UNIVERSITAT DE BARCELONA

Efecto de *Trichoderma asperellum* cepa T34 y compost en plantas de tomate frente estrés biótico

Elena Fernández Gómez

Efecto de *Trichoderma asperellum* cepa T34 y compost en plantas de tomate frente estrés biótico

Tesis Doctoral
Elena Fernández Gómez
Barcelona 2017

UNIVERSITAT DE BARCELONA

Efecto de *Trichoderma asperellum* cepa T34 y compost en plantas de tomate frente estrés biótico

Elena Fernández Gómez

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals

Secció Fisiologia Vegetal

Universitat de Barcelona

Barcelona, 2017

UNIVERSITAT DE BARCELONA

Efecto de *Trichoderma asperellum* cepa T34 y compost en plantas de tomate frente estrés biótico

Memoria presentada por Elena Fernández Gómez para optar al título de Doctor por la *Universitat de Barcelona*. Este trabajo se enmarca dentro del programa de doctorado de Biología Vegetal correspondiente a 2013/2017 del *Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals* de la *Secció Fisiologia Vegetal* de la *Facultat de Biologia* de la *Universitat de Barcelona*. Este trabajo ha sido elaborado bajo la dirección de la Dra. Mª Isabel Trillas Gay y el Dr. Guillem Segarra Braunstein.

Doctoranda

Elena Fernández Gómez

Directores de Tesis

Mª Isabel Trillas Gay

Guillem Segarra Braunstein

Barcelona, Junio de 2017

“Y, aunque vuelvo al principio,
nunca voy hacia atrás.”

Stravaganzza (Raíces)

Agradecimientos

Este ha sido un largo viaje que comenzó con la ilusión de vencer una gris rutina y superar un reto personal. Y casi sin darnos cuenta nos fuimos animando y esta aventura acabó tomando forma. Ahora que está llegando a su desenlace me he detenido a pensar en todo este tiempo, en cada uno de esos coloridos días repletos de alegrías, de lágrimas, de sueños, de dolor, de risas, de frustración, de ilusión, de amistad y de afecto. Y no puedo evitar esbozar una sonrisa porque… ¡ha sido un viaje inolvidable!

Por eso quiero agradeceros a todos los que habéis compartido un tramo de esta travesía y que habéis ayudado a que llegue a hacerse realidad:

En especial a mis directores de tesis Mª Isabel Trillas y Guillem Segarra por todas sus enseñanzas, su orientación y al gran apoyo que me han brindado todos estos años tanto a nivel profesional como personal.

A toda mi familia y amigos, en especial a mis padres Manuel Fernández y Mª Eugenia Gómez y a mi hermano Manel Fernández por su apoyo incondicional. También a los que se marcharon durante este tiempo como Mª Concepción Torres.

A Marc Galera por su gran ayuda y apoyo en tantos momentos que sería imposible mencionarlos todos. Y porque sin él, en los momentos más oscuros la luz no hubiera vuelto a brillar.

A Dolors Sant y Dolors Serret por su ayuda en las medidas de fotosíntesis e isótopos, a Joan Romanyà por su participación en el tercer capítulo y a Saúl Mayorga por el diseño de la portada.

A los compañeros del grupo como Jeorgina Elena, Boubker Bidellaoui-Idrissi Filali, Aixa Iñigo, Virginia Estévez y Mar Fernández.

A todos los compañeros que han pasado por la pecera, a los compañeros del Máster de Agrobiología Ambiental y a todo el Departamento de Biología Vegetal.

Además, al personal de la Universitat de Barcelona del punto de información, de limpieza, de seguridad, de los camps experimentals y de los serveis científico -técnics, al servicio de genómica

funcional del IRB de Barcelona, a la Universidad de Sevilla, a semillas Fitó y a Biocontrol Technologies.

Finalmente me gustaría dar las gracias a los compañeros de Inglés, de Japonés, del laboratorio de "Soluciones Generales" de Oryzon Genomics, y a los compañeros de LabsDivision.

¡Muchas Gracias a todos!

Esta tesis ha sido financiada por fondos de los proyectos AGL2010-21982-C02-02/AGR y AGL2015-66684-R del departamento de Ciencia y Tecnología y el Ministerio de Economía del gobierno español, por la Generalitat de Catalunya AGAUR 2014 SGR 863 "Grup de Fisiologia de les plantes en relació amb l'ambient" y por una beca de la *Universitat de Barcelona* (2014).

Índice

1. Introducción General

1. Introducción General

1.1. Situación actual de fertilizantes y productos fitosanitarios

Actualmente, en la Unión Europea (UE) y consecuentemente en España la protección del suelo agrícola se ha convertido en un objetivo prioritario. Debido principalmente, al uso masivo de fitosanitarios de tipo químico, a la explotación agrícola intensiva y a la dificultad de la rotación de cultivos en las últimas décadas, que han producido el empobrecimiento y la degradación del suelo. Según el RD 824/2005 de fertilizantes es de vital importancia encontrar nuevos productos con adecuada concentración de nutrientes y capacidad fertilizante que no conlleven efectos nocivos para la salud y la seguridad de las personas y del medio ambiente; permitiendo así, un buen abonado que garantice la fertilidad y valor agronómico presente y futuro del suelo (RD 824/2005). El pasado 2016 se presentó un borrador para actualizar la vigente regulación europea sobre el actual Reglamento de abonos (Reglamento CE Nº 2003/2003).

La nueva propuesta 2016/0084 (COD) restringiría el uso de microorganismos como fertilizantes tan solo a 4 grupos: *Azotobacter* spp., hongos micorrícicos, *Rhizobium* spp. y *Azospirillum* spp. La propuesta además detalla las características que han de cumplir los compost para poder ser aceptados como fertilizantes. Estos compost se han de obtener a través de un proceso de compostaje aeróbico procedente de recogida de residuos biológicos (dentro de la Directiva 2008/98/EC), de subproductos de origen animal (categorías 2 y 3 de la Regulación (EC) Nº 1069/2009), de organismos vivos o muertos o de partes de ellos que cumplan unos requisitos concretos o de aditivos compostados. Además afectaría a la Regulación (EC) Nº 1107/2009 sobre la comercialización de productos fitosanitarios.

Otra gran problemática ya conocida desde hace años pero que sigue teniendo un impacto importante en la actualidad, son las elevadas pérdidas económicas que producen las enfermedades de las plantas, aproximadamente $2.2 \cdot 10^5$ millones de dólares al año (Agrios, 2005). En los últimos años la UE ha prohibido y restringido el uso de productos fitosanitarios de origen químico, por ser altamente perjudiciales tanto para la salud humana como para el medio

ambiente, además de producir la aparición de resistencias conllevando la reducción de la eficacia de las materias activas. La vigente Directiva Europea 2009/128/EC para el uso sostenible de los plaguicidas, establece un marco de actuación para reducir los efectos perjudiciales asociados a estos, fomentando la gestión integrada de plagas y enfermedades. El control integrado incluiría reducir el uso de fitosanitarios de origen químico dando prioridad a otros métodos, siempre y cuando, permitan un adecuado control de las plagas y enfermedades. Entre los métodos no químicos encontraríamos: técnicas agronómicas (rotación de cultivos, técnicas de cultivo adecuadas, uso de variedades resistentes o tolerantes, uso equilibrado de fertilización, enmiendas, riego y drenaje, prevención de la propagación de los organismos nocivos, protección de los organismos beneficiosos), métodos físicos, métodos mecánicos o métodos biológicos (agentes de control biológico -ACB-, aceites, etc).

En los últimos años se han invertido grandes esfuerzos en estudiar las alternativas de control biológico y se ha puesto especial interés en el uso de microorganismos como ACBs.

Datos del 2017 indican que en Europa se han aprobado 56 microorganismos para ser empleados como sustancias activas. Entre ellos encontramos levaduras, virus, bacterias y en mayor número hongos (http://ec.europa.eu/food/plant/pesticides/13/04/2017). La exigente legislación y el largo proceso para la aprobación de estos productos limitan el número de nuevas materias activas autorizadas entre ellas los microorganismos (Directiva 91/414 CEE y actual Regulación (CE) 1107/2009). Entre los microorganismo aceptados los más representativos son *Bacillus* spp. y *Trichoderma* spp..

1.2. Microorganismos como Agentes de Control Biológico

En los últimos cuarenta años se han realizado un gran número de estudios y publicaciones científicas utilizando microorganismos como ACBs; ya sean virus, bacterias, hongos o en menor medida levaduras. El control biológico es algo más que una lucha entre dos microorganismos (buenos y malos) es una interacción a tres bandas donde la planta juega un papel muy importante. Estos microorganismos mejoran la salud de la planta con una acción directa sobre el

patógeno y/o indirecta sobre la planta. Entre los mecanismos empleados contra microorganismos se encuentra la antibiosis que se entiende como una inhibición o destrucción de un microorganismo por un producto metabólico de otro, competencia ente ellos que suele ser por nutrientes (carbono, nitrógeno, factores de crecimiento, oxígeno, etc) y espacio; y también fenómenos de parasitismo o depredación (Cook y Baker 1983). En cuanto a las acciones sobre la planta puede darse la promoción de crecimiento mejorando la absorción de elementos minerales del suelo y la activación de los mecanismos de defensa de la planta de forma que cuando resulte atacada tenga una respuesta mayor y más rápida (Heydari y Pessarakli, 2010).

Las estrategias para un eficiente control mediante ACBs sería muy parecida tanto para patógenos edáficos como foliares, es decir, reducir el inóculo del patógeno en el suelo y/o en planta mediante la acción directa de inocular un ACB y/o estimular la microbiota del propio suelo. Además de mejorar los mecanismos de defensa en planta con la utilización de determinadas cepas de ACBs capaces de desencadenar inducción de resistencia y/o promoción de crecimiento. Todo ello verificando que las condiciones ambientales donde se encuentra el cultivo sean adecuadas para el desarrollo del ACB y sus mecanismos de acción (Daguerre et al., 2016).

La utilización de ACBs también es muy útil después de tratamientos químicos autorizados o de una solarización y/o biofumigación, situación en la que la inoculación masiva con ACBs conllevaría la colonización del suelo eliminando poblaciones residuales del patógeno y evitando además la recolonización del patógeno (Galletti et al., 2008).

1.2.1. *Trichoderma* spp.

Trichoderma spp., forma teleomórfica *Hypocrea*, engloba a más de 100 especies de hongos filamentosos pertenecientes a los Ascomycetos (Druzhinina et al., 2011). Principalmente se pueden encontrar en suelos de todo el mundo, sobre la superficie de las plantas, corteza en descomposición y restos orgánicos (Zafra y Cortés-Espinosa, 2015). Las características que han conferido a ciertas cepas de *Trichoderma* la capacidad de ser buenos ACBs son la habilidad de sobrevivir en condiciones desfavorables y variables, una elevada capacidad reproductiva, la capacidad de modificar la rizosfera, la habilidad de competir con otros microorganismos,

eficiencia en el uso de nutrientes, la promoción del crecimiento y la potenciación de las defensas de las plantas (Yedidia et al., 2001; Benítez et al., 2004, Segarra et al., 2009, Trillas y Segarra 2009). La estimulación de las defensas de las planta puedes ser tanto frente a estreses de tipo biótico como abiótico (Shoresh et al., 2010). Los principales modos de acción de *Trichoderma* spp. para el control de enfermedades son competición por espacio y nutrientes, antibiosis, parasitismo e inducción de resistencia sistémica (Benítez et al., 2004; Trillas y Segarra 2009; Segarra et al., 2010; Borrero et al., 2012; Trillas y Segarra, 2012). Esta polivalencia le confiere un amplio espectro de acción frente a diversos patógenos edáficos (Howell et al., 2000; Howell, 2002; Pastrana et al., 2016; Chen et al., 2017) y foliares (De Meyer et al., 1998; Yedidia et al., 2003; Yao et al., 2016).

Por otra parte, su capacidad de estimular el crecimiento de las plantas también ha sido ampliamente documentada (Harman et al., 2004; Harman et al., 2006). Sus principales modos de acción son facilitar la absorción de nutrientes e incrementar la eficiencia en el uso del nitrógeno (NUE) (Shoresh et al., 2010; Altomare y Tringovska, 2011).

El ACB T34 es capaz de controlar enfermedades edáficas como *Rhizoctonia solani* en plantas de pepino (Trillas et al., 2006), *Fusarium oxysporum* f.sp. *lycopersici* en plantas de tomate (Segarra et al., 2010; Borrero et al., 2012), *Fusarium circinatum* en *Pinus radiata* (López-López et al., 2016) y *Phytophthora capsici* en plantas de pepino (Segarra et al., 2013c). Además, es capaz de inducir resistencia sistémica frente a diversos tipos de patógenos foliares como *Pseudomonas syringae* pv. *lachrymans* en plantas de pepino (Segarra et al., 2007a) y *P.syringae* pv. *tomato*, *Hyaloperonospora parasitica* y *Plectosphaerella cucumerina* en Arabidopsis (Segarra et al., 2009). T34 es capaz de controlar unos 20 patógenos distintos, en una gran diversidad de cultivos y ambientes (información confidencial Biocontrol Technologies). Por otra parte, T34 también puede proteger a las plantas frente a estrés de tipo abiótico como se ha demostrado en plantas de tomate sometidas a altas concentraciones de Fe (Segarra et al., 2010). Además, mejora la absorción de nutrientes y promueve el crecimiento de las plantas (De Santiago et al., 2013).

1.3. El compost

El compost se define como “el producto resultante de la descomposición aeróbica de la materia orgánica mediante el proceso de compostaje”. A su vez el proceso de compostaje se define como “descomposición biológica y estabilización de la materia orgánica, bajo condiciones que permitan un desarrollo de temperaturas termófilas como consecuencia de una producción biológica de calor, que da un producto final estable, libre de patógenos, semillas de malas hierbas y que aplicado al terreno produce un beneficio” (Álvarez, 2010).

Según el Ministerio de agricultura y pesca en España en 2008 se produjeron 11 millones de toneladas de residuos de origen agrícola, forestal y de la caza (http://www.mapama.gob.es /13/04/2017). Es de vital importancia reciclar estos residuos para minimizar su impacto sobre el medio ambiente y poder obtener fertilizantes innovadores y alternativos a los inorgánicos convencionales extraídos de minas o de producción química (modelo de economía lineal). La Comisión Europea con la propuesta del reglamento 2016/0084 (COD), pretende reducir estas importaciones mediante el reciclaje de bioresiduos y de otras materias primas secundarias, de acuerdo con un modelo de economía circular, generando así, valor añadido y la creación de empleo local.

La aplicación de compost en el suelo tiene un efecto positivo sobre su estructura, productividad y fertilidad debido a su contenido en materia orgánica, nitrógeno, fósforo y elementos traza (Chowdury et al., 2013). La materia orgánica mejora la capacidad hídrica del suelo y su agregación (estabilidad). Además, el compost proporciona carga microbiana al suelo que mejora su actividad biológica, que favorecerá el intercambio catiónico mediante la solubilización de nutrientes (Senesi, 1989). Por otra parte también favorece la degradación de pesticidas y otros compuestos orgánicos (Chowdury et al., 2013).

Actualmente, las principales aplicaciones del compost son: abono natural en agricultura extensiva y ecológica, fertilizante en labores de jardinería, formulación de sustratos, restauración de suelos degradados por proyectos de obra pública o debido a actividades extractivas (http://www.gencat.cat /13/04/2017) y control de enfermedades de las plantas en los compost

que presentan supresividad natural como se especifica en el Dossier Tècnic N74 (http://www.ruralcat.net / 31/05/2017)

1.3.1. Supresividad natural de los compost y mejora nutricional en plantas

Los compost se empezaron a utilizar en Estados Unidos de América a partir de los años 60 por ser considerados un fertilizante de calidad, con un alto aporte de nutrientes y un sustrato económico no contaminante (Hoitink et al., 1997). Posteriormente, se observó que la comunidad microbiana estable del compost le otorgaba una capacidad supresora de enfermedades (Hoitink et al., 1997). En un primer momento se llevaron a cabo estudios con enfermedades edáficas que evidenciaron una interacción antagonista entre los patógenos y los microorganismos beneficiosos. En un amplio número de estudios se ha determinado que esta interacción era debida a fenómenos de competición, antibiosis e hiperparasitismo (Hoitink et al., 1997; Hoitink y Boehm, 1999; Cotxarrera et al., 2002; Sant et al., 2010). En cambio, menos estudiada y más reciente ha sido la evaluación del efecto de los compost en enfermedades foliares. Este modelo de estudio se caracteriza por la separación espacial entre el patógeno y la microbiota del compost imposibilitando interacciones antagónicas entre los microorganismos, y a su vez, permitiendo evaluar el papel del compost en la inducción de resistencia en planta (Abbasi et al., 2002; Horst et al., 2005; Kavroulakis et al., 2005, Segarra et al., 2007b; Zhang et al., 1998). Sin embargo, resulta difícil separar la mejora nutritiva que aportan los compost para el crecimiento de las plantas de la reducción de enfermedades observada en los patógenos foliares. Una planta bien nutrida con mayor crecimiento será más resistente a condiciones adversas, ya sean de carácter biótico o abiótico. Por este motivo algunos autores atribuyen estos resultados a una inducción de resistencia sistémica y/o a una mejora nutricional (Horst et al., 2005; De Meyer et al., 1998; Segarra et al., 2007b; Yogev et al., 2010).

Varios estudios han determinado que la mejora nutricional que aportan los compost contribuye a la promoción del crecimiento de las plantas produciendo una mayor biomasa (Gallardo-Lara y Nogales, 1987; Bugbee y Frinck, 1989). Sin embargo, otras características de los compost como el pH y conductividad eléctrica (CE) pueden ser demasiado elevadas para el óptimo cultivo de muchas especies. Por este motivo, suele ser necesario realizar lavados o formulados con otros

sustratos para mejorar las propiedades de los compost (Cotxarrera et al., 2002; Sant et al., 2010; Avilés y Borrero 2017).

1.3.2. El alperujo

El alperujo ha sido clasificado dentro de las oleazas por el Catálogo Europeo de Residuos con el código CER 020301 entre los "Residuos de lavado, limpieza, pelado, centrifugación y separación".

El alperujo, por tanto, es un residuo muy contaminante procedente de la industria agroalimentaria del aceite de oliva. Es el principal residuo producido mediante el sistema de dos fases en almazara (Moreno y Moral, 2008). Está compuesto principalmente de alpechín (aguas de vegetación) y restos sólidos y grasos de la aceituna. Se caracteriza por ser rico en nutrientes (K y N), tener carácter ácido, elevada humedad, alto contenido en materia orgánica de tipo ligno-celulósica, alto contenido en grasas y carbohidratos, y una pequeña fracción de fenoles hidrosolubles (Alburquerque et al., 2004).

Según datos de la FAO, en 2014 España fue el principal productor mundial de aceite de oliva con una producción anual de $1.71 \cdot 10^6$ T (http://faostat.fao.org /13/04/2017) y, en consecuencia, también de alperujo.

La principales alternativas estudiadas para reutilizar el alperujo son el compostaje (Alburquerque et al., 2004) y la producción de biocombustible (Lama-Muñoz et al., 2014). Estudios recientes proponen el uso de extractos acuosos de alperujo como sustrato para la producción de carotenoides (Borroni et al., 2017) y una nueva aproximación para obtener energía del alperujo mediante carbonización hidrotermal (Benavente et al., 2017).

El proceso de compostaje permite mejorar las propiedades del alperujo para ser compatible con el crecimiento vegetal y ser reutilizado como sustrato, enmienda del suelo o fertilizante (Chowdhury et al., 2013; Avilés y Borrero, 2017). Aún así, tras el proceso de compostaje es necesario evaluar si existen problemas de fitotoxicidad asociados al compost producido (Chowdhury et al., 2015; De Corato et al., 2016). En el RD 506/2013, 2013 se recogen las

características que ha de cumplir el compost de alperujo para ser utilizado como enmienda orgánica en la producción de fertilizantes.

Además, algunos compost de alperujo son capaces de reducir las enfermedades producidas por patógenos edáficos (Reis y Coelho, 2011; Avilés y Borrero, 2017) y foliares (Segarra et al., 2013a, 2013b), sin embargo, la información existente al respecto es escasa.

1.4. Inducción de resistencia sistémica

La inducción de resistencia sistémica es un estado de las plantas en que su capacidad defensiva innata ha sido potenciada contra futuros encuentros con un amplio espectro de atacantes (Choudhary y Prakash, 2007). Al tratarse de una respuesta sistémica, las partes de la planta alejadas de la zona en contacto con el inductor también pueden ser inducidas. Las plantas pueden adquirir dicho estado tras la infección de un patógeno, tras ser colonizas por microorganismos beneficiosos, en respuesta al ataque de insectos herbívoros y tras ser expuestas a ciertos compuestos químicos (Pieterse et al., 2014). Los efectores que desencadenan la inducción de resistencia pueden proceder de la propia planta, activados en respuesta al daño producido (patrones moleculares asociados a daño -DAMPs-), y/o del atacante, pudiendo ser específicos del atacante o de amplio espectro (patrones moleculares asociados a patógenos-PAMPs- o patrones moleculares asociados a herbívoros -HAMPs) (Boller y Felix, 2009; Wu y Baldwin, 2010).

Existen dos vías de resistencia sistémica dependiendo del tipo de inductor y las moléculas de señalización implicadas: la resistencia sistémica adquirida (SAR) y la resistencia sistémica inducida (ISR) (Taiz y Zeiger, 2010).

La vía SAR se activa durante el ataque de un patógeno tras el reconocimiento de efectores, se producen cambios de expresión y se envía una señal desde la zona afectada que recorre toda la planta, confiriéndole así, resistencia ante un amplio espectro de patógenos (Pieterse et al., 2014). SAR requiere de la acción de la molécula señal ácido salicílico (SA) y está asociada al

acumulo de proteínas PR (Phatogenesis-Related) (Durrant y Dong, 2004; Ryals et al., 1996; Van Loon, 1997). Existen 17 familias de proteínas PR con diversas funciones y algunas de ellas con actividad antipatogénica como por ejemplo actividad glucanasa o quitinasa (Enoki y Suzuki, 2016). Sin embargo, varios estudios sugieren que la inducción de SAR puede producir un efecto negativo en el crecimiento de las plantas conllevando una reducción en la producción (Denánce et al., 2013; Walters y Heil, 2007).

La vía ISR se desencadena por la interacción entre la planta y microorganismos no patogénicos. La principal molécula señal que interviene en este proceso es el ácido jasmónico (JA) (Pieterse et al., 1996). Sin embargo, estudios en *Arabidopsis thaliana* con mutantes evidencian la implicación de otra molécula señal en la vía ISR: el etileno (ET) (Van Loon et al., 1998). En este tipo de interacción no se observa en un primer momento sobreexpresión de genes relacionados con mecanismos de defensa(Van Wees, 1999), probablemente porque conduciría a una fuerte inversión de recursos que repercutiría negativamente en la planta (Heil, 2002). Sin embargo, ante el ataque de un patógeno la planta es capaz de responder con mayor fuerza y rapidez minimizando el efecto de la enfermedad. Este fenómeno se denomina “priming” y puede desencadenarse por SAR, ISR y estrés abiótico (Conrath et al., 2006).

Estudios con *Trichoderma harzianum* cepa T-203 observaron que el ACB puede activar proteínas PR (Yedidia et al., 2000). Además, estudios con T34 muestran que es capaz de inducir ISR o SAR dependiendo de las poblaciones de T34 en las raíces (Segarra et al., 2009). Según Tjamos et al. (2005) la inducción por ISR depende de la combinación planta, ACB y patógeno. Estudios recientes muestran que cepas de *Trichoderma* spp. alteran la expresión génica en plantas, principalmente activando genes de respuesta a estrés y defensa pero siempre sin afectar negativamente al crecimiento de la planta (Alfano et al., 2007; Segarra et al., 2007a; Brotman et al., 2012; Mathys et al., 2012).

Los fenómenos de ISR han sido estudiados en organismos beneficiosos como bacterias promotoras del crecimiento (PGPR), principalmente en cepas de *Pseudomonas*, *Serratia* y *Bacillus*; hongos promotores del crecimiento, principalmente en cepas de *Trichoderma*, *F.*

oxysporum y *Piriformosa indica*; y también en hongos simbiontes de micorrizas arbusculares (Pieterse et al., 2014).

Además en insectos herbívoros se ha observado la inducción de resistencia por herbívoros (HIR) mediante el reconocimiento de DAMPs, HAMPs y/o efectores específicos (Wu y Baldwin, 2009). La vía desencadena la producción de inhibidores de proteinasas que inhiben enzimas intestinales de los insectos y la producción de compuestos volátiles que atraen a depredadores naturales del herbívoro (Wu y Baldwin, 2010). JA es la principal hormona implicada en desencadenar las respuestas asociadas a herbívoros y heridas (Reymond et al., 2000; Li et al., 2002; Reymond et al., 2004).

1.5. *Botrytis cinerea*

Botrytis cinerea Persoon, forma teleomorfica *Botryotinia fuckeliana* (de Bary) es un patógeno foliar capaz de infectar a más de 200 especies de plantas, causando un elevado impacto económico por los severos daños producidos en pre y post cosecha, siendo altamente destructivo en tejidos dañados, maduros o senescentes (Williamson et al., 2007). Por estos motivos, fue clasificado en segunda posición entre los hongos fitopatógenos de mayor importancia desde el punto de vista científico y económico (Dean et al., 2012).

Es un hongo necrotrófico capaz de sobrevivir saprofíticamente sobre restos vegetales en descomposición. Además, puede permanecer durante largos periodos en forma de esclerocio en estado quiescente ante condiciones desfavorables como temperaturas superiores a 25 °C, humedad relativa baja, escasez de nutrientes y ausencia de lámina de agua en hoja (Elad et al., 1993).

B. cinerea produce la enfermedad denominada moho gris que se caracteriza por la producción de enzimas degradadoras de la pared celular y compuestos tóxicos que propician el desencadenamiento de una oleada oxidativa que culmina en la muerte celular programada del huésped (Williamson et al., 2007).

La enfermedad en hojas de tomate cursa la siguiente sucesión de síntomas visibles: clorosis, puntos necróticos húmedos, mancha necrótica húmeda que se va extendiendo por los foliolos con aparición de densas masas gris-parduzco de conidios, la hoja acaba momificándose y finalmente puede llegar a escindirse. Si la infección se extiende por el resto de tejidos y órganos puede producir la muerte de la planta.

Los métodos habituales para el control de la enfermedad son fungicidas de amplio espectro y fungicidas específicos (botriticidas), sin embargo, *B. cinerea* ha desarrollado resistencia a muchos de ellos (Leroch et al. 2011; De Ward et al., 2006). En consecuencia, para reducir el uso de productos químicos se está optando por fomentar las prácticas culturales y el control biológico.

Según Williamson et al. (2007), las prácticas culturales más eficientes para el control de *B. cinerea* son: reducir el gradiente térmico en invernaderos, crear un "canopy" abierto para mejorar el movimiento de aire y la penetración de la luz, incrementar la ventilación en el invernadero, utilizar plásticos con filtros de ultravioleta cercano, control de la temperatura durante la post-cosecha, eliminación de restos vegetales para reducir el inóculo, uso de mantillo o "mulching", adecuada separación de las plantas y evitar heridas (insectos, manipulación, …).

En el control biológico de *B. cinerea* se han utilizado microorganismos como ACBs y en menor media se han empleando compost.

Los principales modos de acción de los microorganismos en el control biológico de *B. cinerea* son el parasitismo, la antibiosis, la competición por nutrientes y espacio, la producción de enzimas, la inhibición de la esporulación, inhibición del desarrollo del tubo germinativo y la inducción de resistencia (Elad. et al, 2007; Jacommetti et al., 2010). Según Elad et al. (2007) existe un gran número de microorganismos capaces de controlar las enfermedades causadas por *Botrytis* spp., sin embargo, la reproducibilidad de su control en diferentes zonas y estaciones ha sido muy baja. Los autores sugieren que *Trichoderma* spp. seria uno de los mejores grupos para su control por su capacidad de supervivencia sobre las plantas en condiciones de campo, ser compatible con el control químico, su complejo modo de acción sobre diferentes formas de la enfermedad y su capacidad de inducir resistencia.

Por otra parte, los principales mecanismos de acción de los compost para reducir la enfermedad son la mejora nutricional que aportan a la plantas y el desencadenamiento de la inducción de resistencia (Horst et al., 2005; Segarra et al., 2007b).

El hecho de que *B. cinerea* sea un patógeno foliar permite estudiar fenómenos de inducción de resistencia gracias a la separación espacial entre los microorganismos beneficiosos (raíces) y el patógeno (hojas) sin la necesidad de utilizar sistemas de "Split root" típicamente utilizados para estudiar inducción en el caso de patógenos edáficos (Yogev et al., 2010).

1.6. El cultivo del tomate

El cultivo del tomate *Lycopersicon esculentum* (Miller 1768) o *Solanum lycopersicum* (Linné 1753) adquirió elevada importancia económica a nivel mundial a partir del siglo XIX, llegando en la actualidad a ocupar la décima posición en producción entre las materias primas agrícolas relacionadas con la alimentación y con un valor bruto asociado de $9.6\ 10^4$ millones de dólares, según datos del 2013 procedentes de la *Food and Agriculture Organitazion* (FAO). En el ranking mundial de productores de tomate, España ocupa el octavo lugar con una producción promedio entre 2005 y 2014 de $4.24 \cdot 10^6$ T anuales (http://faostat.fao.org /13/04/2017).

El tomate es una especie susceptible a la enfermedad moho gris causada por *B. cinerea* que provoca pérdidas durante la producción en invernadero y en postcosecha.

A parte de su importancia económica y susceptibilidad a *B. cinerea*, el tomate ha sido seleccionado para la elaboración de esta tesis por ser parcialmente tolerante a la salinidad, debido a que su producción solo desciende al alcanzar el valor umbral de conductividad eléctrica (CE) 2.5 $mS \cdot cm^{-1}$ (Maas y Hoffman, 1977) y esto nos permite utilizar materiales con elevada conductividad eléctrica como los compost.

Gran número de estudios sobre interacción entre planta-patógeno se han realizado con plantas modelo como *A. thaliana* debido a sus ventajas para trabajar a nivel de laboratorio como son su ciclo de vida corto, su pequeño tamaño, la disponibilidad de la secuenciación completa de su

pequeño genoma o la amplia colección de mutantes existentes (Andargie y Li, 2016). Sin embargo, es necesario potenciar el uso de otras especies como los cultivos de elevada importancia económica que pueden permitir una aproximación más cercana a la realidad en campo. La secuenciación del genoma del tomate en 2012 (Tomato Genome Consortium, 2012) junto a la comercialización de herramientas como los microarrays de tomate (aún considerados mycroarrays raros) están propiciando en los últimos años el uso del tomate para estudios de expresión génica.

Por otra parte, muchos estudios relacionados con la resistencia hacia patógenos se centran en las interacciones entre los ACB y patógenos, dejando en un plano secundario a la planta o incluso atribuyéndole un papel pasivo. Sin embargo, las plantas pueden mantener interacciones negativas (relacionadas con patogénesis) y positivas (relacionadas con simbiosis y protección) con otras plantas, con microorganismos y con invertebrados (Haichar et al., 2014). En estas interacciones juegan un papel importante los exudados de las raíces (Bais et al., 2006). Estos exudados contienen agua, iones, amino ácidos, ácidos orgánicos, azúcares, enzimas y compuestos fenólicos (Bertin et al., 2003) y suponen una gran fuente de carbono y energía para los microorganismos de la rizosfera. La calidad y cantidad de estos exudados depende de factores externos (bióticos y abióticos) y características de la propia planta (especie, genotipos dentro de la especie, crecimiento de la raíz y etapa del desarrollo de la planta) (Badri y Vivanco, 2009).

Algunos componentes de los exudados de las raíces como los ácidos orgánicos, influyen sobre la colonización de las raíces, la quimiotaxis y la motilidad del ACB *Bacillus amyloquefaciens* (Tan et al., 2013), la adhesión y formación de biofilm en *Bacillus subtilis* FB17 (Rudrappa at al., 2008) y sobre la actividad antifúngica de las PGPR *Pseudomonas Chlororaphis* SPB1217 y *Pseudomonas fluorescens* SPB2137 (Kravchenko et al., 2003). Varios estudios han determinado que la presencia de ACBs o patógenos modifica de diferente forma la secreción de exudados (Kamilova et al., 2006; Steinkellner et al., 2008). Incluso existen evidencias de que se modifica el patrón de exudados de *A. thaliana* ante la cepa salvaje del ACB *Pseudomonas putida* KT2440 y la cepa mutante defectiva en inducción de resistencia (Matilla et al., 2010). Además, según Zhang et al. (2013), la interacción entre plantas de pepino y el ACB *T. harzianum* T-E5 conlleva una

modificación en los exudados radiculares que, a su vez, inhiben la germinación del patógeno edáfico *F. oxysporum* f. sp. *cucumerinum*.

Los estudios realizados hasta el momento sobre exudados apuntan a que tienen un papel fundamental en fomentar la colonización de las raíces por parte de ciertos microorganismos e inhibir la de otros. Sin embargo, aunque existen evidencias de que influyen en la defensa de las plantas sobre enfermedades aéreas no está claro el papel que podrían desempeñar sobre la inducción de resistencia.

2. Objetivos

2. Objetivos

El objetivo general de la tesis es estudiar el efecto del agente de control biológico *Trichoderma asperellum*, cepa T34 y compost de alperujo empleados como medio de cultivo en el crecimiento de plantas de tomate y frente a la enfermedad producida por el patógeno foliar *Botrytis cinerea*.

Los objetivos específicos de esta tesis son:

1. Evaluar el crecimiento de las plantas crecidas en compost de alperujo.
2. Evaluar el crecimiento de las plantas crecidas en perlita enriquecida con T34.
3. Evaluar el efecto supresor del compost de alperujo en las plantas frente *B. cinerea.*
4. Evaluar el efecto supresor de la perlita enriquecida con T34 en las plantas frente *B. cinerea*
5. Evaluar el efecto de *B. cinerea* sobre el patrón de exudados de las plantas.
6. Evaluar el efecto de los exudados sobres las poblaciones de T34 y su relación con la inducción de resistencia sistémica.
7. Evaluar el efecto de T34 sobre la modulación de la expresión génica de las plantas y su relación con la promoción del crecimiento y los mecanismos de inducción de resistencia.
8. Evaluar el efecto de la luz y la solución nutritiva en el crecimiento de las plantas cultivadas en presencia y ausencia de T34.

3. Informe de impacto de los artículos

La Dra. Mª Isabel Trillas Gay y el Dr. Guillem Segarra Braunstein como directores de la Tesis que lleva por título: **"Efecto de *Trichoderma asperellum* cepa T34 y compost en plantas de tomate frente estrés biótico"**

INFORMAN sobre el índice de impacto y la participación de la doctoranda en cada uno de los artículos incluidos en la memoria de esta Tesis Doctoral.

Capítulo I. Artículo: "**Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato**" publicado en la revista ***Biological Control*** de la Editorial Elsevier con un índice de impacto 2.012. En este estudio se evaluó la inducción de resistencia en plantas de tomate cultivadas en un sustrato orgánico con elevada actividad microbiológica (compost de alperujo maduroe de 2 años de estabilización) comparado con un sustrato mineral de nula actividad microbiológica (perlita), sin inocular o inoculado con *T. asperellum* cepa T34. La separación espacial entre el patógeno foliar (*B. cinerea*) y los microorganismos del compost o el agente de control biológico, permitió evaluar los fenómenos de inducción.

Este estudio es una contribución original a la inducción de resistencia en planta por parte de los compost como sustrato de cultivo, a la vez que aporta nuevos conocimientos en los mecanismos de interacción entre planta y hongos beneficiosos. La doctoranda contribuyó activamente en la realización de todas las fases del desarrollo experimental y en la redacción de este artículo, desde el diseño conceptual hasta la ejecución física de los ensayos y el análisis de los datos. En esta primera fase de la tesis la tutorización es más elevada, como corresponde.

Capítulo II. Artículo: "**Increased rhizosphere populations of *Trichoderma asperellum* strain T34 caused by secretion pattern of root exudates in tomato plants inoculated with *Botrytis cinerea***" publicado en la revista ***Plant Pathology*** de la editorial Wiley Online Library (British Society for Plant Pathology) con un índice de impacto de 2.383. En este estudio se evaluaron los exudados de las raíces secretados por las plantas de tomate y su relación con la inducción de resistencia en un sustrato de perlita inoculado con *T. asperellum* cepaT34 frente al patógeno

foliar *B. cinerea*. Los resultados obtenidos mostraron que las plantas de tomate infectadas con *B. cinerea* en las hojas indujeron un cambio en el patrón de secreción de los exudados de las raíces.

Este estudio es una contribución original en la comunicación raíz / organismo beneficioso / planta / organismo patógeno. La doctoranda contribuyó activamente en la realización de todas las fases de ejecución física de los ensayos, desde el diseño experiemntal hasta la redacción del artículo, y el análisis de los datos. En esta segunda fase de la tesis la doctoranda es más propositiva, como corresponde.

Capítulo III. Artículo: "***Trichoderma asperellum* strain T34 on the rhizosphere induce on tomato plants growth promotion and up-regutate expression of genes related to response to stimulus, reponse to stress and proteolisis"**. Se ha elaborado un manuscrito para ser enviado a la revista ***PLOS ONE*** de la editorial Public Library of Science (PLoS) con un índice de impacto de 4.411. En este estudio se evaluó el efecto de *T. asperellum* cepa T34 sobre el crecimiento de las plantas en condiciones de alta y baja luz y altos y bajos nutrientes en la solución nutritiva. Además, se seleccionaron las plantas de tomate crecidas en alta nutrición y alta luz para estudiar el efecto de *T. asperellum* cepa T34 en la modulación de la expresión génica de las hojas de tomate.

Este estudio nos permite profundizar sobre el conocimiento del efecto a medio término de *T. asperellum*, cepa T34 en diferentes condiciones ambientales y su interacción con la planta. Aunque estos microorganismos se consideren fundamentalmente fitosanitarios su papel secundario en la promoción del crecimiento y absorción de nutrientes es relevante desde el punto de vista agronómico a la vez que diferencial sobre los tratamientos fitosanitarios clásicos. La doctoranda contribuyó activamente en la realización de todas las fases del estudio y escritura del artículo, asoliendo el estado de madurez que corresponde a un doctorando que está terminando su tesis.

4. Resultados

4. Resultados

4.1. Capítulo I: Efecto fisiológico de la inducción de resistencia por compost o *Trichoderma asperellum* cepa T34 frente a *Botrytis cinerea* en tomate

Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato

Fernández E, Segarra G, Trillas MI, 2014. Biological Control 78, 77–85.

Resumen

Ciertos tipos de compost empleados como medios de cultivo pueden inducir en plantas respuestas de resistencia frente a patógenos foliares. La inducción de resistencia en ocasiones puede estar asociada con una reducción en el crecimiento y en el rendimiento de las plantas. El objetivo de este estudio fue determinar si las plantas cultivadas en compost de alperujo habían mejorado su resistencia frente a *B. cinerea* en detrimento de su crecimiento o del rendimiento fisiológico. Las plantas de tomate cultivadas en compost maduro de alperujo tenían aproximadamente un 60% menos de severidad de la enfermedad que las plantas cultivadas en perlita. Como referencia, las plantas cultivadas en perlita enriquecida con el agente de control biológico inductor de resistencia T34 tuvieron un 35% menos de severidad de la enfermedad que las plantas cultivadas en perlita. La vía SA / ABA estuvo implicada en la resistencia sistémica inducida por compost. En cambio, no lo estuvo en la inducida por perlita enriquecida con T34. Las medidas fisiológicas del estado hídrico, la relación raíz / brote, los isótopos estables de C y la fluorescencia de las clorofilas mostraron que las plantas cultivadas en compost estaban próximas a una situación de estrés. Sin embargo, el crecimiento medido como biomasa y altura de las plantas cultivadas en compost fue mayor que en las plantas cultivadas en perlita, lo que sugiere que las plantas en compost fueron cultivadas en una situación de euestrés. Las plantas de tomate cultivadas en perlita enriquecida con T34 tuvieron un mejor crecimiento, medido como área foliar total, biomasa, altura y absorción de nutrientes, que las plantas cultivadas en perlita.

Las medidas fisiológicas mostraron que las plantas cultivadas en perlita o perlita enriquecida con T34 no mostraron ninguna situación de estrés abiótico.

Biological Control 78 (2014) 77–85

Contents lists available at ScienceDirect

Biological Control

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

Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato

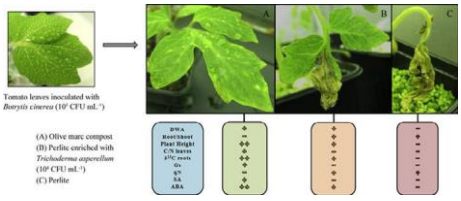
E. Fernández *, G. Segarra, M.I. Trillas

Unitat de Fisiologia Vegetal, Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Catalonia, Spain

highlights

- ·Compost triggered eustress in tomato plants, improving growth and health.
- ·Compost induced systemic resistance linked to SA pathway/ABA.
- ·*Trichoderma*-enriched perlite improved plant growth and innate disease resistance.
- ·Different mechanisms of induced resistance are involved in compost and *Trichoderma*.

graphical abstract



article info

Article history:
Received 17 March 2014
Accepted 21 June 2014 Available online 10 August 2014

Keywords:
Eustress
Induced plant disease resistance
Olive marc compost
Solanum lycopersicum
Stable isotopes

abstract

Certain types of compost used as growth media can induce resistance to foliar pathogens in above-ground parts of a plant. The induction of resistance can sometimes be associated with growth impairment and yield reduction. The objective of this study was to establish whether plants grown in olive marc compost had enhanced resistance against *Botrytis cinerea* at the cost of growth or physiological performance.

Tomato plants grown in mature olive marc compost had approximately 60% less disease severity than plants grown in perlite. As a reference, plants grown in perlite enriched with the known inducer of resistance *Trichoderma asperellum* strain T34 (T34) had 35% less disease severity than plants grown in perlite. The salicylic acid (SA) pathway/abscisic acid (ABA) is involved in compost induced systemic resistance. Instead, perlite enriched with T34 is not linked to SA pathway/ABA. Physiological measures of water status, root/shoot ratio, stable isotopes of C and chlorophyll fluorescence showed that the plants grown in compost were close to a stress situation. However, growth measured as biomass and plant height of plants grown in compost was higher than in plants grown in perlite suggesting that plants in compost were not grown in a stress situation, but in a eustress. Tomato plants grown in perlite enriched with T34 had better growth, measured as total leaf area, biomass, height and nutrient uptake, than plants grown in perlite. Physiological measures showed that plants grown either in perlite or perlite enriched with T34 did not show any abiotic stress situation.

1. Introduction

Botrytis cinerea Persoon: Fries, teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel is a plant pathogenic fungus of economic relevance, since it can infect over 200 plant species. This fungus is also known as grey mold and is one of the most extensively studied necrotrophic fungal pathogens. According to Dean et al. (2012), this pathogen was rated the second most important in an international survey of fungal pathologists. Specific fungicide (botryticide) applications and broad spectrum fungicides are the most common method to control this disease. However, fungicide resistance is

* Corresponding author. Fax: +34 934112842.
E-mail address: efernago13@gmail.com (E. Fernández).

http://dx.doi.org/10.1016/j.biocontrol.2014.06.012 1049-9644/

becoming an important problem (De Ward et al., 2006; Leroch et al., 2011). Moreover, Directive 2009/128/EC will implement integrated pest and disease management in Europe by 2014.

The tomato (*Lycopersicon esculentum* [Miller, 1768], *Solanum lycopersicum* [Linné, 1753]) crop is the eighth largest in the world in terms of food and agricultural commodities production, according to the Food and Agricultural Organization (FAO) (http://faostat.fao.org/site/339/default.aspx). Spain occupies ninth position in the world in value and production of tomato. This crop is susceptible to *B. cinerea*, which leads to losses during production (greenhouse) and post-harvest. The tomato crop was also selected for this study because it is classified as moderately tolerant to salinity (yield decline at a threshold value of 2.5 mS cm^{-1}) (Maas and Hoffman, 1977). The growth medium evaluated in this study is alperujo compost characterized by basic pH and high electrical conductivity (EC) that may not be optimal for plant cultivation. Therefore, some compost requires formulation with other materials like peat, perlite, coconut fiber, etc. (Cotxarrera et al., 2002). Spain generates a large amount of horticultural residues, such as alperujo, which is waste from the olive oil industry. Alperujo is highly contaminating, acidic, and rich in nutrients (K and N) and lignocellulosic organic matter and has a high fat; carbohydrate and water-soluble phenol content (Alburquerque et al., 2004). Alperujo is a mixture of dregs (liquid that emerges from the olive paste) and marc (pits, skins and pulp). Alperujo can be composted by the addition of olive tree leaves and manure and can be used in agriculture as growth media, amendments or fertilizer. Composts can promote plant growth by nutrition improvement (Bugbee and Finck, 1989; Gallardo-Lara and Nogales, 1987; Zhang et al., 2012).

Hoitink et al. (1997) proposed that some types of compost naturally suppress certain plant pathogens. The first studies showed that microorganisms played an important role, with antagonistic interactions (competition, hyperparasitism and antibiosis) between the pathogens and the beneficial microorganisms (Cotxarrera et al., 2002; Hoitink et al., 1997; Hoitink and Boehm, 1999; Sant et al., 2010). Moreover, some authors have claimed that certain types of compost used as growth media can induce resistance to foliar pathogens in above-ground parts of a plant, as the microbial populations of the compost are spatially separated (Abbasi et al., 2002; Horst et al., 2005; Kavroulakis et al., 2005; Yogev et al., 2010). This spatially separation is an indirect evidence of the induction of plant resistance and also could be attributed to improved nutrition in plants (Abbasi et al., 2002; De Meyer et al., 1998; Horst et al., 2005; Segarra et al., 2007b; Yogev et al., 2010). A significant increase in peroxidase activity was observed in plants grown in compost vs. plants grown in peat (Zhang et al., 1998), which suggests that compost affects a plant's defense mechanisms. Knowledge about the induction of systemic resistance has been accumulating in recent years, mostly in microbials (plant growth promoting rhizobacteria [PGPR]- and fungal biological control agents) (Durrant and Dong, 2004; Mathys et al., 2012; Pieterse et al., 1996; Ryals et al., 1996; Segarra et al., 2009; Van Loon, 1997; Van Loon et al., 1998). In this study, we used *Trichoderma asperellum* strain T34 as a reference, as it is known to induce priming and systemic resistance (ISR) against other bacterial and fungal diseases, also in other necrotrophic fungi in Arabidopsis plants (Segarra et al., 2009). Moreover, the induction of resistance by T34 is a concentration depending phenomena and plants use the route of ISR or SAR (Segarra et al., 2006, 2007a).

The main phytohormones involved in plants defense mechanisms are salicylic acid (SA), jasmonic acid (JA), ethylene (ET) and abscisic acid (ABA) (Pieterse et al., 2012; Vos et al., 2013). Mainly, SA signaling pathway is related with plant resistance against biotrophic and hemibiotrophic pathogens (Vos et al., 2013). However, plant resistance against necrotrophic pathogens as *B. cinerea*, is related with JA signaling pathway (Birkenbihl and Somssich, 2011; Vos et al., 2013).Recently, we showed that mature compost from olive oil residues induced resistance against *B. cinerea* in *Arabidopsis thaliana* and that the enhanced resistance was mainly related to processes mediated by SA and ABA, with responses similar to systemic acquired resistance (SAR) and abiotic stress responses (Segarra et al., 2013b). Various studies suggest that SAR can be associated with growth impairment and yield reduction (Denancé et al., 2013; Walters and Heil, 2007). ABA has recently been reported to cross talk with SA and JA in plant disease and defense (Robert-Seilaniantz et al., 2011). ABA has been regarded more as a modulator rather than a primary hormone in plant defense (Ton et al., 2009). The role of ABA is still unclear, because it sometimes appears to promote disease, while at other times it does the opposite.

The objective of this study was to establish whether tomato plants grown in compost had enhanced resistance against *B. cinerea* at the cost of growth or physiological performance.

2. Materials and methods

2.1. Growth media

The growth media used were: mature olive marc compost (CM) produced at the University of Seville; perlite (P), an inert mineral growth medium obtained from Europerlite was used as a standard substrate; perlite enriched with the biological control agent *T. asperellum* strain T34 (P + T34) was used as a positive control of resistance induction.

CM compost was selected from five different olive marc composts from Andalucía (South Spain) because in a previous study it was demonstrated that did not require formulation to obtain similar germination of *S. lycopersicum* cv. Roma to the perlite control. Compost CM was produced by turned piles and was mature (2 years of stabilization). The composition of CM was olive marc 47% and leaf residues 53% and had a pH of 7.74 and an EC of 0.37 mS cm^{-1}. Perlite was composed of SiO_2 (73%) and Al_2O_3 (13%) and had a pH of 6.69 and an EC of 0.12 mS cm^{-1}. To prepare P + T34, perlite was inoculated with *T. asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1} growth media by dilution on nutrient solution of the concentrated commercial product at 10^9 CFU g^{-1} growth media. After the incubation period the concentration of T34 was about 10 times higher. It was then incubated at a water tension of 1000 Pa (adjusted by weight) at 25 °C for 14 days. In order to standardize initial conditions of microbial biomass, P and 4 °C stored CM were incubated for the same time and in the same conditions as P + T34.

2.2. Plant growth studies

Tomato plants (*S. lycopersicum* cv. Roma) were sown in 15 mL multipots for 15 days. Subsequently, the plants were transplanted to 250 mL pots in each growth media for 20 days (the end of the experiment). Multipots and pots were placed in a growth chamber (25 ± 2 °C, 16 h [h] of light at 180–210 µmol m^{-2} s^{-1} photosynthetic photon flux density [PPFD] and 60–80% relative humidity [RH]). Plants were hand irrigated on the media as needed (50 and 100 mL solution mL^{-1} medium day^{-1} in the multipot and pot period, respectively) with the following nutrient solution: 0.5 g L^{-1} Peter's Foliar Feed 27-15-12 (Scotts), 0.22 g L^{-1} $CaCl_2$ and 0.25 g L^{-1} $MgSO_4$ 7 H_2O.

2.2.1. Plant analysis

The plant biomass analysis was performed twice in two separate studies and 4 replicates per treatment were used in each study. Shoots, leaves and roots were separated and dried (forced

air oven) at 60 °C for 48 h. We determined the dry weight of shoots and leaves (DWA), the dry weight of whole plant (DWT), the plant height (H), the root/shoot ratio, and the percentage of water in each plant.

We determined the leaf mineral composition analysis according to Segarra et al. (2007b), using dried leaves from two separate studies and 3 replicates per treatment in each study. An aliquot of 50 mg per sample was digested with 2 mL of concentrated HNO_3 and 2 mL of H_2O_2 in a Teflon container at 90 °C for 3 days. Analyses of Ca, K, Si, Mg, Fe, P and S were performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using Optima-3200RL (Perkin Elmer). Analyses of Ni, Mo, B, Cu, Zn and Mn were performed by inductively coupled plasma mass spectrometry (ICP-MS) using Elan 6000 (Perkin Elmer). The carbon and nitrogen percentage and the stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) for leaves and roots were determined using an elemental analyzer (EA1108, Series 1, Carlo Erba Instruments) coupled to an isotopic ratio mass spectrometer (IRMS, Delta C, Finnigan MAT). Three leaves and roots per treatments were used from the last study. Leaves and roots were ground separately (to pass through a 1 mm sieve) and aliquots of 0.50 mg were weighed in tin cups and analyzed by the EA-IRMS. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were expressed as δ notation ($\delta^{13}C$ and $\delta^{15}N$, respectively), as described by Coplen (2008):
$\delta = [(\text{Isotope Ratio} / \text{Isotope Ratio}) - 1] 1000$ (‰). The standard used to calculate $\delta^{13}C$ was Vienna Pee Dee Belemnite (VPDB) calcium carbonate, and to calculate $\delta^{15}N$ was N_2 in air. In both measurements, international isotope secondary standards were used to obtain an analytical precision of 0.1‰.

Leaf gas exchange and fluorescence analysis was performed on attached tomato leaves. The youngest fully expanded leaves were used (adaxial side). Four plants per treatment were used from the last study for each measure. An infrared gas analyzer (LI-6400, Li-Cor Inc.) was used to measure net CO_2 assimilation rates (A) and stomatal conductance (Gs), using equations developed by Von Caemmerer and Farquhar (1981). Plants were exposed to decreasing PPFD at 1200, 900, 300 and 85 μmol m^{-2} s^{-1} at 25 °C and 400 μmol CO_2 mol^{-1}. Chlorophyll fluorescence was analyzed with an Imaging-PAM fluorometer (Walz). Plants were first dark-adapted for 20 min and a saturating light pulse was applied to determine the maximum quantum efficiency of Photosystem II (PSII) (F_v/F_m) (F_v, variable fluorescence; F_m, maximum fluorescence yield in the dark-adapted state). Later, every leaf was adapted for 5 min to an actinic light of PPFD at 228 μmol m^{-2} s^{-1} (similar to the mean growth PPFD). Then, a second saturating light pulse was used to calculate: the relative quantum efficiency of PSII electron transport (Φ_{PSII}) estimated from $\Phi_{PSII} = (F_m' - F)/F_m'$ (F_m', maximum fluorescence yield in the light-adapted state; F, fluorescence yield) according to Genty et al. (1989); the coefficient of photochemical quenching (qP) estimated from $qP = (F_m' - F)/(F_m' - F_0')$ (F_0', minimum fluorescence yield in the light-adapted state); and the non-photochemical coefficient (qN) estimated from $qN = (F_m - F_m')/(F_m - F_0')$ (Andrews et al., 1993). The parameter F_0' was estimated using an approximation by Oxborough and Baker (1997).

Total leaf area (TLA), specific leaf weight (SLW) and relative water content (RWC) were used from leaves after monitoring gas exchange measures. TLA was determined using a scanner, Image leaf area measurement software (University of Sheffield, 2003), and dry weight. SLW was calculated from SLW = DW/TLA and the RWC was determined according to Turner (1981).

2.3. Plant disease studies

Tomato plants were grown in the same conditions as mentioned for the plant growth studies up to day 15 of transplantation to 250 mL pots. On that day, plants were placed in mini-tunnels (inside the growth chamber) to establish the best conditions for *B. cinerea* disease. The growth chamber was modified to obtain the following conditions inside the mini-tunnels: 24 °C (day) and 20 °C (night), 16 h light and near 100% RH. Plants were adapted to the new environmental conditions one day before inoculation with the pathogen. The number of plants in each of the three growth media was 14:5 (controls) without pathogen inoculation, and 9 with pathogen-inoculated leaves. The control plants were placed in separate mini-tunnels to the inoculated plants, each of which was grown in a different mini tunnel. The experiment was repeated three times.

2.3.1. Pathogen inoculation

A virulent strain of *B. cinerea* isolated from tomato-infected leaves and stored in silica gel crystals at 4 °C was cultivated in mixed vegetable solid medium for 21 days at 20 °C, 7 days under dark conditions, and 14 days at PPFD 85 μmol m^{-2} s^{-1} and 16 h light. The mixed vegetable medium was prepared by cooking 500 g of a commercial frozen mix of potato, carrot and beans in water. The boiled vegetables and cooking water were homogenized with a kitchen blender, the volume was brought to 1 L and 150 mL of the mixture plus 7.5 g of agar were used to prepare 500 mL of mixed vegetable medium. Conidia were collected from the plates in an inoculation buffer containing 0.5 mg mL^{-1} glucose and 0.5 mg mL^{-1} KH_2PO_4 (De Meyer et al., 1998). Twelve mL of the buffer was used per plate; the resulting suspension was filtered through two cotton gauzes. The concentration of *B. cinerea* was adjusted to 10^5 conidia mL^{-1} by hemocytometer counting. Finally, a drop of Tween 20 was added to the inoculum (0.005%) to promote uniform dispersion of the inoculum on plants leaves. Two expanded leaves from each plant were sprayed with approximately 550 μL per leaf with a low pressure plastic hand sprayer.

2.3.2. Assessment of disease

The severity and incidence of disease was examined 7, 10 and 14 days post-inoculation. Severity was evaluated using the following score for each leaf: 0 (asymptomatic), 1 (chlorotic spots), 2 (necrotic specks), 3 (necrotic spots), 4 (dead leaf). The area under the disease progress curve (AUDPC) per leaf was calculated by disease severity as described by Shaner and Finney (1977). The AUDPC was standardized by dividing with the total area of the graph (total days of observing disease symptoms multiplied by the maximum degree of disease). Disease incidence was evaluated as: 0, healthy; 1, infected. Disease incidence was calculated as the percentage of diseased leaves.

2.3.3. Plant hormone analysis

The plant hormones SA, ABA and JA were quantified as follows. The first fully expanded tomato leaf of each plant was sampled on day 0, 1, 3 and 5 post-pathogen inoculation. Three plants per treat-ment were used from the last study. The leaf from each plant was collected separately and quick-frozen in liquid N_2. Frozen samples were ground under liquid N_2 with a mortar and pestle. A total of 50 mg of the resulting powder was first extracted and twice re-extracted with methanol:isopropanol:acetic acid (20:79:1, v/v/v) (Müller and Munné-Bosch, 2011). The extracted samples were quantified according to Segarra et al. (2006), using the following transitions: 137/93, 263/153 and 209/59 for SA, ABA and JA, respectively.

2.4. Experimental design and statistical analysis

Data were analyzed by IBM SPSS Statistics 19 version statistical software. Data from plant growth studies that were performed twice (DWA, DWT, Root/Shoot, H, Total H_2O, mineral composition)

were analyzed using a multifactorial ANOVA ($p < 0.05$). The factor experiment and the interaction with the factor treatment were not significant, so data from the various experiments were pooled. Hence, data was analyzed with a unifactorial ANOVA to study the factor treatment for each growth and physiological parameter analyzed (DWA, DWT, root/shoot, H, Total H_2O, mineral composition, C/N ratio, $\delta^{13}C$, $\delta^{15}N$, A, Gs F_v/F_m, Φ_{PSII}, qP, qN, TLA, SLW and RWC). When significant differences were observed ($p < 0.05$), the Duncan's multiple range test was performed ($p < 0.05$). Data from disease assessments (DS, DI and AUDPC) that were performed three times were also analyzed using a multifactorial ANOVA ($p < 0.05$). The factor experiment and the interaction with the factor treatment were not significant. Therefore, data from the three experiments were also pooled. Consequently, data from disease assessments (DS, DI and AUDPC) and plant hormone analyses (SA, ABA and JA) were analyzed with a unifactorial ANOVA to study the factor treatment over the evaluation days. When significant differences were observed ($p < 0.05$), the Duncan's multiple range test was performed ($p < 0.05$). In cases in which normal distribution and homogeneity of variances were not found, the Kruskal– Wallis test was performed ($p < 0.05$). Relationships between DS, DI and AUDPC and plant physiological parameters and plant hormone content were analyzed with the Pearson correlation coefficient ($p < 0.05$).

3. Results

3.1. Effect of substrate on plant growth and physiological performance

The overall plant growth measured as DWA and DWT was higher in plants grown in P + T34 and in CM than in P alone (Table 1). The root/shoot ratio was higher in P and in P + T34 than in CM. Plants grown in P + T34 developed a higher TLA than in the other treatments, and the SLW (g m^{-2}) was the same for all leaves (data not shown). The lowest plant height was found in plants grown in P, followed by P + T34. Plants grown in CM were the highest. Leaf water status, measured as RWC, was higher in leaves grown on P and P + T34 than in CM. A similar pattern was obtained when the water content was measured in the whole plant (roots, shoots and leaves) (Table 1).

The Ca composition in leaves was higher in plants grown in CM than in the rest of the growth media (Table 2). The overall mineral composition of Mg, P, B and Cu was higher in plants grown in P + T34, followed by P, whilst the lowest values were found in plants grown in CM. Plants grown in P + T34 and P had the highest Fe, Mn and Mo levels in leaves. The lowest Fe, Mn and Mo levels were obtained in plants grown in CM No significant differences among treatments were observed in K, S, Si and Zn levels in leaves (Table 2). The ratio C/N in the leaves was higher in plants grown in CM than in P + T34 and P. The ratio C/N in the roots was the same for plants in all plant growth media (Table 2).

The $\delta^{13}C$ of leaves of plants grown in CM was less negative than in plants grown in P + T34 and P (Table 3). The $\delta^{13}C$ of roots was also less negative in CM, followed by P + T34, whilst the most negative values were found in P. The $\delta^{15}N$ of leaves was higher in plants grown in CM than in the rest of the growth media. The $\delta^{15}N$ of roots was higher in plants grown in P + T34, followed by P and the lowest values were found in compost CM (Table 3).

Leaves of tomato plants grown in the different growth media showed an increase in the net CO_2 assimilation rate (A), according to an increase in PPFD from 85 to saturation levels 1200 µmol m^{-2} s^{-1}. No significant differences among treatments were observed below 1200 µmol m^{-2} s^{-1} PPFD. Plants grown in CM showed the highest rate (Fig. 1). The results for A are in

Table 1

Effect of growth medium (P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost) on various physiological parameters of tomato plants.

Growth Medium	DWA[a] (g)	DWT[b] (g)	Root/Shoot	TLA[c] (cm^2)	H[d] (cm)	RWC[e]	Total H_2O (%)
P	0.14 ± 0.009a	0.17 ± 0.011a	0.23 ± 0.010b	112.52 ± 9.31a	7.66 ± 0.36a	90.57 ± 0.88b	95.21 ± 0.05b
P + T34	0.25 ± 0.010b	0.31 ± 0.011c	0.23 ± 0.018b	181.52 ± 9.13b	9.62 ± 0.30b	89.55 ± 1.64b	95.07 ± 0.10b
CM	0.23 ± 0.016b	0.26 ± 0.020b	0.12 ± 0.009a	99.65 ± 9.49a	10.60 ± 0.28c	81.66 ± 3.41a	94.10 ± 0.28a

Values of DWA, DWT, Root/shoot and total H_2O are means ± standard error of 8 plants per treatment collected from two separate studies (4 replicates each study). Values of TLA, H and RWC are means ± standard error of 4 plants per treatment collected from one of the studies. Different letters show significant differences $p < 0.05$ on Duncan's multiple range test.

[a] Aerial dry weight (leaves and shoots).
[b] Total dry weight.
[c] Total leaf area.
[d] Plant height.
[e] Relative water content.

Table 2

Effect of growth medium (P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost) on mineral composition of fully expanded tomato leaves.

Growth Medium	Macronutrient (mg planta^{-1})						
	K	Ca	Mg	P	S	Si	Fe
P	7.75 ± 0.90a	4.89 ± 0.27a	0.92 ± 0.02b	1.95 ± 0.05b	1.01 ± 0.10a	0.08 ± 0.01a	0.03 ± 0.00b
P + T34	9.84 ± 1.33a	6.12 ± 0.56a	1.21 ± 0.08c	2.52 ± 0.08c	1.25 ± 0.13a	0.09 ± 0.03a	0.04 ± 0.00b
CM	8.10 ± 0.62a	7.81 ± 0.33b	0.76 ± 0.02a	1.21 ± 0.05a	1.32 ± 0.08a	0.03 ± 0.01a	0.02 ± 0.00a
	Micronutrient (µg planta^{-1})					C/N	
	B	Mn	Zn	Cu	Mo	Leaves	Roots
P	13.53 ± 0.29b	30.92 ± 3.88b	12.20 ± 2.04a	5.73 ± 0.20b	0.53 ± 0.02b	5.79 ± 0.14a	7.93 ± 0.10a
P + T34	18.42 ± 1.74c	40.06 ± 6.82b	16.55 ± 1.93a	7.89 ± 0.39c	0.60 ± 0.04b	6.31 ± 0.19a	8.05 ± 0.37a
CM	6.90 ± 0.33a	14.81 ± 2.00a	13.81 ± 0.94a	3.46 ± 0.34a	0.24 ± 0.02a	7.19 ± 0.16b	7.27 ± 0.23a

Values of macronutrients and micronutrients are means ± standard error of 6 leaves per treatment collected from two separated studies (3 replicates per treatment in each study). Values of C/N are means ± standard error of 3 leaves and roots per treatment collected from one of the studies. Different letters show significant differences $p < 0.05$ on a Duncan's multiple range test.

Table 3
Effect of growth medium (P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost) on stable isotope ratios of carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ express as $\delta^{15}N$) of leaves and roots of tomato plants.

Growth Medium	$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
	Leaves	Roots	Leaves	Roots
P	35.30 ± 0.17a	34.53 ± 0.10a	3.14 ± 0.12a	1.94 ± 0.37b
P + T34	34.97 ± 0.15a	34.14 ± 0.08b	2.71 ± 0.21a	3.39 ± 0.26c
CM	34.28 ± 0.11b	33.41 ± 0.13c	1.22 ± 0.21b	0.34 ± 0.40a

Values are means ± standard error of 3 leaves and roots per treatment collected from one of the studies. Different letters show significant differences $p < 0.05$ on a Duncan's multiple range test.

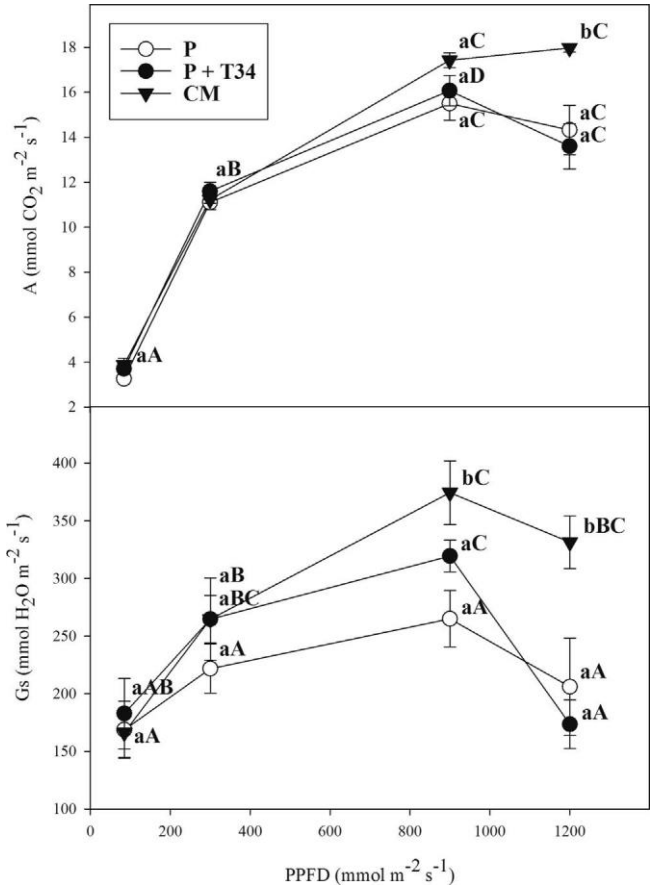


Fig. 1. Photosynthesis response curves to light in fully expanded tomato plant leaves. Measurements were made on 20 days post-seeding, in plants grown on three growth media: perlite (P), perlite + Trichoderma asperellum strain T34 (P + T34) (10^4 CFU mL^{-1}) and compost (CM). Curves were performed at 25 °C, 400 µmol mol^{-1} CO_2 and at a decreasing photosynthetic photon flux density (PPFD) of 1200, 900, 300 and 85 µmol photon m^{-2} s^{-1}. (A) Net CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}) (B) stomatal conductance (Gs, mmol H_2O m^{-2} s^{-1}). Values are means ± standard error of 4 leaves per treatment collected from one of the studies. Different lower case letters show significant differences between treatments and different capital letters show significant difference between days within treatments ($p < 0.05$) on an Duncan's multiple range test.

agreement with the Gs. The highest Gs and A values were observed in plants grown in CM. The lowest levels of Gs and A were observed in plants grown in P and P + T34. At 1200 µmol m^{-2} s^{-1} of PPFD plants grown in P + T34 showed a significant decrease measured in both A and Gs (Fig. 1).

The fluorescence analysis showed that the highest values of F_v/F_m were observed in plants grown in P + T34, followed by plants grown in P alone, whilst the lowest values were observed in plants

Table 4
Effect of growth medium (P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost) on chlorophyll fluorescence of fully expanded tomato leaves.

Growth Medium	F_v/F_m [a]	Φ_{PSII} [b]	qP [c]	qN [d]
P	0.808 ± 0.001b	0.497 ± 0.016a	0.703 ± 0.013a	0.507 ± 0.034b
P + T34	0.820 ± 0.005c	0.514 ± 0.003a	0.710 ± 0.007a	0.502 ± 0.010b
CM	0.793 ± 0.002a	0.533 ± 0.009a	0.740 ± 0.013a	0.402 ± 0.013a

Values are means ± standard error of 4 leaves per treatment collected from one of the studies. Different letters show significant differences $p < 0.05$ on a Duncan's multiple range test.

[a] Maximum quantum efficiency of Photosystem II (PSII).
[b] Relative quantum efficiency of PSII.
[c] Coefficient of photochemical quenching.
[d] Coefficient of non photochemical quenching.

grown in CM (Table 4). No significant differences among treatments were observed in Φ_{PSII} and qP. The highest values of qN were in plants grown in P and P + T34, being the lowest values in CM plants (Table 4).

3.2. Effect of substrate on Botrytis disease control

Tomato plants grown on compost CM showed the lowest levels of disease, measured as disease severity (DS) (lower than 1, on a scale from 0 to 4), disease incidence (DI) (from 57.5 to 72.5%) from 7 to 14 days and AUDPC (0.20 ± 0.03) (Table 5). Plants growing in P showed a DS from 2.07 to 3.36, a DI of around 100% and a AUDPC of 0.72 ± 0.02. P + T34 improved the suppression to levels between those of CM and P, as shown in the values attained for DS and AUDPC (Table 5). Negative correlations ($p < 0.05$) were attained for disease (DS on day 14 and AUDPC) and plant growth parameters such as the DWA, H, C/N shoots, $\delta^{13}C$ of roots and Gs at 1200 and 900 µmol m^{-2} s^{-1} of PPFD. Moreover, negative correlations ($p < 0.05$) were attained for DI on day 14 and C/N shoots. Positive correlations ($p < 0.05$) were attained for disease (DS on day 14 and AUDPC) and plant growth parameters such as root/shoot ratio and qN. Moreover, positive correlations ($p < 0.05$) were attained for DI on day 14 and root/shoot ratio. The hormone quantification of SA, ABA and JA did not show significant differences between treatments on day 0 (previous to inoculation of the leaves with the pathogen) (Table 6). The SA concentration of infected leaves of plants grown in compost CM significantly increased on day 1 post-inoculation, and was higher than in plants grown in P and P + T34. On days 3 and 5 post-inoculation, the SA levels of CM still were higher than in the rest of treatments. On day 5, leaves of plants grown in P and P + T34 decreased similarly or below the levels of day 0. The ABA concentration in leaves of plants grown in compost CM on day 1 was higher than the rest of treatments, followed by P, whilst the lowest values were found in leaves of plants grown in P + T34. On days 3 and 5, ABA levels of all treatments decreased similarly or below the levels of day 0 and there were no differences among treatments (Table 6). The JA concentration was the same along the days of the study and for all treatments (Table 6). Negative correlations ($p < 0.05$) were attained for disease (DS and DI on day 14 and AUDPC) and plant hormone status, such as SA on days 1 and 3 and ABA on day 1. Furthermore, negative correlations ($p < 0.05$) were attained for DI on day 14 and plant ABA status on day 5.

4. Discussion

The beneficial effect of composts on plant growth is well-documented (Arthur et al., 2012; Gallardo-Lara and Nogales, 1987; Zhang et al., 2012) and was also observed in this study on tomato

Table 5
Disease severity (DS), disease incidence (DI) (%) and area under disease progress curve (AUDPC) based on DS caused by the pathogen *Botrytis cinerea* (10^5 CFU mL^{-1}) in tomato plants in three independent bioassays. Plants were grown in three growth media: P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost. Disease was evaluated at 7, 10 and 14 days post-inoculation (dpi).

Growth Medium	DS[a]			DI[b] (%)			AUDPC[c]
	7 dpi	10 dpi	14 dpi	7 dpi	10 dpi	14 dpi	
P	2.07 ± 0.08cA	2.93 ± 0.12cB	3.36 ± 0.11cC	100.0 ± 0.00bA	100.0 ± 0.00bA	100.0 ± 0.00bA	0.72 ± 0.02c
P + T34	1.46 ± 0.08bA	1.77 ± 0.09bB	1.94 ± 0.11bB	97.1 ± 2.86bA	97.1 ± 2.86bA	97.1 ± 2.86bA	0.44 ± 0.02b
CM	0.62 ± 0.12aA	0.78 ± 0.13aA	0.94 ± 0.15aA	57.5 ± 7.91aA	65.0 ± 7.64aA	72.5 ± 7.15aA	0.20 ± 0.03a

Values are means ± standard error of 35–54 leaves per treatment collected from three separated studies (6–18 replicates per treatment in each study). Different lower case letters show significant differences between treatments and different capital letters show significant difference between days (dpi) within treatments ($p < 0.05$) on an Duncan's multiple range test.

[a] DS evaluated with a scale of five grades: 0, asymptomatic; 1, chlorotic leaf; 2, necrotic specks; 3, necrotic spot; 4, dead leaf.
[b] DI was evaluated as: 0, healthy; 1, infected. DI was calculated as the percentage of diseased leaves.
[c] AUDPC was standardized by dividing with the total area of the graph (total days of observing disease symptoms per maximum degree of disease).

Table 6
Effect of growth medium (P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^4 CFU mL^{-1}; CM, olive marc compost) on hormone (SA, salicylic acid, ABA, abscisic acid and JA, jasmonic acid) content in leaves of tomato plants that were not inoculated (0) or inoculated (1, 3, 5 days post-inoculation) with *Botrytis cinerea* (10^5 CFU mL^{-1}).

Hormone	Growth Medium	Days post-inoculation			
		0	1	3	5
SA (ng g^{-1} FW)	P	39.50 ± 2.65aBC	47.53 ± 4.53aC	33.83 ± 3.37aB	22.53 ± 1.03aA
	P + T34	47.10 ± 6.82aBC	52.53 ± 3.92aC	33.57 ± 5.70aAB	25.10 ± 0.59aA
	CM	49.83 ± 6.20aAB	103.75 ± 5.35bC	65.90 ± 10.31bBC	35.87 ± 4.23bA
ABA (ng g^{-1} FW)	P	161.90 ± 12.49aC	142.37 ± 2.52aC	94.17 ± 12.15aB	58.63 ± 5.42aA
	P + T34	151.93 ± 11.31aC	107.30 ± 4.29bB	79.27 ± 18.41aAB	51.97 ± 10.94aA
	CM	251.50 ± 40.55aB	345.25 ± 1.05cC	130.50 ± 6.57aA	115.37 ± 8.47bA
JA (ng g^{-1} FW)	P	1.00 ± 0.10aA	0.40 ± 0.06aA	1.03 ± 0.34aA	0.57 ± 0.12aA
	P + T34	0.83 ± 0.58aA	1.50 ± 0.58aA	0.80 ± 0.11aA	0.77 ± 0.47aA
	CM	1.60 ± 0.55aA	0.90 ± 0.10aA	0.67 ± 0.12aA	0.73 ± 0.14aA

Values are means ± standard error of 2–3 leaves per treatment collected from one of the studies. Different lower case letters show significant differences between treatments and different capital letters show significant difference between days within treatments for each plant hormone ($p < 0.05$) on a Duncan's multiple range test.

plants grown in compost, compared to those grown in perlite. The high pH (7.7) of CM could explain the lower levels of several mineral elements (Mg, P, Fe, Mn, Zn and Cu) in the plants. No difference was observed in the leaf nutrients when the same compost CM was used in *A. thaliana* plants (Segarra et al., 2013a). The highest levels of Ca in compost CM could explain, in part, the involvement of this element in the reduction of gray mold disease caused by *B. cinerea*. Indeed, high levels of Ca in leaves have been associated with foliar disease resistance (Wójcik and Lewandowski, 2003). In several studies, Ca is involved in biotic and abiotic stress responses (Segarra et al., 2007b), callose synthesis (Trillas et al., 2000), pectin binding molecules (Carpita and McCann, 2000), SA (Schneider-Müller et al., 1994) and phytoalexin synthesis (Vögeli et al., 1992).

The lower water status (RWC and total water) of plants grown in compost could be due to the EC of compost, which might have an inhibitory effect on Botrytis development. According to Mayak et al. (2004), RWC is an adequate indicator of water status in plants and is characterized by a decrease in stress conditions (drought and salinity). The pH and EC of olive marc compost were similar to those of other kinds of compost and other samples of the same type (Borrero et al., 2004; Cotxarrera et al., 2002; Segarra et al., 2007b). In one study, the RWC of tomato plants grown with high irradiation in a high EC solution was reduced to 81.5% (Claussen, 2005).

All measures of plant biomass were higher for tomato plants grown in P + T34 than in P alone; and the results were similar to those of plants grown in compost. The use of beneficial microorganisms in the rhizosphere (bacteria and fungus) can facilitate solubility, enhance the availability of nutrients to plants from the nutrient solution (Altomare et al., 1999), and protect against biotic stress. The most relevant characteristic of plants grown in P + T34 was the greater investment in leaf area. This could explain the greater accumulation of most of the elements, especially Mg, P, B and Cu in the leaves. In particular, Mg, P and Cu are involved in key reactions in leaves energetic processes. The increased uptake of Mg^{2+} and P was also observed in tomato shoots and roots grown in soil amended with *T. harzianum* strain T447 (Azarmi et al., 2011). Whereas in a study with *T. harzianum* strain T-203 increased uptake of Cu was observed in roots and not leaves of cucumber plants (Yedidia et al., 2001). Moreover, the principal function of B is a structural role related to the stability of the cell wall (O'Neill et al., 2004). Accordingly greater amount of B could improve plant resistance to *B. cinerea* attack.

The highest C/N ratio of plants grown in compost showed the lowest content of nitrogen in leaves, which would make the leaves more resistant to attack by the pathogen. Conversely, in a study with tomato plants with high C/N ratio makes plants more susceptible to the primary lesions formation caused by *B. cinerea* (Hoffland et al., 1999). The role of host nitrogen content in the susceptibility to *B. cinerea* is still unclear because there are other factors involved as N source and amount and *B. cinerea* isolates virulence (Lecompte et al., 2010).

Our study of stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ clearly distinguishes the roots of plants grown in P from those grown on P + T34. This suggests that the same C assimilation occurs, but the post-photosynthetic fractionation of stable carbone isotopes between leaves and roots and N assimilation by roots in contact with T34 differs. Makarov (2009) have observed that mycorrhizal fungi are involved in determining the plant $\delta^{15}N$. In a similar way, T34 could be involved in determining tomato plants $\delta^{15}N$

probably by improving the availability of this element to plants. Several studies have shown that $\delta^{13}C$ increase or carbon isotope discrimination decrease in water deficit conditions (Condon et al., 2002; Ehleringer and Cooper, 1988; Farquhar et al., 1982; Yousfi et al., 2009). Similarly, in our study, plants grown in compost were characterized by the lowest water status and also showed the highest $\delta^{13}C$, probably related to a certain degree of stress due to compost EC. In contrast, the effect of water status in $\delta^{15}N$ differs between studies (Handley et al., 1997; Lopes and Araus, 2006). The diverse composition of the growth media could explain the lowest $\delta^{15}N$ in CM. Despite this, according to Mariotti et al. (1982) the $\delta^{15}N$ signature of the source of N is not the only factor determining plant $\delta^{15}N$.

The data for photosynthesis and chlorophyll fluorescence were similar for tomato plants grown in a growth chamber at a similar age (Nogués et al., 2002). Measures of photosynthesis showed that plants grown in CM behave as plants grown at high light intensity (measure carried out at 1200 lmol m^{-2} s^{-1} PPFD). This value contrasts with that found for plants grown in all other treatments, which were saturated at this light level. Measures of chlorophyll fluorescence were similar among treatments and the F_v/F_m ratios in all treatments were over 0.75, which is considered the limit for photoinhibition (Björkman and Demmig, 1987).

All these data confirm that plants were grown properly in all growth media, although plants grown in CM were near to the limits of stress conditions (lowest root/shoot ratio, RWC, total water, F_v/F_m and highest $\delta^{13}C$). Growth parameters and physiological measures of plants grown in CM suggests that the plants had grown in a eustress situation. According to Hideg et al. (2012), eustress is considered mild and acclimative stress that improves growth and health. It is the opposite of distress or severe stress, which exceeds tolerance limits and leads to the death of plants.

Our results showed the suppressive capacity of mature olive marc compost CM in reducing the severity and incidence of *B. cinerea* and in inducing systemic resistance. The induction of systemic resistance was also linked to SA (which only increased after pathogen exposure). Although *B. cinerea* is a necrotrophic pathogen and triggers mainly the JA signaling pathway and not the SA signaling pathway (Birkenbihl and Somssich, 2011; Vos et al., 2013). Indirect evidence of the induction of resistance has been observed previously in composted cannery wastes against anthracnose tomato rot disease (Abbasi et al., 2002); in composted cow manure against *B. cinerea* in *Begonia hiemalis* (Horst et al., 2005); and in grape marc compost, olive marc-cotton gin trash (1:1, v:v) compost, cork compost, municipal organic and yard waste compost and spent mushroom compost against *B. cinerea* in *Cucumis sativus* (Segarra et al., 2007b). In another study, disease reduction was also induced by SAR using composted pine bark mix inoculated with *Trichoderma hamatum* 382 and *Flavobacterium balustinum* 299 and compost water extract against anthracnose and against bacterial speck in cucumber plants (Zhang et al., 1998). The spatial separation between the biological control agent (T34) and the pathogen and the reduction of *Botrytis* disease showed the involvement of the induction of plant resistance, even though an increase in JA levels could not be detected by T34 in the evaluated days of this study. Indeed, T34 applied to the roots has been shown to prime for induced resistance independently of SA (Segarra et al., 2009; Trillas and Segarra, 2009). However, SA increases have only been found when high concentrations (laboratory levels, not field levels) of T34 are applied (10^7 CFU mL^{-1}) (Segarra et al., 2007a).

Various studies suggest that ABA is involved in disease reduction or increase, according to the type of pathogen (Robert-Seilaniantz et al., 2011). ABA-deficient *A. thaliana* mutants were more resistant to *B. cinerea*, but more susceptible to *Pythium irregulare* (Adie et al., 2007). Similarly, ABA-deficient tomato mutants were more resistant to *B. cinerea* (Asselbergh et al., 2007). Ton et al. (2009) describes the role of ABA in plant disease defense more as a modulator rather than a primary hormone. Other studies suggested that the levels of SA, ABA and JA prior to contact with the pathogen had a determining impact on the interaction dynamics between these hormones (Robert-Seilaniantz et al., 2011). Interestingly, ABA levels of plants grown in CM increased after *B. cinerea* exposure. Recent studies from our group corroborate the involvement of both SAR and ABA-dependent/independent abiotic stress responses in *A. thaliana* plants grown in olive marc composts or perlite exposed to *B. cinerea* (Segarra et al., 2013a,b).

5. Conclusions

In conclusion, physiological parameters measured in tomato plants grown in mature olive marc compost showed no negative influence on plant biomass, CO_2 assimilation rate or chlorophyll fluorescence measurements, plants grew in a eustress situation that might have had a positive influence on disease resistance. The compost induction of systemic resistance was also linked to SA pathway/ABA.

Tomato plants growing in perlite enriched with *T. asperellum* strain T34 had better nutrient uptake, better C allocation and N assimilation in roots leading to an improvement in dry weight, height and total leaf area than plants grown in perlite alone. Plants grown in perlite enriched with T34 had no stress effects, measured by the overall physiological parameters. The induction of disease resistance observed in perlite enriched with T34 is not linked to the SA pathway/ABA.

By describing the positive effects and the diverse responses of plants grown in compost and perlite enriched with T34, we are contributing to understanding the role of compost and beneficial organisms that help plants to growth healthier and improve their innate resistance to foliar pathogen attacks.

Acknowledgments

This work was funded by Spain's Ministry of Science and Innovation (AGL2010-21982-C02-02). We are grateful to the elemental analysis laboratory (SCiTUB) and the laboratory of Separation Techniques. We also thank the Experimental Fields Service (University of Barcelona) and J. Ordovás, E. Carmona and M. Avilés (University of Seville) for CM compost. Seeds and *Trichoderma asperellum* strain T34 were kindly provided by Semillas Fitó and Biocontrol Technologies, respectively.

References

Abbasi, P.A., Al-Dahmani, J., Sahin, F., Hoitink, H.A.J., Miller, S.A., 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant Dis. 86, 156–161.

Adie, B.A., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., Solano, R., 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell 19, 1665–1681.

Alburquerque, J.A., Gonzálvez, J., García, D., Cegarra, J., 2004. Agrochemical characterization of ''alperujo'', a solid by-product of the two-phase centrifugation method for olive oil extraction. Bioresour. Technol. 91, 195–200.

Altomare, C., Norwell, W.A., Björkman, T., Harman, G.E., 1999. Solubilization of phosphates and micronutrients by the plant growth–promoting and biocontrol fungus Trichoderma harzianum Rifai 1295–22. Appl. Environ. Microbiol. 65, 2926–2933.

Andrews, J.R., Bredenkamp, G.J., Baker, N.R., 1993. Evaluation of the role of state transitions in determining the efficiency of light utilization for CO_2 assimilation in leaves. Photosynth. Res. 38, 15–26.

Arthur, E., Cornelis, W., Razzaghi, F., 2012. Compost amendment of sandy soil affects soil properties and greenhouse tomato productivity. Compos. Sci. Util. 20, 215– 221.

Asselbergh, B., Curvers, K., França, S.C., Audenaert, K., Vuylsteke, M., Van Breusegem, F., Höfte, M., 2007. Resistance to Botrytis cinerea in sitiens, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. Plant Physiol. 144, 1863–1877.

Azarmi, R., Hajieghrari, B., Giglou, A., 2011. Effect of Trichoderma isolates on tomato seedling growth response and nutrient uptake. Afr. J. Biotechnol. 10, 5850– 5855.

Birkenbihl, R.P., Somssich, I.E., 2011. Transcriptional plant responses critical for resistance towards necrotrophic pathogens. Front. Plant Sci. 2, 76.

Björkman, O., Demmig, B., 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489–504.

Borrero, C., Trillas, M.I., Ordovás, J., Tello, J.C., Avilés, M., 2004. Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. Phytopathology 94, 1094–1101.

Bugbee, G.J., Finck, C.R., 1989. Composted waste as a peat substitute in peat-lite media. HortScience 24, 625–627.

Carpita, N., McCann, M., 2000. The cell wall. In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, pp. 52–108.

Claussen, W., 2005. Proline as a measure of stress in tomato plants. Plant Sci. 168, 241–248.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2002. Improving intrinsic water use efficiency and crop yield. Crop Sci. 42, 122–131.

Coplen, T.B., 2008. Explanatory glossary of terms used in expression of relative isotope ratios and gas ratios. IUPAC Recommendations 2008. International Union of Pure and Applied Chemistry Inorganic Chemistry Division. Commission on Isotopic Abundances and Atomic Weights, 1–27.

Cotxarrera, L., Trillas, M.I., Steinberg, C., Alabouvette, C., 2002. Use of sewage sludge compost and Trichoderma asperellum isolates to suppress Fusarium wilt of tomato. Soil Biol. Biochem. 34, 467–476.

De Meyer, G., Bigirimana, J., Elad, Y., Höfte, M., 1998. Induced systemic resistance in Trichoderma harzianum biocontrol of Botytis cinerea. Eur. J. Plant Pathol. 104, 279–286.

De Ward, M.A., Andrade, A.C., Hayashi, K., Schoonbeek, H.J., Stergiopoulos, I., Zwiers, L.H., 2006. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest Manag. Sci. 62, 195–207.

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430.

Denancé, N., Sánchez-Vallet, A., Goffner, D., Molina, A., 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front. Plant Sci. 4, 155.

Durrant, W.E., Dong, X., 2004. Systemic acquired resistance. Annu. Rev. Phytopathol. 42, 185–209.

Ehleringer, J.R., Cooper, T.A., 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. Oecologia 76, 562–566.

Farquhar, G.D., O'leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Funct. Plant Biol. 9, 121–137.

Gallardo-Lara, F., Nogales, R., 1987. Effect of the application of town refuse compost on the soil-plant system: a review. Biol. Wastes 19, 35–62.

Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. B.B.A. – Gen. Subj. 990, 87–92.

Handley, L.L., Robinson, D., Forster, B.P., Ellis, R.P., Scrimgeour, C.M., Gordon, D.C., Nevo, E., Raven, J.A., 1997. Shoot $d^{15}N$ correlates with genotype and salt stress in barley. Planta 201, 100–102.

Hideg, É., Jansen, M.A., Strid, Å., 2012. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci. 18, 107–115.

Hoffland, E., van Beusichem, M.L., Jeger, M.J., 1999. Nitrogen availability and susceptibility of tomato leaves to Botrytis cinerea. Plant Soil 210, 263–272.

Hoitink, H.A.J., Boehm, M.J., 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu. Rev. Phytopathol. 37, 427–446.

Hoitink, H.A.J., Stone, A.G., Han, D.Y., 1997. Suppression of plant diseases by composts. HortScience 32, 184–187.

Horst, L.E., Locke, J., Krause, C.R., McMahon, R.W., Madden, L.V., Hoitink, H.A.J., 2005. Suppression of Botrytis blight of Begonia by Trichoderma hamatum 382 in peat and compost-amended potting mixes. Plant Dis. 89, 1195–1200.

Kavroulakis, N., Ehaliotis, C., Ntougias, S., Zervakis, G.I., Papadopoulou, K.K., 2005. Local and systemic resistance against fungal pathogens of tomato plants elicited by a compost derived from agricultural residues. Physiol. Mol. Plant Pathol. 66, 163–174.

Lecompte, F., Abro, M.A., Nicot, P.C., 2010. Contrasted responses of Botrytis cinerea isolates developing on tomato plants grown under different nitrogen nutrition regimes. Plant. Pathol. 59, 891–899.

Leroch, M., Kretschmer, M., Hahn, M., 2011. Fungicide resistance phenotypes of Botrytis cinerea isolates from commercial vineyards in South West Germany. J. Phytopathol. 159, 63–65.

Lopes, M.S., Araus, J.L., 2006. Nitrogen source and water regime effects on durum wheat photosynthesis and stable carbon and nitrogen isotope composition. Physiol. Plant. 126, 435–445.

Maas, E.V., Hoffman, G.J., 1977. Crop salt tolerance-current assessment. J. Irrig. Drain. Div. 103, 115–134.

Makarov, M.I., 2009. The nitrogen isotopic composition in soils and plants: its use in environmental studies (a review). Eurasian Soil Sci. 42, 1335–1347.

Mariotti, A., Mariotti, F., Champigny, M.L., Amarger, N., Moyse, A., 1982. Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO^3 by Pearl Millet. Plant Physiol. 69, 880–884.

Mathys, J., De Cremer, K., Timmermans, P., Van Kerckhove, S., Lievens, B., Vanhaecke, M., Cammue, B.P.A., De Coninck, B., 2012. Genome-wide characterization of ISR induced in Arabidopsis thaliana by Trichoderma hamatum T382 against Botrytis cinerea infection. Front. Plant Sci. 3, 108.

Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem. 42, 565–572.

Müller, M., Munné-Bosch, S., 2011. Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Methods 7, 37.

Nogués, S., Cotxarrera, L., Alegre, L., Trillas, M.I., 2002. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytol. 154, 461–470.

O'Neill, M.A., Ishii, T., Albersheim, P., Darvill, A.G., 2004. Rhamnogalacturonan II: structure and function of a borate crosslinked cell wall pectic polysaccharide. Annu. Rev. Plant Biol. 55, 109–139.

Oxborough, K., Baker, N.R., 1997. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components–calculation of qP and F_v^0/F_m^0; without measuring Fo^0. Photosynth. Res. 54, 135–142.

Pieterse, C.M.J., Van Wees, S.C., Hoffland, E., Van Pelt, J.A., Van Loon, L.C., 1996. Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8, 1225–1237.

Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C., 2012. Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28, 489– 521.

Robert-Seilaniantz, A., Grant, M., Jones, J.D.G., 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu. Rev. Phytopathol. 49, 317–343.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., Hun, M.D., 1996. Systemic acquired resistance. Plant Cell 8, 1809–1819.

Sant, D., Casanova, E., Segarra, G., Avilés, M., Reis, M., Trillas, M.I., 2010. Effect of Trichoderma asperellum strain T34 on Fusarium wilt and water usage in carnation grown on compost-based growth medium. Biol. Control 53, 291–296.

Schneider-Müller, S., Kurosaki, F., Nishi, A., 1994. Role of salicylic acid and intracellular Ca^{2+} in the induction of chitinase activity in carrot suspension culture. Physiol. Mol. Plant Pathol. 45, 101–109.

Segarra, G., Jáuregui, O., Casanova, E., Trillas, M.I., 2006. Simultaneous quantitative LC–ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of Cucumis sativus under biotic stress. Phytochemistry 67, 395–401.

Segarra, G., Casanova, E., Bellido, D., Odena, M.A., Oliveira, E., Trillas, M.I., 2007a. Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with Trichoderma asperellum strain T34. Proteomics 7, 3943–3952.

Segarra, G., Casanova, E., Borrero, C., Avilés, M., Trillas, M.I., 2007b. The suppressive effects of composts used as growth media against Botrytis cinerea in cucumber plants. Eur. J. Plant Pathol. 117, 393–402.

Segarra, G., Van der Ent, S., Trillas, M.I., Pieterse, C.M.J., 2009. MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biol. 11, 90–96.

Segarra, G., Elena, G., Trillas, M.I., 2013a. Systemic resistance against Botrytis cinerea in Arabidopsis triggered by an olive marc compost substrate requires functional SA signaling. Physiol. Mol. Plant Pathol. 82, 46–50.

Segarra, G., Santpere, G., Elena, G., Trillas, M.I., 2013b. Enhanced Botrytis cinerea resistance of Arabidopsis plants grown in compost may be explain by increased expression of defense-related genes, as revealed by microarray analysis. PLoS ONE 8, e56075.

Shaner, G., Finney, R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67, 1051–1056.

Ton, J., Flors, V., Mauch-Mani, B., 2009. The multifaceted role of ABA in disease resistance. Trends Plant Sci. 14, 310–317.

Trillas, M.I., Segarra, G., 2009. Interactions between nonpathogenic fungi and plants. Plant innate immunity. Adv. Bot. Res. 51, 321–359.

Trillas, M.I., Cotxarrera, L., Casanova, E., Cortadellas, N., 2000. Ultrastructural changes and localization of chitin and callose in compatible and incompatible interactions between carnation callus and Fusarium oxysporum. Physiol. Mol. Plant Pathol. 56, 107–116.

Turner, N.C., 1981. Techniques and experimental approaches for the measurement of plant water status. Plant Soil 58, 339–366.

Van Loon, L.C., 1997. Induced resistance in plants and the role of pathogenesis-related proteins. Eur. J. Plant Pathol. 103, 753–765.

Van Loon, L.C., Bakker, P.A.H.M., Pieterse, C.M.J., 1998. Systematic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36, 453–483.

Vögeli, U., Vögeli-Lange, R., Chappell, J., 1992. Inhibition of phytoalexin biosynthesis in elicitor-treated tobacco cell-suspension cultures by calcium/calmodulin antagonists. Plant Physiol. 100, 1369–1376.

Von Caemmerer, S.V., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.

Vos, I.A., Pieterse, C.M.J., Wees, S.C.M., 2013. Costs and benefits of hormone-regulated plant defences. Plant. Pathol. 62, 43–55.

Walters, D., Heil, M., 2007. Costs and trade-offs associated with induced resistance. Physiol. Mol. Plant Pathol. 71, 3–17.

Wójcik, P., Lewandowski, M., 2003. Effect of calcium and borum sprays on yield and quality of ''Elsanta'' strawberry. J. Plant Nutr. 26, 671–682.

Yedidia, I., Srivastva, A.K., Kapulnik, Y., Chet, I., 2001. Effect of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. Plant Soil 235, 235–242.

Yogev, A., Raviv, M., Hadar, Y., Cohen, R., Wolf, S., Gil, L., Katan, J., 2010. Induced resistance as a putative component of compost suppressiveness. Biol. Control 54, 46–51.

Yousfi, S., Serret, M.D., Araus, J.L., 2009. Shoot $d^{15}N$ gives a better indication than ion concentration or $d^{13}C$ of genotypic differences in the response of durum wheat to salinity. Funct. Plant Biol. 36, 144–155.

Zhang, W., Han, D.Y., Dick, W.A., Davis, K.R., Hoitink, H.A.J., 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology 88, 450–455.

Zhang, R.H., Duan, Z.Q., Li, Z.G., 2012. Use of spent mushroom substrate as growing media for tomato and cucumber seedlings. Pedosphere 22, 333–342.

Web reference

http://faostat.fao.org/site/339/default.aspx. Accessed 28 May 2013.

4.2. Capítulo II: Poblaciones de *Trichoderma asperellum* cepa T34 de la rizosfera incrementadas por el patrón de secreción de exudados de la raíz en plantas de tomate inoculadas con *Botrytis cinerea*

Increased rhizosphere populations of Trichoderma asperellum strain T34 caused by secretion pattern of root exudates in tomato plants inoculated with Botrytis cinerea

Fernández E, Trillas MI, Segarra G, 2017. Plant Pathology. DOI: 10.1111/ppa.12668

Resumen

Los exudados de las raíces secretados por las plantas pueden modificar la microbiota de la rizosfera aumentando o inhibiendo el crecimiento de los ACBs y / o los patógenos. De forma similar, los microorganismos pueden modificar la secreción de exudados radiculares. El objetivo de este estudio fue analizar el efecto de la infección foliar producida por *B. cinerea* sobre la secreción de exudados en la raíz de tomate y sobre las poblaciones del ACB T34.

Este estudio mostró que el patrón de secreción de exudados radiculares en plantas de tomate estaba influenciado por la infección de *B. cinerea* en las hojas. Se observó un aumento en los niveles de ácido glucónico, mientras que los niveles de sacarosa e inositol disminuyeron. También se observó una disminución en la severidad de *B. cinerea* debido a la inducción de resistencia sistémica desencadenada por T34 en la raíz. Las plantas de tomate infectadas con *B. cinerea* mantuvieron las poblaciones de T34 en las raíces, mientras que las poblaciones de T34 disminuyeron en las plantas no inoculadas con el patógeno. Las muestras expuestas a medios que contenían ácido glucónico (como única fuente de carbono o a la misma concentración encontrada en los exudados de las raíces) mostraron un aumento en el crecimiento in vitro de T34 en comparación con los medios sin ácido glucónico. En conclusión, el cambio en el patrón de

secreción de exudados radiculares causado por la presencia de *B. cinerea*, junto con el aumento del crecimiento de T34 en presencia de ácido glucónico, indican la existencia de una comunicación entre la hoja y la raíz. El resultado de esta comunicación es el aumento de las poblaciones de T34, que a su vez, inducen resistencia a la enfermedad con la consecuente reducción de su severidad.

Plant Pathology (2017) Doi: 10.1111/ppa.12668

Increased rhizosphere populations of *Trichoderma asperellum* strain T34 caused by secretion pattern of root exudates in tomato plants inoculated with *Botrytis cinerea*

E. Fernandez*, M. I. Trillas and G. Segarra

Unitat de Fisiologia Vegetal, Departament de Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Avinguda Diagonal 643, 08028 Barcelona, Catalonia, Spain

Root exudates secreted from plants can modify rhizosphere microbiota by enhancing or inhibiting the growth of biological control agents (BCAs) and/or pathogens. Similarly, microorganisms can modify the secretion of plant root exudates. The aim of this study was to analyse the effect of a *Botrytis cinerea* leaf infection on the secretion of tomato root exudates and on the populations of the BCA *Trichoderma asperellum* strain T34 (T34). This study found that the secretion pattern of root exudates in tomato plants was influenced by *B. cinerea* infection in plant leaves. An increase in the levels of gluconic acid was observed, while levels of sucrose and inositol decreased. A decrease in the severity of *B. cinerea* by the induction of systemic resistance triggered by T34 was also observed. Tomato plants infected with *B. cinerea* maintained the populations of T34 in the roots, while populations of T34 decreased in plants not inoculated with the pathogen. Samples exposed to media containing gluconic acid (as the only carbon source or at the same concentration found in roots exudates) saw an increase in the in vitro growth of T34 compared to media without gluconic acid. In conclusion, a change in the secretion pattern of root exudates caused by *B. cinerea*, together with the enhanced growth of T34 in the presence of gluconic acid, indicates the existence of leaf to root communication. The result of this is enhanced populations of T34, and in turn induced disease resistance and a consequential reduction in disease severity.

Keywords: biological control agent, gluconic acid, induced systemic resistance, organic acids

Introduction

The use of biological control measures has been promoted in recent years due to an increased awareness of the noxious effect on the environment of chemical pesticides and the emergence of resistance to these pesticides. The current European Directive 2009/128/EC addresses the sustainable use of pesticides, which includes promoting the use of integrated pest management and the use of alternative approaches to chemical pesticides such as biological control agents (BCAs).

Botrytis cinerea, teleomorph *Botryotinia fuckeliana*, is a necrotrophic plant pathogen that causes a grey mould disease. *Botrytis cinerea* was ranked as the second most important pathogenic plant fungus due to its economic impact and the capacity to infect more than 200 plant species (Dean et al., 2012). Normally the disease is controlled with fungicides (specific and broad spectrum); however, *B. cinerea* has developed resistance to most of them (Leroch et al., 2011).

Mechanisms to control foliar diseases used by bacterial and fungal BCAs are: (i) direct action of BCA over the pathogen; or (ii) indirect action of BCA through the plant by the induction of systemic resistance and/or the improvement of plant nutrition and growth. The genus *Trichoderma* spp. is one of the most isolated soil fungi and has been used as a biopesticide, a biofertilizer and in soil amendments (Vinale et al., 2008). According to Verma et al. (2007), the typical biocontrol actions of Trichoderma spp. are mycoparasitism, spatial and nutrient competition and antibiosis. Some strains of *Trichoderma* spp. can induce systemic resistance (Pieterse et al., 2014) and improve plant nutrition and growth (Li et al., 2015). *Trichoderma asperellum* strain T34 (T34) induced systemic resistance against different foliar pathogens: in cucumber plants against *Pseudomonas syringae* pv. *lachrymans* (Segarra et al., 2007); in *Arabidopsis* against *Pseudomonas syringae* pv. *tomato*, *Hyaloperonospora parasitica* and *Plectosphaerella cucumerina* (Segarra et al., 2009); and in the tomato plant, it induced systemic resistance against *B. cinerea* as well as increasing plant growth and nutrient uptake (Fernandez et al., 2014).

In this study, given its global economic significance, tomato (*Solanum lycopersicum*) was used; this plant is susceptible to *B. cinerea* and is inducible by T34, which triggers the systemic resistance response against attack by *B. cinerea* (Fernandez et al., 2014).

Plants can maintain positive (symbiotic and protective relationships) and negative (pathogenic associations) interactions with other plants, microbes and invertebrates

*E-mail: efernago13@gmail.com

Plant Pathology

through selective root exudation of specific compounds (Bais et al., 2006; Haichar et al., 2014). According to Bertin et al. (2003), root exudates mainly contain water, ions, amino acids, organic acids, sugars, enzymes and phenolic compounds. Nevertheless, the quality and quantity of root exudation depend on characteristics of the plant itself (root growth, development stage of plants, plant species and genotypes within species) and external biotic and abiotic factors (Badri & Vivanco, 2009).

The presence of pathogens and BCAs – in isolation or together – modifies the way in which root exudates are secreted. This was observed in tomato root exudates in the presence and absence of the pathogen Fusarium oxysporum f. sp. *radicis-lycopersici* (Forl) and the BCA *Pseudomonas fluorescens* WCS365 (Kamilova et al., 2006b). Similarly, root exudates of tomato plants are altered differently by pathogenic and nonpathogenic *F. oxysporum* strains, and consequently, the propagation of every strain in the rhizosphere is affected differently (Steinkellner et al., 2008). Furthermore, exudate profiles of *Arabidopsis* plants change in the presence of the wildtype Pseudomonas putida KT2440 and the mutant PP2561, which is negative in the induction of resistance against the foliar pathogen *P. syringae* pv. *tomato* DC3000 (Pst D3000) (Matilla et al., 2010). Interestingly, *Arabidopsis* plants increase secretions of L-malic acid induced by the foliar pathogen Pst D3000 that promote binding and biofilm formation of the beneficial rhizobacterium *Bacillus subtilis* FB17 on roots (Rudrappa et al., 2008). This demonstrates the importance of leaf to root communication, the versatile interactions between microorganisms and plants through root exudates, and the crucial role they could play in plant defence.

Moreover, there is evidence that tomato plants may secrete certain compounds through exudation to attract beneficial microorganisms. Organic acids from tomato root exudates influence root colonization, chemotaxis and swarming motility by the BCA *Bacillus amylolique-faciens* T-5 (Tan et al., 2013). The antifungal activity against Forl of the plant growth-promoting rhizobacteria *Pseudomonas chlororaphis* SPB1217 and *P. fluorescens* SPB2137 depend on the sugar and organic acid composition of tomato root exudates (Kravchenko et al., 2003).

There is little information available about the interaction between Trichoderma spp. and root exudates. According to Zhang et al. (2013), Trichoderma harzia-num T-E5 modified cucumber root exudates and these exudates reduced the germination of the soil pathogen F. oxysporum f. sp. cucumerinum. Moreover, root exudates of Bengal gram can promote the growth of some isolates of T. harzianum, Trichoderma viride and Trichoderma roseum (Jash & Pan, 2007). Information is scarce on the role of root exudates in plant defence against foliar pathogens, how plants encourage beneficial microorganisms to help them in response to pathogen attack, and the responses that can be triggered in beneficial microorganisms.

The objective of this study was to analyse the effect of *B. cinerea* leaf infection on the secretion pattern of tomato root exudates and its connection with the populations of the BCA *T. asperellum* strain T34 and disease reduction.

Materials and methods

Study in sterile conditions

To obtain root exudates in sterile conditions, the setup of the experiment was adapted from Kamilova et al. (2006a). Tomato cv. Roma seeds were surface sterilized by immersion in 5% HOCl for 3 min and subsequently washed with sterile distilled water (SDW). Seeds were placed in sterile 500 mL glass jars under sterile conditions containing a 2 cm thick layer of sand on the bottom. The sand was saturated with 50 mL nutrient solution (0.5 g L^{-1} Peter's foliar feed 27-15-12 (Scotts), 0.22 g L^{-1} $CaCl_2$ and 0.25 g L^{-1} $MgSO_4.7H_2O$). Jars were kept at 24 °C (day) and 20 °C (night) with 16 h light. Two weeks later, in half of the jars, the seedlings were inoculated with *B. cinerea* adjusted to 10^5 conidia mL^{-1} suspension prepared as described in Fernandez et al. (2014), with the exception that the inoculation buffer did not contain glucose. Two weeks after the inoculation, the nutrient solution was removed from the sand and lyophilized.

Samples for root exudates were processed and analysed by gas chromatography/mass spectrometry (GC/MS) as described by Matthew et al. (2009). Ribitol (100 µg g^{-1}) was used as the internal standard. Retention index was calculated by the use of an alkane standard mix containing 100 µg g^{-1} of C12, C15, C19, C22, C28, C32 and C36.

For GC/MS instrument settings, the samples were randomized, and a splitless injection was used to move 1 µL of derivatized sample into a Shimadzu QP2010 GC-MS gas chromatograph-mass spectrometer. Helium was used as the carrier gas at a constant flow of 1 mL min^{-1}. The inlet temperature was set at 280 °C. The oven temperature was initially set at 70 °C for 1 min, ramped at 1 °C min^{-1} until 76 °C, then ramped at 6 °C min^{-1} until 325 °C, with a final hold of 8 min. A Trace capillary column (TRB-5 ms, 30 m x 0.25 mm x 0.25 µm) was used. The mass selective detector transfer line heater was kept at 300 °C and MS and source temperature at 200 °C. Mass detection range was set from 40 to 900 atomic mass units. For GC/MS metabolite peak identification and quantification, the metabolites were identified by retention index and spectral comparison to pre-run standards or by searching the NIST library. Normalization was performed to the internal standard ribitol. Due to the high number of unidentified peaks, an alternative approach was used to analyse the data output from the GC/MS experiments: peaks were aligned based on retention index, their areas were normalized according to the internal standard and a principal component analysis (PCA) was performed.

Study of *T. asperellum* T34 growth rate

To assess the capacity of gluconic acid to promote T34 growth, the growth media used were: cornmeal dextrose agar (CMD, cornmeal agar and 20 g L^{-1} dextrose; Difco) and three modified versions of synthetic nutrient-poor agar (SNA) described by Nirenberg (1981) with different sugar composition: SNA1 (0.2 g L^{-1} sucrose and 0.2015 g L^{-1} dextrose, pH 5.7); SNA2 (0.2 g L^{-1} sucrose, 0.2 g L^{-1} dextrose and 0.0015 g L^{-1} gluconic acid, pH 5.7); and SNA3 (0.4015 g L^{-1} gluconic acid, pH 4.1). Plastic Petri dishes with a diameter of 9 cm

and 20 mL of fresh medium per plate were used.

First, T34 stored in silica gel crystals at 4 °C was cultivated in CMD for 3 days at 25 °C under dark conditions. Following this, plugs of 5 mm diameter were obtained from the actively growing edge of the colony and were placed mycelium side down at approximately 1.5 cm from the edge of the plate in SNA 1, SNA 2 and SNA 3. Plates were incubated for 5 days at 30 °C under dark conditions. A total of nine plates per growth media were used and the experiment was repeated twice.

The growth rates were evaluated every 24 h from day 2 to 4 by measuring the colony radius from the middle of the inoculum plug. A visual inspection of the colony growth was also made.

Study in growth chamber

To evaluate T34 as an inducer of plant disease resistance against *B. cinerea*, tomato seeds cv. Roma were pre-germinated with SDW and paper for 4 days at 25 ± 3 °C and dark conditions. Germinated seeds were grown for 14 days in 200 mL pots in perlite (Europerlite) and perlite enriched with T34 (P + T34). To prepare P + T34, perlite was inoculated with T34 to achieve an average concentration of 3.85 ± 0.53 x 10^5 CFU cm^{-3} growth media at the point of inoculation with the pathogen (day 0). Pots were placed in a growth chamber (25 ± 2 °C, 16 h light at 100–120 µmol m^{-2} s^{-1} photosynthetic photon flux density and 60–80% relative humidity). Plants were irrigated on the media as required (17.5 mL solution medium/day in the pot) with the following nutrient solution: 0.2 g L^{-1} Peter's Foliar Feed 27-15-12 (Scotts), 0.09 g L^{-1} $CaCl_2$ and 0.05 g L^{-1} $MgSO_4.7H_2O$.

On day 14, plants were placed in mini-tunnels (inside the growth chamber) to establish optimal conditions for *B. cinerea* disease (Fernandez et al., 2014). There were a total of 16 plants in each of the two growth media: eight (control) plants that had not been inoculated with the pathogen, and eight plants with pathogen-inoculated leaves. The control plants were placed in separate mini-tunnels to the inoculated plants, each of which was grown in a different mini-tunnel. The experiment was repeated twice. Two expanded leaves from each plant were sprayed (low pressure plastic hand sprayer) with approximately 550 µL per leaf of inoculation buffer (De Meyer et al., 1998) with or without pathogen, according to the treatment. The pathogen inoculum was adjusted to 10^5 conidia mL^{-1} (Fernández et al., 2014). Before applying the spray, the pots were covered with plastic to prevent contact between *B. cinerea* on leaves and T34 on the growth media. The severity of disease was examined 7, 10 and 14 days post-inoculation. Severity was evaluated using a scale of 0–4 for each leaf: 0, symptomless; 1, chlorotic leaf; 2, necrotic specks; 3, necrotic spot; 4, dead leaf.

In both studies, perlite from the rhizosphere of the pots with P + T34 (control and inoculated with *B. cinerea*) was collected at 0 and 14 days post-inoculation. Three pots from each treatment were sampled. Populations of T34 were calculated by the method of dilution plate (Johnson & Curl, 1972) in water agar (0.7 g L^{-1} agar) and by the selective medium Trichoderma (Chung & Hoitink, 1990). Data corresponded to the average of two dilutions performed for each pot, and three plates of medium Trichoderma for each dilution were used.

Statistical analysis

For all studies, data were analysed by SPSS STATISTICS v. 19 (IBM) software and results are expressed as means standard error (SE). The areas of the collection of aligned peaks were studied using standardized PCA to detect differences in the secretion pattern of exudates between treatments. Values of the first and second components were subjected to analysis of variance (ANOVA) ($P < 0.05$). The quantity of known metabolites was analysed by ANOVA ($P < 0.05$) to study the factor treatment. Data from T34 growth rate studies and from growth chamber studies were analysed with ANOVA to study the factor treatment over the evaluation days and to study within treatments the effect of the evaluation days. When significant differences were observed ($P < 0.05$) and more than two groups were present, the Duncan's multiple range test was performed ($P < 0.05$). In cases when normal distribution and homogeneity of variances were not found, the Kruskal–Wallis test was performed ($P < 0.05$).

Results

In sterile conditions, up to 200 metabolite peaks per chromatogram were detected. The PCA of the peak areas showed that the metabolite composition of root exudates from *B. cinerea*-inoculated plants differed from that of noninoculated control plants (Fig. 1). PCA 1 showed a variability of 52% while that of PCA 2 was 35%. Values from PCA 1 were significantly different ($P < 0.05$) between inoculated and noninoculated samples.

Peaks of 13 exuded metabolites were identified (Table 1). Significant differences were observed in the concentration of three metabolites between plants inoculated and noninoculated with *B. cinerea*. The concentration of gluconic acid increased by almost five times in the presence of *B. cinerea*, which contrasts with the concentrations of inositol and sucrose which decreased by up to three and nine times, respectively.

The growth rates of T34 – measured by the size of the colony radiuses – increased significantly from day 2 to day 4 in the presence of growth media SNA2 and SNA3; this was not the case in the presence of SNA1 (Fig. 2a). Colony radius significantly increased throughout the study period (day 2, 3 and 4).

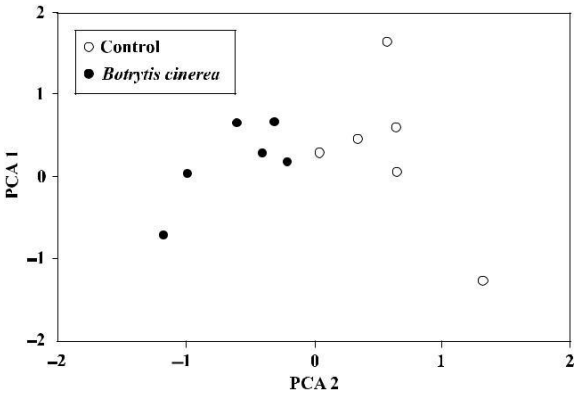


Figure 1 Principal component analysis (PCA 1 and PCA 2) of the peak areas obtained by gas chromatography/mass spectrometry of exuded metabolites from tomato plants inoculated and noninoculated with *Botrytis cinerea*.

Table 1 Metabolite composition (μg per plant) of root exudates of tomato seedlings grown on sand in the absence and presence of the pathogen *Botrytis cinerea* (10^5 CFU mL^{-1})

Metabolite	Control	*B. cinerea*
Arabinose	0.62 ± 0.06	1.17 ± 0.27
Fructose	0.29 ± 0.03	0.26 ± 0.04
Galactaric acid	0.83 ± 0.35	1.29 ± 0.05
Galactose	0.91 ± 0.24	1.42 ± 0.44
Gluconic acid	0.30 ± 0.08	1.48 ± 0.17*
Glucose	0.15 ± 0.04	0.08 ± 0.03
Inositol	3.82 ± 0.11	1.14 ± 0.14*
Maltose	0.69 ± 0.51	0.60 ± 0.08
Mannitol	0.32 ± 0.05	0.20 ± 0.03
Rhamnose	1.52 ± 0.05	1.93 ± 0.98
Ribose	1.32 ± 0.69	0.56 ± 0.03
Sucrose	1.30 ± 0.20	0.14 ± 0.03*
Xylose	0.10 ± 0.03	0.04 ± 0.01

Means ± standard error, n = 6 (from two separate studies).
*indicates significant difference between treatments ($P < 0.05$; ANOVA test).

Over the course of the study period, mycelia of T34 grown in SNA3 were characterized by more defined concentric rings, denser conidial production and higher abundances of pustules than the colonies of T34 grown in SNA1 and SNA2. Moreover, the conidia of T34 grown in SNA3 growth media became greener and darker earlier than in samples grown in SNA1 and SNA2. In terms of pustule abundance, only subtle differences were observed between colonies of T34 grown in SNA1 and SNA2, with samples grown in SNA2 having a slightly higher abundance. Figure 2b–d shows representative images of the growth of T34 colonies on day 4.

When the T34 enriched perlite was compared to non-enriched perlite (Fig. 3) in the growth chamber study, disease severity at day 10 and 14 after pathogen inoculation was 12.59% and 24.34% lower, respectively. Levels of disease severity in the T34 non-enriched perlite samples increased by 49.21% after inoculation (day 7, 10 and 14). Disease severity in the T34 enriched perlite samples remained the same from day 10 to 14 after inoculation.

The populations of T34 were unchanged when the pathogen was inoculated on the leaves and decreased significantly – by up to 50% – in the absence of the pathogen (Fig. 4).

Discussion

The fact that the presence of *B. cinerea* can modify the secretion pattern of root exudates is in agreement with the conclusions of previous studies (Hale & Moore, 1979; Lanoue et al., 2010). Other studies have observed that plants exposed to pathogens show an increase in the content of diverse organic acids in root exudates such as

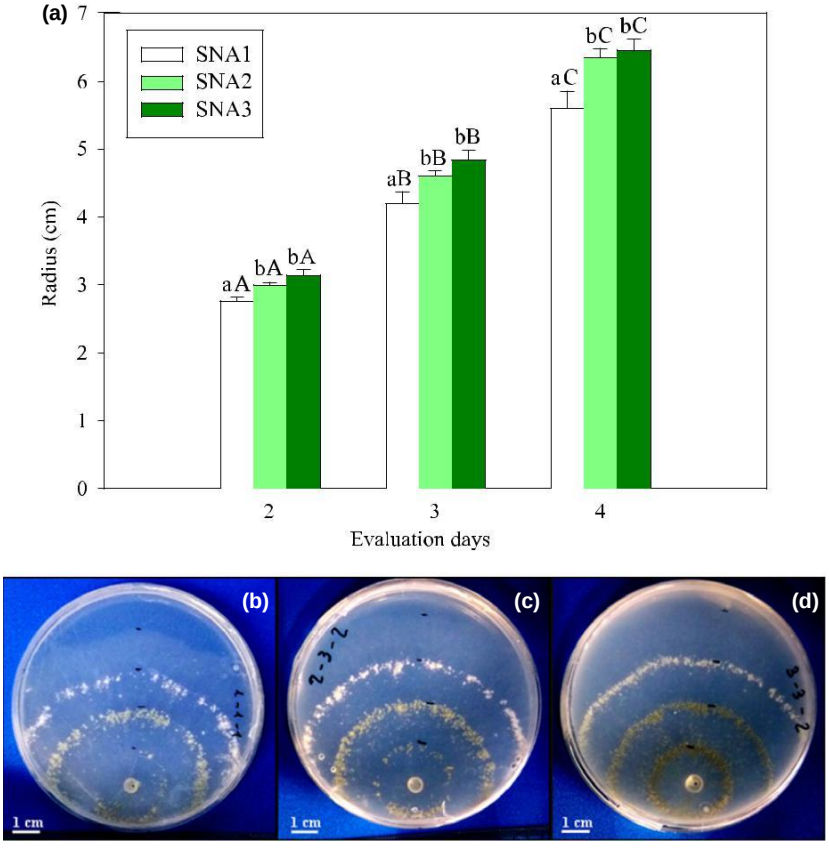


Figure 2 Growth of *Trichoderma asperellum* strain T34 (T34). T34 was grown in synthetic nutrient-poor agar (SNA) with varying sugar compositions: SNA1 (0.2 g L^{-1} sucrose and 0.2015 g L^{-1} dextrose), SNA2 (0.2 g L^{-1} sucrose, 0.2 g L^{-1} dextrose and 0.0015 g L^{-1} gluconic acid) and SNA3 (0.2015 g L^{-1} gluconic acid). (a) Colony radius of T34 from days 2 to 4.

Means ± standard error, n = 18 (collected from two separate studies). Different lower case letters show significant differences between treatments and different upper case letters show significant differences between days within treatments ($P < 0.05$; Duncan's multiple range test). (b–d) Representative images of T34 growth on day 4 in SNA1, SNA2 and SNA3, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

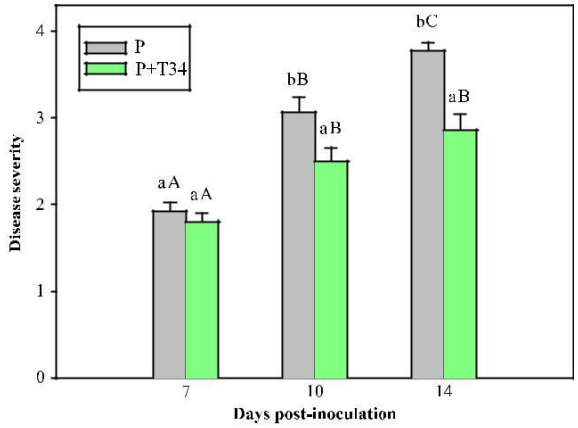


Figure 3 Disease severity caused by the pathogen *Botrytis cinerea* (10^5 CFU mL^{-1}) in tomato plants. Plants were grown in two growth media: P, perlite; P + T34, perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^5 CFU mL^{-1}. Disease was evaluated at 7, 10 and 14 days post-inoculation. Disease severity was evaluated using a scale of 0–4: 0, symptomless; 1, chlorotic leaf; 2, necrotic specks; 3, necrotic spot; 4, dead leaf. Means ± standard error, n = 32 (collected from two separate studies). Different lower case letters show significant differences between treatments and different upper case letters show significant differences between days within treatments ($P < 0.05$; ANOVA test and Duncan's multiple range test, respectively). [Colour figure can be viewed at wileyonlinelibrary. com]

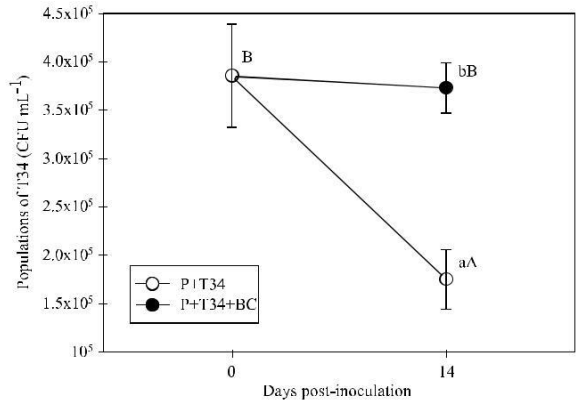


Figure 4 Populations of *Trichoderma asperellum* strain T34 (T34) calculated by the method dilution plate. Tomato plants were grown in P + T34 (perlite enriched with T34 at a concentration of 10^5 CFU mL^{-1}). Two treatments were applied on day 0: P + T34, without pathogen; and P + T34 + BC, inoculated with the pathogen *Botrytis cinerea* (10^5 CFU mL^{-1}). Samples from rhizosphere were collected at 0 and 14 days post-inoculation. Means ± standard error, n = 12 (collected from two separate studies). Different lower case letters show significant differences between treatments and different upper case letters show significant difference between days within treatments ($P < 0.05$; ANOVA test).

succinic acid and malic acid, but not gluconic acid (Kamilova et al., 2006b; Rudrappa et al., 2008). A study by Kamilova et al. (2006b) also noted a decrease in sugar content but not in sucrose content. No information in relation to the effect of pathogens on inositol content of root exudates was found.

The closest similarities to the results here on metabolite changes were found in botrytised aerial plant organs but not in root exudates. According to Ribereau-Gayon et al. (2006), *B. cinerea* infection leads to the accumulation of gluconic acid in grape tissues, which are a characteristic secondary product of sugar degradation when *B. cinerea* emerges outside of the grape. Other authors have shown, through the metabolic profiling of sunflower cotyledons infected by *B. cinerea*, a 90% decrease in sucrose and 75% decrease in inositol 48 h after inoculation (Dulermo et al., 2009). Although these results occurred in aerial organs of the infected plant, it could be hypothesized that results in root exudates may reflect the metabolite status in other plant parts depending on factors, such as the type of pathogen involved.

The production of gluconic acid and the subsequent host tissue acidification enhances the establishment of conditions suitable for the necrotrophic development of *Penicillium expansum* (Hadas et al., 2007). De Cal et al. (2013) also suggest that ambient pH is linked to pathogenicity processes because *Monilinia fructicola* accumulated gluconic acid at the infection site as the main organic acid. For *B. cinerea*, the accumulation of organic acid is also an advantage as it helps reduce the pH, facilitating the degradation of the cell walls of the host by polygalacturonases. In contrast, this function has been traditionally associated with the production of oxalic acid (Elad et al., 2007) rather than gluconic acid.

Some authors report that while gluconic acid provided benefits to certain rhizosphere microorganisms, it is still considered an antifungal agent (Kaur et al., 2006) and the most frequent organic acid produced by rhizobacteria to solubilize insoluble or poorly soluble mineral phosphates (Rodrıguez & Fraga, 1999). However, in the present study, the growth and development of T34 was not negatively affected.

Trichoderma spp. are able to thrive in a wide range of external pH conditions and are more efficient in acidic than in alkaline soils (Benıtez et al., 2004). Nevertheless, the optimum pH varies among isolates from pH 4.0 to 6.8 (Steyaert et al., 2010). In SNA3 the pH was more acidic than the other media and that could have affected the growth. However, in SNA2 the pH was the same as in SNA1 and there was a significant T34 growth increase, indicating that gluconic acid has a pH-independent growth-promoting effect on T34. It should be noted that the dose of gluconic acid used in SNA2 was the same as that obtained in exudates. Lugtenberg et al. (2001) reported the crucial role of organic acids in proliferation and root colonization by BCA P. fluorescens WCS365. Moreover, according to Zhang et al. (2014), some organic acids from cucumber root exudates (oxalic acid, malic acid and citric acid) increase growth and conidial germination of *T. harzianum* T-E5, which is positive for root colonization. *Botrytis cinerea* cultured in vitro with organic acids (citric or malic acid) also had

a higher mycelial production than in media without them (Verhoeff et al., 1988).

T34 colonizing roots triggered the induction of systemic resistance, which reduced disease severity significantly as in previous studies (Fernandez et al., 2014). The ability of T34 to trigger induced systemic resistance (ISR) against other foliar pathogens in *Arabidopsis* plants has also been demonstrated (Segarra et al., 2009). In addition, a study with cucumber plants has shown that the induction of resistance by T34 can take place via ISR or systemic acquired resistance, depending on T34 populations (Segarra et al., 2007). Accordingly, the concentration of T34 in a substrate may play a crucial role in the pathway route of induction of resistance in plants. Therefore, the fact that populations of T34 in the roots were maintained and not decreased in the presence of *B. cinerea* on the leaves could indicate a connection with the reduction of disease severity. Moreover, considering that the presence of *B. cinerea* modifies the secretion pattern of tomato plant exudates, certain compounds within these exudates may stimulate T34 growth. Studies of diverse *Trichoderma* showed that root exudates of some crops can increase the growth of some isolates whereas others have no effect (Jash & Pan, 2007; Bharathi et al., 2008). Exudates secreted by plants provide an environment rich in carbon and energy that can be used by rhizosphere microorganisms, and can communicate specifically to them by producing signals that modulate colonization (Haichar et al., 2014). Furthermore, it is necessary to consider that plants use the ability to recruit microorganisms to obtain the desired benefits of this interaction (Sarma et al., 2015); in this case the benefit might be to help fend off disease. Accordingly, Pieterse et al. (2014) discusses the importance of achieving high population densities to ensure effective ISR. The results obtained in this study indicate the presence of communication from leaf to root via the secretion of exudates that enhance the growth of T34 and in doing so promote resistance to *B. cinerea*.

In conclusion, *B. cinerea* infection on leaves modified the secretion pattern of root exudates in tomato plants. This increased the concentration of gluconic acid and decreased the concentration of sucrose and inositol. This infection also increased populations of T34 and reduced disease severity through induction of systemic resistance. Despite these results, the possibility that an unidentified compound from the root exudates could have increased the growth of T34 cannot be dismissed. The results of this study contribute to the body of research in relation to leaf to root communication, the ways in which plants encourage beneficial microorganism relationships, and the role of root exudates in plant defence against foliar pathogens.

Acknowledgements

This work was supported by Spain's Ministry of Science and Innovation (AGL2010-21982-C02-02) and by a grant from the University of Barcelona (2014). The authors are grateful to Semillas Fito and Biocontrol Technologies for kindly providing the seeds and *T. asperellum* strain T34, respectively. They also thank the Experimental Fields Service (University of Barcelona) and Lourdes Berdie from the Scientific & Technological Centres (University of Barcelona).

References

Badri DV, Vivanco JM, 2009. Regulation and function of root exudates. Plant, Cell & Environment 32, 666–81.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM, 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–66.

Benıtez T, Rincon AM, Limon MC, Codon AC, 2004. Biocontrol mechanisms of Trichoderma strains. International Microbiology 7, 249–60.

Bertin C, Yang X, Weston LA, 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256, 67–83.

Bharathi V, Rao KC, Reddy LK, 2008. Studies on the effect of root exudates of different crops on pigeon pea wilt pathogen (Fusarium udum Butler) and its antagonistic fungi. International Journal of Agriculture Sciences 4, 670–3.

Chung YR, Hoitink HAJ, 1990. Interactions between thermophilic fungi and Trichoderma hamatum in suppression of Rhizoctonia damping-off in a bark compost-amended container medium. Phytopathology 80, 73–7.

De Cal A, Sandın-Espana~ P, Martinez F et al., 2013. Role of gluconic acid and pH modulation in virulence of Monilinia fructicola on peach fruit. Postharvest Biology and Technology 86, 418–23.

De Meyer G, Bigirimana J, Elad Y, Hofte€ M, 1998. Induced systemic resistance in Trichoderma harzianum T39 biocontrol of Botrytis cinerea. European Journal of Plant Pathology 104, 279–86.

Dean R, Van Kan JAL, Pretorius ZA et al., 2012. The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13, 414–30.

Dulermo T, Rascle C, Chinnici G, Gout E, Bligny R, Cotton P, 2009. Dynamic carbon transfer during pathogenesis of sunflower by the necrotrophic fungus Botrytis cinerea: from plant hexoses to mannitol. New Phytologist 183, 1149–62.

Elad Y, Williamson B, Tudzynski P, Delen N, 2007. Botrytis: Biology, Pathology and Control. Dordrecht, Netherlands: Kluwer Academic Publishers.

Fernandez E, Segarra G, Trillas MI, 2014. Physiological effects of the induction of resistance by compost or Trichoderma asperellum strain T34 against Botrytis cinerea in tomato. Biological Control 78, 77–85.

Hadas Y, Goldberg I, Pines O, Prusky D, 2007. Involvement of gluconic acid and glucose oxidase in the pathogenicity of Penicillium expansum in apples. Phytopathology 97, 384–90.

Haichar FEZ, Santaella C, Heulin T, Achouak W, 2014. Root exudates mediated interactions belowground. Soil Biology and Biochemistry 77, 69–80.

Hale MG, Moore LD, 1979. Factors affecting root exudation II: 1970– 1978. In: Brady NCD, ed. Advances in Agronomy, Vol. 31. New York, NY, USA: Academic Press, 93–124.

Jash S, Pan S, 2007. Variability in antagonistic activity and root colonizing behaviour of Trichoderma isolates. Journal of Tropical Agriculture 45, 29–35.

Johnson LF, Curl EA, 1972. Methods for Research on the Ecology of Soil-borne Plant Pathogens. Minneapolis, MN, USA: Burgess.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B, 2006a. Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. Molecular Plant–Microbe Interactions 19, 250–6.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B, 2006b. Effects of the tomato pathogen Fusarium oxysporum f. sp. radicis-lycopersici and of the biocontrol bacterium

Pseudomonas fluorescens WCS365 on the composition of organic acids and sugars in tomato root exudate. Molecular Plant–Microbe Interactions 19, 1121–6.

Kaur R, Macleod J, Foley W, Nayudu M, 2006. Gluconic acid: an antifungal agent produced by Pseudomonas species in biological control of take-all. Phytochemistry 67, 595–604.

Kravchenko LV, Azarova TS, Shaposhnikov AI, Makarova NM, Tikhonovich IA, 2003. Root exudates of tomato plants and their effect on the growth and antifungal activity of Pseudomonas strains. Microbiology 72, 37–41.

Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Rose€ USR, 2010. De novo biosynthesis of defense root exudates in response to Fusarium attack in barley. New Phytologist 185, 577–88.

Leroch M, Kretschmer M, Hahn M, 2011. Fungicide resistance phenotypes of Botrytis cinerea isolates from commercial vineyards in south west Germany. Journal of Phytopathology 159, 63–5.

Li R, Cai F, Pang G, Shen Q, Li R, Chen W, 2015. Solubilisation of phosphate and micronutrients by Trichoderma harzianum and its relationship with the promotion of tomato plant growth. PLoS ONE 10, e0130081.

Lugtenberg BJ, Dekkers L, Bloemberg GV, 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. Annual Review of Phytopathology 39, 461–90.

Matilla MA, Ramos JL, Bakker PAHM et al., 2010. Pseudomonas putida KT2440 causes induced systemic resistance and changes in Arabidopsis root exudation. Environmental Microbiology Reports 2, 381–8.

Matthew T, Zhou W, Rupprecht J et al., 2009. The metabolome of Chlamydomonas reinhardtii following induction of anaerobic H_2 production by sulfur depletion. Journal of Biological Chemistry 284, 23415–25.

Nirenberg HI, 1981. A simplified method for identifying Fusarium spp. occurring on wheat. Canadian Journal of Botany 59, 1599–609.

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM, 2014. Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology 52, 347–75.

Ribereau-Gayon P, Dubourdieu D, Doneche B, Lonvaud A, 2006. The grape and its maturation. Handbook of Enology, the Microbiology of Wine and Vinifications, Vol. 1. Chichester, UK: John Wiley & Sons Ltd, 241–97.

Rodrıguez H, Fraga R, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances 17, 319–39.

Rudrappa T, Czymmek KJ, Pare PW, Bais HP, 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiology 148, 1547–56.

Sarma BK, Yadav SK, Singh S, Singh HB, 2015. Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. Soil Biology and Biochemistry 87, 25–33.

Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas I, 2007. Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with Trichoderma asperellum strain T34. Proteomics 7, 3943–52.

Segarra G, Van der Ent S, Trillas I, Pieterse CMJ, 2009. MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biology 11, 90–6.

Steinkellner S, Mammerler R, Vierheilig H, 2008. Germination of Fusarium oxysporum in root exudates from tomato plants challenged with different Fusarium oxysporum strains. European Journal of Plant Pathology 122, 395–401.

Steyaert JM, Weld RJ, Mendoza-Mendoza A, Stewart A, 2010. Reproduction without sex: conidiation in the filamentous fungus Trichoderma. Microbiology 156, 2887–900.

Tan S, Yang C, Mei X et al., 2013. The effect of organic acids from tomato root exudates on rhizosphere colonization of Bacillus amyloliquefaciens T-5. Applied Soil Ecology 64, 15–22.

Verhoeff K, Leeman M, Peer R, Posthuma L, Schot N, Eijk GW, 1988. Changes in pH and the production of organic acids during colonization of tomato petioles by Botrytis cinerea. Journal of Phytopathology 122, 327–36.

Verma M, Brar SK, Tyagi RD, Surampalli RY, Valero JR, 2007. Antagonistic fungi, Trichoderma spp.: panoply of biological control. Biochemical Engineering Journal 37, 1–20.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M, 2008. Trichoderma–plant–pathogen interactions. Soil Biology and Biochemistry 40, 1–10.

Zhang F, Zhu Z, Yang X, Ran W, Shen Q, 2013. Trichoderma harzianum T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. Applied Soil Ecology 72, 41–8.

Zhang F, Meng X, Yang X, Ran W, Shen Q, 2014. Quantification and role of organic acids in cucumber root exudates in Trichoderma harzianum T-E5 colonization. Plant Physiology and Biochemistry 83, 250–7.

4.3. Capítulo III: *Trichoderma asperellum* cepa T34 en la rizosfera induce promoción del crecimiento en plantas de tomate e incrementa la expresión de genes relacionados con respuesta a estímulos, respuesta a estrés y proteólisis

***Trichoderma asperellum* strain T34 on the rhizosphere induces growth promotion on tomato plants and up-regulates the expression of genes related to response stimulus, response to stress and proteolysis**

Fernández E, Segarra G, Romanyà J, Trillas MI

Resumen

Trichoderma spp. ha sido ampliamente utilizado como ACB debido a que algunas cepas pueden proteger a las plantas frente a estreses de tipo abiótico y biótico, y además, de promover el crecimiento de las plantas. Para lograr estos beneficios, algunas cepas pueden inducir cambios en la expresión génica de las plantas, cuyo resultado diferirá dependiendo de la combinación de planta, ACB y otros factores externos. Los objetivos de este estudio fueron evaluar el efecto de la luz y la disponibilidad de nutrientes en el crecimiento de las plantas de tomate sembradas con o sin T34, su efecto en la expresión génica de las plantas de tomate y su relación con la mejora del crecimiento.

Los resultados del microarray mostraron que la expresión de 64 genes fue significativamente diferente ($P < 0.05$) entre las plantas crecidas en perlita enriquecida o no con T34 (63 se incrementaron y uno se redujo). El análisis de enriquecimiento funcional de los genes que incrementaron su expresión reveló que los procesos biológicos 3 términos GO se enriquecieron significativamente ($P < 0.05$): respuesta a estrés, respuesta a estímulos y proteólisis.

La evaluación del crecimiento de las plantas mostró que el peso seco aumentó en los tratamientos enriquecidos con T34 (10^4 y 10^6 UFC mL^{-1}) en todas las condiciones experimentales evaluadas, excepto en la combinación de solución nutritiva completa y

baja intensidad lumínica (60-80 µmol m^{-2} s^{-1} PPFD-densidad de flujo fotosintética de fotones-). El bajo suministro de nutrientes (dilución 1: 3 de la solución nutritiva completa) combinado con baja y alta luz (300-400 µmol m^{-2} s^{-1} PPFD) mostró los valores más bajos de peso seco y altura y los valores más bajos de área foliar total, respectivamente. El mejor rendimiento se alcanzó en el tratamiento enriquecido con T34 con solución nutritiva completa y alta intensidad lumínica.

En conclusión, T34 en la rizosfera induce cambios en la expresión génica en tomate. Estos cambios promueven el crecimiento de las plantas de tomate por T34 en condiciones favorables (alta disponibilidad de nutrientes y alta intensidad luminosa), y de manera similar con baja disponibilidad de nutrientes (baja y alta intensidad de lumínica).

***Trichoderma asperellum* strain T34 on the rhizosphere induces growth promotion on tomato plants and up-regulates the expression of genes related to response stimulus, response to stress and proteolysis**

Elena Fernández[1]*, Guillem Segarra[1], Joan Romanyà[2], Maria Isabel Trillas[1]

[1]Secció de Fisiologia Vegetal, Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Catalonia, Spain

[2]Departament de Productes Naturals, Biologia Vegetal i Edafologia, Universitat de Barcelona, Barcelona, Catalonia, Spain

*Corresponding author

E-mail: efernago13@gmail.com

Abstract

Trichoderma spp. has been widely used as biological control agent (BCA) because some strains can protect plants front abiotic and biotic stress and promote plant growth. To achieve these benefits some strains can induce changes in plant gene expression whose performance depends on the combination of the plant, the BCA and other external factors. The aims of this study were to evaluate light and nutrient availability effect on the growth of tomato plants sown with or without the BCA *Trichoderma. asperellum* strain T34 (T34), its effect in the gene expression of tomato plants ant its relation with growth improvement.

The results of the microarray analysis showed that expression of 64 genes was significantly different ($P < 0.05$) between plants grown in perlite enriched or not with T34 (63 up-regulated and 1 down-regulated). The functional enrichment analysis of up-regulated genes revealed that 3 GO biological process terms were enriched significantly ($P < 0.05$): response to stress, response to stimulus and proteolysis.

Plants growth evaluation showed that dried weight increased in treatments enriched with T34 (10^4 and 10^6 CFU mL^{-1}) in all environmental conditions tested, except for the combination of complete nutrient solution and low light intensity (60-80 µmol m^{-2} s^{-1} PPFD). Low nutrient supply (1:3 dilution of complete nutrient solution) combined with low and high light (300-400 µmol m^{-2} s^{-1} PPFD) showed the lowest values of dried weight and height and the lowest values of TLA, respectively. The best performance was achieved in T34 enriched treatment with complete nutrient solution and high light.

In conclusion, T34 on rhizosphere induces changes in gene expression on tomato. These changes promote tomato plants growth by T34 under favorable conditions (high nutrient availability and high light intensity).and similarly with low nutrient availability (low and high light intensity).

Introduction

Integrate control of plant diseases have been promoted in the recent years with the aim to reduce the negative impact of chemical pesticides in human health and environment (Directive 2009/128/EC). Integrated pest management control encloses the combined use of cultural practices, mechanical and physical methods, biological control and, as the last resource, chemical methods. In recent years great efforts have been invested in studying biological control alternatives and special interest has been placed in the use of microorganisms as biological control agents (BCAs).

Nowadays, in Europe 56 microorganisms have been accepted as authorized active substances (http://ec.europa.eu/food/plant/pesticides). Among the accepted microorganisms we can find yeast, virus, bacteria and fungi; being *Bacillus* spp. and *Trichoderma* spp. the most representative ones.

Trichoderma spp. includes worldwide distributed filamentous ascomycetes with over than 100 species (http://www.isth.info/). They are frequently found in soils, on the surface of plants, on decomposing wood and bark, on other fungi and on other organic materials (Jaklitsch, 2009; Zafra and Cortés-Espinosa, 2015). Strains of *Trichoderma* spp. have been used as BCAs against a broad spectrum of soil (Howell et al., 2000; Howell, 2002; Pastrana et al., 2016; Chen et al., 2017) and foliar diseases (De Meyer et al., 1998; Yedidia et al., 2003; Yao et al., 2016). The main characteristics that confer good disease control abilities to certain *Trichoderma* strains are survival in unfavourable and variable ambient conditions, a high reproductive capacity, competence ability with stable resident microbial community, efficient nutrient use, plants growth promotion and enhancement of plant defence from both biotic and abiotic stress (Yedidia et al., 2001; Benítez et al., 2004, Segarra et al., 2009, Trillas and Segarra 2009; Shoresh et al., 2010). Moreover, *Trichoderma* spp. competes for nutrients against pathogens (Segarra et al. 2010; Borrero et al., 2012). On the other hand, the main modes of action to control diseases are competition for space and nutrient, antibiosis and parasitism (Benítez et al., 2004, Trillas and Segarra 2009; Trillas and Segarra, 2012). Although the ability to promote growth is widely known (Harman et al., 2004; Harman, 2006), there is scarce information about the influence of some environmental conditions on this growth promotion.

The biological control agent *Trichoderma asperellum* strain T34 (T34) controls both soil (Trillas et al., 2006; Segarra et al., 2010; Segarra et al., 2013) and foliar pathogens (Segarra et al., 2007; Segarra et al., 2009; Fernández el al., 2017). Moreover, T34 can also protect plants against abiotic stress as demonstrated in tomato plants subjected to high concentrations of Fe (Segarra et al., 2010) and improve plant growth and nutrient uptake (De Santiago et al., 2013; Fernández el al., 2014).

The induction of systemic resistance is considered a physiological "state of enhanced defensive capacity" which confers increased basal resistance to a broad spectrum of pathogens (Choudhary and Prakash, 2007). The main pathways are systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is triggered by pathogens, depends on the signaling molecule salicylic acid (SA) and is related to the accumulation of pathogenesis-related (PR) proteins (Van Loon, 1997; Durrant y Dong, 2004). In contrast, ISR is triggered by non-pathogenic microorganisms, depends on jasmonic acid (JA) and ethylene (ET) and is not related to accumulate PR proteins (Pieterse et al., 1996; Pieterse et al., 1998). However, there are evidences about the activation of PR proteins by *Trichoderma harzianum* strain T-203 (Yedidia et al., 2000). Moreover, T34 is able to trigger induction of resistance by ISR or SAR depending on T34 populations on roots (Segarra et al., 2009). Accordingly, some authors suggest that ISR performance depends on the combination of plant, BCA and pathogen (Tjamos et al., 2005).

According to Van Wees (1999), at the first moment of interaction between the plant and the non-pathogenic microorganism it was not observed the overexpression of genes related to defense mechanisms. Nevertheless, when new challenges with pathogens occur plants are primed to respond to stresses more quickly and aggressively minimizing the effect of the disease. This phenomenon is called "priming" and can be triggered by SAR, ISR and abiotic stress (Conrath et al., 2006).

Recent studies have focused on elucidating the gene expression changes triggered by certain *Trichoderma* strains in plants to evaluate the pathways and mechanisms involved in induced systemic resistance and priming (Alfano et al., 2007; Brotman et al., 2012; Mathys et al., 2012). All of these studies agree that *Trichoderma* spp. alters gene expression of plants mainly up-regulating gens related with stress responses and defense without compromising plant growth. Moreover, cucumber plants after 24 h of

interaction with T34 (T34 10^7 CFU mL^{-1}) differently modulate protein expression (17 up-regulated and 11 down-regulated proteins) related to stress and defence, energy and metabolism, secondary metabolism and protein synthesis and folding (Segarra et al., 2007). Nevertheless, lower populations of T34 easier to maintain on rhizosphere may modulate differently plants gene expression and it could be interesting to study these effects.

We hypothesized that the inoculation of T34 will change the gene expression of tomato plants grown under different light and nutrient availability.

The objectives of this study were to analyse the effect of low and high light and nutrient availability on the growth of tomato plants sown with or without the BCA *T. asperellum* strain T34, its effect in the gene expression of tomato plants ant its relation with growth improvement.

Materials and methods

Plant material

Tomato, *Solanum lycopersicum* cv. Roma, seeds were pre-germinated with sterile distilled water (H_2O_d) and paper for 4 days at 25 ± 3 °C and dark conditions. Germinated seeds were sown for 18 days in 600 mL pots in perlite (Europerlite) (P) and perlite enriched with T34 at a concentration of 10^4 CFU mL^{-1} growth media (P+T34 10^4) and 10^6 CFU mL^{-1} growth media (P+T34 10^6). T34 enriched perlite was prepared by dilution from the commercial product T34 Biocontrol® (10^9 CFU g^{-1} growth media). Plants were grown in a growth chamber at 25 ± 2 °C, 60-80% relative humidity and 16 h of light. Two light conditions were evaluated 60-80 µmol m^{-2} s^{-1} photosynthetic photon flux density -PPFD- (Low) and 300-400 µmol m^{-2} s^{-1} PPFD (High). Plants were irrigated on the media with a 25.5 mL nutrient solution day^{-1} pot^{-1} and with H_2O_d on alternate days. Two regime of nutrient solutions were applied: complete nutrient solution 0.5 g L^{-1} Peter's foliar feed 27-15-12 from Scotts, 0.22 g L^{-1} $CaCl_2$ and 0.25 g L^{-1} $MgSO_4 \cdot 7\ H_2O$ (C) and 1:3 diluted nutrient solution (complete nutrient solution:H_2O_d, v/v) (1/4).

The study was performed twice, the first time to evaluated plant growth and the second to evaluated gene expression. In each study 6 plants per experimental condition were sown.

At the end of each study, populations of T34 were calculated as described previously (Fernández et al., 2017).

Mycroarray analaysis

To evaluate tomato plants gene expression, plants grown at High PPFD and complete nutrient solution were selected. Leaves from 3 plants grown on perlite alone and from 3 plants grown in P+T34 10^6 were sampled, quick-frozen in liquid N_2 and ground. RNA was extracted from samples with the RNeasy Plant Mini Kit (Qiagen) following the Kit protocol. Extracted RNA quality and quantity was evaluated with an Agilent 2100 Bioanalyzer and a NanoDrop ND-1000 spectrophotometer.

Sample preparation and hybridization with the GeneChip tomato Genome Array were carried out at the Functional Genomics Core of the Institute for Research in Biomedicine of Barcelona (Spain).

Microarray data analysis of the Affymetrix arrays was performed by running the default settings in AltAnalyze software (Emig et al., 2010). Specifically microarrays were normalized by using RMA (Robust Microarray Analysis) with a 2-fold cut-off and an adjusted (Benjamini-Hochberg) $P < 0.05$.

Functional enrichment

To identify enriched Gene Ontology (GO) terms of gens with different expression the agriGO tool was used (Du et al., 2010). The singular enrichment analysis (SEA) was performed by comparing the reference list of the microarray probe set (Tomato Affymetrix Genome Array) with the different expressed gens set. The statistical method used in SEA was the Fisher test. Multiple comparison correction was performed by the Benjamini-Yekutieli method.

Plant growth evaluation

To evaluate the effect of T34, light and nutrient solution on tomato plants growth; shoots, leaves and roots of six plants per experimental condition were sampled. Samples were dried (forced air oven) at 60°C for 48 h and dry weight of shoots and leaves (DWA), dry weight of whole plant (DWT), plant height, root/shoot ratio, and percentage of water in each plant were determined. Total leaf areas (TLA) of leaves were calculated by analyzing leaves photos with the ImageJ v 1.48 software.

Data from physiological parameters were analysed by IBM SPSS Statistics 19 version statistical software and results are expressed as means ± standard error (SE). Data were analysed with ANOVA to study the factors growth media, light and nutrient solution. When significant differences were observed ($P < 0.05$) and more than two groups were present, the Duncan's multiple range test was performed ($P < 0.05$). In cases when normal distribution and homogeneity of variances were not found, the Kruskal-Wallis test was performed ($P < 0.05$).

Results

Mycroarray analaysis

Mycroarray analysis revealed that expression of 64 genes was significantly different ($P < 0.05$) in plants grown in P+T34 10^6 compared to P alone. Among these 64 genes 63 were up-regulated and 1 gene was down-regulated (Table 1).

Functional enrichment

The functional enrichment analysis of up-regulated genes between plants grown in P+T34 10^6 and perlite showed that from the category of biological process 3 GO terms were enriched significantly ($P < 0.05$) (Fig 1). The most enriched term was related to the function response to stress, followed by response to stimulus and, finally, the lowest enriched function was proteolysis.

Plant growth

Higher application of nutrient solution and/or light intensity for each growth medium was related to a higher DWA and DWT performance. In addition, DWA and DWT show an increase in treatments with T34 in all environmental conditions tested, except for the combination of Low light intensity and C nutrient solution, where no significant differences ($P < 0.05$) were found (Table 2). Moreover, plants grown on P+T34 10^6 at High light and C nutrient solution showed an intermediated performance compare with P+T34 10^4 as a result of a minor investment in roots.

Among nutrient solution conditions differences only were observed in low light intensity, being higher in plants irrigated with 1/4 of nutrient solution (Table 2). In addition to this, plants grown P+T34 10^6 and those conditions showed higher root/shoot than those grown with High light intensity. On the other hand, plants grown in P+T34 10^4 and P at High light and C nutrient solution conditions, showed higher root/shoot ratio than those grown in Low light conditions. Root /shoot plant performance only showed differences among growth media at Low light and C nutrient solution conditions. The lowest values were observed in plants grown in P+T34 10^4 and P, but P values were no significantly different ($P < 0.05$) than those from P+T34 10^6.

Table 1. Gene expression changes in leaves of tomato plants grown in perlite (P) and perlite enriched with *Trichoderma asperellum* strain T34 at a concentration of 10^6 CFU mL^{-1} (P+T34 10^6).

Gene ID	Gene Symbol	Gene description	Fold change (P+T34 10^6-P)	Ajusted p-value
Up-regulated genes				
Les.3740.1.S1_at	LOC100125890 /// LOC544087	inhibitor of yeast proteinase A /// cathepsin D inhibitor protein	33.69	0.0099
Les.3621.1.S1_at	LOC543954	wound-induced proteinase inhibitor 1	26.76	0.0122
Les.1675.1.S1_at	LOC101262660 /// LOC543955	reticulon-like protein B1 /// wound-induced proteinase inhibitor II prepeptide	23.64	0.0109
Les.3974.1.A1_at	mcpi	metallocarboxypeptidase inhibitor	12.08	0.0200
Les.5597.1.S1_at	LOC101244079	protein disulfide-isomerase-like	11.55	0.0064
LesAffx.1.1.S1_at	ARG2	arginase 2	11.24	0.0125
Les.2173.1.A1_at	---	---	10.81	0.0125
Les.4022.1.S1_at	cevi57	proteinase inhibitor II	10.20	0.0072
Les.4317.1.S1_at	LOC543875	asparagine synthetase [glutamine-hydrolyzing]	9.02	0.0149
Les.4820.1.S1_x_at	LOC543570 /// TMC	multicystatin /// multicystatin	8.44	0.0238
Les.2971.1.S1_at	LOC101246961	wound-induced proteinase inhibitor 1	8.29	0.0125
Les.3286.1.S1_at	CHRDi	inducible plastid-lipid associated protein	7.51	0.0172

Les.3299.2.A1_s_at	ARG2	arginase 2	7.47	0.0139
Les.840.1.A1_at	LOC101266266	proteinase inhibitor I-B-like	7.35	0.0064
Les.3070.2.A1_at	LOC101264133	acid phosphatase 1	6.48	0.0119
Les.3021.1.S1_at	LOC101265368	proteinase inhibitor I-B	5.90	0.0097
Les.84.1.S1_at	lap2	leucine aminopeptidase	5.56	0.0206
Les.3940.2.A1_at	LOC101247557	wound-induced proteinase inhibitor 1	4.95	0.0245
Les.3618.1.S1_at	LOC544001	uncharacterized LOC544001	4.88	0.0399
Les.3266.2.S1_at	LOC101268129	acetylornithine deacetylase	4.77	0.0181
Les.4299.1.S1_at	LOC101259064	polyphenol oxidase F, chloroplastic	4.45	0.0172
Les.3619.1.S1_at	ER1	proteinase inhibitor I	4.27	0.0149
Les.3266.3.S1_at	LOC101268129	acetylornithine deacetylase	4.16	0.0125
Les.3663.1.S1_at	9612	probable pectate lyase P18	3.24	0.0099
Les.2946.2.S1_at	LOC101055528	Hop-interacting protein THI101	3.19	0.0239
LesAffx.53591.1.S1_at	LOC101260654	myb-related protein Myb4-like	3.13	0.0473
Les.3623.1.S1_at	SAR2	GTPase	3.02	0.0125
Les.4693.1.S1_at	P4	pathogenesis-related protein P4	2.82	0.0198
Les.4281.3.S1_at	LOC101250072	apyrase-like	2.81	0.0141
Les.4460.1.S1_at	PR-P2	pathogenesis-related protein P2	2.78	0.0149
Les.3742.1.S1_at	Prg1	Pto-responsive gene 1 protein	2.76	0.0200
Les.2971.2.A1_at	LOC101246961	wound-induced proteinase inhibitor 1	2.75	0.0200
LesAffx.10091.1.S1_at	LOC101260610	acetyl-CoA-benzylalcohol acetyltransferase	2.74	0.0072

LesAffx.37715.1.A1_at	---	---	2.70	0.0165
Les.3686.1.S1_at	THT7-8	N-hydroxycinnamoyl-CoA:tyramine N-hydroxycinnamoyl transferase THT7-8	2.69	0.0149
Les.2852.1.S1_at	NP24	protein NP24	2.66	0.0330
Les.5855.1.S1_at	LOC101246594	probable 2-oxoglutarate-dependent dioxygenase AOP1	2.62	0.0414
LesAffx.18686.1.S1_at	LOC101246036	inorganic pyrophosphatase 1-like	2.54	0.0200
LesAffx.43793.1.S1_at	LOC101252298	putative ER lumen protein-retaining receptor C28H8.4	2.51	0.0125
LesAffx.69261.1.S1_at	LOC101266414	xylem cysteine proteinase 1	2.44	0.0353
Les.435.1.S1_at	LOC101253788	acidic endochitinase	2.44	0.0262
Les.4496.1.S1_at	LOC101246381	pathogenesis-related protein STH-2-like	2.37	0.0305
Les.647.1.A1_at	---	---	2.37	0.0125
LesAffx.43793.1.A1_at	---	---	2.35	0.0125
Les.2068.1.A1_at	LOC101267900	serine carboxypeptidase-like	2.32	0.0240
Les.5251.1.S1_at	LOC101250358	vacuolar-sorting receptor 6-like	2.28	0.0072
Les.4953.1.S1_at	LOC101255600	aquaporin TIP1-3-like	2.21	0.0156
Les.3101.1.S1_at	LOC101262921	protein disulfide-isomerase-like	2.20	0.0081
LesAffx.65682.1.A1_at	---	---	2.20	0.0187
Les.3515.1.S1_at	LOC544223	wound-inducible carboxypeptidase	2.15	0.0151
Les.3071.2.S1_at	LOC101261722	alpha-aminoadipic semialdehyde synthase	2.13	0.0156
Les.5309.1.S1_at	LOC101268729	alanine--glyoxylate aminotransferase 2 homolog 2, mitochondrial-like	2.13	0.0470

LesAffx.16769.1.S1_at	LOC101250259	dirigent protein 22-like	2.13	0.0179
LesAffx.22812.2.S1_at	LOC101250202	probable E3 ubiquitin-protein ligase RNF217	2.12	0.0410
Les.2090.1.S1_at	LOC101262362	thioredoxin M3, chloroplastic	2.11	0.0122
LesAffx.13252.2.S1_at	LOC101245358	GDSL esterase/lipase At3g26430-like	2.11	0.0458
LesAffx.805.1.S1_at	LOC101246562	dnaJ protein ERDJ3B	2.09	0.0098
Les.2090.3.A1_at	LOC101262362	thioredoxin M3, chloroplastic	2.07	0.0258
LesAffx.3610.1.S1_at	---	---	2.06	0.0242
Les.2591.1.S1_at	LOC543987	beta-1,3-glucanase	2.05	0.0200
LesAffx.33402.1.S1_at	---	---	2.04	0.0172
LesAffx.4763.1.S1_at	LOC101256176	putative methyltransferase DDB_G0268948	2.02	0.0486
LesAffx.71535.1.S1_at	LOC101262552	glutathione S-transferase L3	2.01	0.0271
Down-regulated genes				
Les.4044.1.S1_at	---	---	-2.02	0.0149

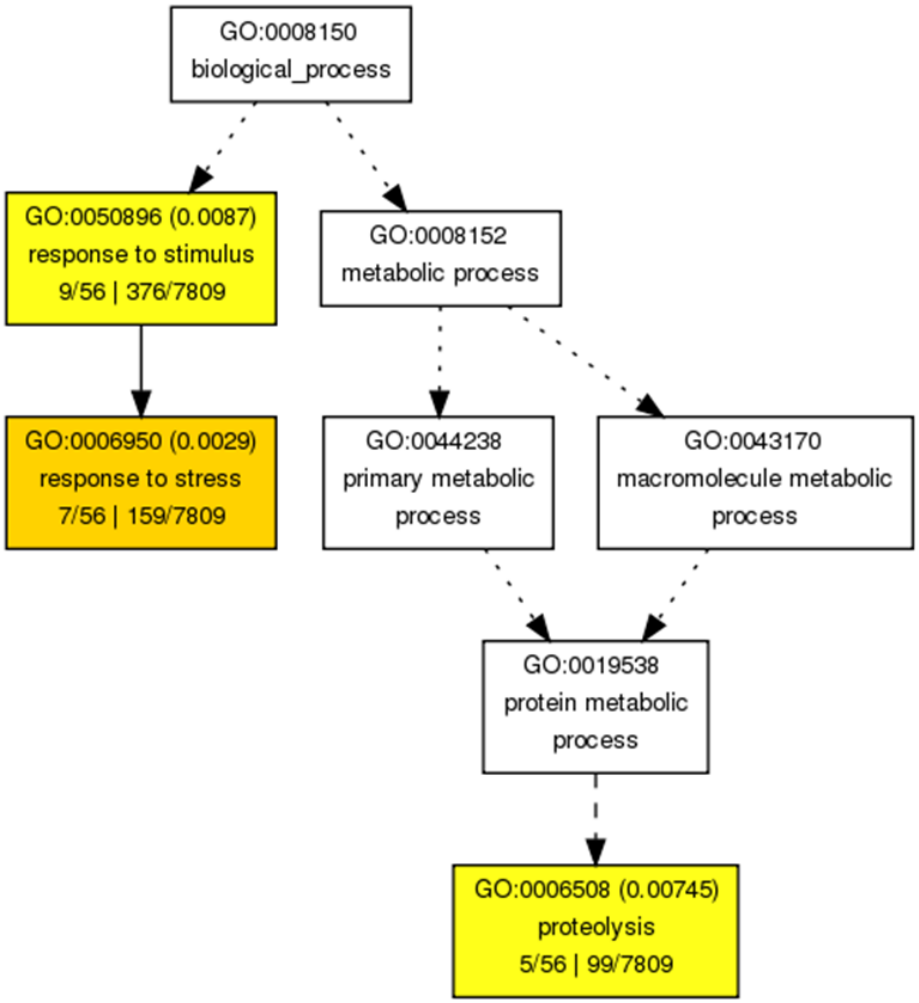


Figure 1. Enrichment GO terms of up-regulated tomato gens represented in a hierarchical tree graph by singular enrichment analysis (AgriGO). The boxes information shows each GO term ID, GO term definition and statistical information. The enrichment level of every term is represented by the boxes colours that indicates significant differences ($P < 0.05$), the darker and redder colour the more enriched terms and white boxes indicate non-significant differences. The types of lines indicate the number of enriched terms at the end of the lines: zero (dotted lines), one (dashed lines) or two (solid lines).

At High light conditions, higher TLA were observed in plants grown in P+T34 10^4 and P+T34 10^6, however plants grown in P+T34 10^4 and irrigated with C nutrient solution were no significantly different from plants grown in P (Table 2). Nevertheless, the highest values of TLA at low light intensity were achieved in P and P+T34 10^4 growth media with C nutrient solution.

Irrigation with C nutrient solution increase TLA of plants grown in each growth media for all the experimental conditions (Table2). On the other hand, irrigated plants with 1/4 of nutrient solution showed the lowest TLA for P and P+T34 10^4 treatments at High light conditions. Positive effect of High light intensity and C nutrient solution irrigation on TLA only was observed in plants grown in P+T34 10^6.

Height increase by higher light conditions only was observed in P+T34 10^6 at High light intensity and C nutrient solution (Table 2). On the other hand, the higher concentration of nutrient solution the higher height was performed for all growth media. Height increase among growth media only was recorded in plants grown on P+T34 10^6 at High light intensity and 1/4 of nutrient solution.

Plant water content

Plant grown at High light intensity showed the lowest content of Total H_2O regardless of the growth media and nutrient solution (Table 2). Moreover, P+T34 10^6 showed the highest Total H_2O content and P showed an intermediated performance, only with C nutrient solution. At Low light intensity plants grown in P+T34 10^4 and P showed higher Total H_2O, but P treatments didn't show significant differences ($P < 0.05$) with plants grown in P+T34 10^6 when 1/4 of nutrient solution was applied. On the other hand, higher concentration of nutrient solution increases Total H_2O at Low light (only in P a P+T34 10^4) and High light conditions (only in P+T34 10^6).

Table 2 Effect of photosynthetic photon flux density (PPFD) (Low, 60-80 µmol photon m^{-2} s^{-1}; High, 300-400 µmol photon m^{-2} s^{-1}), nutrient solution (1/4, diluted Peters foliar feed ; C, complet) and growth medium (P, perlite; P+T34 10^4, perlite enriched with 10^4 CFU mL^{-1} of *Trichoderma asperellum* strain T34; P+T34 10^6, perlite enriched with 10^6 CFU mL^{-1} of *T. asperellum* strain T34), on various physiological parameters of tomato plants

PPFD	Nutrient Solution	Growth Medium	DWA[a] (g)	DWT[b] (g)	Root/Shoot
Low	1/4	P	0.015 ± 0.001 a A α	0.021 ± 0.001 a A α	0.430 ± 0.057 a B α
		P + T34 10^4	0.020 ± 0.001 b A α	0.028 ± 0.002 b A α	0.412 ± 0.026 a B α
		P + T34 10^6	0.019 ± 0.001 b A α	0.028 ± 0.001 b A α	0.489 ± 0.036 a B β
	C	P	0.035 ± 0.001 a B α	0.044 ± 0.001 a B α	0.261 ± 0.015 ab A α
		P + T34 10^4	0.042 ± 0.004 a B α	0.049 ± 0.004 a B α	0.178 ± 0.033 a A α
		P + T34 10^6	0.033 ± 0.003 a B α	0.043 ± 0.003 a B α	0.321 ± 0.038 b A α
High	1/4	P	0.027 ± 0.002 a A β	0.036 ± 0.003 a A β	0.324 ± 0.047 a A α
		P + T34 10^4	0.036 ± 0.002 b A β	0.049 ± 0.003 b A β	0.385 ± 0.033 a A α
		P + T34 10^6	0.041 ± 0.002 b A β	0.056 ± 0.003 b A β	0.365 ± 0.017 a A α
	C	P	0.096 ± 0.006 a B β	0.130 ± 0.005 a B β	0.359 ± 0.046 a A β
		P + T34 10^4	0.139 ± 0.009 b B β	0.184 ± 0.012 b B β	0.325 ± 0.007 a A β
		P + T34 10^6	0.124 ± 0.010 b B β	0.155 ± 0.012 ab B β	0.305 ± 0.027 a A α

PPFD	Nutrient Solution	Growth Medium	TLA[c] (cm^2)	Height (cm)	Total H_2O (%)
Low	1/4	P	7.27 ± 0.53 a A β	3.03 ± 0.16 a A α	95.73 ± 0.10 ab A β
		P + T34 10^4	10.66 ± 1.43 a A β	3.33 ± 0.14 a A α	96.03 ± 0.08 b A β
		P + T34 10^6	8.57 ± 0.41 a A α	3.12 ± 0.17 a A α	95.55 ± 0.13 a A β
	C	P	18.33 ± 0.78 b B α	4.10 ± 0.22 a B α	96.08 ± 0.11 b B β
		P + T34 10^4	21.59 ± 1.53 b B α	4.20 ± 0.21 a B α	96.31 ± 0.03 b B β
		P + T34 10^6	14.09 ± 1.47 a B α	3.90 ± 0.21 a B α	95.41 ± 0.07 a A β
High	1/4	P	3.90 ± 0.47 a A α	2.73 ± 0.22 a A α	92.25 ± 0.22 a A α
		P + T34 10^4	5.43 ± 0.32 b A α	3.07 ± 0.07 ab A α	92.07 ± 0.43 a A α
		P + T34 10^6	9.17 ± 0.32 c A α	3.43 ± 0.14 b A α	93.49 ± 0.28 b A α
	C	P	19.17 ± 1.17 a B α	4.22 ± 0.18 a B α	93.60 ± 0.65 ab A α
		P + T34 10^4	22.85 ± 1.97 ab B α	4.33 ± 0.22 a B α	92.76 ± 0.08 a A α
		P + T34 10^6	27.37 ± 1.73 b B β	4.67 ± 0.16 a B β	94.28 ± 0.14 b B α

[a] Aerial dry weight (leaves and shoots)

[b] Total dry weight

[c] Total leaf area

Values are means ± standard error of 6 plants per treatment. Different lower case letters show significant differences between growth medium for the same PPFD and nutrient solution condition ($p<0.05$) on Duncan's multiple range test. Different capital letters show significant differences between nutrient solutions for the same growth medium and PPFD condition ($p<0.05$) on ANOVA test. Different greek letters show significant differences between PPFD for the same growth medium and nutrient solution condition ($p<0.05$) on ANOVA test.

Discussion

T34 presence on roots induced gene expression changes of tomato leaves, up-regulating the expression of genes categorized as response to stress, response to stimulus and proteolysis.

Similarly, Alfano et al. (2007) identified 45 genes differently modulated by *Trichoderma hamatum* T382 (T382) ($7 \cdot 10^5$ CFU g^{-1} growth media) after a 5 week challenge in tomato plants whose functions were associated with biotic or abiotic stress, RNA, DNA and protein metabolism, signaling and transport. On the other hand, in a short term challenges (2 days) between T382 and *Arabidopsis thaliana* plants, up-regulating genes were related to defense involved in a SAR-like response, anthocyanins biosynthesis and metabolism, catabolism of cell wall macromolecules, biosynthesis of ATP, protein phosphorylation, signal transduction, toxin catabolism and calcium ion homeostasis (Mathys et al., 2012). Segarra et al. (2007) also observed a SAR-like reaction in cucumber plants in a short term challenge (24 h) with T34 (10^7 CFU mL^{-1}) that modified the expression of proteins involve in ROS scavenging, stress response, isoprenoid and ethylene biosynthesis, photosynthesis, photorespiration and carbohydrate metabolism. Nevertheless, in the current study the main genes up-regulated were related to JA signaling pathway and only a few were related to SA pathway. Differences between the expression of both studies may attributed to the different duration of the challenge between plants and T34 (1 and 18 days) and its populations on the rhizosphere at the sampling time (10^7 CFU mL^{-1} and 2.5 10^5 CFU mL^{-1}). It is known that induction of resistance pathway triggered by T34 depends on the size of populations of T34 and is linked at 10^5 CFU mL^{-1} with the increased expression of the ISR marker LOX 2 (Segarra et al., 2007; Segarra et al., 2009). Moreover, Mathys et al. (2012) noticed that T382 up regulated the expression of SA-markers like PR-1, PR-2 and PR-5 that decrease after 5 days of challenge with Arabidopsis, while the ET/JA-marker PDF1.2a was not differently modulated. In contrast, *Trichoderma asperelloides* T203 after 3 days challenge enhanced in Arabidopsis plants the expression of gens related with JA/ET pathways (TAT3, eto3 and etr1) and SA pathway (PR-5) (Brotman et al., 2012). These suggest that gene expression modulation by *Trichoderma* spp may depend on the interaction between the plant and the BCA in addition to

the duration of these interactions. Moreover, a pathogen addition alters these interactions and leads to a re-modulation of gene expression (Brotman et al., 2012; Mathys et al., 2012).

In our experiment, among the 30 more up-regulated genes in response to T34 challenge we found genes related to biotic and abiotic stress and plant defense, N, P and protein metabolism, energy, transport and cell wall signaling.

About the 40% of the 30 more up-regulated genes were related with proteinase inhibitor activity. This group of protein is characterized by forming complexes with enzymes leading to a reversible loss of their catalytic activity (Lawskoski and Kato, 1980). Proteolytic enzymes inhibitors play an important role in plant protection from unfavorable conditions (biotic and abiotic stress) and in proteolytic process regulation (cleaving nonfunctional proteins, activating proteins synthesis and recycling nitrogen) (Mosolov and Valueva, 2011). In the literature, proteinase inhibitors are linked to the JA signaling pathway and have been extensively related with biotic stress associated with herbivorous insects and wounds that inhibit digestive enzymes of insect guts (Farmer and Ryan, 1990; Wu and Baldwin, 2010; Pieterse et al., 2014). The following genes associated with the JA signaling pathway were up-regulated in the present study and in a previous study with the presence of *Tetranychus urticae*: cathepsin D inhibitor protein, wound-induced proteinase inhibitor II prepeptide, metallocarboxypeptidase inhibitor, multicystatin and leucine aminopeptidase (Nachappa et al., 2013). The Leucine aminopeptidase is a wound response protein that has an important role in insect defense (Scranton et al., 2013). Moreover, proteinase inhibitors enhanced expression has been related to abiotic stress tolerance such as heat (Sadder et al., 2014) and salinity (Shan et al., 2008). Interestingly, a tomato double mutant accumulator of anthocyanins also increase the expression of 5 of the same proteinase inhibitors genes and 2 genes encoding for arginases (Povero et al., 2011). Arginases have been also related to plant defense and are induced by wounding, JA and by the foliar pathogen *Pseudomonas syringae* pv. tomato (Chen et al., 2004). According to Gould et al. (2004) anthocyanins are involved in stress tolerance to drought, UV-B, heavy metals, resistance to herbivores and pathogens, mainly by scavenging free radicals and reactive oxygen species avoiding photooxidative damage.

On the other hand, among the over-expressed genes linked with protein metabolism we also found a gene related with disulfide bonds formation and isomerization between specific cysteines and chaperone activity the protein disulfide-isomerase-like gen (Wilkinson and Gilbert, 2004).

In the current study, others up-regulated genes related to plant defense and stress response were the β-1,3-glucanase belonging to the protein family PR-2 linked with SA signaling pathway and a chitinase from PR-4 family whose expression in Arabidopsis plants was induced by pathogens in a JA-dependent pathway (Thomma et al., 1998). The β-1,3-glucanases and chitinases produce a synergistic inhibition of fungal pathogens growth by hydrolyzing fungal cell wall (Jongedijk et al., 1995; Enoki and Suzuki, 2016). Previous studies also reported that PR proteins expression was promoted by *Trichoderma* spp. (Yedidia et al., 2000; Alfano et al., 2007; Brotman et al., 2012; Mathys et al., 2012). Moreover, Chloroplastic polyphenol oxidase F was also up-regulated. According to, Boeckx et al. (2015) polyphenol oxidases catalyse the oxidation of monophenols and o-diphenols to o-quinones and are related to defense against herbivores, pathogens and abiotic stress but its functions are still unclear.

In addition the expression of Plastid-lipid associated protein (PAP), also called plastoglobulins or fibrillins, were up-regulated by the presence of T34. PAP are involved in photosystem protection from oxidative stress, plant resistance to biotic and abiotic stress, plastoglobule structural development, hormonal responses and sequestration of hydrophobic compounds like pigment accumulation in chromoplast (Singh and MacNellis, 2011; Besagni and Kessler, 2013).

Myb-related protein Myb4-like was also up-regulated. MYB transcription factors are involved in the regulation of plant stress responses (Roy, 2016). In rice MYB4 modified metabolite accumulation and improved adaptation to drought, cold and freezing (Vannini et al., 2003; Pascuali et al., 2008) and in Arabidopsis plants is related to the accumulation of UV protective compounds (Anbawat et al., 2013).

Genes related to cell wall signaling and responses to stress were also enhanced like the probable pectate lyase P18 involved in cell wall degradation and elicit defense responses (Bruce and West, 1982) and the extensin like protein Hop-interacting protein THI101 involve in cell wall modification and plant defense (Wei and Shirsat, 2006).

Some genes related to energy, P metabolism and transport were up-regulated like acid phosphatase 1, apyrase-like and GTPase. Acid phosphatases hydrolyze phosphate esters compounds (pH lower than 7.0) producing inorganic phosphate (Pi) (Vincent et al., 1992) and are involved in production, transport and recycling of Pi, in cellular metabolism and energy transduction processes (Khan et al., 2016). Apyrases hydrolyze the γ- and the β-phosphate on ATP or ADP (Plesner, 1995) and are involved in phosphate transport (Thomas et al., 1999). SAR2 GTPase belongs to a GTPase subfamily whose function is considered to be related to vesicular transport (Davies, 1994).

Some genes related with N metabolisms were also up-regulated like asparagine synthetase (AS) [glutamine-hydrolyzing] and N^2-acetylornithine deacetylase (NOAD).

AS catalyze the formation of Asparagine and Glutamate from Aspartate and Glutamine linked with the glutamine synthetase (GS)–glutamate synthase (GOGAT) cycle. Asparagine has a high N:C ratio and plays a key role in N storage and transport (Taiz and Zeiger, 2010). N assimilation into asparagine is promoted under energy-limiting conditions when GS-GOGAT cycle is inhibited (Lam et al., 1996) like under salinity stress conditions (Renau-Morata et al., 2017).

NOAD is involved in the linear pathway of the ornithine and acetate synthesis, despite of being described in bacteria, NOAD activity has not been demonstrated in plants (Slocum, 2005). Nevertheless, a recent study with *A. thaliana* proved that down-regulation of NOAD is associated with a decrease of ornithine content, demonstrating a link between them in plants (Molesini et al., 2015). Ornithine is related with the synthesis of polyamines involved in different processes of growth and development, citrulline involved in drought tolerance and arginine which is accumulated under stress conditions and is considered a good N storage for its high N:C ratio (Kalamaki et al., 2009; Molesini et al., 2015).

Proteomics studies of *Trichoderma* spp. found expression changes in genes related to carbohydrate metabolisms but these changes were not observed in the current study (Segarra et al., 2007; Shoresh et al., 2010).

The modulation of all these genes may lead to a new conformation of leaves proteins by T34 that may help plants to cope with a wide range of unfavorable conditions. Probably this protective response has been triggered because light conditions were not optimal for the growth of the

tomato plants with the irrigation regime applied, although being the treatment with greater growth of those evaluated. Some of these genes like the genes involved in P and N metabolism and transport may be related with the growth enhancement induced by T34 in tomato plants. Some authors suggest that plant N content plays a key role in *Trichoderma* spp. plant growth promotion (Shoresh et al., 2010; Harman, 2011). Brotman et al. (2012) suggest that greater nitrogen use efficiency is crucial for growth promotion and may be determinate by the accumulation of aminoacids induced by *T. asperelloides* T203. The accumulation of the polyamine prutescine also has been related with plant growth promotion by mycorrhiza that is linked to ornithine synthesis (Sarjala et al., 2010).

The beneficial effect of *Trichoderma* spp. on plant growth has been previously described in different plant species (Yedidia et al., 2001; Harman et al., 2004; Chagas et al., 2016; Lee et al., 2016). Some of these studies have been performed under restricting growth conditions like pathogen attack (Shaw et al., 2016), plant culture without applying fertilization (Pascale et al., 2017), high levels of heavy metals and pesticides application (Mishra et al., 2016), irrigating with arsenic contaminated water (Caporale et al., 2014) and salt stress (Qi and Zhao, 2013). Some authors suggest that growth promotion by *Trichoderma* spp. occurs especially in suboptimal conditions (Harman, 2006; Pascale et al., 2017). In the present study, T34 enrichment in the growth media promotes plant growth of tomato plants under both limiting and favorable conditions.

Reduced nutrient supply in the nutrient solution combined with Low and High light intensity was considered limiting conditions because plants performance was the lowest for some evaluated parameters (dried weight, height and/or TLA). The limiting factor may be nutrient supply because at low light and complete nutrient solution T34 did not enhance plants growth. Some mechanisms that could promote growth under these conditions may be to facilitate nutrient uptake (Altomare and Tringovska, 2011) and to increase nitrogen use efficiency (Shoresh et al., 2010). Nevertheless, in a previous study T34 did not induce changes in C/N ratio of tomato plants and stable isotopic signature of N on leaves (Fernández et al., 2014). We hypothesized that plants grown with low nutrient supply suffered more at Hight light intensity than at Low light by the lower water status and TLA. T34 also may play a protective role on that situation by over-

expressing genes related with oxidative damage protection like PAP. Moreover, T382 increase production of anthocyanins in leaves that also protect cells from oxidative damage (Mathys et al. 2012) and anthocyanins accumulation seems to be related to the enhanced expression of proteinase inhibitors genes that were also induced by T34 (Povero et al., 2011). A previous study evidenced that T34 can protect plants from abiotic stress such as Fe toxic effects, and also, improve plant height and dry weights of tomato plants growing with higher Fe concentrations on the nutrient solution (1000 µM) (Segarra et al., 2010).

Complete nutrient solution and Hight light intensity was considered tomato growth favorable conditions because plants achieved the highest dry weight values of the study. It is interesting that T34 promotes plant growth but treatment P+T34 10^4 invested more in roots and P+T34 10^6 instead invested more in leaf area. Tomato plants probably did not require greater investment in roots to improve nutrient uptake due to the higher populations of T34 in rhizosphere.

In a previous study with tomato plants, T34 also improved plant growth measured as TLA, height, DWA, DWT and nutrient uptake (Fernández et al., 2014). In this study the plants were even higher and heavier than in the current study, probably due to a higher irrigation (50-100 mL of the same complete nutrient solution day^{-1}) and a lower light intensity (180-210 µmol m^{-2} s^{-1} PPFD) that made plants to invest in height and consequently in stems. According to Santiago et al. (2013) T34 also improve growth in cucumber plants and increase total accumulation of Fe and Cu in aerial parts.

It is important to highlight that growth promotion occurs in both treatments of T34 (P+T34 10^4 and P+T34 10^6). Populations of T34 at the end of the study in treatment P+T34 10^4 were 5.67 10^3 ± 3.40 10^2 this result indicates that application of high doses of T34 are not necessary to enhance tomato plant growth in favorable and limiting conditions of light and nutrients. Although *Trichoderma* spp. is an ubiquitous microorganism found in soils around the world (Jacklitsch, 2009) it is important to apply it with the appropriate concentration to avoid displacement of other rhizosphere beneficial microorganisms.

In conclusion, T34 ($2.5 \cdot 10^5$ CFU g^{-1} mL) induce changes in tomato gene expression of plants after 18 days of interaction up-regulating genes involved in response to stress, response to stimulus and proteolysis. This gene modulation may increase tolerance to unfavorable conditions and

promote tomato plants growth. T34 growth promotion on tomato plant is achieved with low nutrient availability (Low and High light intensity) and under favorable conditions (high nutrient availability and Hight light intensity).

Acknowledgements

We are grateful to Semillas Fitó and Biocontrol Technologies for kindly providing the tomato seeds and *Trichoderma asperellum* strain T34, respectively. We also thank José Ignacio Pons from the Functional Genomics Core (Institute for Research in Biomedicine of Barcelona) and the Experimental Fields Service (University of Barcelona).

References

Alfano G, Ivey MLL, Cakir C, Bos JIB, Miller SA, et al. (2007) Systemic Modulation of Gene Expression in Tomato by *Trichoderma hamatum* 382. Phytopathology 97: 429–437.

Altomare C, Tringovska I (2011) Beneficial soil microorganisms, an ecological alternative for soil fertility management. In Genetics, biofuels and local farming systems (pp. 161–214). Springer Netherlands.

Ambawat S, Sharma P, Yadav NR, Yadav RC (2013) MYB transcription factor genes as regulators for plant responses : an overview. Physiol Mol Biol Plants 19: 307–321.

Benítez T, Rincón AM, Limón MC, Codón AC. (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7: 249–260.

Besagni C, Kessler F (2013) A mechanism implicating plastoglobules in thylakoid disassembly during senescence and nitrogen starvation. Planta 237: 463–470.

Boeckx T, Winters AL, Webb KJ, Kingston-smith AH (2015) Polyphenol oxidase in leaves : is there any significance to the chloroplastic localization ? J Exp Bot 66:3571–3579.

Borrero C, Trillas MI, Delgado A, Avile M (2012) Effect of ammonium ⁄ nitrate ratio in nutrient solution on control of *Fusarium* wilt of tomato by *Trichoderma asperellum* T34. Plant Pathol 61: 132–139.

Brotman Y, Lisec J, Méret M, Chet I, Willmitzer L, Viterbo A (2012) Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. Microbiology 158: 139–146.

Bruce RJ, West CA (1982) Elicitation of casbene synthetase activity in castor bean The role of pectic fragments of the plant cell wall in elicitation by a fungal endopolygalacturonase. Plant Physiol 69: 1181–1188.

Caporale AG, Sommella A, Lorito M, Lombardi N, Azam SMGG, et al. (2014) *Trichoderma* spp . alleviate phytotoxicity in lettuce plants (*Lactuca sativa* L .) irrigated with arsenic-contaminated water. J Plant Physiol 171: 1378–1384.

Chagas LF, de Castro HG, Colonia BS, Filho MR (2016) Efficiency of the inoculation of *Trichoderma asperellum* UFT-201 in cowpea production components under growth conditions in field. Revista de Ciências Agrárias 39: 413–421.

Chen H, Mccaig BC, Melotto M, He SY, Howe GA (2004) Regulation of Plant Arginase by Wounding , Jasmonate , and the Phytotoxin Coronatine. J Biol Chem 279: 45998–46007.

Chen S, Zhao H, Wang Z, Zheng C (2017) *Trichoderma harzianum* -induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content , cell viability and cell cycle-related genes expression in cucumber roots. Eur J Plant Pathol 147: 43–53.

Choudhary DK, Prakash A (2007) Induced systemic resistance (ISR) in plants : mechanism of action. Indian J Microbiol 47: 289–297.

Conrath PGU, Beckers GJM, Flors V, García-agustín P, Jakab G, et al. (2006) Priming : Getting Ready for Battle. Mol Plant Microbe In 19:1062–1071.

Davies C (1994) Cloning and characterization of a tomato GTPase-like gene related to yeast and Arabidopsis genes involved in vesicular transport. Plant Mol Biol 24: 525–531.

De Meyer G, Bigirimana J, Elad Y, Monica H (1998) Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. Eur J Plant Pathol 104: 279–286.

De Santiago A, García-lópez AM, Manuel J, Avilés M, Delgado A (2013) Effect of *Trichoderma asperellum* strain T34 and glucose addition on iron nutrition in cucumber grown on calcareous soils. Soil Biol Biochem 57: 598–605.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) AgriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res, gkq310.

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42: 185–209.

Enoki S, Suzuki S (2016) Pathogenesis-Related Proteins in Grape. In: Antonio Morata, eds. Grape and Wine Biotechnology. InTech, DOI: 10.5772/64873.

Emig D, Salomonis N, Baumbach J, Lengauer T, Conklin BR, Albrecht M (2010) AltAnalyze and DomainGraph: analyzing and visualizing exon expression data. Nucleic Acids Res 38: W755–W762.

Farmer EE, Ryan CA (1990) Interplant communication : Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. P Natl A Sci 87: 7713–7716.

Fernández E, Segarra G, Trillas MI (2014) Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato. Biol Control 78: 77–85.

Fernández E, Trillas MI, Segarra, G (2017) The secretion pattern of root exudates in tomato plants inoculated with Botrytis cinerea results in higher populations of *Trichoderma asperellum* strain T34 in the rhizosphere. Plant Pathol DOI: 10.1111/ppa.12668.

Gould KS (2004) Nature's Swiss Army Knife : The Diverse Protective Roles of Anthocyanins in Leaves. BioMed Res Int 5: 314–320.

Harman GE, Petzoldt R, Comis A, Chen J (2004) Interactions Between *Trichoderma harzianum* Strain T22 and Maize Inbred Line Mo17 and Effects of These Interactions on Diseases Caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology 94: 147–153.

Harman GE (2006) Overview of Mechanisms and Uses of *Trichoderma* spp . Phytopathology 96: 190–94.

Harman GE (2011) *Trichoderma* — not just for biocontrol anymore Phytoparasitica 39, 103–108.

Howell CR, Hanson LE, Stipanovic RD, Puckhaber LS (2000) Induction of Terpenoid Synthesis in Cotton Roots and Control of *Rhizoctonia solani* by Seed Treatment with *Trichoderma virens*. Phytopathology 90: 248–252.

Howell CR (2002) Cotton Seedling Preemergence Damping-Off Incited by *Rhizopus oryzae* and *Pythium* spp. and Its Biological Control with *Trichoderma* spp. Phytopathology 92: 177–180.

Jaklitsch WM (2009) European species of Hypocrea Part I. The green-spored species. Stud Mycol 63: 1–91.

Jongedijk E, Tigelaar H, Van Roekel JS, Bres-Vloemans SA, DekkerI, et al. (1995) Synergistic activity of chitinases and β-1, 3-glucanases enhances fungal resistance in transgenic tomato plants. Euphytica 85: 173–180.

Kalamaki MS, Alexandrou D, Lazari D, Merkouropoulos G, Fotopoulos V, Pateraki I, Aggelis A, Carrillo-lo A (2009) Over-expression of a tomato N -acetyl- L -glutamate synthase gene (SlNAGS1) in *Arabidopsis thaliana* results in high ornithine levels and increased tolerance in salt and drought stresses. J Exp Bot 60: 1859–1871.

Khan S, Khan S, Batool S, Ahmed, M (2016) Purification and biochemical characterisation of acid phosphatase-I from seeds of Nelumbo nucifera. Nat Prod Res 30: 570–573.

Lam H, Coschigano KT, Oliveira IC, Coruzzi GM (1996) The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annu Rev Plant Biol 47, 569–593.

Laskowski Jr M, Kato I (1980) Protein inhibitors of proteinases. Annu Rev Biochem, 49: 593–626.

Lee S, Yap M, Behringer G, Hung R, Bennett JW (2016) Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biol Biotechnol 3: 1–14.

Mathys J, De Cremer K, Timmermans P, Van Kerckhove S, Lievens B, Vanhaecke M, Cammue BPA, De Coninck B (2012) Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. Front Plant Sci 3: 1–25.

Mishra N, Khan SS, Sundari SK (2016) Native isolate of *Trichoderma*. Wold J Microbiol Biotechnol 32:1–23.

Molesini B, Mennella G, Martini F, Francese G, Pandolfini T (2015) Involvement of the Putative N -Acetylornithine Deacetylase from *Arabidopsis thaliana* in Flowering and Fruit Development. Plant Cell Physiol 56: 1084–1096.

Mosolov VV, Valueva TA (2011) Inhibitors of Proteolytic Enzymes under Abiotic Stresses in Plants (Review). Applied Biochem Micro 47: 501–507.

Nachappa P, Margolies DC, Nechols JR, Whitfield AE, Rotenberg D (2013) Tomato Spotted Wilt Virus Benefits a Non-Vector Arthropod , *Tetranychus Urticae*, by Modulating Different Plant Responses in Tomato. PLoS ONE 8: 1–14.

Pascale A, Vinale F, Manganiello G, Nigro M, Lanzuise S, et al. (2017). *Trichoderma* and its secondary metabolites improve yield and quality of grapes. Crop Prot 92: 176–181.

Pasquali G, Biricolti S, Locatelli F, Baldoni E, Mattana M (2008) Osmyb4 expression improves adaptive responses to drought and cold stress in transgenic apples. Plant Cell Rep 27, 1677–1686.

Pastrana AM, Basallote-Ureba MJ, Aguado A, Akdi K, Capote N (2016) Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and *Bacillus* spp. Phytopathol Mediterr 55: 109–120.

Pieterse CMJ, Wees SCM Van, Hoffland E, Pelt JA Van (1996) Systemic Resistance in Arabidopsis lnduced by Biocontrol s lndependent of Salicylic Acid Accumulation and Bacteria 1 Pat hogenesis-Related Gene Expression. Plant Cell 8: 1225–1237.

Pieterse CMJ, Wees SCM Van, Pelt JA Van, Knoester M, Laan R, et al. (1998) A Novel Signaling Pathway Controlling Induced Systemic Resistance in *Arabidopsis*. Plant Cell 10: 1571–1580.

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker Pa HM (2014) Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52: 347–75.

Plesner, L (1995) Ecto-ATPases: identities and functions. Int Rev Cytol 158: 141–214.

Povero G, Gonzali S, Bassolino L, Mazzucato A, Perata P (2011) Transcriptional analysis in high-anthocyanin tomatoes reveals synergistic effect of Aft and atv genes. J Plant Physiol 168: 270–279.

Qi W, Zhao L (2013) Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. J Basic Microb 53: 355–364.

Renau-Morata B, Molina RV, Carrillo L, Cebolla-Cornejo J, Sánchez-Perales M, et al., (2017) Ectopic Expression of CDF3 genes in Tomato Enhances Biomass Production and Yield under Salinity Stress Conditions. Front Plant Sci 8.

Roy S (2016) Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signal Behav 11: 1–7.

Sadder MT, Alsadon A, Wahb-Allah M (2014) Transcriptomic analysis of tomato lines reveals putative stress-specific biomarkers. Turk J Agric For 38: 700–715.

Sarjala T, Niemi K, Häggman H (2010) Mycorrhiza formation is not needed for early growth induction and growth-related changes in polyamines in Scots pine seedlings in vitro. Plant Physiol Biochem 48: 596–601.

Scranton MA, Fowler JH, Girke T, Walling LL (2013) Microarray Analysis of Tomato's Early and Late Wound Response Reveals New Regulatory Targets for Leucine Aminopeptidase A. PLoS ONE 8: 1–18.

Segarra G, Casanova E, Bellido D, Odena MA (2007) Proteome , salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. Proteomics 7: 3943–3952.

Segarra G, Van der Ent S, Trillas I, Pieterse CMJ (2009) MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biol 11: 90–6.

Segarra G, Casanova E, Avilés M, Trillas I (2010) *Trichoderma asperellum* Strain T34 Controls *Fusarium* Wilt Disease in Tomato Plants in Soilless Culture Through Competition for Iron. Microbial Ecology 59: 141–149.

Segarra G, Avilés M, Casanova E, Borrero C, Trillas I (2013) Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34. Phytopathol Mediterr, 77–83.

Shan LEI, Li C, Chen F, Zhao S, Xia G, Wrsi A, The P (2008) A Bowman-Birk type protease inhibitor is involved in the tolerance to salt stress in wheat. Plant, Cell Environment 31: 1128–1137.

Shaw S, Le Cocq K, Paszkiewicz K, Moore K, Winsbury R, et al. (2016) Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. Mol Plant Pathol 17: 1425–1441.

Shoresh M, Harman GE, Mastouri F (2010) Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annu Rev Phytopathol 48: 21–43.

Singh DK, Mcnellis TW (2011) Fibrillin protein function : the tip of the iceberg ? Trends Plant Sci 16: 432–441.

Slocum RD (2005) Genes, enzymes and regulation of arginine biosynthesis in plants. Plant Physiol Biochem 43: 729–745.

Taiz L, Zeiger E, 2010. Plant physiology: International Edition. Massachusetts: Sinauer Associates Inc.

Thomas C, Sun Y, Naus K, Lloyd A, Roux S (1999). Apyrase Functions in Plant Phosphate Nutrition and Mobilizes Phosphate from Extracellular ATP 1. Plant Physiol 119: 543–551.

Thomma BP, Eggermont K, Penninckx IA, Mauch-Mani B, Vogelsang R, et al. (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proceedings of the National Academy of Sciences 95: 15107–15111.

Tjamos SE, Flemetakis E, Paplomatas EJ, Katinakis P (2005) Induction of Resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the Biocontrol Agent K-165 and Pathogenesis-Related Proteins Gene Expression. Mol Plant Microbe In 18: 555–561.

Trillas MI, Casanova E, Cotxarrera L, Ordovás J, Borrero C, Avilés M (2006) Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biol Control 39: 32–38.

Trillas MI, Segarra G (2009) Interactions between nonpathogenic fungi and plants. Adv Bot Res 51: 321–359.

Trillas, M. I., Segarra, G. (2012). Overview on the different mode of actions of Trichoderma spp. Conference in Trichoderma spp. For the use in Plant Protection Products: similarities and differences. The OECD Biopestices Steering Group.

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol 103: 753–765.

Van Wees SCM, Luijendijk M, Smoorenburg I, Van Loon LC (1999) Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis is not associated with a direct effect on

expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene Atvsp upon challenge. Plant Mol Biol 41: 537–549.

Vanhaecke M, Bruno P, Cammue A, Coninck B De (2012) Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against Botrytis cinerea infection. Front Plant Sci 3: 1–25.

Vannini C, Locatelli F, Bracale M, Magnani E, Marsoni M, et al. (2004) Overexpression of the rice Osmyb4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J 37: 115–127.

Vincent JB, Crowder MW, Averill BA (1992) Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. Trends Biochem Sci 17: 105–110.

Wei, GUO, Shirsat AH (2006) Extensin over-expression in *Arabidopsis* limits pathogen invasiveness. Molecular Plant Pathol 7: 579–592.

Wilkinson B, Gilbert HF (2004) Protein disulfide isomerase. BBA-Proteins Proteom 1699: 35–44.

Wu J, Baldwin IT (2010) New Insights into Plant Responses to the Attack from Insect Herbivores. Annu Rev Gen 44: 1–24

Yao Y, Li Y, Chen Z, Zheng B, Zhang L (2016) Biological Control of Potato Late Blight Using Isolates of *Trichoderma*. Am J Potato Res 93: 33–42.

Yedidia I, Benhamou N, Kapulnik Y, Chet I (2000) Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiol Biochem 38: 863–873.

Yedidia I, Srivastva AK, Kapulnik Y, Chet I (2001) Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil 235: 235–242.

Yedidia I, Shoresh M, Kerem Z, Benhamou N, Kapulnik Y, Chet I (2003) Concomitant Induction of Systemic Resistance to *Pseudomonas syringae* pv . lachrymans in Cucumber by *Trichoderma asperellum* (T-203) and Accumulation of Phytoalexins. Appl Environ Microb 69: 7343–7353.

Zafra G, Cortés-Espinosa, DV (2015) Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species : a mini review. Enviro Sci and Pollut R 22: 19426–19433.

Web references:

[http://ec.europa.eu/food/plant/pesticides] Accessed 13 April 2017

[http://www.isth.info/] Accessed 13 April 2017

5. Discusión General

5. Discussión General

5.1. Efecto del compost de alperujo CM en el crecimiento y rendimiento fisiológico de las plantas de tomate

El efecto beneficioso de los compost en el crecimiento de las plantas ha sido ampliamente documentado (Gallardo-Lara y Nogales, 1987; Arthur et al., 2012; Zhang et al., 2012) y también fue observado en este estudio en plantas de tomate crecidas en compost, comparadas con aquellas crecidas en perlita. El elevado pH (7.7) del compost de alperujo (CM) podría explicar los niveles más bajos de varios de los elementos minerales (Mg, P, Fe, Mn, Zn y Cu) en las plantas. Cuando el mismo compost fue utilizado en plantas de *A. thaliana* no se observaron diferencias en la composición de nutrientes en hojas (Segarra et al., 2013a). Los niveles más elevados de Ca en compost CM podrían explicar, en parte, la implicación de este elemento en la reducción de la enfermedad moho gris causada por *B. cinerea*. De hecho, los elevados niveles de Ca en hojas han sido asociados con resitencia a enfermedades foliares (Wójcik y Lewandowski, 2003). En diversos estudios, el Ca está implicado en respuestas a estrés biotíco y abiótico (Segarra et al., 2007b), síntesis de calosa (Trillas et al., 2000), moléculas de unión a pectinas (Carpita y McCann, 2000), SA (Schneider-Müller et al., 1994) y fitoalexinas (Vögeli et al., 1992).

Los niveles más bajos de agua (conenido hídrico relativo -RWC- y agua total) de las plantas cultivadas en compost podrían deberse a la CE del compost, que podría tener un efecto inhibidor sobre el desarrollo de Botrytis. Según Mayak et al. (2004), el RWC es un indicador del estado hídrico de las plantas y se caracteriza por descender en condiciones de estrés (sequía y salinidad). Así mismo, Claussen (2005) mostró una reducción del 81.5% de RWC en plantas de tomate cultivadas a alta irradianciay elevada CE en la solución nutritiva. Por otra parte, los valores de pH y CE del compost de alperujo fueron similares a otros tipos de compost y a muestras de diferentes lotes del mismo tipo (Borrero et al., 2004; Cotxarrera et al., 2002; Segarra et al., 2007b).

La relación C / N más elevada en las plantas cultivadas en compost indicó un contenido más bajo de nitrógeno en las hojas, lo que provocaría que las hojas fueran más resistentes al ataque del patógeno. Por el contrario, en un estudio con plantas de tomate con alta relación C / N, las plantas eran más susceptibles a la formación de lesiones primarias causadas por *B. cinerea* (Hoffland et al., 1999). El papel del contenido de nitrógeno del huésped en la susceptibilidad a *B. cinerea* todavía no está claro porque hay otros factores implicados como la fuente y cantidad de N y la virulencia de los aislados de *B. cinerea* (Lecompte et al., 2010).

Varios estudios han demostrado que el aumento de la relación de isótopos estables de carbono $^{13}C/^{12}C$ ($\delta^{13}C$) o la discriminación de isótopos de carbono disminuyen en las condiciones de déficit hídrico (Condon et al., 200;, Ehleringer y Cooper, 1988; Farquhar et al., 1982). Del mismo modo, en nuestro estudio, las plantas cultivadas en compost se caracterizaron por el estado hídrico más bajo y también mostraron el $\delta^{13}C$ más alto, probablemente relacionado con cierto grado de estrés debido a la CE del compost. Por el contrario, el efecto del estado hídrico en la relación de isótopos estables de nitrógeno $^{15}N/^{14}N$ ($\delta^{15}N$) difiere entre estudios (Handley et al., 1997; Lopes y Araus, 2006). La composición diversa de los medios de crecimiento podría explicar el menor $\delta^{15}N$ en CM. A pesar de esto, según Mariotti et al. (1982) la firma de $\delta^{15}N$ de la fuente de N no es el único factor que determina el $\delta^{15}N$ de la planta.

Los datos de fotosíntesis y fluorescencia de clorofilas fueron similares a los de otro estudio con plantas de tomate cultivadas en fitotrón de una edad similar (Nogués et al., 2002). Las medidas de fotosíntesis mostraron que las plantas cultivadas en CM se comportaron como plantas cultivadas a alta intensidad lumínica (medida realizada a 1200 µmol m^{-2} s^{-1} PPFD). Este valor contrasta con el encontrado en las plantas cultivadas en el resto de tratamientos, que se saturaron a esta intensidad lumínica. Las medidas de fluorescencia de las clorofilas fueron similares entre tratamientos y el rendimiento cuántico máximo del fotosistema II (Fv / Fm) fue superior a 0.75 en todos los tratamientos, lo que se considera el límite para la fotoinhibición (Björkman y Demmig, 1987).

5.2. Efecto de la perlita enriquecida con T34 en el crecimiento y rendimiento fisiológico de las plantas de tomate

El efecto beneficioso de *Trichoderma* spp. sobre el crecimiento de diversas especies vegetales ha sido descrito previamente (Yedidia et al., 2001; Harman et al., 2004; Chagas et al., 2016; Lee et al., 2016). Algunos de estos estudios se han realizado bajo condiciones restrictivas para el crecimiento de las plantas como el ataque de patógenos (Shaw et al., 2016), no aplicar fertilización al cultivo (Pascale et al., 2017), altos niveles de metales pesados y pesticidas (Mishra et al., 2016), riego con agua contaminada con arsénico (Caporale et al., 2014) y estrés salino (Qi y Zhao, 2013). Algunos autores sugieren que la promoción del crecimiento por *Trichoderma* spp. Se produce especialmente en estas condiciones no óptimas (Harman, 2006; Pascale et al., 2017). Tanto en el Capítulo I y III el enriquecimiento de la perlita con T34 ha promovido el crecimiento de las plantas de tomate en condiciones favorables y en el Capítulo III en condiciones no óptimas.

En el capítulo I, todas las medidas de biomasa fueron superiores en las plantas de tomate cultivadas en perlita enriquecida con T34 (P + T34) que en perlita sola; y los resultados fueron similares a los de las plantas cultivadas en compost. El uso de microorganismos beneficiosos en la rizosfera (bacterias y hongos) puede facilitar la solubilidad, aumentar la disponibilidad de nutrientes para las plantas a partir de la solución nutritiva (Altomare et al., 1999) y proteger contra el estrés biótico. La característica más relevante de las plantas cultivadas en P + T34 fue la mayor inversión en área foliar. Esto podría explicar la mayor acumulación de la mayoría de los elementos, especialmente Mg, P, B y Cu en las hojas. En particular, Mg, P y Cu están implicados en reacciones clave en los procesos energéticos de las hojas. La mayor absorción de Mg^{2+} y P también se observó en brotes y raíces de tomate cultivadas en suelo modificado con *T. harzianum* cepa T447 (Azarmi et al., 2011). Mientras que en un estudio con *T. harzianum* cepa T-203 se observó una mayor absorción de Cu en las raíces y no en las hojas de las plantas de pepino (Yedidia et al., 2001). Además, la función principal del B es un papel estructural relacionado con la estabilidad de la pared celular (O'Neill et al., 2004). Por consiguiente, una mayor cantidad de B podría mejorar la resistencia de la planta al ataque de *B. cinerea*.

El estudio de isótopos estables carbono y nitrógeno permitió distinguir claramente las raíces de plantas cultivadas en perlita de las cultivadas en P + T34. Esto sugiere que se produce la misma asimilación de C, pero el fraccionamiento post-fotosintético de isótopos de carbono estables entre hojas y raíces y la asimilación de N por las raíces en contacto con T34 difiere. Makarov (2009) ha observado que los hongos micorrízicos están implicados en la determinación del $\delta^{15}N$ en la planta. De manera similar, T34 podría estar implicado en la determinación del $\delta^{15}N$ en plantas de tomate probablemente mejorando la disponibilidad de este elemento a las plantas.

Según los datos de fotosíntesis las plantas cultivas en P + T34 se saturaron a elevada intensidad lumínica (1200 µmol m^{-2} s^{-1} PPFD) de manera similar que las cultivadas en perlita.

En el Capítulo III, el reducido suministro de nutrientes en la solución nutritiva combinado con la intensidad lumínica baja y alta se consideró no óptimo o limitante porque algunos de los parámetros evaluados mostraron un rendimiento más bajo (peso seco, altura y / o área folia total-TLA-). El factor limitante pudo ser el suministro de nutrientes porque a baja luz y solución nutritiva completa T34 no favoreció el crecimiento de las plantas. Algunos mecanismos que podrían promover el crecimiento en estas condiciones podrían ser facilitar la absorción de nutrientes (Altomare y Tringovska, 2011; De Santiago et al., 2013) y aumentar la eficiencia del uso de nitrógeno (Shoresh et al., 2010). Sin embargo, en el Capítulo I T34 no indujo cambios en la relación C / N de tomate y solo se observaron diferencias a nivel de raíz de $\delta^{15}N$.

Hipotetizamos que las plantas con un bajo suministro de nutrientes han sufrido más con elevada intensidad lumínica que a baja por el menor contenido hídrico y menor TLA. T34 también podría desempeñar un papel protector en esa situación sobre-expresando genes relacionados con la protección a daño oxidativo como, por ejemplo, las proteínas asociadas a lípidos plastídicos (PAP). Asimismo, T382 incrementa la producción de antocianinas en hojas que también protegen a las células del daño oxidativo (Mathys et al., 2012) y la acumulación de antocianinas parece estar relacionada con la expresión aumentada de inhibidores de proteinasas (Povero et al., 2011), genes que también fueron inducidos por T34 en el Capítulo III. Un estudio previo evidenció que T34 puede proteger a las plantas frente a estrés abiótico a la vez que promueve el crecimiento, como es el caso de plantas de tomate con altas concentraciones de Fe (Segarra et

al., 2010). La solución nutritiva completa combinada con elevada intensidad lumínica fueron consideradas condiciones favorables para el crecimiento del tomate, ya que, las plantas alcanzaron los valores de peso seco más elevados en el Capítulo III. Sin embargo, en el capítulo I, las plantas fueron aún más altas y más pesadas, probablemente debido a un mayor riego (50-100 mL de la misma solución nutritiva completa día^{-1}) y a una menor intensidad de luz (180-210 µmol m^{-2} S^{-1} PPFD) favoreciendo la inversión en altura y la consecuentemente inversión en tallos. Es importante destacar que la promoción del crecimiento se produjo en ambos tratamientos de T34 (P + T34 10^4 y P + T34 10^6), pero es interesante remarcar que el tratamiento P + T34 10^4 invirtió más en las raíces y el tratamiento P + T34 10^6 invirtió más en área foliar.

Las poblaciones de T34 al final del estudio en el tratamiento P + T34 10^4 fueron 5.67 10^3 ± 3.40 10^2, este resultado indica que la aplicación de altas dosis de T34 no son necesarias para potenciar el crecimiento de las plantas de tomate en condiciones favorables y condiciones no óptimas de luz y nutrientes. Aunque *Trichoderma* spp. incluye microorganismos ampliamente distribuidos en suelos de todo el mundo (Jacklitsch, 2009), es importante aplicarlo a la concentración apropiada para evitar el desplazamiento de otros microorganismos beneficiosos de la rizosfera.

5.3. Evidencias de Euestrés en las plantas de tomate crecidas en compost CM

Todos los datos confirmaron que las plantas crecieron adecuadamente en todos los medios de cultivo, aunque las plantas cultivadas en CM estaban cerca de los límites de las condiciones de estrés (inferior relación raíz / brote, inferior RWC, inferior agua total, inferior Fv / Fm y mayor δ^{13}C). Los parámetros de crecimiento y las medidas fisiológicas de las plantas cultivadas en CM sugieren que las plantas habían crecido en una situación de euestrés. Según Hideg et al. (2012), el euestrés es considerado un leve y aclimatativo estrés que mejora el crecimiento y la salud. Es lo opuesto a “distress” o estrés severo, que excede los límites de tolerancia y conduce a la muerte de las plantas.

5.4. Efecto del compost de alperujo en el control de *B. cinerea* y en los niveles hormonales de las plantas de tomate

Nuestros resultados mostraron la capacidad supresiva del compost de alperujo CM en la reducción de la severidad y la incidencia de *B. cinerea* y en la inducción de resistencia sistémica. La evidencia indirecta de la inducción de resistencia ha sido previamente observada en compost de residuos de conserveras contra la antracnosis en plantas de tomate (Abbasi et al., 2002); en compost de estiércol de vaca frente a *B. cinerea* en *Begonia hiemalis* (Horst et al., 2005); y en compost de orujo, compost de residuos de desmontadora de algodón (1:1, v: v), compost de corcho, compost de residuos orgánicos municipales y de jardineria y compost agotado de champiñón contra *B. cinerea* en *Cucumis sativus* (Segarra et al., 2007b). La inducción de resistencia sistémica desencadenada por el compost CM estuvo relacionada con SA (que sólo aumentó después de la exposición al patógeno) y ABA. Los patógenos necrotróficos como *B. cinerea* desencadenan principalmente la vía de señalización JA y no la vía de señalización SA (Birkenbihl y Somssich, 2011; Vos et al., 2013). Sin embargo, Zhang et al. (1998) también observaron reducción de la enfermedad inducida por SAR en compost de corteza de pino inoculada con *Trichoderma hamatum* 382 y *Flavobacterium balustinum* 299 y en extracto de "te de compost" contra la antracnosis y contra la mancha bacteriana en plantas de pepino. Varios estudios sugieren que el ABA está implicado en la reducción o aumento de la enfermedad, según el tipo de patógeno (Robert-Seilaniatz et al., 2011). Según Adie et al. (2007), los mutantes de *A. thaliana* deficientes en ABA eran más resistentes a *B. cinerea*, pero más susceptibles a *Pythium irregulare.* De forma similar, los mutantes de tomate deficientes en ABA eran más resistentes a *B. cinerea* (Asselbergh et al., 2007). Ton et al. (2009) describen que el ABA en la defensa de las plantas tiene un papel de tipo modulador en lugar de tratarse de una hormona primaria. Otros estudios sugirieron que los niveles de SA, ABA y JA antes del contacto con el patógeno tendrían un impacto determinante en la dinámica de interacción entre estas hormonas (Robert-Seilaniatz et al., 2011). Curiosamente, los niveles de ABA de plantas cultivadas en CM aumentaron después de la exposición a *B. cinerea*. Estudios recientes de nuestro grupo corroboran la participación de ambas respuestas de SAR y ABA dependiente / independiente estrés abiótico en plantas de *A.*

thaliana cultivadas en compost alperujo o perlita expuestas a *B. cinerea* (Segarra et al., 2013a, 2013b).

5.5. Efecto de perlita enriquecida con T34 en el control de *B. cinerea* y en los niveles hormonales de las plantas de tomate

La separación espacial entre el agente de control biológico T34 y el patógeno, conjuntamente con la reducción de la enfermedad de Botrytis mostraron la implicación de la inducción de resistencia de las plantas. La reducción de la severidad de la enfermedad varió entre estudios siendo del 35% en el Capítulo I y del 24% en el Capítulo II. Este hecho es atribuible a que las plantas en el Capítulo II crecieron con menor intensidad lumínica y menor aporte de nutrientes en la solución nutritiva dando lugar a plantas de menor tamaño (resultados no mostrados), propiciando así, mejores condiciones para el desarrollo de la enfermedad.

El incremento en los niveles de JA en las plantas crecidas con T34 no pudo ser detectado en los días evaluados en el estudio del Capítulo I. En un estudio previo con cotiledones de pepino pudo detectarse un pico de JA durante las 6 horas posteriores a la inoculación con el patógeno (Segarra et al., 2007a). La dificultad en la detección del incremento de JA puede deberse a su breve duración temporal y/o a que su intervención en ISR se base en un incremento de sensibilidad o “primining” en lugar de un aument o de su producción como sugieren algunos autores (Van Wees et al., 1999; Pieterse et al., 2000). De hecho, se ha demostrado que T34 aplicado a las raíces prepara a la planta para la inducción de resistencia independientemente de SA (Segarra et al., 2009; Trillas y Segarra, 2009). Sin embargo, se han observado aumentos en los niveles de SA sólo cuando se aplican concentraciones elevadas de T34 (10^7 UFC mL^{-1}) (Segarra et al., 2007a).

5.6. Efecto de *B. cinerea* sobre los exudados de las plantas de tomate

La presencia de *B. cinerea* modificó el patrón de exudados de las raíces de tomate, concordando así, con las conclusiones previas de otros estudios que indicaban que los patógenos pueden modificar el patrón de exudado de los huéspedes (Hale y Moore, 1979; Lanoue et al., 2010). Otros estudios han observado que las plantas expuestas a patógenos muestran un incremento en el contenido de diversos ácidos orgánicos en sus exudados radiculares como el ácido succínico y málico, pero no del ácido glucónico (Kamilova et al., 2006; Rudrappa et al., 2008). En el estudio de Kamilova et al. (2006) también detectaron un descenso en el contenido de azúcares pero no en el contenido de sacarosa. En la bibliografía consultada no se ha encontrado información relacionada con el efecto de los patógenos en el contenido de inositol en los exudados de las raíces.

Las mayores similitudes, con nuestros estudios, por lo que se refiere a cambios del contenido de metabolitos (incremento de ácido glucónico, descenso de sacarosa e inositol) fueron observadas en partes aéreas botrytitizadas pero no en los exudados de las raíces. Según Ribéreau-Gayon et al. (2006) la infección de *B. cinerea* conduce a la acumulación de ácido glucónico en los tejidos de las uvas, el cual, es un producto secundario característico de la degradación de azúcares cuando *B. cinerea* emerge del interior de las uvas. Otros autores han mostrado que en el perfil metabólico de cotiledones de girasol infectados por *B. cinerea* se produce un descenso del 90 % sacarosa y del 75 % de inositol a las 48 h post inoculación (Dulermo et al., 2009). A pesar de que estos resultados se produzcan en órganos aéreos infectados de las plantas, podríamos hipotetizar que los resultados en los exudados de las raíces es posible que reflejen el estado de los metabolitos en otras partes de la planta y que este estado dependa de factores tales como el tipo de patógeno implicado.

5.7. Papel del ácido glucónico y efecto sobre el crecimiento *in vitro* de T34

La producción de ácido glucónico y la subsiguiente acidificación del tejido huésped favorece el establecimiento de condiciones óptimas para el desarrollo necrotrófico de *Penicillium expansum*

(Hadas et al., 2007). De Cal et al. (2013) también sugiere que el pH del medio está relacionado con procesos de patogénesis porque *Monilinia fructicola* acumula en la zona de la infección ácido glucónico como principal ácido orgánico. Para *B. cinerea* la acumulación de ácidos orgánicos también supondría una ventaja, ya que, le ayuda a reducir el pH, facilitando la degradación de la pared celular del huésped mediante polygalacturonasas. Sin embargo, en la bibliografía esta función ha estado tradicionalmente asociada con la producción de ácido oxálico (Elad et al., 2007) en lugar de ácido glucónico.

Por otra parte, el ácido glucónico es el ácido orgánico producido con mayor frecuencia por las rizobacterias con la finalidad de solubilizar fosfatos minerales insolubles o poco solubles (Rodríguez y Fraga, 1999). Algunos autores evidencían que a pesar de que el ácido glucónico proporciona ventajas a ciertos microorganismos de la rizosfera, éste es también considerado un agente antifúngico (Kaur et al., 2006). Sin embargo, en nuestro estudio el crecimiento y el desarrollo de T34 no se vió afectado negativamente, sinó al contrario.

Trichoderma spp. es capaz de prosperar en un amplio rango de condiciones externas de pH y es más eficiente en suelos ácidos que en alcalinos (Benítez et al., 2004). Aún así, el pH óptimo varía entre pH 4.0 y 6.8 dependiendo del aislado (Steayaert et al., 2010). El pH del medio de cultivo SNA3 era más ácido que los otros medios empleados y esto podría haber afectado al crecimiento de T34. Sin embargo, en SNA2 el pH era el mismo que en SNA1 y hubo un incremento significativo en el crecimiento de T34, indicando que el ácido glucónico tiene un efecto promotor del crecimiento en T34 independiente del pH. Debe observarse que la dosis de ácido glucónico utilizada en SNA2 es la misma que la obtenida en los exudados de las raíces de las plantas de tomate. Lugtenberg et al. (2001) describieron el papel crucial de los ácidos orgánicos en la proliferación y colonización de la raíz por el ACB *P. fluorescens* WCS365. Además, según Zhang et al. (2014) algunos ácidos orgánicos de los exudados radiculares de pepino (ácido oxalico, ácido málico y ácido cítrico) incrementan el crecimiento y la germinación de conidios de *T. harzianum* T-E5, y correlacionan positivamente con la colonización de las raíces. Además, *B. cinerea* cultivada *in vitro* con ácidos orgánicos (ácido cítrico y málico) también incrementó la producción de micelio respecto a medios sin ellos (Verhoeff et al., 1988).

5.8. Relación entre la presencia de *B. cinerea* en hojas de tomate, inducción de resistencia sistémica y las poblaciones de T34 en las raíces

Las raíces colonizadas por T34 desencadenaron la inducción de resistencia sistémica reduciendo la severidad de la enfermedad. Según Segarra et al. (2007a) la inducción de resistencia por T34 puede tener lugar vía ISR o SAR dependiendo de las poblaciones de T34. Por consiguiente, la concentración de T34 en el substrato puede desempeñar un papel crucial en la ruta de inducción de resistencia desencadenada en las plantas. El hecho de que las poblaciones de T34 en las raíces se mantuvieran y no descendieran en presencia de *B. cinerea* en las hojas, podría indicar una conexión con la reducción de la severidad de la enfermedad. Además, considerando que la presencia de *B. cinerea* modifica el patrón de secreción de exudados de las plantas de tomate, es posible que ciertos compuestos de estos exudados puedan estimular el crecimiento de T34. Estudios de diversos *Trichoderma* spp. mostraron que los exudados de las raíces de algunos cultivos pueden incrementar el crecimiento de algunos aislados mientras que sobre otros no tienen efecto (Jash y Pan, 2007; Bharathi et al., 2008). Los exudados secretados por las plantas proveen un ambiente rico en carbono y energía, que puede ser utilizados por los microorganismos de la rizosfera y las plantas pueden comunicarse específicamente con ellos mediante la producción de señales moduladoras de la colonización (Haichar et al., 2014). Además, es necesario tener en consideración que las plantas usan esta habilidad para reclutar microorganismos y obtener beneficios de esta interacción (Sarma et al., 2015). Por consiguiente, Pieterse et al. (2014) remarcan la importancia de alcanzar elevadas densidades de población para asegurar un ISR efectivo. Los resultados obtenidos en este estudio describen una comunicación entre la hoja y la raíz vía donde la infección del patógeno en la hoja conduce a una modificación de la secreción de exudados que potencian el crecimiento de T34 y este, a su vez, promueve la resistencia frente a *B. cinerea*.

5.9. Efecto de la presencia de T34 sobre la expresión génica en hojas de tomate

La presencia de T34 en las raíces indujo cambios en la expresión génica de las hojas de tomate, incrementando la expresión de genes categorizados con funciones de respuesta a estrés, respuesta a estímulos y proteólisis.

De manera similar, Alfano et al. (2007) identificaron 45 genes que fueron modulados diferencialmente por la presencia de *T. hamatum* T382 (T382) (7 10^5 UFC g^{-1}) después de 5 semanas de interacción con las plantas de tomate y cuyas funciones estaban asociadas con estrés biótico o abiótico, ARN, ADN y metabolismo de proteínas, señalización y transporte. Por otra parte, durante una interacción de corta duración (2 días) entre T382 y plantas de *A. thaliana*, se incrementó la expresión de genes relacionados con defensa implicados en respuesta de tipo SAR, biosíntesis y metabolismo de antocianinas, catabolismo de macromoléculas de la pared celular, biosíntesis de ATP, fosforilación de proteínas, transducción de señal, catabolismo de toxinas y homeostasis del ión calcio (Mathys et al., 2012). Segarra et al. (2007a) también observaron una reacción de tipo SAR en plantas de pepino durante un corto encuentro (24 h) con T34 (107 UFC mL^{-1}) que modificó la expresión de proteínas relacionadas con protección frente a radicales libres de oxígeno, respuesta a estrés, biosíntesis de isoprenoides y etileno, fotosíntesis, fotorespiración y metabolismo de carbohidratos. Sin embargo, en el presente estudio los principales genes que incrementaron su expresión estaban relacionados con la vía de señalización del JA y sólo unos pocos estaban relacionados con la vía del SA. Las diferencias de expresión entre ambos estudios pueden atribuirse a la diferente duración de la exposición a T34 (1 y 18 días) y al tamaño de las poblaciones de T34 en la rizosfera en el momento del muestreo (10^7 UFC mL^{-1} y $2.5 \cdot 10^5$ UFC mL^{-1}). Se sabe que la vía de inducción de resistencia desencadenada por T34 depende del tamaño de sus poblaciones y que a 10^5 UFC mL^{-1} está relacionada con el aumento de la expresión del marcador de ISR LOX 2 (Segarra et al., 2007a; Segarra et al., 2009). Además, Mathys et al. (2012) observaron que T382 potenciaba en *A. thaliana* la expresión de marcadores de la vía del SA como PR-1, PR-2 y PR-5 durante los 5 primeros días de interacción y luego descendían; mientras que el marcador de ET / JA PDF1.2a no vio modificada su expresión. Por el contrario, *Trichoderma asperelloides* T203 tras 3 días de interacción con plantas de *A.*

thaliana sí que se incrementó la expresión de genes relacionados con las vías de JA / ET (TAT3, eto3 y etr1) y la vía de SA (PR-5) (Brotman et al., 2012). Esto sugiere que la modulación de la expresión génica por *Trichoderma* spp. puede depender de la interacción entre la planta y el ACB, además de la duración de estas interacciones. Por otra parte, la adición de patógenos altera estas interacciones y conduce a una re-modulación de la expresión génica (Brotman et al., 2012; Mathys et al., 2012).

En el Capítulo III se observó que entre los 30 genes que más incrementaron su expresión en respuesta a la presencia de T34, encontramos genes relacionados con estrés (biótico y abiótico) y defensa de las plantas, metabolismo de N, P y proteínas, energía, transporte y señalización de la pared celular. Aproximadamente el 40% de estos genes estaban relacionados con la actividad de inhibición de proteinasas. Este grupo de proteínas se caracteriza por formar complejos con enzimas que conducen a una pérdida reversible de su actividad catalítica (Lawskoski y Kato, 1980). Los inhibidores de las enzimas proteolíticas desempeñan un papel importante en la protección de las plantas frente a condiciones desfavorables (estrés biótico y abiótico) y en la regulación del proceso proteolítico (escindiendo proteínas no funcionales, activando la síntesis de proteínas y reciclando nitrógeno) (Mosolov y Valueva, 2011). En la literatura, los inhibidores de proteinasas están vinculados a la vía de señalización JA y han sido extensamente relacionados con estrés biótico asociado con insectos herbívoros y heridas, por su capacidad de inhibir las enzimas digestivas de los insectos (Farmer y Ryan, 1990; Pieterse et al., 2014). En un estudio previo, ante la presencia de *Tetranychus urticae* se incrementaba la expresión de 5 genes (inhibidores de proteinasas y una leucina aminopeptidasa) asociados a la vía de señalización del JA (Nachappa et al., 2013). Estos mismos genes también fueron regulados positivamente en nuestro estudio. La leucina aminopeptidasa es inducible en respuesta a heridas y tiene un papel importante en la defensa frente a insectos (Scranton et al., 2013). Además, el incremento de la expresión de inhibidores de proteinasas ha sido relacionado con la tolerancia a estrés abiótico como el calor (Sadder et al., 2014) y la salinidad (Shan et al., 2008). Es interesante remarcar que un doble mutante de tomate acumulador de antocianinas también aumentó la expresión de 5 de los mismos genes inhibidores de proteinasas y 2 genes que codificanam para arginasas (Povero et al., 2011). Las arginasas también se han relacionado con la defensa de las plantas y su

expresión puede ser inducida por heridas, JA o por el patógeno foliar *P. syringae* pv. Tomate (Chen et al., 2004). Según Gould et al. (2004) las antocianinas están relacionadas en la tolerancia a estrés como la sequía, UV-B, metales pesados, resistencia a herbívoros y patógenos, principalmente por su capacidad de eliminar radicales libres y especies reactivas de oxígeno evitando el daño por fotooxidación. Por otro lado, entre los genes relacionados con el metabolismo proteico también se incrementó la expresión de un gen relacionado con la formación de enlaces disulfuro, isomerización entre cisteínas específicas y con actividad chaperona (Wilkinson y Gilbert, 2004).

Otros genes relacionados con la defensa de las plantas y respuestas a estrés cuya expresión fue regulada positivamente fueron una β-1,3-glucanasa perteneciente a la familia de proteínas PR-2 unida a la vía de señalización del SA y una quitinasa de la familia PR-4 cuya expresión en plantas de Arabidopsis fue inducida por patógenos en una vía dependiente a JA (Thomma et al., 1998). Las β-1,3-glucanasas y las quitinasas producen una inhibición sinérgica del crecimiento de patógenos fúngicos mediante la degradación de la pared celular de los hongos (Jongedijk et al., 1995; Enoki y Suzuki, 2016). Estudios previos también han descrito la promoción de la expresión de proteínas PR en planta por *Trichoderma* spp. (Yedidia et al., 2000; Alfano et al., 2007; Brotman et al., 2012; Mathys et al., 2012). Por otra parte, una polifenol oxidasa cloroplástica también fue regulada positivamente. Estas enzimas catalizan la oxidación de monofenoles y o-difenoles a o-quinonas y están relacionados con la defensa frente a herbívoros, patógenos y estrés abiótico, aunque su función aún no ha sido esclarecida (Boeckx et al., 2015). Asimismo, la expresión de PAP, también llamadas plastoglobulinas o fibrilinas, fueron reguladas positivamente por la presencia de T34. Las PAP están implicadas en la protección del fotosistema frente a estrés oxidativo, estrés biótico y abiótico, en el desarrollo estructural de los plastoglóbulos, en respuestas hormonales y en el secuestro de compuestos hidrófobos como la acumulación de pigmentos en el cromoplasto (Singh y MacNellis, 2011; Besagni y Kessler, 2013).

La proteína Myb4-like también fue regulada positivamente. Los factores de transcripción MYB están involucrados en la regulación de las respuestas de estrés de las plantas (Roy, 2016). En arroz, MYB4 modifica el acúmulo de metabolitos modificados y mejora la adaptación a la sequía,

al frío y a la congelación (Vannini et al., 2003; Pasquali et al., 2008). En *A. thaliana* favorece la acumulación de compuestos protectores de UV (Anbawat et al., 2013).

Genes relacionados con la señalización de la pared celular y respuestas al estrés mediante degradación o modificación de la pared (pectato liasas y extensinas) (Bruce y West, 1982; Wei y Shirsat, 2006) también incrementaron su expreión.

Algunos genes relacionados con la energía y con el metabolismo y el transporte de P también fueron regulados positivamente como genes que codifican para fosfatasas ácidas, apyrasas y GTPasas. Las fosfatasas ácidas hidrolizan compuestos con ésteres de fosfato (pH inferior a 7.0) produciendo fosfato inorgánico (Pi) (Vincent et al., 1992) y participan en la producción, el transporte y el reciclado de Pi en el metabolismo celular y en los procesos de transducción de energía (Khan et al., 2016). Las apyrasas hidrolizan los enlaces γ- y el β-fosfato en ATP o ADP (Plesner, 1995) y están implicados en el transporte de fosfato (Thomas et al., 1999). La GTPasa *SAR2* pertenece a una subfamilia cuya función está relacionada con el transporte vesicular (Davies, 1994). Algunos genes relacionados con el metabolismo del N también incrementaron su expresión como el de la asparagina sintetasa (glutamina-hidrolizante) (AS) y de la N_2-acetilornitina desacetilasa (NOAD). Las AS catalizan la formación de asparagina y glutamato a partir de aspartato y glutamina y se encuentran vinculadas al ciclo glutamina sintetasa (GS) - glutamato sintasa (GOGAT). La asparagina tiene una alta relación N:C y desempeña un papel clave en el almacenamiento y transporte de N (Taiz y Zeiger, 2010). La asimilación del N en asparagina aumenta en condiciones limitantes de energía cuando se inhibe el ciclo GS-GOGAT (Lam et al., 1996) como en condiciones de estrés salino (Renau-Morata et al., 2017). NOAD interviene en la vía lineal de síntesis de ornitina y acetato, que a pesar de estar descrita en bacterias no se ha podido demostrar en plantas (Slocum, 2005). Sin embargo, un estudio reciente con *A. thaliana* demostró que la regulación negativa de NOAD estaba asociada a una disminución del contenido de ornitina, demostrando un vínculo entre ambas en las plantas (Molesini et al., 2015). La ornitina está relacionada con la síntesis de poliaminas implicadas en diferentes procesos de crecimiento y desarrollo, con la citrulina implicada en la tolerancia a la

sequía y con la arginina que se acumula en condiciones de estrés y se considera un buen almacenamiento de N por su alta relación N:C (Kalamaki et al. 2009, Molesini et al., 2015).

Estudios de proteómica de *Trichoderma* spp. observaron cambios en la expresión de genes relacionados con el metabolismo de carbohidratos, sin embargo, estos cambios no se observaron en el presente estudio (Segarra et al., 2007; Shoresh et al., 2010).

La modulación de todos estos genes inducida por T34 puede conducir a una nueva conformación proteica en las hojas de tomate que podría ayudar a las plantas a hacer frente a una amplia gama de condiciones desfavorables. Algunos de estos genes como los genes implicados en el metabolismo y transporte de P y N podrían estar relacionados con la promoción del crecimiento inducida por T34. Algunos autores sugieren que el contenido de N de las plantas desempeña un papel clave en la promoción del crecimiento por parte de *Trichoderma* spp. (Shoresh et al., 2010; Harman, 2011). Brotman et al. (2012) sugieren que una mayor eficiencia en el uso del N es crucial para la promoción del crecimiento y la acumulación de aminoácidos inducida por *T. asperelloides* T203 podría tener un papel importante. Asimismo, la acumulación de la poliamina prutescina, que está relacionada con la síntesis de ornitina, también se ha relacionado con la promoción del crecimiento inducida por micorrizas (Sarjala et al., 2010).

6. Conclusiones

6. Conclusiones

1. Las plantas de tomate crecidas en compost maduro de alperujo no mostraron efectos negativos sobre la biomasa o la tasa de asimilación de CO_2, sin embargo, otros parámetros fisiológicos (inferior relación raíz / brote, inferior RWC, inferior agua total, inferior Fv / Fm y mayor $\delta^{13}C$) indicaron que las plantas se encontraban en una situación de euestrés. El euestrés pudo tener un efecto positivo en la reducción de la enfermedad causada por *B. cinerea*.

2. Las plantas de tomate crecidas en perlita enriquecida con T34 mostraron una mejor absorción de nutrientes, una mejor distribución de C y N en las raíces conllevando un incremento en el peso seco, altura y área foliar total respecto de las plantas crecidas sólo en perlita. Las plantas de tomate crecidas en perlita enriquecida con T34 no mostraron ningún tipo de estrés.

3. La inducción de resistencia sistémica desencadenada por el compost maduro de alperujo conllevó una reducción de la enfermedad del 60 % y estuvo asociada a las vías de señalización de SA y de ABA.

4. La inducción de resistencia sistémica desencadenada por T34 conllevó una reducción de la enfermedad del 24-35 % y no estuvo asociada a las vías de señalización de SA y de ABA.

5. La infección producida por *B. cinerea* en las hojas modificó el patrón de exudados radiculares de las plantas de tomate. Estos cambios implicaron el incremento de la concentración de ácido glucónico y la reducción de la concentración de glucosa e inositol.

6. La infección de *B. cinera* en las hojas evitó que las poblaciones de T34 en las raíces descendieran y, éstas a su vez, mediante la inducción de resistencia sistémica redujeron la severidad de la enfermedad. El ácido glucónico tuvo un efecto promotor del crecimiento *in vitro* de T34 independiente del pH, pudiendo estimular el crecimiento de T34 en raíces de plantas de tomate y ser responsable del mantenimiento de sus poblaciones. Sin embargo, no

se puede descartar que otros compuestos sin identificar hayan participado en la promoción del crecimiento de T34.

7. La presencia de T34 en las raíces indujo cambios en la expresión génica de las plantas de tomate tras 18 días de interacción. Los genes que incrementaron su expresión estaban relacionados con las funciones de respuesta estrés, respuesta a estímulos y proteólisis. La modulación génica diferencial inducida por T34 puede incrementar la tolerancia a condiciones desfavorables y promover el crecimiento de las plantas de tomate.

8. La promoción del crecimiento en plantas de tomate por T34 se alcanzó con baja disponibilidad de nutrientes combinado con baja o alta intensidad lumínica y también en condiciones favorables (alta disponibilidad de nutrientes y alta intensidad lumínica).

7. Bibliografía

7.

7. Bilbiografía

Abbasi PA, Al-Dahmani J, Sahin F, Hoitink HAJ, Miller SA, 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant Disease **86**, 156–161.

Adie BA, Pérez-Pérez J, Pérez-Pérez MM, Godoy M, Sánchez-Serrano JJ, Schmelz EA, Solano R, 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in *Arabidopsis*. The Plant Cell **19**, 1665–1681.

Agrios GN, 2005. Plant Pathology. Fifht Edition. New York-London: Elsevier Academic Press.

Alburquerque JA, Gonzálvez J, García D, Cegarra J, 2004. Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. Bioresource Technology **91**, 195–200.

Alfano G, Ivey MLL, Cakir C, Bos JIB, Miller SA, Madden L V, Kamoun S, Hoitink HAJ, 2007. Systemic Modulation of Gene Expression in Tomato by *Trichoderma hamatum* 382. Phytopathology **97,** 429–437.

Altomare C, Norwell WA, Björkman T, Harman GE, 1999. Solubilization of phosphates and micronutrients by the plant growth–promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295–22. Applied and Environmental Microbiology **65**, 2926–2933.

Altomare C, Tringovska I, 2011. Beneficial soil microorganisms, an ecological alternative for soil fertility management. In: Genetics, biofuels and local farming systems. Netherlands: Springer, 161–214.

Alvarez JM, 2010. Manual de compostaje. Junta de Andalucia, Colección de Manuales Técnicos.

Ambawat S, Sharma P, Yadav NR, Yadav RC, 2013. MYB transcription factor genes as regulators for plant responses : an overview. Physiology and Molecular Biology of Plants **19**, 307–321.

Andargie M, Li J, 2016. *Arabidopsis thaliana*: a model host plant to study plant–pathogen interaction using rice false smut isolates of *Ustilaginoidea virens*. Frontiers in plant science **7**.

Arthur E, Cornelis W, Razzaghi F, 2012. Compost amendment of sandy soil affects soil properties and greenhouse tomato productivity. Compost Science and Utilization **20**, 215–221.

Asselbergh B, Curvers K, França SC, Audenaert K, Vuylsteke M, Van Breusegem F, Höfte M, 2007. Resistance to *Botrytis cinerea* in *sitiens*, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. Plant Physiology **144**, 1863–1877.

Aviles M, Borrero C, 2017. Identifying characteristics of Verticillium wilt suppressiveness in olive mill composts. Plant Disease. https://doi.org/10.1094/PDIS-08-16-1172-RE

Azarmi R, Hajieghrari B, Giglou A, 2013. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. African Journal of Biotechnology **10**, 5850–5855.

Badri DV, Vivanco JM, 2009. Regulation and function of root exudates. Plant, Cell and Environment **32**, 666–81.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM, 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology **57**, 233–66.

Benavente V, Fullana A, Berge ND, 2017. Life cycle analysis of hydrothermal carbonization of olive mill waste: Comparison with current management approaches. Journal of Cleaner Production **142**, 2637–2648.

Benítez T, Rincón AM, Limón MC, Codón AC, 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology **7**, 249–60.

Bertin C, Yang X, Weston LA, 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil **256**, 67–83.

Besagni C, Kessler F, 2013. A mechanism implicating plastoglobules in thylakoid disassembly during senescence and nitrogen starvation. Planta **237**, 463–470.

Bharathi V, Rao KC, Reddy LK, 2008. Studies on the effect of root exudates of different crops on pigeon pea wilt pathogen (*Fusarium udum* Butler) and its antagonistic fungi. International Journal of Agriculture Sciences **4**, 670–3.

Birkenbihl RP, Somssich IE, 2011. Transcriptional plant responses critical for resistance towards necrotrophic pathogens. Frontiers in Plant Science **2**, 76.

Björkman, O, Demmig, B, 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta **170**, 489–504.

Boeckx T, Winters AL, Webb KJ, Kingston-smith AH, 2015. Polyphenol oxidase in leaves : is there any significance to the chloroplastic localization ? Journal of Experimental Botany **66**, 3571–3579.

Boller T, Felix G, 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annual Review of Plant Biology **60**, 379–406

Borrero C, Trillas MI, Ordovás J, Tello JC, Avilés M, 2004. Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. Phytopathology **94**, 1094–1101.

Borrero C, Trillas MI, Delgado A, Avile M, 2012. Effect of ammonium ⁄ nitrate ratio in nutrient solution on control of Fusarium wilt of tomato by *Trichoderma asperellum* T34. Plant Pathology **61**, 132–139.

Borroni V, Gonzalez MT, Carelli, AA, 2017. Bioproduction of carotenoid compounds using two-phase olive mill waste as the substrate. Process Biochemistry **21**, 465–473

Brotman Y, Lisec J, Méret M, Chet I, Willmitzer L, Viterbo A, 2012. Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. Microbiology **158**, 139–146.

Bruce RJ, West CA, 1982. Elicitation of casbene synthetase activity in castor bean The role of pectic fragments of the plant cell wall in elicitation by a fungal endopolygalacturonase. Plant Physiology **69**, 1181–1188.

Bugbee GJ, Finck CR, 1989. Composted waste as a peat substitute in peat-lite media. HortScience **24**, 625–627.

Caporale AG, Sommella A, Lorito M, Lombardi N, Azam SMGG, Pigna M, Ruocco M, 2014. *Trichoderma* spp . alleviate phytotoxicity in lettuce plants (*Lactuca sativa* L.) irrigated with arsenic-contaminated water. Journal of Plant Physiology **171,** 1378–1384.

Carpita N, McCann M, 2000. The cell wall. In: Buchanan B, Gruissem W, Jones R, eds. Biochemistry and molecular biology of plants. Rockville: American Society of Plant Physiologists, 52–108.

Chagas LF, de Castro HG, Colonia BS, Filho MR, 2016. Efficiency of the inoculation of *Trichoderma asperellum* UFT-201 in cowpea production components under growth conditions in field. Revista de Ciências Agrárias **39**, 413–421.

Chen H, Mccaig BC, Melotto M, He SY, Howe GA, 2004. Regulation of Plant Arginase by Wounding , Jasmonate , and the Phytotoxin Coronatine. Journal of Biological Chemistry **279**, 45998–46007.

Chen S, Zhao H, Wang Z, Zheng C, 2017. *Trichoderma harzianum* -induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content, cell viability and cell cycle-related genes expression in cucumber roots. European Journal of Plant Pathology **147**, 43–53.

Choudhary DK, Prakash A, 2007. Induced systemic resistance (ISR) in plants : mechanism of action. Indian Journal of Microbiology **47**, 289–297.

Chowdhury AKMMB, Akratos CS, Vayenas DV, Pavlou S, 2013. Olive mill waste composting: A review. International Biodeterioration and Biodegradation **85**, 108–119.

Chowdhury AKMMB, Konstantinou F, Damati A, Akratos CS, Vlastos D, Tekerlekopoulou AG, Vayenas DV, 2015. Is physicochemical evaluation enough to characterize olive mill waste compost as soil amendment? The case of genotoxicity and cytotoxicity evaluation. Journal of Cleaner Production **93**, 94–102.

Claussen W, 2005. Proline as a measure of stress in tomato plants. Plant Science **168**, 241–248.

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD, 2002. Improving intrinsic water use efficiency and crop yield. Crop Science **42**, 122–131.

Conrath PGU, Beckers GJM, Flors V, García-agustín P, Jakab G, Mauch F, Newman M, Pieterse CMJ, Poinssot B, Pozo MJ, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-mani B, 2006. Priming : Getting Ready for Battle. Molecular Plant-Microbe Interactions **19**, 1062–1071.

Cotxarrera L, Trillas MI, Steinberg C, Alabouvette C, 2002. Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress Fusarium wilt of tomato. Soil Biology and Biochemistry **34**, 467–476.

Cook RJ, Baker KF, 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society.

Daguerre Y, Edel-Hermann V, Steinberg C, 2016. Fungal Genes and Metabolites Associated with the Biocontrol of Soil-borne Plant Pathogenic Fungi. In: Fungal metabolites, 1–7.

Davies C, 1994. Cloning and characterization of a tomato GTPase-like gene related to yeast and Arabidopsis genes involved in vesicular transport. Plant Molecular Biology **24**, 525–531.

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD, 2012. The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology **13**, 414–430.

De Cal A, Sandín-España P, Martinez F, Egüen B, Chien-Ming C, Lee MH, Melgarejo P, Prusky D, 2013. Role of gluconic acid and pH modulation in virulence of Monilinia fructicola on peach fruit. Postharvest Biology and Technology **86**, 418–23.

De Corato U, Viola E, Arcieri G, Valerio V, Zimbardi F, 2016. Use of composted agro-energy co-products and agricultural residues against soil-borne pathogens in horticultural soil-less systems. Scientia Horticulturae **210**, 166–179.

De Meyer G, Bigirimana J, Elad Y, Höfte M, 1998. Induced systemic resistance in *Trichoderma harzianum* biocontrol of *Botytis cinerea*. European Journal of Plant Pathology **104**, 279–286.

Denancé N, Sánchez-Vallet A, Goffner D, Molina A, 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Frontiers in Plant Science **4**, 1–12.

De Santiago A, García-López AM, Quintero JM, Delgado A, 2013. Effect of *Trichoderma asperellum* strain T34 and glucose addition on iron nutrition in cucumber grown on calcareous soils. Soil Biology and Biochemistry **57**, 598–605.

De Ward MA, Andrade AC, Hayashi K, Schoonbeek HJ, Stergiopoulos I, Zwiers LH, 2006. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest Management Science **62**, 195–207.

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP, 2011. *Trichoderma*: the genomics of opportunistic success. Nature reviews. Microbiology **9**, 749.

Dulermo T, Rascle C, Chinnici G, Gout E, Bligny R, Cotton P, 2009. Dynamic carbon transfer during pathogenesis of sunflower by the necrotrophic fungus *Botrytis cinerea* : from plant hexoses to mannitol. New Phytologist **183**, 1149–62.

Durrant WE, Dong X, 2004. Systemic acquired resistance. Annual Review of Phytopathology **42**, 185–209.

Ehleringer JR, Cooper TA, 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. Oecologia **76**, 562–566.

Elad Y, Yunis H, 1993. Effect of microclimate and nutrients on development of cucumber gray mold (*Botrytis cinerea*). Phytoparasitica **21**, 257–268.

Elad Y, Williamson B, Tudzynski P, Delen N, 2007. *Botrytis: Biology, Pathology and Control.* Dordrecht: Kluwer Academic Publishers.

Enoki S, Suzuki S, 2016. Pathogenesis-Related Proteins in Grape. In: Antonio Morata Grape, eds. and Wine Biotechnology. InTech, DOI: 10.5772/64873.

Farmer EE, Ryan CA, 1990. Interplant communication : Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proceedings of the National Academy of Sciences **87,** 7713–7716.

Farquhar GD, O'leary MH, Berry JA, 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology **9**, 121–137.

Fernández E, Segarra G, Trillas MI, 2014. Physiological effects of the induction of resistance by compost or *Trichoderma asperellum* strain T34 against *Botrytis cinerea* in tomato. Biological Control **78**, 77–85.

Fernández E, Trillas MI, Segarra G, 2017. Increased rhizosphere populations of *Trichoderma asperellum* strain T34 caused by secretion pattern of root exudates in tomato plants inoculated with *Botrytis cinerea.*Plant Pathology. DOI: 10.1111/ppa.12668

Gallardo-Lara F, Nogales R, 1987. Effect of the application of Town Refuse Compost on the soil-plant system: A review. Biological Wastes **19**, 35–62.

Galletti S, Sala E, Leoni O, Burzi PL, Cerato C, 2008. *Trichoderma* spp. tolerance to Brassica carinata seed meal for a combined use in biofumigation. Biological Control **45**, 319–327.

Gould KS, 2004. Nature's Swiss Army Knife : The Diverse Protective Roles of Anthocyanins in Leaves. BioMed Research International **5**, 314–320.

Hadas Y, Goldberg I, Pines O, Prusky D, 2007. Involvement of Gluconic Acid and Glucose Oxidase in the Pathogenicity of *Penicillium expansum* in Apples. Phytopathology **97**, 384–90.

Haichar FEZ, Santaella C, Heulin T, Achouak W, 2014. Root exudates mediated interactions belowground. Soil Biology and Biochemistry **77**, 69–80.

Hale MG, Moore LD, 1979: Factors affecting root exudation II: 1970-1978. In: Brady NCD, eds. *Advances in Agronomy*. Vol. 31. New York - London - Toronto - Sydney - San Francisco: Academic Press, 93–124.

Handley LL, Robinson D, Forster BP, Ellis RP, Scrimgeour CM, Gordon DC, Nevo E, Raven JA, 1997. Shoot $\delta^{15}N$ correlates with genotype and salt stress in barley. Planta **201**, 100–102.

Harman GE, Petzoldt R, Comis A, Chen J, 2004. Interactions Between *Trichoderma harzianum* Strain T22 and Maize Inbred Line Mo17 and Effects of These Interactions on Diseases Caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology **94**, 147–153.

Harman GE, 2006. Overview of Mechanisms and Uses of *Trichoderma* spp . Phytopathology **96**, 190–94.

Harman GE, 2011. *Trichoderma* — not just for biocontrol anymore. Phytoparasitica **39**, 103–108.

Heil M, 2002. Ecological costs of induced resistance. Current Opinion in Plant Biology **5**, 345–350.

Heydari A, Pessarakli M, 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences **10**, 273–290.

Hideg É, Jansen MA, Strid Å, 2012. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates?. Trends in Plant Science **18**, 107–115.

Hoffland E, van Beusichem ML, Jeger MJ, 1999. Nitrogen availability and susceptibility of tomato leaves to *Botrytis cinerea*. Plant and Soil **210**, 263–272.

Hoitink HAJ, Stone AG, Han DY, 1997. Suppression of plant diseases by composts. HortScience **32**, 184–187.

Hoitink HAJ, Boehm MJ, 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annual Review of Phytopathology **37**, 427–446.

Horst LE, Locke J, Krause CR, McMahon RW, Madden LV, Hoitink HAJ, 2005. Suppression of Botrytis blight of Begonia by *Trichoderma hamatum* 382 in peat and compost-amended potting mixes. Plant Disease **89**, 1195–1200.

Howell CR, Hanson LE, Stipanovic RD, Puckhaber LS, 2000. Induction of Terpenoid Synthesis in Cotton Roots and Control of *Rhizoctonia solani* by Seed Treatment with *Trichoderma virens*. Phytopathology **90**, 248–252.

Howell CR, 2002. Cotton Seedling Preemergence Damping-Off Incited by *Rhizopus oryzae* and *Pythium* spp. and Its Biological Control with *Trichoderma* spp . Phytopathology **92**, 177–180.

Jacometti MA, Wratten SD, Walter M, 2010. Review: alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. Australian Journal of Grape and Wine Research **16**, 154–172.

Jaklitsch WM, 2009. European species of Hypocrea Part I. The green-spored species. Studies in Mycology **63**, 1–91.

Jash S, Pan S, 2007. Variability in antagonistic activity and root colonizing behaviour of *Trichoderma* Isolates. Journal of Tropical Agriculture **45**, 29–35.

Jongedijk E, Tigelaar H, Van Roekel JS, Bres-Vloemans SA, Dekker I., van den Elzen PJ, Cornelissen BJ, Melchers, LS, 1995. Synergistic activity of chitinases and β-1, 3-glucanases enhances fungal resistance in transgenic tomato plants. Euphytica **85**, 173–180.

Kalamaki MS, Alexandrou D, Lazari D, Merkouropoulos G, Fotopoulos V, Pateraki I, Aggelis A, Carrillo-lo A, 2009. Over-expression of a tomato N -acetyl- L -glutamate synthase gene (SlNAGS1) in *Arabidopsis thaliana* results in high ornithine levels and increased tolerance in salt and drought stresses. Journal of Experimental Botany **60**, 1859–1871.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B, 2006. Effects of the Tomato Pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and of the Biocontrol Bacterium *Pseudomonas fluorescens* WCS365 on the Composition of Organic Acids and Sugars in Tomato Root Exudate. Molecular Plant Microbe Interactions **19**, 1121–6.

Kaur R, Macleod J, Foley W, Nayudu M, 2006. Gluconic acid: an antifungal agent produced by *Pseudomonas* species in biological control of take-all. Phytochemistry **67**, 595–604.

Kavroulakis N, Ehaliotis C, Ntougias S, Zervakis GI, Papadopoulou KK, 2005. Local and systemic resistance against fungal pathogens of tomato plants elicited by a compost derived from agricultural residues. Physiological and Molecular Plant Pathology **66**, 163–174.

Khan S, Khan S, Batool S, Ahmed, M, 2016. Purification and biochemical characterisation of acid phosphatase-I from seeds of *Nelumbo nucifera*. Natural Product Research **30**, 570–573.

Kravchenko LV, Azarova TS, Shaposhnikov AI, Makarova NM, Tikhonovich IA, 2003. Root Exudates of Tomato Plants and Their Effect on the Growth and Antifungal Activity of *Pseudomonas* Strains. Microbiology **72**, 37–41.

Lam H, Coschigano KT, Oliveira IC, Coruzzi GM, 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annual Review of Plant Biology **47**, 569–593.

Lama-Muñoz A, Álvarez-Mateos P, Rodríguez-Gutiérrez G, Durán-Barrantes MM, Fernández-Bolaños J, 2014. Biodiesel production from olive–pomace oil of steam-treated alperujo. Biomass and Bioenergy **67**, 443–450.

Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse USR, 2010. De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. New Phytologist **185**, 577–88.

Laskowski Jr M, Kato I, 1980. Protein inhibitors of proteinases. Annual Review of Biochemistry **49**, 593–626.

Lecompte F, Abro MA, Nicot PC, 2010. Contrasted responses of *Botrytis cinerea* isolates developing on tomato plants grown under different nitrogen nutrition regimes. Plant Pathology **59**, 891–899.

Lee S, Yap M, Behringer G, Hung R, Bennett JW, 2016. Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biology and Biotechnology **3**, 1–14.

Leroch M, Kretschmer M, Hahn M, 2011. Fungicide resistance phenotypes of *Botrytis cinerea* isolates from commercial vineyards in South West Germany. Journal of Phytopathology **159**, 63–65.

Li L, Li C, Lee GI, Howe GA, 2002. Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. Proceedings of the National Academy of Sciences **99**, 6416–6421.

Lopes MS, Araus JL, 2006. Nitrogen source and water regime effects on durum wheat photosynthesis and stable carbon and nitrogen isotope composition. Physiologia Plantarum **126**, 435–445.

López-López N, Segarra G, Vergara O, López-Fabal A, Trillas MI, 2016. Compost from forest cleaning green waste and *Trichoderma asperellum* strain T34 reduced incidence of *Fusarium circinatum* in *Pinus radiata* seedlings. Biological Control, **95**, 31–39.

Lugtenberg BJ, Dekkers L, Bloemberg GV, 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annual Review of Phytopathology **39**, 461–90

Maas EV, Hoffman GJ, 1977. Crop Salt Tolerance\-Current Assessment. Journal of the Irrigation and Drainage Division **103**, 115–134.

Makarov MI, 2009. The nitrogen isotopic composition in soils and plants: its use in environmental studies (a review). Eurasian Soil Science **42**, 1335–1347.

Mariotti A, Mariotti F, Champigny ML, Amarger N, Moyse A, 1982. Nitrogen isotope fractionation associated with nitrate reductase activity and uptake of NO^{3-} by Pearl Millet. Plant Physiology **69**, 880–884.

Mathys J, De Cremer K, Timmermans P, Van Kerckhove S, Lievens B, Vanhaecke M, Cammue BPA, De Coninck B, 2012. Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. Frontiers in Plant Science **3**, 1–25.

Matilla MA, Ramos JL, Bakker PAHM, Doornbos R, Badri DV, Vivanco JM, Ramos-González MI, 2010. *Pseudomonas putida* KT2440 causes induced systemic resistance and changes in *Arabidopsis* root exudation. Environmental Microbiology Reports **2**, 381–8.

Mayak S, Tirosh T, Glick BR, 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiology and Biochemistry **42**, 565–572.

Mishra N, Khan SS., Sundari SK, 2016. Native isolate of *Trichoderma*. World Journal of Microbiology and Biotechnology **32**, 1–23.

Molesini B, Mennella G, Martini F, Francese G, Pandolfini T, 2015. Involvement of the Putative N -Acetylornithine Deacetylase from *Arabidopsis thaliana* in Flowering and Fruit Development. Plant and Cell Physiology **56**, 1084–1096.

Moreno J, Moral R, 2008. Compostaje. Madrid: Mundi-Prensa.

Mosolov VV, Valueva TA, 2011. Inhibitors of Proteolytic Enzymes under Abiotic Stresses in Plants (Review). Applied Biochemistry and Microbiology **47**, 501–507.

Nachappa P, Margolies DC, Nechols JR, Whitfield AE, Rotenberg D, 2013. Tomato Spotted Wilt Virus Benefits a Non-Vector Arthropod , *Tetranychus Urticae* , by Modulating Different Plant Responses in Tomato. PLoS ONE **8**, 1–14.

Nogués S, Cotxarrera L, Alegre L, Trillas MI, 2002. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytologist **154**, 461–470.

O'Neill MA, Ishii T, Albersheim P, Darvill AG, 2004. Rhamnogalacturonan II: structure and function of a borate crosslinked cell wall pectic polysaccharide. Annual Review of Plant Biology **55**, 109–39.

Pascale A, Vinale F, Manganiello G, Nigro M, Lanzuise S, Ruocco M, Marra R, Lombardi N, Woo SL, Lorito M, 2017. *Trichoderma* and its secondary metabolites improve yield and quality of grapes. Crop Protection **92**, 176–181.

Pasquali G, Biricolti S, Locatelli F, Baldoni E, Mattana M, 2008. Osmyb4 expression improves adaptive responses to drought and cold stress in transgenic apples. Plant Cell Reports **27**, 1677–1686.

Pastrana AM, Basallote-Ureba MJ, Aguado A, Akdi K, Capote N, 2016. Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and *Bacillus* spp. Phytopathologia Mediterranea **55**, 109–120.

Pieterse CMJ, Van Wees SC, Hoffland E, Van Pelt JA, Van Loon LC, 1996. Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. The Plant **8**, 1225–1237.

Pieterse CM, Van Pelt JA, Ton J, Parchmann S, Mueller MJ, Buchala AJ, Métraux J-P, Van Loon LC, 2000. Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiological and Molecular Plant Pathology **57**, 123–134.

Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM, 2014. Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology **52**, 347–75.

Plesner L, 1995. Ecto-ATPases: identities and functions. International Review of Cytology **158**, 141–214.

Povero G, Gonzali S, Bassolino L, Mazzucato A, Perata P, 2011. Transcriptional analysis in high-anthocyanin tomatoes reveals synergistic effect of Aft and atv genes. Journal of Plant Physiology **168**, 270–279.

Qi W, Zhao L, 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress Journal of basic microbiology **53**, 355–364.

Reis M, Coelho L, 2011. Controlling *Rhizoctonia solani* in cucumber using compost of agro-industrial residues. In International Symposium on Growing Media, Composting and Substrate Analysis 1013 (pp. 499–505).

Renau-Morata B, Molina RV, Carrillo L, Cebolla-Cornejo J, Sánchez-Perales M, Pollmann S, Medina J, 2017. Ectopic Expression of CDF3 genes in Tomato Enhances Biomass Production and Yield under Salinity Stress Conditions. Frontiers in Plant Science **8**.

Reymond P, Weber H, Damond M, Farmer EE, 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. The Plant Cell **12**, 707–19.

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE, 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. The Plant Cell **16**, 3132–47.

Ribéreau-Gayon P, Dubourdieu D, Donèche B, Lonvaud A, 2006. The grape and its maturation. In: *Handbook of enology, the microbiology of wine and vinifications*. Vol. 1. Chichester, UK: John Wiley & Sons Ltd, 241–297.

Robert-Seilaniantz A, Grant M, Jones JDG, 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annual Review of Phytopatology **49**, 317–343.

Rodríguez H, Fraga R, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances **17**, 319–39

Roy S, 2016. Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signalling and Behavior **11**, 1–7.

Rudrappa T, Czymmek KJ, Paré PW, Bais HP, 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiology **148**, 1547–56.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hun MD, 1996. Systemic acquired resistance. The Plant Cell **8**, 1809–1819.

Sadder MT, Alsadon A, Wahb-Allah M, 2014. Transcriptomic analysis of tomato lines reveals putative stress-specific biomarkers. Turkish Journal of Agriculture and Forestry, **38,** 700–715.

Sant D, Casanova E, Segarra G, Avilés M, Reis M, Trillas MI, 2010. Effect of *Trichoderma asperellum* strain T34 on Fusarium wilt and water usage in carnation grown on compost-based growth medium. Biological Control **53**, 291–296.

Sarjala T, Niemi K, Häggman H, 2010. Plant Physiology and Biochemistry Mycorrhiza formation is not needed for early growth induction and growth-related changes in polyamines in Scots pine seedlings in vitro. Plant Physiology and Biochemestry **48,** 596–601.

Sarma BK, Yadav SK, Singh S, Singh HB, 2015. Microbial consortium-mediated plant defense against phytopathogens: Readdressing for enhancing efficacy. Soil Biology and Biochemistry **87**, 25–33.

Schneider-Müller S, Kurosaki F, Nishi A, 1994. Role of salicylic acid and intracellular Ca^{2+} in the induction of chitinase activity in carrot suspension culture. Physiological and Molecular Plant Pathology **45**, 101–109.

Scranton MA, Fowler JH, Girke T, Walling LL, 2013. Microarray Analysis of Tomato's Early and Late Wound Response Reveals New Regulatory Targets for Leucine Aminopeptidase A. PLoS ONE **8,** 1–18.

Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas MI, 2007a. Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. Proteomics **7**, 3943–3952.

Segarra G, Casanova E, Borrero C, Avilés M, Trillas MI, 2007b. The suppressive effects of composts used as growth media against *Botrytis cinerea* in cucumber plants. European Journal of Plant Pathology **117**, 393–402.

Segarra G, Van der Ent S, Trillas MI, Pieterse CMJ, 2009. MYB72, a node of convergence in induced systemic resistance triggered by a fungal and a bacterial beneficial microbe. Plant Biology **11**, 90–96.

Segarra G, Casanova E, Avilés M, Trillas MI, 2010. *Trichoderma asperellum* strain T34 controls *Fusarium* wilt disease in tomato plants in soilless culture through competition for iron. Microbial ecology **59**, 141–149.

Segarra G, Elena G, Trillas MI, 2013a. Systemic resistance against *Botrytis cinerea* in Arabidopsis triggered by an olive marc compost substrate requires functional SA signaling. Physiological and Molecular Plant Pathology **82**, 46–50.

Segarra G, Santpere G, Elena G, Trillas MI, 2013b. Enhanced *Botrytis cinerea* resistance of *Arabidopsis* plants grown in compost may be explain by increased expression of defense-related genes, as revealed by microarray analysis. PLoS ONE **8**, e56075.

Segarra G, Avilés M, Casanova E, Borrero C, Trillas I, 2013c. Effectiveness of biological control of *Phytophthora capsici* in pepper by *Trichoderma asperellum* strain T34. Phytopathologia Mediterranea, 77–83.

Senesi N, 1989. Composted materials as organic fertilizers. Science of the Total Environment **81**, 521–542.

Shan LEI, Li C, Chen F, Zhao S, Xia G, 2008. A Bowman-Birk type protease inhibitor is involved in the tolerance to salt stress in wheat. Plant, Cell and Environment **31**, 1128–1137.

Shaw S, Le Cocq K, Paszkiewicz K, Moore K, Winsbury R, de Torres Zabala, M, Grant MR, 2016. Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. Molecular Plant Pathology **17**, 1425–1441.

Shoresh M, Harman GE, Mastouri F, 2010. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annual Review of Phytopathology **48**, 21–43.

Singh DK, Mcnellis TW, 2011. Fibrillin protein function : the tip of the iceberg ? Trends in Plant Sciences **16,** 432–441.

Slocum RD, 2005. Genes , enzymes and regulation of arginine biosynthesis in plants. Plant Physiology and Biochemistry **43**, 729–745.

Steinkellner S, Mammerler R, Vierheilig H, 2008. Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. European Journal of Plant Pathology **122**, 395–401.

Steyaert JM, Weld RJ, Mendoza-Mendoza A, Stewart A, 2010. Reproduction without sex: conidiation in the filamentous fungus *Trichoderma*. Microbiology **156**, 2887–900.

Tan S, Yang C, Mei X, Shen S, Raza W, Shen Q, Xu Y, 2013. The effect of organic acids from tomato root exudates on rhizosphere colonization of *Bacillus amyloliquefaciens* T-5. Applied Soil Ecology **64**, 15–22.

Taiz L, Zeiger E, 2010. Plant physiology: International Edition. Massachusetts: Sinauer Associates Inc.

Thomas C, Sun Y, Naus K, Lloyd A, Roux S. 1999. Apyrase Functions in Plant Phosphate Nutrition and Mobilizes Phosphate from Extracellular ATP 1. Plant Physiology **119**, 543–551.

Thomma BP, Eggermont K, Penninckx IA, Mauch-Mani B, Vogelsang R, Cammue BP, Broekaert WF, 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proceedings of the National Academy of Sciences **95**, 15107–15111.

Tjamos SE, Flemetakis E, Paplomatas EJ, Katinakis P, 2005. Induction of Resistance to *Verticillium dahliae* in *Arabidopsis thaliana* by the Biocontrol Agent K-165 and Pathogenesis-Related Proteins Gene Expression. Molecular Plant-Microbe Interactions **18**, 555–561.

Tomato Genome Consortium, 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature **485**, 635–641.

Ton J, Flors V, Mauch-Mani B, 2009. The multifaceted role of ABA in disease resistance. Trends in Plant Science **14**, 310–317.

Trillas MI, Cotxarrera L, Casanova E, Cortadellas N, 2000. Ultrastructural changes and localization of chitin and callose in compatible and incompatible interactions between carnation callus and *Fusarium oxysporum*. Physiological and Molecular Plant Pathology **56**, 107–116.

Trillas MI, Casanova E, Cotxarrera L, Ordovás J, Borrero C, Avilés M, 2006. Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. Biological Control **39**, 32–38.

Trillas MI, Segarra G, 2009. Interactions between nonpathogenic fungi and plants. Plant innate immunity. Advances in Botanical Research **51**, 321–359.

Trillas MI, Segarra G, 2012. Overview on the different mode of actions of *Trichoderma* spp. Conference in *Trichoderma* spp. For the use in Plant Protection Products: similarities and differences. The OECD Biopestices Steering Group.

Van Loon LC, 1997. Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology **103**, 753–765.

Van Loon LC, Bakker PAHM, Pieterse CMJ, 1998. Systematic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology **36**, 453–483.

Van Wees, SCM, Luijendijk M, Smoorenburg I, Van Loon LC, Pieterse CMJ, 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene Atvsp upon challenge. Plant Molecular Biology **41**, 537–549.

Vannini C, Locatelli F, Bracale M, Magnani E, Marsoni M, Osnato M, Strutturale B, 2004. Overexpression of the rice Osmyb4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. The Plant Journal **37,** 115–127.

Verhoeff K, Leeman M, Peer R, Posthuma L, Schot N, Eijk GW, 1988. Changes in pH and the Production of Organic Acids During Colonization of Tomato Petioles by *Botrytis cinerea*. Journal of Phytopathology **122**, 327–36.

Vincent JB, Crowder MW, Averill BA, 1992. Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. Trends in Biochemical Sciences **17**, 105–110.

Vögeli U, Vögeli-Lange R, Chappell J, 1992. Inhibition of phytoalexin biosynthesis in elicitor-treated tobacco cell-suspension cultures by calcium/calmodulin antagonists. Plant Physiology **100**, 1369–1376.

Vos IA, Pieterse CMJ, Wees SCM, 2013. Costs and benefits of hormone-regulated plant defences. Plant Pathololology **62**, 43–55.

Walters D, Heil M, 2007. Costs and trade-offs associated with induced resistance. Physiological and Molecular Plant Pathology **71**, 3–17.

Wei, GUO, Shirsat AH, 2006. Extensin over-expression in Arabidopsis limits pathogen invasiveness. Molecular Plant Pathology **7,** 579–592.

Wilkinson B, Gilbert HF, 2004. Protein disulfide isomerase. Biochimica et Biophysica Acta - Proteins and Proteomics **1699**, 35–44.

Williamson B, Tudzynski B, Tudzynski P, Van Kan JAL, 2007. *Botrytis cinerea*: The cause of grey mould disease. Molecular Plant Pathology **8**, 561–580

Wójcik P, Lewandowski M, 2003. Effect of calcium and borum sprays on yield and quality of "Elsanta" strawberry. Journal of Plant Nutrition **26**, 671–682.

Wu J, Baldwin IT, 2009. Herbivory-induced signalling in plants: perception and action. Plant, Cell and Environment **32**, 1161–1174.

Wu J, Baldwin IT, 2010. New Insights into Plant Responses to the Attack from Insect Herbivores. Annual Review of Genetics **44**, 1–24

Yao Y, Li Y, Chen Z, Zheng B, Zhang L, 2016. Biological Control of Potato Late Blight Using Isolates of *Trichoderma*. American Journal of Potato Research **93**, 33–42.

Yedidia I, Benhamou N, Kapulnik Y, Chet I, 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiology and Biochemistry **38**, 863–873.

Yedidia I, Srivastva AK, Kapulnik Y, Chet I, 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil **235**, 235–242.

Yedidia I, Shoresh M, Kerem Z, Benhamou N, Kapulnik Y, Chet I, 2003. Concomitant Induction of Systemic Resistance to *Pseudomonas syringae* pv. lachrymans in Cucumber by *Trichoderma asperellum* (T-203) and Accumulation of Phytoalexins. Applied and Environmental Microbiology **69,** 7343–7353.

Yogev A, Raviv M, Hadar Y, Cohen R, Wolf S, Gil L, Katan J, 2010. Induced resistance as a putative component of compost suppressiveness. Biological Control **54**, 46–51.

Zafra G, Cortés-Espinosa, DV, 2015. Biodegradation of polycyclic aromatic hydrocarbons by *Trichoderma* species : a mini review. Environmental Science and Pollution Research **22**, 19426–19433.

Zhang W, Han DY, Dick WA, Davis KR, Hoitink HAJ, 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and *Arabidopsis*. Phytopathology **88**, 450–455.

Zhang RH, Duan ZQ, Li ZG, 2012. Use of Spent Mushroom Substrate as Growing Media for Tomato and Cucumber Seedlings. Pedosphere **22**, 333–342.

Zhang F, Zhu Z, Yang X, Ran W, Shen Q, 2013. *Trichoderma harzianum* T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere. Applied Soil Ecology **72**, 41–8.

Zhang F, Meng X, Yang X, Ran W, Shen Q, 2014. Quantification and role of organic acids in cucumber root exudates in *Trichoderma harzianum* T-E5 colonization. Plant Physiology and Biochemistry **83**, 250–7.

Referencias web:

http://ec.europa.eu/food/plant/pesticides Último acceso: 13/04/2017

http://faostat.fao.org Último acceso: 13/04/2017

http://www.gencat.cat Último acceso: 13/04/2017

http://www.mapama.gob.es Último acceso: 13/04/2017

http://www.ruralcat.net Último acceso: 31/05/2017)