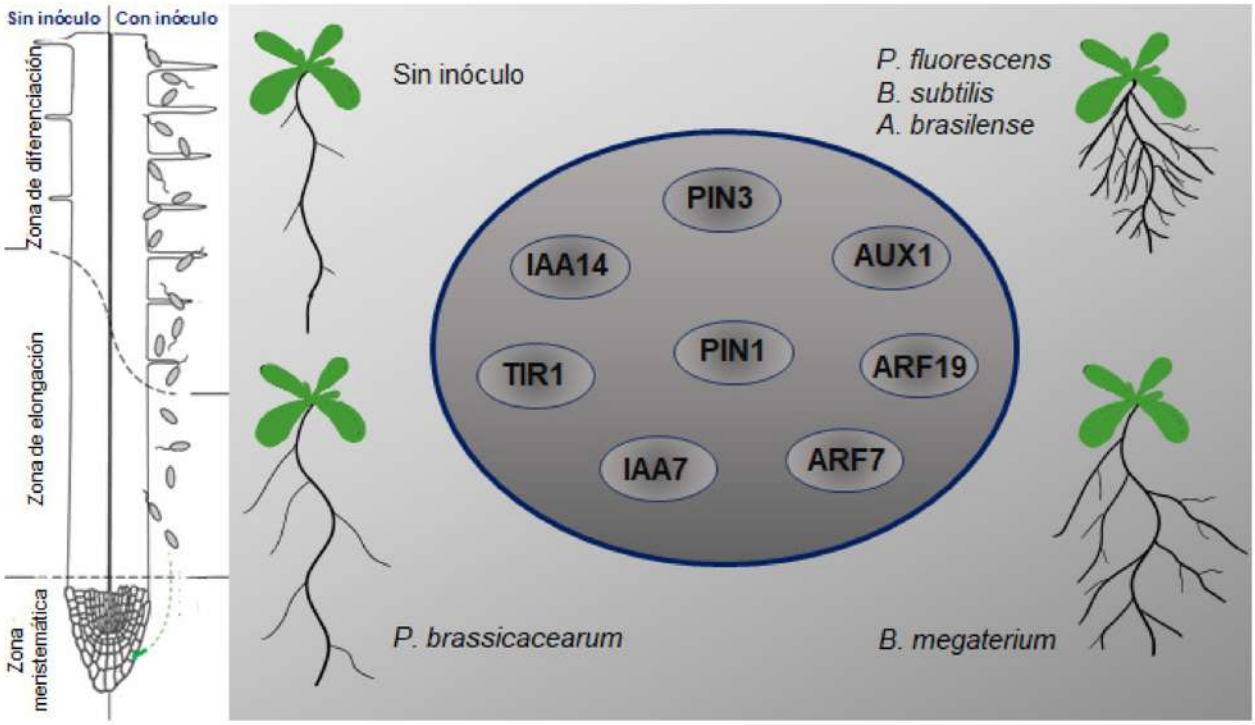


Figura 3. Las rizobacterias modifican la arquitectura radicular mediante la activación de elementos auxínicos. En la izquierda se ilustra el cambio ocurrido en las tres zonas principales de la raíz primaria en condiciones axénicas o ante la influencia de rizobacterias. Los esquemas de la derecha muestran las principales modificaciones de la arquitectura radicular en plántulas de *Arabidopsis* inoculadas, así como los elementos de la respuesta a auxinas involucrados. Las proteínas PIN y AUX participan en el transporte de auxinas, en tanto que las proteínas denominadas TIR1, IAA y ARF están involucrados en la regulación de la transcripción (Modificada de Verbon y Liberman, 2016).

et al., 2007; Ortiz-Castro *et al.,* 2009; Contesto *et al.,* 2010; Zamioudis *et al.,* 2013; Spaepen *et al.,* 2014). *Bacillus megaterium* incrementa el número y la longitud de las raíces laterales (López-Bucio *et al.,* 2007); los compuestos orgánicos volátiles liberados por *Bacillus subtilis* GB03 (Zhang *et al.,* 2007) y *Pseudomonas fluorescens* WCS417 reprimen el crecimiento de la raíz primaria y estimulan el desarrollo de las raíces laterales (Zamioudis *et al.,* 2013); y, *Azospirillum brasilense* y *Serratia marcescens* incrementan la formación de raíces laterales (Spaepen *et al.,* 2007; Shi *et al.,* 2010). *Phyllobacterium brassicacearum* no afecta la longitud de la raíz primaria ni el número de raíces laterales, sin embargo, la velocidad de ramificación incrementa notablemente (Contesto *et al.,* 2010).

Phyllobacterium brassicacearum STM196 induce la expresión del marcador *DR5::GUS* en el haz vascular y en la punta de la raíz (Casimiro *et al.,* 2001; Contesto *et al.,* 2010). Experimentos con una bacteria mutante de *Azospirillum brasilense* designada como FAJ0009, la cual está afectada en la capacidad de producir auxinas (Costacurta *et al.,* 1994), demostraron que *A. brasilense* altera el sistema radicular a través de la producción de dichas fitohormonas (Spaepen *et al.,* 2014).

Pseudomonas fluorescens WCS417 induce la división celular en el meristemo como consecuencia de un incremento en la expresión de genes de respuesta a auxinas, como se observó con los genes reporteros *DR5::vYFP* (Laskowski *et al.,* 2008) y *pAUX1::AUX1-YFP* (Swarup *et al.,* 2001). Las plantas inoculadas con WCS417 manifiestan una fuerte respuesta a auxinas en las células de la cofia lateral de la raíz y en la zona meristemática (Zamioudis *et al.,* 2013). *P. fluorescens* aparentemente no produce auxinas, por lo que probablemente, podría producir compuestos con afinidad a los receptores de la familia TIR1, como en *P. aeruginosa,* cuya producción de ciclodipeptidos promueve la ramificación de la raíz (Zamioudis *et al.,* 2013; Ortiz-Castro *et al.,* 2011).

Bacillus megaterium UMCV1 reduce la división celular en la raíz primaria, al disminuir la respuesta a auxinas, lo cual fue evidenciado en plantas que expresan *DR5::GUS*, sin embargo, se incrementó la expresión de este marcador en los primordios de las raíces laterales, lo que sugiere que la estimulación en el crecimiento de las raíces laterales podría estar mediada por una redistribución hormonal (López-Bucio *et al.,* 2007). Por otra parte, los compuestos volátiles emitidos por *Bacillus subtillis* GB03 incrementan la acumulación de auxinas en las raíces y en el follaje, contribuyendo de esta manera con el aumento en la producción de

biomasa foliar y radical (Zhang *et al.*, 2007).

Phyllobacterium brassicacearum STM196 estimula la expresión de varias enzimas de la vía de biosíntesis de auxinas en el follaje con el consecuente aumento en el crecimiento de las raíces laterales (Contesto *et al.*, 2010). Spaepen y col. (2014), mediante estudios de expresión global de genes en plantas de *Arabidopsis* inoculadas con una cepa silvestre (Sp245) y una mutante (FAJ0009) de *Azospirillum brasilense* que no produce auxinas, observaron que el número de genes expresados aumenta con el tiempo y que la bacteria silvestre produce una respuesta mayor que la mutante.

Conclusiones

La información recabada en años recientes sugiere que tanto la producción local de auxinas en el meristemo como su transporte son esenciales para la simbiosis planta-bacteria. Los compuestos orgánicos volátiles liberados por *Bacillus subtilis* GB03, incrementaron la expresión de enzimas que participan en la biosíntesis de auxinas en *Arabidopsis* y el uso de un inhibidor del transporte reveló que la mayor producción de biomasa vegetal por la inoculación con GB03 depende de una redistribución hormonal en los tejidos (Zhang *et al.*, 2007). Aunque la inoculación de *Arabidopsis* con *Bacillus megaterium* UMCV1 modificó la concentración de auxinas tanto en la raíz primaria como en los primordios de la raíz lateral, de acuerdo con un reporte por López-Bucio y col. (2007), la promoción del crecimiento es independiente de la vía de señalización de auxinas, por lo que se plantean varios y diversos posibles mecanismos de fitoestimulación por las rizobacterias. En este escenario, se han identificado ciclodipéptidos en extractos de cultivos de *Pseudomonas aeruginosa* que son similares a las auxinas producidas por las plantas y que modulan la arquitectura de la raíz involucrando los mismos mecanismos de señalización (Ortiz-Castro *et al.*, 2011).

Las auxinas modulan la organogénesis vegetal y coordinan las transiciones del desarrollo como la floración y la fructificación, aspectos que dependen de un suministro adecuado de nutrientes. En el ápice de la raíz se concentra el ácido indol-3-acético, principal auxina natural, debido a los flujos provenientes del follaje y por su producción local en la cofia y en el meristemo. El desarrollo de las raíces depende de las auxinas producidas por bacterias benéficas, como se comprobó con el uso de plantas transgénicas que emplean genes de respuesta a auxinas y mutantes afectadas en la vía de biosíntesis, transporte y señalización de estas fitohormonas. Resulta evidente su participación como una señal clave en las interacciones planta-bacteria y en el establecimiento de simbiosis efectivas y perdurables en el reino vegetal.

Referencias

Atkinson JA, Rasmussen A, Traini R, Voß U, *et al.* (2014) Branching out in roots: uncovering form, function, and regulation. *Plant Physiology.* 166: 538-550.

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. *Trends in Plant Science.* 9: 26-32.

Bansal T, Alaniz RC, Wood TK, Jayaraman A (2010) The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proceedings of the National Academy of Sciences.* 107: 228-233.

Barea JM, Pozo MJ, Azcón R, Azcón-Aguilar C (2005) Microbial cooperation in the rhizosphere. *Journal of Experimental Botany.* 56: 1761-1778.

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. *Biology and Fertility of Soils.* 47: 729-43.

Brumos J, Robles LM, Yun J, Vu TC, *et al.* (2018) local auxin biosynthesis is a key regulator of plant development. *Developmental Cell.* 47: 306-318.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology.* 64: 807-838.

Chamam A, Sanguin H, Bel-Ivert F, Meiffren G, Comte G, Wisniewski-Dyé F, *et al.* (2013) Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum-Oryzasativa* association. *Phytochemistry.* 87: 65-77.

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, *et al.* (2001) Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell.* 13: 843-852.

Chaiwanon J, Wang W, Zhu J-Y, Oh E, Wang Z-Y (2016) Information integration and communication in plant growth regulation. *Cell.* 164: 1257-68.

Contesto C, Milesi S, Mantelin S, Zancarini A, Desbrosses G, Varoquaux F, Bellini C, Kowalczyk M, Touraine B (2010) The auxin-signaling pathway is required for the lateral root response of Arabidopsis to the rhizobacterium *Phyllobacterium brassicacearum*. *Planta.* 232: 1455-1470.

Costacurta A, Keijers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. *Molecular and General Genetics.* 243: 463-472.

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low nutrient environments. *Plant and Soil.* 245: 35-47.

De Weger LA, Van der Vlugt CI, Wijfjes AH, Bakker PA, *et al.* (1987) Flagella of a plant-growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. *Journal of Bacteriology.* 169: 2769-73.

Du Y, Scheres B (2018) Lateral root formation and the multiple roles of auxin. *Journal of Experimental Botany.* 69: 155-167.

Gourion B, Berrabah F, Ratet P, Stacey G (2015) Rhizobium-legume symbioses: the crucial role of plant immunity. *Trends in Plant Science.* 20: 186-194.

Kim J, Lee HW (2013) Direct activation of *EXPANSIN14* by LBD18 in the gene regulatory network of lateral root

formation in *Arabidopsis*. *Plant Signaling & Behavior*. 8: e22979.

Kong Y, Zhu Y, Gao C, She W, *et al.* (2013) Tissue-specific expression of SMALL AUXIN UP RNA41 differentially regulates cell expansion and root meristem patterning in Arabidopsis. *Plant Cell Physiology*. 54: 609-621.

Laskowski M, Grieneisen VA, Hofhuis H, Hove CA, *et al.* (2008) Root system architecture from coupling cell shape to auxin transport. *PLoS Biology*. 6: e307.

Lebeis SL, Rott M, Dangl JL, Schulze-Lefert P (2012) Culturing a plant microbiome community at the cross-Rhodes. *New Phytologist*. 196: 341-44.

Lee JH, Lee J (2010) Indole as an intercellular signal in microbial community. *FEMS Microbiology Reviews*. 34: 426-444.

Lee JH, Cho MH, Lee J (2011) 3-indolylacetonitrile decreases *Escherichia coli* O157:H7 biofilm formation and *Pseudomonas aeruginosa* virulence. *Enviromental Microbiology*. 13: 62-73.

Lee HW, Kim J (2013) *EXPANSINA17* up-regulated by LBD18/ASL20 promotes lateral root formation during the auxin response. *Plant and Cell Physiology*. 54: 1600-1611.

Lemanceau P, Bauer P, Kraemer S, Briat JF (2009) Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. *Plant and Soil* 321: 513-35.

Leyser O (2018) Auxing signaling. *Plant Physiology*. 176: 465-479.

López-Bucio J, Campos-Cuevas JC, Hernández-Calderón E, Velásquez- Becerra C, *et al.* (2007) *Bacillus megaterium* rhizobacteria promote growth and alter rootsystem architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*. 20: 207-217.

Marschner P, Crowley D, Rengel Z (2011) Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis model and research methods. *Soil Biology Biochemistry*. 43: 883-94.

Marhavý P, Vanstraelen M, De Rybel B, Zhaojun D, *et al.* (2013) Auxin reflux between the endodermis and pericycle promotes lateral root initiation. *The EMBO Journal*. 32: 149-158.

Motte H, Vanneste S, Beeckman T (2019) Molecular and enviromental regulation of root development. *Annual Review of Plant Biology*. 70: 465-88.

Olatunji D, Geelen, Verstraeten I (2017) Control of Endogenous auxin levels in plant root development. *International Journal of Molecular Sciences*. 18: 2587.

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. *Plant Signaling & Behavior*. 4: 701-712.

Ortiz-Castro R, Diaz-Perez C, Martinez-Trujillo M, del Rio RE, *et al.* (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proceedings of the National Academy of Sciences*.108: 7253-7258.

Ortiz-Castro R, López-Bucio J (2019) Review: Phytostimulation and root architectural responses to quorum sensing signals and related molecules from rhizobacteria. *Plant Science*. 284: 135-142.

Park JE, Park JY, Kim YS, Sraswick PE, *et al.* (2007b) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in *Arabidopsis*. *Journal of Biological Chemistry*. 282: 10036-10046.

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*. 42: 207-220.

Petrášek J, Friml J (2009) Auxin transport routes in plant development. *Development*. 136: 2675-2688.

Porco S, Larrieu A, Du Y, Gaudinier A, *et al.* (2016) Lateral root emergence in Arabidopsis is dependent on transcription factor LBD29 regulation of auxin influx carrier LAX3. *Development*. 143: 3340-3349.

Qiu T, Chen Y, Li M, Kong Y, Zhu Y, *et al.* (2013) The tissue-specific and developmentally regulated expression patterns of the SAUR41 subfamily of SMALL AUXIN UP RNA genes: potential implications. *Plant Signal & Behavior*. 8: e25283.

Ryu CM, Farag MA, Hu CH, Reddy MS, *et al.* (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiology*. 134: 1017-26.

Saini S, Sharma I, Kaur N, Pati PK (2013) Auxin: a master regulator in plant root development. *Plant Cell Reports*. 32: 741-757.

Salwan R, Sharma A, Sharma V (2019) Microbes mediated plant stress tolerance in saline agricultural ecosystem. *Plant and Soil*. 1-2.

Shi CL, Park HB, Lee JS, Ryu S, Ryu CM (2010) Inhibition of primary roots and stimulation of lateral root development in *Arabidopsis thaliana* by the rhizobacterium *Serratia marcescens* is through both auxin dependent and independent signaling pathways. *Molecules and Cells*. 29: 251-258.

Spaepen S, Vanderleyden J, Remans R (2007a) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*. 31: 425-448.

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*. 3: a001438.

Spaepen S, Bossuyt S, Engelen K, Marchal K, Vanderleyden J (2014) Phenotypical and molecular responses of *Arabidopsis thaliana* roots as a result of inoculation with the auxin-producing bacterium *Azospirillum brasilense*. *New Phytologist*. 201: 850-861.

Strader L, Zhao Y (2016) Auxin perception and downstream events. *Current Opinion in Plant Biology*. 33: 8-14.

Sukumar P, Legue V, Vayssieres A, Martin F, *et al.* (2013) Involvement of auxin pathways in modulating root architecture during beneficial plant–microorganism interactions. *Plant Cell and Enviroment*. 36: 909-919.

Swarup R, Friml J, Marchant A, Ljung K, *et al.* (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes & Development*. 15: 2648-2653.

Urquiaga S, Xavier RP, de Morais RF, Batista RB, *et*

al. (2012) Evidence from field nitrogen balance and ^{15}N natural abundance data for the contribution of biological N_2 fixation to Brazilian sugarcane varieties. *Plant and Soil.* 365: 5-21.

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, *et al.* (2013) Plant growth-promoting rhizobacteria and root system functioning *Frontiers in Plant Science.* 4, 356.

Vande-Broek A, Lambrecht M, Vanderleyden J (1998) Bacterial chemotactic motility is important for the initiation of wheat root colonization by *Azospirillum brasilense. Microbiology.* 144: 2599-2606.

Vega NM, Allison KR, Samuels AN, Klempner MS, Collins JJ (2013) *Salmonella typhimurium* intercepts *Escherichia coli* signaling to enhance antibiotic tolerance. *Proceedings of the National Academy of Sciences.* 110: 14420-14425.

Verbon EH, Liberman LM (2016) Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in Plant Science.* 21: 218-229.

Vokou D, Vareli K, Zarali E, Karamanoli K, *et al.* (2012) Exploring biodiversity in the bacterial community of the Mediterranean phyllosphere and its relationship with airborne bacteria. *Molecular Plant Microbe Interactions* 64: 714-24.

Waters CM, Bassler BL (2005) Quorum Sensing: Cell-to-Cell Communication in Bacteria *Annual Review of Cell and Developmental Biology.* 21: 319-346.

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CM (2013) Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. *bacteria. Plant Physiology.* 162: 304-318.

Zhang H, Kim MS, Krishna- machair V, Payton P, *et al.* (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis Planta.* 226: 839-851.

9. DISCUSIÓN Y CONCLUSIONES

La cofia representa una innovación crucial a lo largo de la evolución de las plantas terrestres, ya que protege el meristemo radicular y funciona como un órgano sensorial que percibe y transmite las señales ambientales, lo que provoca cambios en la dirección del crecimiento de la raíz (Hetherington y Dolan, 2018b; Kumpf y Novack, 2015). Previamente, se demostró que la rizobacteria benéfica *Achromobacter* sp. 5B1 interrumpe el crecimiento gravitrópico, permitiéndole a las raíces explorar el sustrato en busca de los recursos del entorno, como resultado de la alteración en el transporte de las auxinas y el establecimiento de la distribución asimétrica de las mismas (Jiménez-Vázquez *et al*., 2020). En este trabajo, se demostró que esta respuesta es específica, ya que las bacterias promotoras del crecimiento vegetal *Bacillus* sp. LC390 y *Micrococcus luteus* LS570 (García-Cárdenas *et al*., 2022; 2023) no desviaron el crecimiento de las raíces del vector de gravedad. Además, se descubrió una función para los factores de transcripción FEZ y SMB, localizados en la cofia, en la regulación del comportamiento de la raíz agravitrópico en respuesta a *Achromobacter* sp. 5B1 mediante la modulación en la expresión de los transportadores de las auxinas PIN.

Los movimientos radiculares en torno a los estímulos ambientales se conocen como tropismos y la detección de las señales que los dirigen suele ocurrir en la cofia. Para investigar la posible función de este órgano sensorial con la capacidad de responder a las señales de *Achromobacter* sp. 5B1 y desencadenar el comportamiento agravitrópico de la raíz, se evaluó el efecto de la colonización bacteriana en la raíz primaria de plántulas de *Arabidopsis*, cuyas cofias se removieron quirúrgicamente, así como la respuesta en las mutantes con pérdida de función *fez-2* y *smb-3*. Curiosamente, las raíces de plantas normales sin cofia no desviaron el crecimiento en contacto directo con las bacterias, por su parte las mutantes mostraron fenotipos contrastantes: *fez-2* mostró un fenotipo de superenrollamiento; mientras que, las raíces de *smb-3* crecieron hacia el vector de gravedad, lo que indica que el crecimiento direccional de la raíz en respuesta a *Achromobacter* sp. 5B1 está regulado por estos factores de transcripción localizados en la cofia. Esto es comparable con un estudio reciente en el que SMB

desencadena la curvatura halotrópica de la raíz para escapar de las zonas con sal, ya que las raíces de la mutante *smb-3* no pueden activar la curvatura halotrópica para evitar el estrés salino (Zheng *et al*., 2024). Estos datos sugieren que la cofia puede desempeñar una función fundamental en la adaptación al estrés abiótico y en la respuesta a las bacterias asociadas a la planta mediante movimientos discretos y la modificación en la dirección de crecimiento de la raíz.

Los patrones de crecimiento de la raíz denominados "*waving*" y, "*skewing*" y la formación de giros resultan de una combinación de la circumnutación, el tigmotropismo y los movimientos circulares intrínsecos de las puntas de las raíces (Migliaccio y Piconese, 2001; Massaa y Gilroy, 2003; Porat *et al*., 2024). Los resultados mostraron una mayor frecuencia de ondulación en las raíces *fez-2*, mientras que, las raíces de *smb-3* crecieron en línea recta a lo largo del eje gravitacional. Las plántulas tipo silvestre y la mutante *fez-2* inoculadas con la bacteria exhibieron una notable ondulación y enrollamiento radicular, posiblemente asociado a una interacción entre la respuesta agravitrópica causada por *Achromobacter* sp. 5B1, la respuesta gravitrópica de la raíz y el contacto con el medio. La elongación diferencial de las células conduce a la formación de curvaturas para superar obstáculos en el suelo y responder a los cambios ambientales (Sato *et al*., 2015; Su *et al*., 2017). Para investigar más a fondo si el comportamiento agravitrópico de la raíz producido por *Achromobacter* sp. 5B1 involucra la elongación diferencial de las células, se realizaron mediciones de células de la epidermis y la corteza de la zona de elongación de la raíz de plántulas tipo silvestre, *fez-2* y *smb-3*. En la raíz de *fez-2*, las células mostraron una diferencia de crecimiento entre el lado convexo y cóncavo de la curvatura de la raíz; en contraste, en la raíz de *smb-3*, la longitud de las células se mantuvo similar en ambos lados de la raíz. Esto sugiere que los estímulos bióticos como la presencia de *Achromobacter* sp. 5B1, impulsan movimientos radiculares a través del crecimiento celular diferencial de las células y la participación de elementos localizados en la cofia.

Además, se evaluó si la estructura de la cofia podría influir en los patrones de crecimiento de la raíz en respuesta a *Achromobacter* sp. 5B1 y se descubrió que

la bacteria incremento el área de la cofia en los tres genotipos. Los datos indican que, si bien el inóculo bacteriano aumenta el tamaño de la cofia, posiblemente la forma y la estructura de la cofia que exhiben las mutantes *fez-2* y *smb-3* inoculadas con la bacteria podrían desencadenar el enrollamiento radicular o guiar el crecimiento hacia la gravedad, respectivamente. En conjunto con las diferencias aparentes en el tamaño de la cofia que muestran las mutantes *fez-2* y *smb-3*, un reporte previo señaló que la forma de la cofia influye en la capacidad de penetración de la raíz a través del medio: la mutante *fez-2* exhibe una cofia puntiaguda, lo que reduce su capacidad de penetración, mientras que en la mutante *smb-3* muestra una cofia con forma rectangular, lo que aumenta la capacidad de penetración de las puntas de sus raíces (Roue *et al*., 2020).

FEZ y SMB se distribuyen específicamente en la cofia e interactúan entre sí para el preciso control de los procesos de división y diferenciación celular (Willemsen *et al*., 2008; Bennett *et al*., 2010). Para evaluar si estos factores de transcripción podrían desempeñar una función en la respuesta a *Achromobacter* sp. 5B1, se evaluó su expresión en el ápice de la raíz de plántulas inoculadas con la bacteria. La expresión de *FEZ:GFP* y *SMB:GFP* mostró una tendencia opuesta; FEZ disminuyó y SMB aumentó en las células de la cofia, lo que indica su participación en el comportamiento radicular en co-cultivo con *Achromobacter* sp. 5B1. Estos resultados sugieren que la bacteria reprime la división celular y mejora la diferenciación celular en la cofia, lo que podría contribuir al crecimiento radicular direccional.

Previamente, se observó la formación de un gradiente lateral de auxinas en la punta de la raíz asociado con el crecimiento direccional de la raíz en respuesta a *Achromobacter* sp. 5B1 (Jiménez-Vázquez *et al*., 2020). Para confirmar la participación de la cofia en el comportamiento agravitrópico radicular en co-inoculación con la bacteria, analizamos la respuesta auxínica en la punta de la raíz de las mutantes *fez-2* y *smb-3*. Los datos mostraron una reducción en la expresión de *DR5:GFP* en la cofia de la mutante *fez-2* sin inoculo bacteriano, comparado con las plántulas de tipo silvestre. En las raíces de la mutante *fez-2* co-cultivadas con *Achromobacter* sp. 5B1, aunque la señal de fluorescencia dirigida por *DR5* fue

visible ligeramente, las auxinas se movilizaron hacia la capa lateral. Por el contrario, en las raíces de la mutante *smb-3*, se interrumpió la distribución asimétrica de las auxinas. En este contexto, se reportó que, en condiciones de deficiencia de nutrientes, como condiciones de bajo fosfato, SMB actúa como un modulador negativo de la señalización de las auxinas (Ravelo-Ortega *et al*., 2022). Evidencia reciente reveló la función crucial de SMB en el establecimiento del gradiente lateral de las auxinas en la cofia para desencadenar la curvatura halotrópica de la raíz (Zheng *et al*., 2024). Por lo tanto, es posible que la redistribución de las auxinas hacia la cofia lateral y las células de la epidermis, podría reducir la acumulación de las auxinas en la cofia y contribuir a la alteración en el gravitropismo de la raíz en respuesta a *Achromobacter* sp. 5B1. De acuerdo con Zhang *et al*. (2019), las auxinas median el gravitropismo radicular mediante la regulación en la acumulación de los gránulos de almidón en las células de la columnela, responsables de la percepción y la respuesta a la gravedad. Estos hallazgos indican que los factores de transcripción FEZ y SMB regulan la acumulación de las auxinas en la punta de la raíz. Esencialmente, SMB es necesario para el establecimiento de la distribución asimétrica de las auxinas a lo largo de la cofia para desencadenar el crecimiento direccional de la raíz en interacción con *Achromobacter* sp. 5B1.

La localización polar de los transportadores de las auxinas PIN coordina el transporte direccional de las auxinas célula a célula para regular las respuestas trópicas y el desarrollo (Han *et al*., 2021). La evidencia aquí mostrada indica que FEZ y SMB regulan las proteínas PIN, por su parte, la interacción con *Achromobacter* sp. 5B1 mostró un efecto sinérgico que contribuye a los cambios en la abundancia y la localización de los transportadores de las auxinas en la punta de la raíz. Las mutaciones en FEZ y SMB causaron cambios en la expresión de los transportadores de las auxinas PIN de una manera específica. En la mutante *fez-2*, la expresión de PIN1, PIN3 y PIN4 se redujo, mientras que, la expresión de PIN2 y PIN7 no cambió en plántulas sin el inóculo; sin embargo, en co-cultivo con la bacteria, la expresión de las proteínas PIN evaluadas se reprimió. La mutación en SMB incremento la represión de los transportadores de eflujo de las auxinas PIN excepto PIN7; con respecto a la colonización bacteriana de la raíz, la expresión de

los PINs disminuyó en la punta de la raíz, excepto en las células de la columnela. Por lo tanto, SMB es necesaria para la modulación específica de los transportadores de las auxinas PIN localizados en la cofia, activando la distribución asimétrica de las auxinas. Zheng *et al*. (2024) demostraron que SMB induce la expresión de la permeasa de las auxinas AUX1 en la cofia lateral para generar un gradiente lateral de las auxinas que desencadena la respuesta halotrópica de la raíz. Otro reporte evidenció que el transporte polar de las auxinas mediado por PIN promueve la acumulación de las auxinas en el lado cóncavo de la curvatura de la raíz durante el crecimiento lejos de la barrera física durante la evasión de obstáculos (Lee *et al*., 2020). Esta compleja regulación de las proteínas PIN sugiere que *Achromobacter* sp. 5B1 induce cambios en los transportadores de las auxinas para redirigir el flujo de las auxinas en la punta de la raíz a través de elementos endógenos de la cofia y promover la respuesta trópica causada por la rizobacteria.

Finalmente, a partir de esta compleja interacción planta-bacteria, los resultados revelaron que los elementos endógenos de la cofia, incluyendo FEZ y SMB, regulan los patrones de crecimiento de la raíz en respuesta a *Achromobacter* sp. 5B1, mediante la modulación del transporte polar de las auxinas mediado por PIN, para promover la redirección del flujo de las auxinas. En esencia, SMB es crucial para establecer la distribución asimétrica de las auxinas a través de la cofia, lo que desencadena el crecimiento direccional de la raíz. Los hallazgos aquí mostrados, sugieren que la punta de la raíz percibe y responde a *Achromobacter* sp. 5B1 a través de factores de transcripción relacionados con la cofia, lo que proporciona un marco para explorar el entorno heterogéneo de la rizosfera mediante movimientos dinámicos, con el fin de alcanzar áreas con disponibilidad de agua y nutrientes. La demostración de esta hipótesis, requiere una mayor investigación.

10. REFERENCES

Abdel-Rahman, H.M., Salem, A.A., Moustafa, M.M., & El-Garhy, H.A. (2017). A novice *Achromobacter* sp. EMCC1936 strain acts as a plant-growth-promoting agent. *Acta Physiologiae Plantarum*, *39*, 1-15.

Arabidopsis Genome Initiative genome analysis (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature*, *408*(6814), 796-815.

Arnaud, C., Bonnot C, Desnos, T. & Nussaume, L. (2010) The root cap at the forefront. *Comptes Rendus Biologies,* 333, 335-343.

Atieno, M., Herrmann, L., Nguyen, H. T., Phan, H. T., Nguyen, N. K., Srean, P., Than, M.M., *et al*., (2020). Assessment of biofertilizer use for sustainable agriculture in the Great Mekong Region. *Journal of environmental management*, 275, 111300.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, *9*, 1473.

Bailly, A., Groenhagen, U., Schulz, S., Geisler, M., Eberl, L., & Weisskopf, L. (2014). The inter-kingdom volatile signal indole promotes root development by interfering with auxin signalling. *The Plant Journal*, *80*(5), 758-771.

Baldwin, K.L., Strohm, A.K., & Masson, P.H. (2013). Gravity sensing and signal transduction in vascular plant primary roots. *American Journal of Botany*, *100*(1), 126-142.

Banda, J., Bellande, K., von Wangenheim, D., Goh, T., Guyomarc'h, S., Laplaze, L., & Bennett, M. J. (2019). Lateral root formation in Arabidopsis: a well-ordered LRexit. *Trends in Plant Science*, *24*(9), 826-839.

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*, *13*(3), 1140.

Beauregard, P.B. (2015). Not just sweet talkers: how roots stimulate their colonization by beneficial bacteria. In *Advances in Botanical Research* (Vol. 75, pp. 1-20). Academic Press.

Benková, E., & Bielach, A. (2003). Lateral root organogenesis from cell to organ. *Current Opinion in Plant Biology*, *13*(6), 677-683.

Bennett, T., Sanchez-Perez, G.F., Campilho, A., Willemsen, V., Snel, B. & Scheres, B. (2010). SOMBRERO, BEARSKIN1, and BEARSKIN2 regulate root cap maturation in Arabidopsis. *The Plant Cell*, 22, 64-54.

Bennett, T., van den Toorn, A., Willemsen, V. & Scheres, B. (2014). Precise control of plant stem cell activity through parallel regulatory inputs. *Development*, 141, 4055-4064.

Blancaflor, E.B. (2013). Regulation of plant gravity sensing and signaling by the actin cytoskeleton. *American Journal of Botany*, 100(1), 143-152.

Blancaflor, E.B., Fasano, J. & Gilroy, S. (1999). Laser ablation of root cap cells: implications for models of graviperception. *Advances in Space Research*, 24(6), 731-738.

Blancaflor, E.B., & Masson, P.H. (2003). Plant gravitropism. Unraveling the ups and downs of a complex process. *Plant Physiology*, *133*(4), 1677-1690.

Badri, D.V. & Vivanco, J.M. (2009). Regulation and function of root exudates. *Plant, Cell & Environment,* 32(6), 666-681.

Cassán, F., & Diaz-Zorita, M. (2016). *Azospirillum* sp. in current agriculture: From the laboratory to the field. *Soil Biology and Biochemistry*, *103*, 117-130.

Chang, J., Li, X., Fu, W., Wang, J., Yong, Y., Shi, H., Ding, Z., Kui, X., He, K., & Li, J. (2019). Asymmetric distribution of cytokinins determines root hydrotropism in Arabidopsis thaliana. *Cell Research*, *29*(12), 984-993.

Cheng, C.Y., Krishnakumar, V., Chan, A.P., Thibaud-Nissen, F., Schobel, S., & Town, C.D. (2017). Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *The Plant Journal*, 89(4), 789-804.

Chialva, M., Lanfranco, L., & Bonfante, P. (2022). The plant microbiota: composition, functions, and engineering. *Current Opinion in Biotechnology*, *73*, 135-142.

Darwin, C., & Darwin, F.E. (1888). The' Power of movement in plants. London: John Murray.

De Rybel, B., Vassileva, V., Parizot, B., Demeulenaere, M., Grunewald, W., Audenaert, D. *et al*. (2010). A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Current Biology*, *20*(19), 1697-1706.

De Smet, I., Tetsumura, T., De Rybel, B., Frey, N. F. D., Laplaze, L., Casimiro, I., Swarup, R., *et al*. (2007). Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. *Development*, 134 (4): 681–690.

De Smet, I., Lau, S., Mayer, U., & Jürgens, G. (2010). Embryogenesis–the humble beginnings of plant life. *The Plant Journal*, *61*(6), 959-970.

Dietrich, D. (2018). Hydrotropism: how roots search for water. *Journal of Experimental Botany*, *69*(11), 2759-2771.

Ding, Z. & Friml, J. (2010) Auxin regulates distal stem cell differentiation in Arabidopsis roots. *Proceedings of the National Academic of Sciences USA*, 107, 12046-12051.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P. *et al*. (1993). Cellular organisation of the Arabidopsis thaliana root. *Development.* 119, 71-84.

Driouich, A., Follet-Gueye, M. L., Vicré-Gibouin, M., & Hawes, M. (2013). Root border cells and secretions as critical elements in plant host defense. *Current Opinion in Plant Biology*, *16*(4), 489-495.

Driouich, A., Gaudry, A., Pawlak, B. & Moore, J.P. (2021). Root cap-derived cells and mucilage: a protective network at the root tip. *Protoplasma,* 258:1179-1185.

Driouich, A., Smith, C., Ropitaux, M., Chambard, M., Boulogne, I., Bernard, S., Follet-Gueye, M.L., Vicré, M., & Moore, J. (2019). Root extracellular traps versus neutrophil extracellular traps in host defence, a case of functional convergence? *Biological Reviews*, *94*(5), 1685-1700.

Du, Y., & Scheres, B. (2018). Lateral root formation and the multiple roles of auxin. *Journal of Experimental Botany*, *69*(2), 155-167.

Dubreuil, C., Jin, X., Grönlund, A. & Fischer, U. (2018). A local auxin gradient regulates root cap self-renewal and size homeostasis. *Current Biology*, *28*(16), 2581-2587.

Dubrovsky, J.G., Doerner, P. W., Colón-Carmona, A., & Rost, T.L. (2000). Pericycle cell proliferation and lateral root initiation in Arabidopsis. *Plant Physiology*, *124*(4), 1648-1657.

Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F., & Hashem, A. (2017). Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, *8*, 2104.

Fendrych, M., Van Hautegem, T., Van Durme, M., Olvera-Carrillo, Y., Huysmans, M., Karimi, M., *et al*. (2014). Programmed cell death controlled by ANAC033/SOMBRERO determines root cap organ size in Arabidopsis. *Current Biology*, 24, 931-940.

Feng, Q., De Rycke, R., Dagdas, Y., & Nowack, M.K. (2022). Autophagy promotes programmed cell death and corpse clearance in specific cell types of the Arabidopsis root cap. *Current Biology*, *32*(9), 2110-2119.

Fujii, N., Miyabayashi, S., Sugita, T., Kobayashi, A., Yamazaki, C., Miyazawa, Y., Kamada, M., *et al*. (2018). Root-tip-mediated inhibition of hydrotropism is accompanied with the suppression of asymmetric expression of auxin-inducible genes in response to moisture gradients in cucumber roots. *PLOS ONE*, *13*(1), e0189827.

Galván-Ampudia, C.S., Julkowska, M.M., Darwish, E., Gandullo, J., Korver, R.A., Brunoud, G., *et al*. (2013). Halotropism is a response of plant roots to avoid a saline environment. *Current Biology*, *23*(20), 2044-2050.

Ganesh, A., Shukla, V., Mohapatra, A., George, A.P., Bhukya, D.P.N., Das, K.K. *et al*. (2022). Root cap to soil interface: A driving force toward plant adaptation and development. *Plant and Cell Physiology*, 63(8), 1038-1051.

García-Cárdenas, E., Ortiz-Castro, R., Ruiz-Herrera, L.F. *et al*. (2022). *Micrococcus luteus* LS570 promotes root branching in Arabidopsis via decreasing apical dominance of the primary root and an enhanced auxin response. *Protoplasma,* 259(5), 1139-1155.

García-Cárdenas, E., Ortiz-Castro, R., Ruiz-Herrera, L.F. *et al.* (2023). *Bacillus* sp. LC390B from the maize rhizosphere improves plant biomass, root elongation, and branching and requires the phytochromes PHYA and PHYB for phytostimulation. *Journal of Plant Growth Regulation* 42, 3056–3070.

Goh, T., Joi, S., Mimura, T., & Fukaki, H. (2012). The establishment of asymmetry in Arabidopsis lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development*, *139*(5), 883-893.

Haberlandt, G. (1900). 31. G. Haberlandt: Ueber die Perception des geotropischen Reizes. *Berichte der Deutschen Botanischen Gesellschaft*, *18*(6), 261-272.

Han, H., Adamowski, M., Qi, L., Alotaibi, S.S. & Friml, J. (2021). PIN-mediated polar auxin transport regulations in plant tropic responses. *New Phytologist*, *232*(2), 510-522.

Hartman, K., & Tringe, S.G. (2019). Interactions between plants and soil shaping the root microbiome under abiotic stress. *Biochemical Journal*, *476*(19), 2705-2724.

Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. *Bacteria*, *3*(2), 59-75.

Hawes, M., Allen, C., Turgeon, B. G., Curlango-Rivera, G., Minh Tran, T., Huskey, D. A., & Xiong, Z. (2016). Root border cells and their role in plant defense. *Annual Review of Phytopathology*, *54*(1), 143-161.

Hetherington, A.J., & Dolan, L. (2018). Stepwise and independent origins of roots among land plants. *Nature*, *561*(7722), 235-238.

Hodson, M.J., & Mayer, A.M. (1987). Salt-induced changes in the distribution of amyloplasts in the root cap of excised pea roots in culture. *Annals of Botany*, *59*(5), 499-503.

Hu, F., Fang, D., Zhang, W., Dong, K., Ye, Z., & Cao, J. (2024). Lateral root primordium: Formation, influencing factors and regulation. *Plant Physiology and Biochemistry*, *207*, 108429.

Huysmans, M., Buono, R.A., Skorzinski, N., Radio, M.C., De Winter, F. *et al*. (2018) NAC transcription factors ANAC087 and ANAC046 control distinct aspects of programmed cell death in the Arabidopsis columella and lateral root cap. *The Plant Cell*, 30, 2197-2213.

Jha, P., & Kumar, A. (2009). Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microbial Ecology*, *58*, 179-188.

Jiménez-Vázquez, K.R., García-Cárdenas, E., Barrera-Ortiz, S. *et al.* (2020). The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. *The Plant Journal*, 103:1639-1654.

Jiménez-Vázquez, K.R., López-Hernández, J., García-Cárdenas, E. *et al*. (2023). The plant growth promoting rhizobacterium *Achromobacter* sp. 5B1 rescues Arabidopsis seedlings from alkaline stress by enhancing root organogenesis and hormonal responses. *Microbiological Research*, 281,127594.

Jones, D.L., Nguyen, C., & Finlay, R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soi*l, 321, 5–33.

Kader, M. A., & Lindberg, S. (2008). Cellular traits for sodium tolerance in rice (Oryza sativa L.). *Plant Biotechnology*, *25*(3), 247-255.

Kamiya, M., Higashio, S. Y., Isomoto, A., Kim, J. M., Seki, M., Miyashima, S., & Nakajima, K. (2016). Control of root cap maturation and cell detachment by BEARSKIN transcription factors in Arabidopsis. *Development*, *143*(21), 4063-4072.

Kanno, S., Arrighi, J. F., Chiarenza, S., Bayle, V., Berthome, R., Peret, B. *et al*. (2016). A novel role for the root cap in phosphate uptake and homeostasis. *Elife*, *5*, e14577.

Kesavan, P.C., & Swaminathan, M.S. (2018). Modern technologies for sustainable food and nutrition security. *Current Science*, *115*(10), 1876-1883.

Khoso, M.A., Wang, M., Zhou, Z., Huang, Y., Li, S., Zhang, Y. *et al*. (2024). Bacillus altitudinis AD13− 4 Enhances Saline–Alkali Stress Tolerance of Alfalfa and Affects Composition of Rhizosphere Soil Microbial Community. *International Journal of Molecular Sciences*, *25*(11), 5785.

Kiss, J.Z., Hertel, R., & Sack, F.D. (1989). Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. *Planta*, *177*(2), 198-206.

Kiss, J.Z., Guisinger, M.M., Miller, A.J., & Stackhouse, K.S. (1997). Reduced gravitropism in hypocotyls of starch-deficient mutants of Arabidopsis. *Plant and Cell Physiology*, *38*(5), 518-525.

Korver, R.A., van den Berg, T., Meyer, A.J., Galvan-Ampudia, C.S., Ten Tusscher, K.H., & Testerink, C. (2020). Halotropism requires phospholipase Dζ1-mediated modulation of cellular polarity of auxin transport carriers. *Plant, Cell & Environment*, *43*(1), 143-158.

Kumar, N., & Lyer-Pascuzzi, A.S. (2020). Shedding the last layer: mechanisms of root cap cell release. *Plants*, 9:308.

Kumpf RP, & Nowack MK. (2015). The root cap: a short story of life and death. *Journal of Experimental Botany*. *66*(19), 5651-5662.

Lee, H.J., Kim, H.S., Park, J.M., Cho, H.S., & Jeon, J.H. (2020). PIN-mediated polar auxin transport facilitates root− obstacle avoidance. *New Phytologist*, *225*(3), 1285-1296.

Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., ... & Sheen, J. (2013). Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. *Nature Biotechnology*, *31*(8), 688-691.

Li, Y., Shao, J., Xie, Y., Jia, L., Fu, Y., Xu, Z., Zhang, N., Feng, H., Xun, W., *et al*. (2021). Volatile copounds from beneficial rhizobacteria Bacillus spp. promote periodic lateral root development in Arabidopsis. *Plant Cell & Environment*, 44: 1663–1678.

Lijima, M., Morita, S., & Barlow, P.W. (2008). Structure and function of the root cap. *Plant Production Science*, *11*(1), 17-27.

Ling, N., Wang, T., & Kuzyakov, Y. (2022). Rhizosphere bacteriome structure and functions. *Nature Communications*, *13*(1), 836.

López-Ruiz, B.A., Zluhan-Martínez, E., Sánchez, M.D.L.P., Álvarez-Buylla, E.R., & Garay-Arroyo, A. (2020). Interplay between hormones and several abiotic stress conditions on *Arabidopsis thaliana* primary root development. *Cells*, *9*(12), 2576.

Luijten, M., & Heidstra, R. (2009). Arabidopsis root development. *Annual Plant Reviews Volume 37: root development*, *37*, 1-38.

Massa, G.D., & Gilroy, S. (2003). Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *The Plant Journal*, *33*(3), 435-445.

Mazumder, A., Gaur, V.S., Kole, P.C., & Mondal, T.K. (2024). Root halotropism in plants: tolerance or escape? *Rhizosphere*, 101002.

Maeda, K., Kunieda, T., Tamura, K., Hatano, K., Hara-Nishimura, I., & Shimada, T. (2019). Identification of periplasmic root-cap mucilage in developing columella cells of Arabidopsis thaliana. *Plant and Cell Physiology*, *60*(6), 1296-1303

Malamy, J.E., & Benfey, P.N. (1997). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development*, *124*(1), 33-44.

Marhavý, P., & Benková, E. (2015). Real-time analysis of lateral root organogenesis in Arabidopsis. *Bio-protocol*, *5*(8), e1446-e1446.

Méndez-Gómez, M., Barrera-Ortiz, S., Castro-Mercado, E. *et al*. (2020). The nature of the interaction *Azospirillum-Arabidopsis* determines the molecular and morphological changes in root and plant growth promotion. *Protoplasma*, 258:179-189.

Migliaccio, F., & Piconese, S. (2001). Spiralizations and tropisms in Arabidopsis roots. *Trends in Plant Science*, *6*(12), 561-565.

Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., Simon, M.D., Werck-Reichhart, D., & Ausubel, F.M. (2010). Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *The Plant Cell*, *22*(3), 973-990.

Miyazawa, Y., & Takahashi, H. (2020). Molecular mechanisms mediating root hydrotropism: what we have observed since the rediscovery of hydrotropism. *Journal of Plant Research*, *133*(1), 3-14.

Monshausen, G.B., & Gilroy, S. (2009). The exploring root—root growth responses to local environmental conditions. *Current Opinion in Plant Biology*, *12*(6), 766-772.

Motte, H., Vanneste, S., Beeckman, T. (2019). Molecular and enviromental regulation of root development. *Annual Review of Plant Biology.* 70, 465-88.

Muthert, L.W.F., Izzo, GL., Zanten, M.V. (2020). Root Tropisms: Investigations on Earth and in Space to Unravel Plant Growth Direction. *Frontiers in Plant Science*. 10, 1807.

Nakamura, M., Nishimura, T., & Morita, M.T. (2019). Gravity sensing and signal conversion in plant gravitropism. *Journal of Experimental Botany*, *70*(14), 3495-3506.

Nascimento, F.X., Glick, B.R., & Rossi, M.J. (2019). Isolation and characterization of novel soil-and plant-associated bacteria with multiple phytohormone-degrading activities using a targeted methodology. *Access Microbiology*, *1*(7), e000053.

Nascimento, F.X., Glick, B.R., & Rossi, M.J. (2021). Multiple plant hormone catabolism activities: an adaptation to a plant-associated lifestyle by Achromobacter spp. *Environmental Microbiology Reports*, *13*(4), 533-539.

Němec, B. (1900). 28. Bohumil Němec: Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen. *Berichte der deutschen botanischen Gesellschaft*, *18*(6), 241-245.

Odell, R.E., Dumlao, M.R., Samar, D., & Silk, W.K. (2008). Stage-dependent border cell and carbon flow from roots to rhizosphere. *American Journal of Botany*, *95*(4), 441-446.

Oldroyd, G.E. (2013). Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology*, *11*(4), 252-263.

Olvera-Carrillo, Y., Van Bel, M., Van Hautegem, T., Fendrych, M., Huysmans, M., Simaskova, M., *et al*. (2015). A conserved core of programmed cell death indicator genes discriminates developmentally and environmentally induced programmed cell death in plants. *Plant physiology*, *169*(4), 2684-2699.

Ortiz-Castro, R., Martínez-Trujillo, M., López-Bucio, J. (2008). N-acyl-Lhomoserine lactones: a class of bacterial quorum-sensing signals alter post-embryonic root development in Arabidopsis thaliana. *Plant Cell and Environment*, 2008, 31:1497–1509. 25.

Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Río, R. E., Campos-García, J., & López-Bucio, J. (2011). Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proceedings of the National Academy of Sciences*, *108*(17), 7253-7258.

Ortiz-Castro, R. & López-Bucio, J. (2019). Review: phytostimulation and root architectural responses to quorum-sensing signals and related molecules from rhizobacteria. *Plant Science*. 284, 135-142.

Ortiz-Castro, R., Campos-García, J., & López-Bucio, J. (2020). Pseudomonas putida and Pseudomonas fluorescens influence Arabidopsis root system architecture through an auxin response mediated by bioactive cyclodipeptides. *Journal of Plant Growth Regulation*, *39*(1), 254-265.

Ovečka, M., Luptovčiak, I., Komis, G., Šamajová, O., Samakovli, D., & Šamaj, J. (2020). Spatiotemporal pattern of ectopic cell divisions contributes to mis-shaped phenotype of primary and lateral roots of katanin1 mutant. *Frontiers in Plant Science*, *11*, 734.

Palacio-Rodríguez, R., Coria-Arellano, J.L., López-Bucio, J., Sánchez-Salas, J., Muro-Pérez, G., Castañeda-Gaytán, G., & Sáenz-Mata, J. (2017). Halophilic rhizobacteria from Distichlis spicata promote growth and improve salt tolerance in heterologous plant hosts. *Symbiosis*, *73*, 179-189.

Pantigoso, H.A., Newberger, D., & Vivanco, J.M. (2022). The rhizosphere microbiome: Plant–microbial interactions for resource acquisition. *Journal of Applied Microbiology*, *133*(5), 2864-2876.

Pérez-Flores, P., Valencia-Cantero, E., Altamirano-Hernández, J., Pelagio-Flores, R., López-Bucio, J., García-Juárez, P., Macías-Rodríguez, L. (2017). *Bacillus methylotrophicus* M4-96 isolated from maize (*Zea mays*) rhizoplane increases growth and auxin content in *Arabidopsis thaliana* via emission of volatiles. *Protoplasma*, 254: 2201–2213.

Petricka, J.J., Winter, C.M., & Benfey, P.N. (2012). Control of Arabidopsis root development. *Annual Review of Plant Biology*, *63*(1), 563-590.

Pi, L., Aichinger, E., van der Graaff, E., Llavata-Peris, C. I., Weijers, D., Hennig, L. *et al*. (2015). Organizer-derived WOX5 signal maintains root columella stem cells through chromatin-mediated repression of CDF4 expression. *Developmental Cell*, *33*(5), 576-588.

Ponce, G., Corkidi, G., Eapen, D., Lledías, F., Cárdenas, L., & Cassab, G. (2017). Root hydrotropism and thigmotropism in Arabidopsis thaliana are differentially controlled by redox status. *Plant Signaling & Behavior*, *12*(4), e1305536.

Porat, A., Tekinalp, A., Bhosale, Y., Gazzola, M. & Meroz, Y. (2024). On the mechanical origins of waving, coiling, and skewing in *Arabidopsis thaliana* roots. *Proceedings of the National Academy of Sciences*, 121(11), e2312761121.

Provart, N.J., J. Alonso, S.M., Assmann, D., Bergmann, S.M., *et al*. (2016). 50 years of Arabidopsis research: highlights and future directions. *New Phytologist*. 209: 921–94.

Raj, G., Shadab, M., Deka, S., Das, M., Baruah, J., Bharali, R., & Talukdar, N.C. (2019). Seed interior microbiome of rice genotypes indigenous to three agroecosystems of Indo-Burma biodiversity hotspot. *BMC Genomics*, *20*, 1-16.

Rakusová, H., Fendrych, M., & Friml, J. (2015). Intracellular trafficking and PIN-mediated cell polarity during tropic responses in plants. *Current Opinion in Plant Biology*, *23*, 116-123.

Ranjan, A., Sinha, R., Singla-Pareek, S.L., Pareek, A., & Singh, A.K. (2022). Shaping the root system architecture in plants for adaptation to drought stress. *Physiologia Plantarum*, *174*(2), e13651.

Ravelo-Ortega, G., Pelagio-Flores, R., López-Bucio, J., Campos-García, J., Reyes de la Cruz, H., & López-Bucio, J.S. (2022). Early sensing of phosphate deprivation triggers the formation of extra root cap cell layers via SOMBRERO through a process antagonized by auxin signaling. *Plant Molecular Biology*, 1-15.

Ravelo-Ortega, G., Raya-González, J. & López-Bucio, J. (2023). Compounds from rhizosphere microbes that promote plant growth. *Current Opinion in Plant Biology*, 73, 102336.

Rengasamy, P. (2006a). Soil salinity and sodicity. *In: Stevens, D. (Ed.), Growing Crops with Reclaimed Wastewater*. CSIRO, Victoria, 125-138.

Ropitaux, M., Bernard, S., Schapman, D., Follet-Gueye, M. L., Vicré, M., Boulogne, I., & Driouich, A. (2020). Root border cells and mucilage secretions of soybean, Glycine max (Merr) L.: characterization and role in interactions with the oomycete Phytophthora parasitica. *Cells*, *9*(10), 2215.

Roue, J., Chauvet, H., Brunel-Michac, N., Bizet, F., Moulia, B., Badel, E. & Legue, V. (2020). Root cap size and shape influence responses to the physical strength of the growth medium in Arabidopsis thaliana primary roots. *Journal of Experimental Botany*, *71*(1), 126-137.

Roychoudhry, S., & Kepinski, S. (2022). Auxin in root development. *Cold Spring Harbor Perspectives in Biology*, *14*(4), a039933.

Rüger, L., Ganther, M., Freudenthal, J., Jansa, J., Heintz-Buschart, A., Tarkka, M. T., & Bonkowski, M. (2023). Root cap is an important determinant of rhizosphere microbiome assembly. *New Phytologist*, *239*(4), 1434-1448.

Sánchez-López, Á. M., Baslam, M., De Diego, N., Muñoz, F. J., Bahaji, A., Almagro, G. *et al*. (2016). Volatile compounds emittedby diverse phytopathogenic microorganisms promote plant growth andflowering through cytokinin action. *Plant, Cell & Environment*, 39,2592–2608.

Sato, E.M., Hijazi, H., Bennett, M.J., Vissenberg, K. & Swarup, R. (2015). New insights into root gravitropic signalling. *Journal of Experimental Botany,* 66(8), 2155-2165.

Shi CL, Von Wangenheim D, Herrmann U, Wildhagen M, *et al.* (2018). The dynamics of root cap sloughing in Arabidopsis is regulated by peptide signalling. *Nature Plants*. *4*(8), 596-604.

Smith, S., & De Smet, I. (2012). Root system architecture: insights from Arabidopsis and cereal crops. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1595), 1441-1452.

Spaepen, S., Bossuyt, S., Engelen, K., Marchal, K., & Vanderleyden, J. (2014). Phenotypical and molecular responses of *Arabidopsis thaliana* roots as a result of inoculation with the auxin-producing bacterium *Azospirillum brasilense*. *New Phytologist*, *201*(3), 850-861.

Stoeckle, D., Thellmann, M., & Vermeer, J.E. (2018). Breakout—lateral root emergence in *Arabidopsis thaliana*. *Current Opinion in Plant Biology*, *41*, 67-72.

Su, S.H., Gibbs, N.M., Jancewicz, A.L., & Masson, P.H. (2017). Molecular mechanisms of root gravitropism. *Current Biology*, *27*(17), R964-R972.

Tanaka, K., Gilroy, S., Jones, A. M., & Stacey, G. (2010). Extracellular ATP signaling in plants. *Trends in Cell Biology*, *20*(10), 601-608.

Teixeira, J.A.S. & Tusscher, K.H.T. (2019). The Systems Biology of Lateral Root Formation: Connecting the dots. *Molecular Plant.* 12, 784-803.

Tian, H., Jia, Y., Niu, T., Yu, Q., & Ding, Z. (2014). The key players of the primary root growth and development also function in lateral roots in Arabidopsis. *Plant Cell Reports*, *33*, 745-753.

Tran, T. M., MacIntyre, A., Hawes, M., & Allen, C. (2016). Escaping underground nets: extracellular DNases degrade plant extracellular traps and contribute to virulence of the plant pathogenic bacterium Ralstonia solanacearum. *PLoS Pathogens*, *12*(6), e1005686.

Tsugeki, Ryuji., Fedoroff N. V. (1999) Genetic ablation of root cap cells in Arabidopsis. *Proceedings of the National Academy of Sciences USA*, 96: 12941-12946.

van den Berg, T., Korver, R. A., Testerink, C., & Ten Tusscher, K.H. (2016). Modeling halotropism: a key role for root tip architecture and reflux loop remodeling in redistributing auxin. *Development*, *143*(18), 3350-3362.

Verbon EH, Liberman LM (2016) Beneficial microbes affect endogenous mechanisms controlling root development. *Trends in Plant Science.* 21: 218-229.

Verhage, L. (2020). Underground allies: how bacteria stimulate plant growth by altering root development. *The Plant Journal*, *103*(5), 1637-1638.

Vitha, S., Yang, M., Sack, F.D., & Kiss, J.Z. (2007). Gravitropism in the starch excess mutant of Arabidopsis thaliana. *American Journal of Botany*, *94*(4), 590-598.

von Wangenheim, D., Fangerau, J., Schmitz, A., Smith, R. S., Leitte, H., Stelzer, E. H., & Maizel, A. (2016). Rules and self-organizing properties of post-embryonic plant organ cell division patterns. *Current Biology*, *26*(4), 439-449.

Waidmann, S., Sarkel, E., & Kleine-Vehn, J. (2020). Same same, but different: growth responses of primary and lateral roots. *Journal of Experimental Botany*, *71*(8), 2397-2411.

Weiller, F., Moore, J. P., Young, P., Driouich, A., & Vivier, M. A. (2017). The Brassicaceae species Heliophila coronopifolia produces root border-like cells that protect the root tip and secrete defensin peptides. *Annals of Botany*, *119*(5), 803-813.

Weise, S.E., & Kiss, J.Z. (1999). Gravitropism of inflorescence stems in starch-deficient mutants of Arabidopsis. *International Journal of Plant Sciences*, *160*(3), 521-527.

Wen, F., VanEtten, H.D., Tsaprailis, G., & Hawes, M.C. (2007). Extracellular proteins in pea root tip and border cell exudates. *Plant Physiology*, *143*(2), 773-783.

Willemsen, V., Bauch, M., Bennett, T., Campilho, A., Wolkenfelt, H., Xu, J., ... & Scheres, B. (2008). The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in Arabidopsis root stem cells. *Developmental cell*, *15*(6), 913-922.

Woodward, A.W., & Bartel, B. (2018). Biology in bloom: a primer on the Arabidopsis thaliana model system. *Genetics*, *208*(4), 1337-1349.

Xuan, W., De Gernier, H., & Beeckman, T. (2020). The dynamic nature and regulation of the root clock. *Development*, *147*(3).

Yalamanchili, K., Vermeer, J. E., Scheres, B., & Willemsen, V. (2024). Shaping root architecture: towards understanding the mechanisms involved in lateral root development. *Biology Direct*, *19*(1), 87.

Yamazaki, K., Ohmori, Y., & Fujiwara, T. (2020). A positive tropism of rice roots toward a nutrient source. *Plant and Cell Physiology*, *61*(3), 546-553.

Zhang, Y., He, P., Ma, X., Yang, Z., Pang, C., Yu, J., *et al*. (2019). Auxin-mediated statolith production for root gravitropism. *New Phytologist,* 224(2), 761-774.

Zheng, L., Hu, Y., Yang, T., Wang, Z., Wang, D., Jia, L. *et al*. (2024). A root cap-localized NAC transcription factor controls root halotropic response to salt stress in Arabidopsis. *Nature Communications*, *15*(1), 2061.

Formato de Declaración de Originalidad y Uso de Inteligencia Artificial

Coordinación General de Estudios de Posgrado
Universidad Michoacana de San Nicolás de Hidalgo

A quien corresponda,

Por este medio, quien abajo firma, bajo protesta de decir verdad, declara lo siguiente:

- Que presenta para revisión de originalidad el manuscrito cuyos detalles se especifican abajo.
- Que todas las fuentes consultadas para la elaboración del manuscrito están debidamente identificadas dentro del cuerpo del texto, e incluidas en la lista de referencias.
- Que, en caso de haber usado un sistema de inteligencia artificial, en cualquier etapa del desarrollo de su trabajo, lo ha especificado en la tabla que se encuentra en este documento.
- Que conoce la normativa de la Universidad Michoacana de San Nicolás de Hidalgo, en particular los Incisos IX y XII del artículo 85, y los artículos 88 y 101 del Estatuto Universitario de la UMSNH, además del transitorio tercero del Reglamento General para los Estudios de Posgrado de la UMSNH.

Datos del manuscrito que se presenta a revisión		
Programa educativo	Doctorado en Ciencias en Biología Experimental	
Título del trabajo	"Participación de los genes *FEZ* y *SOMBRERO* en el crecimiento de la raíz de *Arabidopsis* en interacción con *Achromobacter* sp. 5B1"	
	Nombre	**Correo electrónico**
Autor/es	M.C. Kirán Rubí Jiménez Vázquez	kiran.jimenez@umich.mx
Director	D.C. José López Bucio	jbucio@umich.mx
Codirector		
Coordinador del programa	D.C. Ernesto García Pineda	egpineda@umich.mx

Uso de Inteligencia Artificial		
Rubro	**Uso (sí/no)**	**Descripción**
Asistencia en la redacción	No	N/A

Formato de Declaración de Originalidad y Uso de Inteligencia Artificial

Coordinación General de Estudios de Posgrado
Universidad Michoacana de San Nicolás de Hidalgo

Uso de Inteligencia Artificial		
Rubro	**Uso (sí/no)**	**Descripción**
Traducción al español	No	N/A
Traducción a otra lengua	No	N/A
Revisión y corrección de estilo	No	N/A
Análisis de datos	No	N/A
Búsqueda y organización de información	No	N/A
Formateo de las referencias bibliográficas	No	N/A
Generación de contenido multimedia	No	N/A
Otro		

Datos del solicitante	
Nombre y firma	M.C. Kirán Rubí Jiménez Vázquez
Lugar y fecha	Morelia, Michoacán a 07 de mayo de 2025

Kirán Rubí Jiménez Vázquez

Participación de los genes FEZ y SOMBRERO en el crecimiento de la raíz de Arabidopsis en interacción

Universidad Michoacana de San Nicolás de Hidalgo

Detalles del documento

Identificador de la entrega
trn:oid:::3117:457315199

Fecha de entrega
9 may 2025, 8:47 a.m. GMT-6

Fecha de descarga
9 may 2025, 9:00 a.m. GMT-6

Nombre de archivo
Participación de los genes FEZ y SOMBRERO en el crecimiento de la raíz de Arabidopsis en interac....pdf

Tamaño de archivo
32.9 MB

152 Páginas

25.845 Palabras

144.731 Caracteres

47% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Top Sources

41% Internet sources
38% Publications
0% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.