REPORTE DE ESTANCIAS ERASMUS+ 2020
En CIRAD-REUNION

Nombres y apellidos: Rayza M. González Rodríguez

ECTI: Centro de Bioplantas.

UNIVERSIDAD: Ciego de Ávila, Máximo Gómez Báez

País: Francia, isla Reunión **CES/EXT**: CIRAD REUNION

Período de las visitas: mi-junio – mi-agosto 2022, y mi-marzo – mi mayo 2023

Universidad(es) o institución(es) visitadas:

-Laboratorios de la sede CIRAD, así como los laboratorios del Polo científico de Protección de Plantas (3P).

Actividades desarrolladas:

Se trabajó en la evaluación y diseño de experimentos de evaluación de resistencia a la fusariosis en conjunto con la doctorante Jessica.

Se trabajó en el montaje y análisis del experimento de evaluación histológica de la inoculación de Fusarium en vitroplantas resistentes y susceptibles de banano.

Se realizó el montaje del efecto de la inoculación de inductores de resistencia frente al patógeno Fusiarium y la evaluación de la expresión de genes en diferentes tiempos de inducción.

Se realizaron varios procedimientos para regenerar las cepas de *Phytophthora nicotianae,* con el empleo de diferentes métodos microbiológicos a nivel de laboratorio.

Se realizó el ensayo para conocer el efecto de la aplicación de borax en la infección de Phytopthhora a nivel de casas de cultivo.

Se participó en la preparación de la ponencia a presentar en el Congreso internacional de de Piñicultura, del 15-18 Mayo.

Se asesoró el trabajo del técnico del laboratorio para el mantenimiento de las cepas con las que se trabaja en el proyecto ERASMUS.

Se trabajó en la actualización, diseño y análisis de la experimentación a desarrollar por la doctorante Jessica Mendoza.

Aunque no todos los resultados fueron satisfactorios, durante las estancias se ejecutaron varias acciones que permitieron valorar y ajustar los futuros experimentos y planificar la presentación de nuevos proyectos para continuar la colaboración científica y la ejecución de las acciones aún pendientes en el programa doctoral que se ejecuta en el marco de este proyecto.

Se fortalecieron las relaciones de trabajo y se pudo aprovechar más eficiente las potencialidades y laboratorios del Polo de protección de Plantas para la realización de diferentes determinaciones biológicas.

Dra Rayza M. González Rodríguez
Centro de Bioplantas.
UNICA

Determinación del efecto biológico del empleo de inductores sobre la incidencia de Phytophthora parasitica en vitroplantas de pina cv MD2 y Queen victoria.

Rayza M. González R[1]., Alain Soler[2], Jessica Mendoza[1], Tara Pappalardo[2], Adrien Négrier[2], Georget Tullus[2]

1. Centro Bioplantas, Universidad de Ciego de Avila, Cuba.
2. CIRAD UPR GECO. Saint Pierre, Reunion, Francia

INTRODUCCIÓN

A nivel mundial, la piña (*Ananas comosus* (L.) Merril) es la especie económica más importante de la familia Bromeliaceae. Es uno de los principales frutales del mundo, cultivada con el fin de satisfacer necesidades alimenticias de la población y constituye un importante renglón para la producción de conservas y venta de fruta fresca para la captación de divisas. La producción de piña a nivel mundial se encuentra distribuida en 83 países, destacando Nigeria y Tailandia con una producción de 132,000.00 y 103,413.00 toneladas respectivamente (FAOSTAT, 2013).

En los rendimientos del cultivo las afectaciones de plagas y enfermedades son de gran importancia, las de origen fúngico ocupan un lugar significativo, se destacan en el ámbito mundial las causadas por *Fusarium subglutinans, Phytophthora nicotianae,* y *Chalara paradoxa* (Rohrbach y Schmitt, 1994). Una de las enfermedades de mayor impacto en la piña híbrido MD2, es la pudrición del corazón de la piña o pudrición del cogollo, provocado por *Phytophthora* sp., que ocasiona pérdidas considerables de la producción, siendo inconstante para las distintas áreas de producción; de no efectuarse un buen manejo integrado y fitosanitario podría llegar a afectar hasta el 100% de la producción. Los hongos verdaderos y los Oomicetos constituyen el grupo más importante de agentes causantes de enfermedades en las plantas, pero *Phytophthora* y otros oomicetos tienen unas características biológicas que no son comunes en los hongos verdaderos: la mayor parte del ciclo vital es diploide y poseen zoosporas biflageladas, mientras que en los hongos verdaderos es fundamentalmente haploide o dicariótico (Cavalier-Smith, 1998; García-Jiménez *et al*., 2010; Sánchez *et al*., 2010).

Una de las estrategias más prometedoras en el control de los agentes patógenos de plantas es el manejo de la resistencia inducida. En los últimos años ha habido un gran número de estudios destinados a comprender cuál es la mejor forma de emplear esta estrategia para lograr la protección de cultivos (Walters et al., 2013). Esta resistencia puede ser inducida en plantas tanto a nivel local como sistémico, y, a pesar de que rara vez se consigue el control completo del patógeno, la principal ventaja de esta estrategia es que protege a las plantas contra una amplia gama de patógenos (Kuc, 1982; Díaz et al., 2005). Este tipo de resistencia a la infección por patógenos puede ser inducida en las plantas por una amplia gama de agentes bióticos y abióticos (da Rocha & Hammerschmidt, 2005; Lyon, 2007; Walters et al.,2013). Entre los agentes bióticos encontramos una gran variedad de microorganismos que son utilizados en la inducción de resistencia, incluyendo virus, bacterias, levaduras y hongos, siendo algunos patógenos de plantas y otros no (Díaz et al., 2005).

En términos generales, la resistencia inducida se puede dividir en dos tipos principales, la resistencia sistémica adquirida (SAR) y la resistencia sistémica inducida (ISR). Ambas respuestas, junto con otras formas de resistencia inducida, representan una forma atractiva para el manejo de enfermedades de los cultivos de manera sostenible en el marco de un sistema de agricultura convencional (Vallad & Goodman, 2004).

El objetivo de este trabajo fue evaluar el efecto de la estimulación de defensas con inductores (SA,AJ,MeSA) sobre los síntomas de infección de *P. parasitica* (I% y S%) en vitroplantas de piña cvs MD2 y Queen Victoria (QV).

Materiales y métodos

Generalidades

Para desarrollar la experimentación se emplearon vitroplantas de los cultivares MD2 y QV. sembradas en fase de aclimatización. Se utilizaron 10 vitroplantas por cada tratamiento.
Se emplearon los inductores ácido salicílico (SA), metil salicilato (MeSA) y metil jasmonato (MeJA) a una concentración de 0.25 mM según resultados previos.

Preparación del inóculo de *P. parasítica*

Se utilizó un cultivo de esporangios y zoosporas de la cepa Reunión, previamente sembrado en medio agar V8+ betasitosterol, al cual se le realizó el siguiente protocolo para la liberación de zoosporas (Figura 1). Posteriormente se colectó la suspensión de zoosporas y esporangios de 3 placas para la inoculación de las vitroplantas.

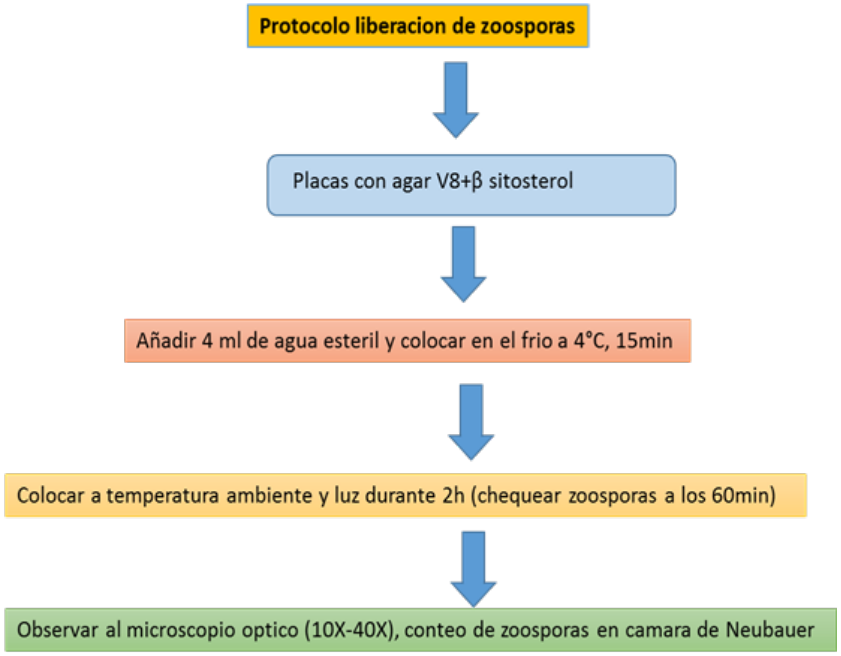


Figura 1. Protocolo para la liberación de zoosporas

En este caso se utilizó para la inoculación una concentración de esporangios de 1x 10^6 esporangios.mL^{-1} para optimizar el proceso debido a dificultades de visualización de las zoosporas al microscopio óptico.

Aplicación de inductores e inoculación del patógeno.

De cada inductor se añadió 5mL por planta previo a la inoculación del patógeno, tres aplicaciones cada 3 días. En el caso de Sa y MeSa se aplicaron en el sustrato alrededor de cada vitroplanta, y para el MeJA se aplicó directamente en las hojas.
De la suspensión obtenida previamente del patógeno, se inoculó a cada planta evaluada 1mL por planta en el cogollo a los 10 días posteriores a la inducción de las

vitroplantas.(Figura 2). Para favorecer la infección se realizó a cada planta, una pequeña herida de 0.5 mm en una de las hojas más jóvenes.


a)


b)

Figura 2. a) Procedimiento de inoculación de las vitroplantas, y aplicación de inductores.b) Diseño experimental.

De manera general se utilizó el siguiente diseño experimental:

Diseño experimental:
1. Vitroplantas no inducidas ni inoculadas. CONTROL (-)
2. Plantas inoculadas con Phytophthora CONTROL (+)
3. Plantas inoculadas + inducidas con SA
4. Plantas inoculadas + inducidas con MeSA
5. Plantas inoculadas + inducidas con MeJA.

Las plantas se mantuvieron bajo cobertor y riego diario para favorecer y mantener la humedad necesaria y temperatura adecuada para lograr la infección del patógeno.
Para la evaluación de los síntomas se utilizó una escala de muestreo (Segarra et al. 2013) para determinar el estadio de la infección en cada tratamiento evaluado.

Escala de síntomas

0: plantas sanas

1+: inicio de la infección. (infeccion solo visible al nivel de la punteadura de la hoja)

2+: infección de varias hojas. (infeccion progresando en 2 o 3 hojas contiguas a la primera)

3+: infección completa del corazón.

4+: plantas con pudrición total.

El muestreo se realizó durante cuatro semanas, con una frecuencia de observación cada dos días. Se evaluaron los indicadores incidencia (%I) y severidad (%S) de la infección.

%I= [(No. plantas dañadas)/(Total de plantas)]x 100

$$\%S = \Sigma\ [(GdxNP)/(EMxTP)]\ x100$$

GD= grado de daño, NP= número de plantas dañadas, EM= grado de daño máximo de la escala de severidad y TP= número de plantas del tratamiento (Shanmugam y Kanoujia, 2011; Li et al., 2012)

Resultados y discusión

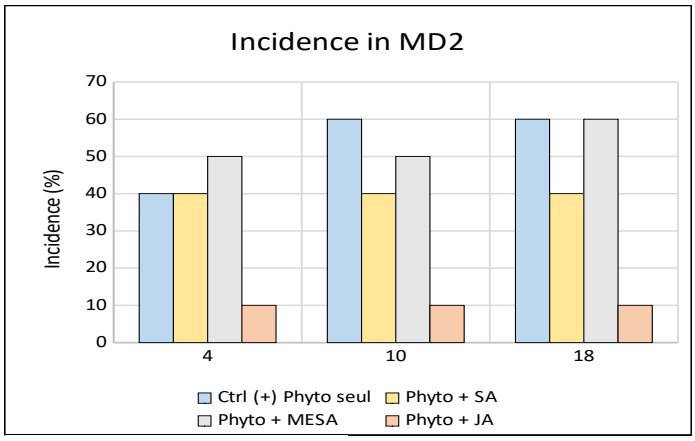
En la figura 3 se observan el % de incidencia de la enfermedad en los dos cultivares evaluados.

Los resultados muestran que hubo diferencias en la respuesta de cada uno de los tratamientos respecto a las variedades en estudio. En el cv MD2, los tratamientos con SA y MeSA mostraron un 50% de incidencia de la enfermedad similar al control +. Mientras que el tratamiento con MeJa ejerció un control de la incidencia de la enfermedad por debajo de los valores del control + desde los primeros días y durante todo el experimento. El interés en las moléculas estimuladoras de los mecanismos naturales de defensa de la planta, de aplicación exógena, surgió por su contribución al control de plagas, ya que presentan el potencial de disminuir y/o evitar el riesgo de emergencia de poblaciones de plagas resistentes a productos químicos, contrarrestar parcialmente los daños químicos ocasionados a la planta por los plaguicidas y finalmente originar aumento del rendimiento de las cosechas. El primer activador de resistencia de origen químico, el probenazol, se registró en Japón en 1975, y desde entonces se desarrollaron gran cantidad de compuestos de este tipo orientados a la protección de los cultivos. Muchos de ellos, fueron registrados como productos comerciales, incluyendo el ASM (Acibenzolar-S-metil), registrado como Bion y Actigard (Syngenta). Los inductores de resistencia químicos mostraron ser efectivos contra hongos, bacterias y virus (Edreva,2004). Estos compuestos, normalmente, inducen la SAR.

En el cv QV existió un menor % de incidencia de la enfermedad en todos los tratamientos con respecto al control +, excepto en el tratamiento estimulado con SA. Las plantas esdtimuladas con MeJa mostraron los menores valores de incidencia de la enfermedad, lo que sugiera que este inductor activó los mecanismos prematuros de defensa en las vitroplantas, lo que permitió que no se desarrollara la enfermedad.

Estas sustancias inductoras son descompuestas biológicamente y el espectro de patógenos que pueden ser repelidos es muy amplio, su aplicación en la liberación de la respuesta sistémica tiene un buen potencial en la protección de las plantas. La investigación intensa de la respuesta sistémica adquirida, en particular su genética molecular, pronto mostrará que la respuesta sistémica puede ser aplicada exitosamente, quizá combinada con otras medidas de protección (Camarena-Gutiérrez, 2007).

En contraste con la var MD2, en el caso de QV los inductores MeSa y SA ejercieron un control sobre las plantas estimuladas e inoculadas con % de incidencia menores al control+, lo que se puede inferir además que el cv también ejerce una función determinante en la respuesta al desarrollo de la enfermedad, ya que el cv MD2 es mucho más sensible a enfermedades que QV.



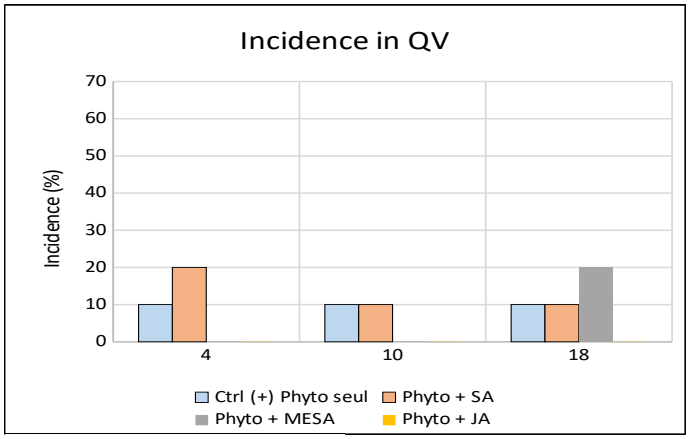
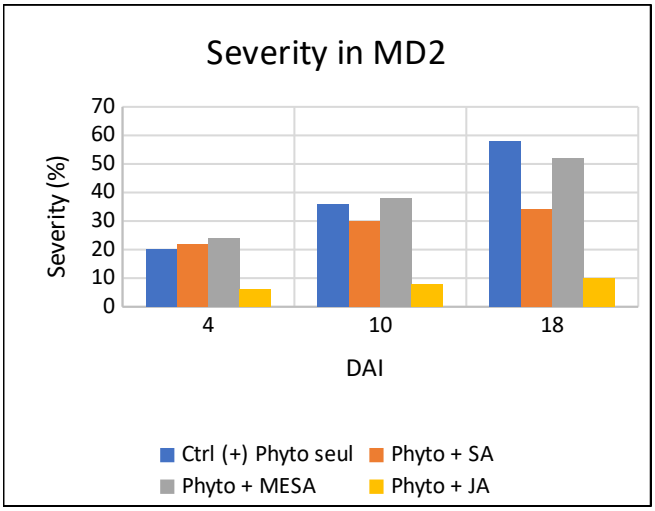


Figura 3: Porcentaje de incidencia de la enfermedad en vitroplantas estimuladas con inductores e inoculadas con *P parasitica*. Datos a los 4,10 y 18 días posterior a la inoculación.

En la figura 4 se observan los resultados del efecto de los inductores en las plantas inoculadas con respecto al severidad de la enfermedad causada por el patógeno. En el cv MD2 se pudo apreciar que en el control + la severidad de la enfermedad aumento hasta un 60% en los 18 días post inoculación. En los tratamientos estimulados la severidad fue menor que en el control+ lo que permitió una disminución de los daños en las vitroplantas. El tratamiento estimulado con JA mostró los más bajos porcentajes de severidad.



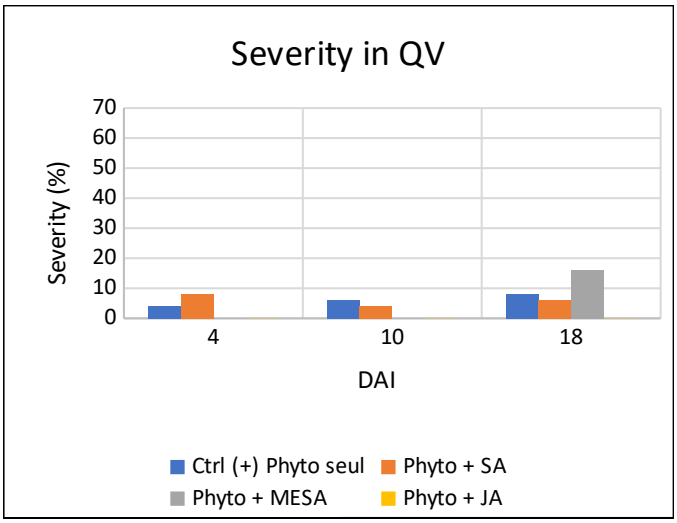


Figura 4. Porcentaje de severidad de la inoculación de *P. parasitica* en plantas estimuladas con inductores. Datos a los 4,10 y 18 días posterior a la inoculación.

Estos resultados están en correspondencia con los referido por Kuc, (2001), donde plantea que esta resistencia, rara vez evita la aparición de la enfermedad, sino que más bien reduce su severidad. En comparación con el control químico tradicional, la resistencia inducida por agentes bióticos o abióticos no tiene efectos inmediatos drásticos sobre la reducción de la enfermedad, pero si tiene un efecto mucho más duradero. La reacción de resistencia comienza muy pronto después de la aplicación del agente inductor y los beneficios duran, en general, entre tres semanas y dos meses, aunque existen reportes de duración de la resistencia hasta por seis meses (Kuc, 2001).
En el caso del cv QV los porcentajes de severidad se mantuvieron por debajo del 20% en todos los tratamientos, lo que refuerza la hipótesis de que las características del cv podría contribuir a disminuir el efecto del patógeno.

Figura 5 se muestran algunos de los resultados de los tratamientos a los 25 días posterior a la inoculación.

Control+ cv (MD2) inoc+JA (MD2) inoc+JA (QV)

Figura 5. Resultados fotográficos de las plantas inoculadas (control+ MD2), plantas inoculadas y estimuladas con JA (cv MD2 y QV) a los 25 días post inoculación.

Estos resultados abren una perspectiva futura para la utilización de los inductores de resistencia en el control de *P. parasítica* en el cultivo de la piña que podría constituir una alternativa amigable con el medio ambiente. Los mismos se aplican en bajas dosis por lo que su factibilidad podrá ser evaluada en estudios posteriores. Profundizar en estos resultados y en los genes de defensa que se activan durante la estimulación temprana constituye objetivos futuros a desarrollar.

Conclusiones

1. Los inductores de resistencia ejercieron un control sobre la incidencia y severidad del patógeno P parasitica.

2. Las plantas estimuladas con JA presentaron los mas bajos porcentajes de incidencia y severidad de la enfermedad.
3.El cv QV presentó un mejor control de los % de incidencia y severidad de la enfermedad.

Referencias bibliograficas

-Camarena-Gutiérrez G, R de la Torre Almaráz. (2007). Resistencia sistémica adquirida en plantas: Estado actual. Revista Chapingo Serie Ciencias Forestales y del Ambiente, 13(2): 157-162.

-Edreva A. A novel strategy for plant protection: Induced resistance. (2004). Journal of Cell and Molecular Biology. 3: 61-69.

-García-Jiménez, J., Monte, E., Trapero, A. (2010). *Los hongos y Oomicetos fitopatógenos* Valencia, España: SEF-Phytoma.

-Kuc J. Concepts and direction of induced systemic resistance in plants its application.(2001). European Journal of Plant Pathology. ; 107: 7-12.

-Li CH, Shi L, Han Q, Hu HL, Zhao MW, Tang CM and Li SP. (2012). Biocontrol of verticillium wilt and colonization of cotton plants by an endophytic bacterial isolate. Journal of Applied Microbiology 113:641-651. DOI: 10.1111/j.1365-2672.2012.05371.x

-Rohrbach KG, Schmitt DP. (1994). Pineapple. Compendium of Tropical Fruits Diseases, Part IV, The American Phytopathological Society, EE. UU.

-Sánchez, M., Andicoberry, S., & Trapero, A. (2004). Patogenicidad de *Phytophthora* spp. causantes de podredumbre radical de Quercus ilex ssp. ballota en viveros forestales. Bol. San. Veg. Plagas, 30, 239-255.

-Segarra G, Aviles M, Casanova E, Borrero A and Trillas I. (2013). Effectiveness of biological control of Phytophthora capsici in pepper by Trichoderma asperellum strain T34. Phytopathology Mediterranea 52(1):77-83. http://hdl.handle.net/11441/30458

-Shanmugam V and Kanoujia N. (2011). Biological management of vascular wilt of tomato caused by Fusarium oxysporum f. sp. lycospersici by plant growth-promoting rhizobacterial mixture. Biological Control 57:85-93. https://doi.org/10.1016/j.biocontrol.2011.02.001

Synthetic report research for the 2 mobilities in Cirad- Reunion with Erasmus+ project (2 months in 2021 and 2 months in 2022)

Ermis Yanes Paz

Introduction

Dr Alain Soler has been successfully using rattle box (Crotalaria) in crop rotation strategies to combat nematodes and increase fertility. Crotalaria apparently suppresses nematodes growth by secreting toxic or inhibitory compounds to the soil (Halbrendt, 1996).

Methyl Jasmonate and salicylic acid have been used in other crops as an agroecological alternative to the use of chemical plant protection products due to their role in the induction of the synthesis of secondary metabolites involved in plant defense (Singh and Dwivedi., 2018). Therefore, they could also play a role in Moissonia control.

The use rattle box in La Reunion as a service plant has been affected by the presence in this area of a very aggressive pest: *Moissonia importunitas (Ratnadass et al., 2018).*

In this context the aims of this work were i) to stablish a system for the multiplication of *Moissonia importunitas* under controlled conditions and ii) To evaluate the differential expression of genes in *Crotalaria juncea* plants infested with Moissonia treated or not with SA and JA.

Materials and methods

Multiplication of *Moissonia importunitas*

The insects were chased using plastic bags in a field of *Crotalaria juncea* infested with the pest. Then, 10 insects were placed in cages containing 9 plants of *C. juncea* in the greenhouse. Plants were watered daily.

The number of insects was counted 16 and 25 days to evaluate the multiplication.

RNA profiling

Evaluation of differential expression of genes

Four cages each containing three replicates of three plants each of *Crotalaria juncea* were placed under greenhouse conditions. A mixture of peat moss and vermiculite was used as a substrate. Plants were watered daily. Four different treatments were applied. 1. Water control (plants sprayed with water), 2. Water+ Moissonia, 3. SA+ Moissonia, 4. JA+ Moissonia

The plants were either sprayed with water or with 1mM Salicylic acid or 0.1mM Methyl Jasmonate according to the treatment and 10 insects per cage were added to all treatments four days after treatment except for the water control.

RNA isolation and cDNA synthesis

Four days after adding the insects, aerial part of the plant was collected in carbonic ice and frozen at -80 °C until use. RNA was isolated using the Qiagen RNeasy Plant minikit according to the manufacturer's instructions. RNA quality was checked using standard agarose gels stained with ethidium bromide and visualized under UV light. RNA concentration was checked using nanodrop.

Results

Life cycle of *Moissonia importunitas is completed in 16-24 days Banerjee and Kakoti (1969). Therefore, we counted the number of insects after 16 and 25 days after the initial addition of the insects to Crotalaria juncea plants. Table 1 shows the results.*

Table 1. Multiplication of *Moissonnia importunitas* under greenhouse conditions. The values shown are the mean value of three cages ± standard error

Initial number	At 16 days	At 25 days
10 insects	23±3 insects	42±5 insects

After 15 days of the initial infestation of Crotalaria with Moissonia, the number has doubled when insects in the first instars were observed, after 25 days the number of insects was about four times the initial number.

Après 15 jours de l'infestation initiale de Crotalaria avec Moissonia, le nombre a doublé lorsque les insectes des premiers stades ont été observés, après 25 jours, le nombre d'insectes était environ quatre fois le nombre initial.

Figure 1. Cages containing 9 plants of *Crotalaria juncea* used for the multiplication of Moissonia.

RNA profiling

The objectives of this experiment are to identify differentially expressed genes in Crotalaria plants treated or not with defense inducers infested with Moissonia in relation to untreated plants and to obtain sequences for these genes to be able to study them later by qPCR and other techniques.

By identifying genes which are either over or under expressed under the insect's attack and those which are induced by the application of defense response inducers such as SA and Me-JA we could be able to study the defense mechanisms of Crotalaria to the insect and evaluating which defense inducers could have a better effect in controlling the pests. Moreover, based on these results genotypes with higher levels of resistance to the pest could be selected.

The availability of sequences for many of these genes will be also an important result of this study since Crotalaria genome is not available so far.

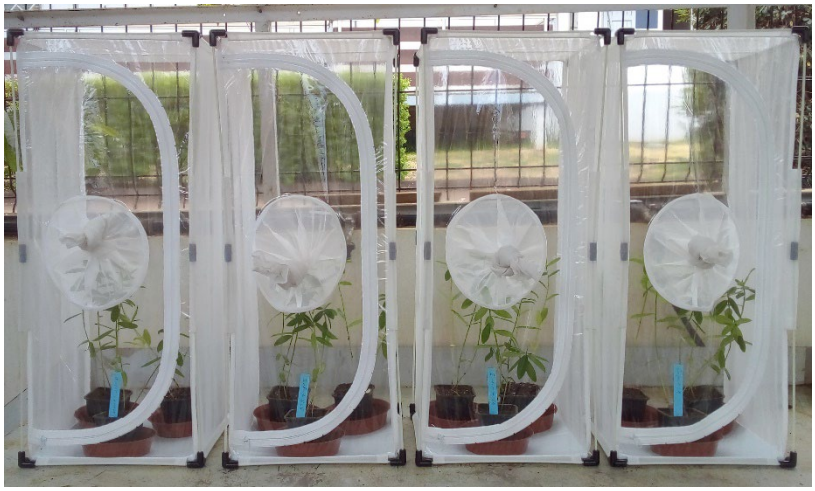


Figure 2. Cages containing three replicates of three plants each of Crotalaria plants. From left to right Water Control, Water+Moissonia, JA+Moissonia, SA+Moissonia. Treatment with defense inducers were done in separate places and cages were separated to avoid plant-plant communication.

Figure 3. *Crotalaria juncea* plants after four days of *Moissonia importunitas* insfestation. a-water control, b-water+Moissonia, c-SA+Moissonia and d-JA+Moissonia

As observed in figure 3 all treatments infested with Moissonia were affected by the pest although a slightly lower level in SA-treated plants which might be indicative of a role for SA in pest control. Surprisingly JA treated plants were severely affected by Moissonia as shown in the figure. The results of RNA-seq could show which pathway is activated in Crotalaria plants challenged with Moissonia.

Figure 4 shows a gel picture of isolated RNA from some of the samples showing a good integrity of RNA. The next step will be sending for RNAseq analysis, bioinformatic analysis and interpretation of results.

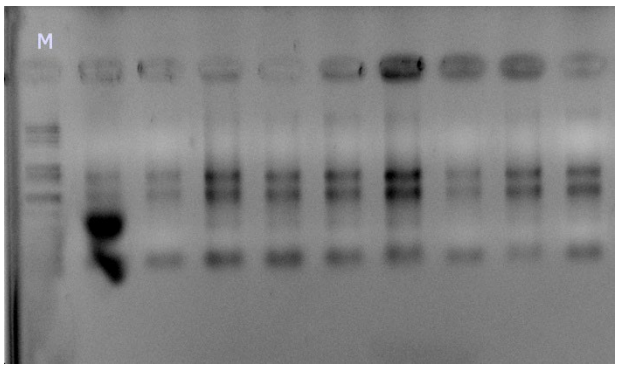


Figure 4. Gel picture of RNA isolated from *Crotalaria juncea* four days after Moissonia insfestation.

Table 2. Concentration and quality of RNA samples from *Crotalaria juncea* four days after infestation with *Moissonia*

Sample ID	Description	Conc.	Units	A260/280
M1	Water+Moissonia Rep 1	39.75	ng/ul	2.37
M2	Water+Moissonia Rep 2	38.85	ng/ul	1.95
M3	Water+Moissonia Rep 3	76.99	ng/ul	2.22
S1	SA+Moissonia Rep 1	59.75	ng/ul	2.13
S2	SA+Moissonia Rep 2	83.83	ng/ul	2.18
S3	SA+Moissonia Rep 3	127.6	ng/ul	2.11
J1	JA+Moissonia Rep 1	38.88	ng/ul	1.63
J2	JA+Moissonia Rep 2	51.69	ng/ul	1.84
J3	JA+Moissonia Rep 3	73.27	ng/ul	2.26
W1	Water Control rep 1	24.25	ng/ul	2.03
W2	Water Control rep 2	26.51	ng/ul	1.66
W3	Water Control rep 3	57.18	ng/ul	1.96

References

Halbrendt J.M 1996. Allelopathy in the management of plant-parasitic nematodes. Jounal of Nematology 28:8-14

Ratnadass A.; Soler A.;Chabanne A.;Tullus RG; Techer P.; Le Bellec F.; Marnotte P.; Streito JC.; Matocq, A 2018. First report of Moissonia importunitas as a pest of rattle box (Crotalaria spp) in Réunion Island (Hemiptera, Miridae). Bulleting of the etomological society of France, 123 (1): pp 59-64.

Singh A.; Dwivedi, P 2018. Methyl jasmonate and Salicilyc acid as potent elicitors for secondary metabolite production in medicinal plants: A review.Journal of Pharmacology and Phytochemistry 7(1): 750-757.

Banerjee J and Kakoti N.N 1969. Biology, population cycle and control of Ragmus importunitas distant. Indian Journal of entomology 30 (4): 257-262.

REPORTE DE 2 ESTANCIAS en CIRAD Réunion en 2022 y 2023

Nombre y Apellidos: Jéssica Mendoza Rodríguez

Estancias: Dos estancias de 180 días cada una.

- La primera estancia inició el 6 de enero del 2022 y concluyó 6 de julio del 2022
- La segunda estancia inició el 7 de octubre del 2022 y concluyó 7 de abril del 2023

Área de la UNICA a la que pertenece: Centro de Bioplantas

Objetivo general

Cumplimiento a tareas comprometidas con el proyecto internacional ERASMUS+ (Unión Europea y Agreenium/ Cirad) en el desarrollo de sistemas innovadores y la inducción de resistencia sistémicas de cultivos de interés agrícola. Desarrollo de experimentos que tributan a la tesis doctoral de inducción de resistencia mediada por la ruta del ácido salicílico o ácido jasmónico. Además del manejo integrado en la búsqueda de control a fitopatógenos.

En las dos estancias trabajó en los laboratorios de **Agrofisiología UR GECO** y **Pôle De Protection Des Plantes,** (CIRAD) en Isla Reunión (Francia). Participó en la planificación y el diseño de diferentes experimentos de inducción de resistencia sistémica adquirida (SAR) e inducida (ISR) mediada la señalización por la ruta del ácido salicílico (SA) y el ácido jasmónico en dos frutales (Piña y Banano) de interés económico para la Isla Reunión y Cuba.

Se realizaron ensayos de inoculación y experimentos de síntomas con el hongo *Fusarium oxysporum* f sp. *cubense* en cuatro cultivares de bananos: dos susceptibles (Fressynette y Mignonne) y dos resistentes (Hibrido 938 y Cavendish) en fase de aclimatización. Simultáneamente, se desarrolló y diseñó un experimento sobre el timing en curso de los genes de bananos en dos variedades: Cavendish y Fressynette.

Se recibió formación y realizó trabajos de extracción de ARN a los cuatro cultivares de bananos utilizando protocolo de Qiagen, donde se logró optimizar el protocolo de extracción de ARN de banana al realizar ajustes y correcciones. También se comprobó la calidad y pureza de ARN y el ADN en Nanodrop y Qubit 4 (espectrofotómetro y fluorímetro respectivamente). Adicionalmente, recibió formación en preparación, corrida y revelación de geles de

electroforesis. Asimismo, realizó la síntesis de la primera cadena cADN mediante RT_PCR y determinó expresiones relativas de seis genes: *pal, npr1, pr1, pr3, ICS* y *cys* utilizados como marcadores moleculares de resistencia. Además, se colaboró en la escritura y corrección del artículo: **Systemic resistance as a potential tool to control *Fusarium oxysporum* f. sp. *cubense* race 1 of banana.**

Las tareas y objetivos planteados anteriormente son parte fundamental de la tesis de doctorado del estudiante. Aunque no todos los experimentos concluyeron la estancia contribuyó a la formación y capacitación del estudiante en técnicas y equipos novedosos en el campo de la genética molecular.

Systemic resistance as a potential tool to control *Fusarium oxysporum* f. sp. *cubense* race 1 of banana

J. Mendoza Rodríguez[1], N. Portal[1], L. Bolaños Hernández[1], L. Pérez Bonachea[1], E. Yanes Paz[1], R. González Rodríguez[1], R. Santos[2] and A. Soler[3]

[1]Centro Bioplantas, Universidad de Ciego de Avila, Cuba, [2]Universidad Estatal de Bolivar, Guaranda 020150, Ecuador, [3]CIRAD, UPR GECO, F-97455 Saint-Pierre, Réunion, France. GECO, Univ Montpellier, CIRAD, Montpellier, France.

Abstract

Banana is a fruit consumed by millions of people worldwide with a production of 150 million tons mostly consumed in areas of production. The export production (about 20 million tons) is based on Cavendish variety but is jeopardized by a wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4). Integrated Pest Management strategies consist of designing banana production systems based on crop rotation and/or association with cover crops to restore ecosystem services such as organic matter content and turnover, disease suppression and regulation. The stimulation of systemic resistances to strengthen banana tolerance to pathogens in agroecosystems without pesticide is one of the strategies being considered. In a previous study of the interaction of banana with *Foc* race 1, gene expression was measured after *Foc* race1 extract was applied to leaves. Changes in the expression of genes involved in signaling pathways and defenses were measured in susceptible and tolerant banana varieties after application of the extract of *Foc* race1. In the present study, the direct interaction between banana and *Foc* race 1 was used to evaluate a potential use of systemic resistances of banana to control Fusarium wilt. Following inducer application of salicylic acid, methyl-salicylate, acibenzolar-S methyl, or methyl-jasmonate, *Foc* race 1 was inoculated on banana vitroplants. Comparisons of expression of wilt symptoms and of genes related to SAR and ISR signaling pathways and defense were made on banana varieties susceptible or naturally tolerant to *Foc* race 1 or/and *Foc* TR4. Jasmonates, ISR inducers, were more effective than acibenzolar-S methyl, SAR inducer, to reduce severity and incidence of wilt symptoms at 80-and 160-days post inoculation of *Foc* race 1. Methyl-salicylate and salicylic acid, SAR inducers, were effective inducing short-term regulation of different molecular markers, such as expression of PAL, NPR1 and ICS, all related to salicylic acid control and SAR signaling pathway, and PR3, PR1 and CYS, related to defenses. The results suggest that both SAR and ISR are involved in the banana response against Fusarium wilt and might support disease management strategies

INTRODUCTION

Global banana production reaches 150 million tons including dessert and cooking bananas. The majority of banana is exported to Europe and USA and is predominantly Cavendish from Latin America. The original wild species of bananas are all diploid (AA and BB) all from Southeast Asia. Natural mutations and selection produced diploids groups as AA, like the sweet fig or Frayssinette, and AB (about 290 cultivars, mostly produced in Southeast Asia). Breeding have produced three groups of triploids, AAA, AAB and ABB (Lescot, 2018). The same author stated that there are at least 650 cultivars, and among these groups are most of the sweet dessert varieties and cooking varieties.

Bananas are more or less susceptible to Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) due to its different races. Guo et al. (2014) reported Tropical Race 4 (TR4), a new variant to be aggressive and to have a wide host range including the

Cavendish group, and banana cultivars groups both susceptible and resistant to race 1 and 2 in any tropical condition. In the sixties, *Foc* race 1 led to a shift from the susceptible variety Gros Michel to Cavendish on the World trade market. Nevertheless, Cavendish is susceptible to TR4, jeopardizing again the world banana industry. An ecological management may include stimulation of natural defenses of plants against pests and diseases (Systemic resistances).

Plants cells can detect the presence of pathogens with membrane receptors capable of recognizing different pathogen-associated molecular patterns called PAMPs (Pai Li et al.,2020, Boller et al., 2009). Damage caused to plant cells by the pathogens (DAMPs) is also recognized (Zipfel, 2014). Detection of these patterns activates pattern-triggered immunity (PTI), (Klessig, 2018). Plants have developed efficient systemic signaling pathways that depend on different hormones including jasmonate and ethylene, or salicylate, specific transcription factors, and different signal molecules (Pai Li et al., 2020). The jasmonate pathway, which leads to jasmonate accumulation, is associated with induced systemic resistance (ISR) against necrotrophic pathogens, and during interactions with non-pathogenic microorganisms (bacteria or mycorrhizal fungi). The salicylate pathway, which leads to salicylate accumulation, is associated with systemic acquired resistance (SAR) against biotrophic pathogens. After a primary infection or elicitation, these long-distance signaling pathways activate immune responses in all uninfected tissues of the plant, the mechanism being called priming (Holmes et al., 2019; Villena et al., 2018). When plant defenses are primed, they enter a state of enhanced alert allowing a faster and stronger reaction to a pathogen attack compared to unstimulated (or unprimed) plants (Conrath, 2011, Mehari et al., 2015). Consequently, the plant activates genes of defense as well as enzymatic activities linked to oxidative burst and defenses. The aim of this preliminary study was to characterize systemic defenses against *Foc* race 1 in different banana varieties through biological effects and molecular markers.

MATERIAL AND METHODS

Plant materials

Symptoms evaluation after SAR or ISR stimulation were performed on a *Foc* race 1 susceptible variety Manzano (AAB), and a *Foc* race 1 tolerant variety Grande Naine (Cavendish AAA). Plants were acclimated at 25.5°C ± 2°C, 80% ± 3% of relative humidity, and under light 400 ± 25 µmol $m^{-2}s^{-1}$ for 90 days. Molecular tests for SAR were performed on 3 varieties: a variety susceptible to *Foc* race 1 (Frayssinette AA), and two varieties tolerant to *Foc* race 1 (Cavendish cv902 and a Cirad hybrid, (AAA)). The last one being also tolerant to TR4.

Systemic resistances stimulation (SAR & ISR)

For symptoms evaluation, two technical formulations of inducers of systemic resistance were used before *Foc* race1 inoculation: 0.1mM and 1mM of S-Methyl acibenzolar (ASM) (Actigard, Syngenta), and 0.1mM and 1mM of methyl-jasmonate from *Botryodiplodia theobromae* (Biojas, ICIDCA, Cuba). Three applications at three days interval on 90-day old vitroplants. For molecular markers evaluation, SAR stimulations were done with one application of salicylic acid (SA) or methyl salicylate (MESA) both at 0.1mM (Sigma) on 90-day old vitroplants

Fusarium strains and inoculation

Local *Foc* race1 isolates from Réunion island and Cuba were used. Inoculum was prepared in Petri dishes from a monosporic culture on PDA medium and incubated for 10 days at 25°C. Then, spore suspensions were produced in liquid medium Potato Dextrose incubated at 28°C with magnetic stirring for 20 days. Gauze filtrate was centrifuged at 4000g /15', then pellets containing spores were resuspended in liquid medium and adjusted to 10^6 $CFU.ml^{-1}$ for inoculation on roots. Banana root tips were partially removed with a surgical blade and the base of the plants immersed in 70ml of the Foc spore suspension for about 15 min to allow complete absorption of the suspension by the soil substrate covering plant roots. Root tips immersed in water served as controls. The timing between stimulations and inoculations was

3 days for all treatments. The timing between inoculation and collection of roots was 12h and 48h, respectively for both the susceptible and resistant cultivars. A total of 10 plants were inoculated per treatment, each plant acting as a replicate.

Symptom evaluation

Fusarium wilt symptoms were evaluated at 60 and 180 days after inoculation (dpi). Symptoms were scored on a scale of 1 to 5 for both the foliar and internal corm damage symptoms (Fig.1). Disease severity was evaluated with a formula according to Sherwood and Hagedorn (1958). Incidence was measured as a percentage of plants with disease symptoms.

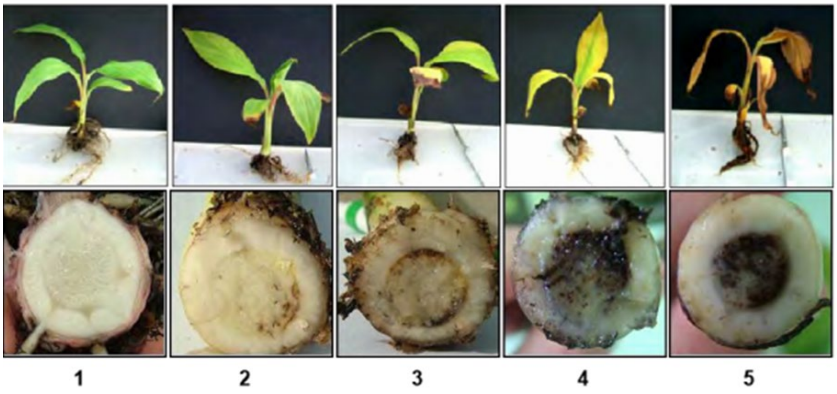


Figure 1. Scale of damages (1 to 5) induced by Foc race 1 on a susceptible variety. (Perez Vicente et al., 2014).

Evaluation of molecular markers

Roots were crushed in liquid Nitrogen and maintained at -80°C until RNA extraction, that was done using RNeasy Plant Mini-Kit (Qiagen) but modifying the initial step of the protocol specifically for bananas due to a high content of phenolic compounds and latex in roots. Insoluble PVPP (30mg) were added to 150mg of deep-frozen material and immediately mixed with 450µl of lyse Buffer (RLT) containing 10µl.ml-1 of beta mercapto-ethanol. The original RLT provided with the Kit was modified by adding 0.54% of IGEPAL, 0.81% of SDS, 18.1% PVP40 (1g in 10ml) all from Sigma. Then the following steps of RNA extraction were done as indicated by Qiagen including an on-column DNase step. Quality of RNA was tested with electrophoresis (1% agarose in TAE buffer) and concentration/purity with spectrophotometric analyses (Nanodrop). cDNAs was obtained by Reverse Transcription (Qiagen KIT Quantitech), and for gene expression by RTqPCR (Fast SybrGreen, on Stepone analyzer by Applied Biosystems) for 6 molecular markers tested, MaPAL, MaICS2 (linked to SA synthesis), MaNPR1 (transcription factor linked to SAR signaling pathways and level control of SA), MaPR1, MaPR3 (two pathogenesis proteins linked to defense), and MaCYS (Cystatin, a protease inhibitor) linked to defense, and one reference gene RNA26S (Table 1). Relative expression of genes was calculated with the 2-ΔΔCt method.

Table 1. List of primers used for markers of gene expression of systemic defenses

Gene	Forward	Reverse
MaARN 26S (reference gene)	GTAAACGGCGGGAGTCACTA	TCCCTTTGGTCTGTGGTTTC
MaNPR1like	GTCGGCATTGTACCAACACA	CAGTGCAGGAGTCAGCAAAA
MaPR1	TCCGGCCTTATTTCACATTC	GCCATCTTCATCATCTGCAA
MaPR3	GTCACCACCAACATCATCAA	CCAGCAAGTCGCAGTACCTC
MaCYS (Cystatin)	CGATTGGCTTTTACCGTGCG	TGAATGAACGTCCGCCTTCC
MaICS (Isochorismate synth)	CCAGCATTTATCAGCCCAAT	AACAGGGCCAGCATACATTC
MaPAL	CCATCGGCAAACTCATGTTC	GTCCAAGCTCGGGTTTCTTC

Design of the experiment for evaluation of molecular markers

Treatments for evaluation of molecular markers included: i) unstimulated & not inoculated (NS-NI, Ctrl(-)), ii) stimulated - not inoculated (S-NI, only stimulation), iii) unstimulated - inoculated (NS-I, Ctrl(+)), and iv) stimulated - inoculated (S-I, test). Each treatment had six biological replicates and each PCR analysis was repeated twice. The samples were taken 12h and 48h after inoculation with Foc race 1 for both the susceptible and tolerant varieties. Results were normalized against untreated plants (NS-NI), and the expression of the 6 molecular markers compared after Foc race 1 inoculation on stimulated and unstimulated plants with SA or MESA.

Statistics

Symptoms were analyzed with One-Way Anova and Tukey test ($p \leq 0,05$) on data transformed as $y=2 \arcsin(\sqrt{(y/100)})$. Molecular data were analyzed with Kruskal & Wallis and Dunn tests, (n=6) using XLstat software.

RESULTS AND DISCUSSION

Fusarium wilt symptom evaluation

On the susceptible Manzano, methyl-jasmonate formulated as Biojas significantly reduced ($p<0.05$) both severity and incidence of Foc race 1, compared to control across both concentrations (0.1mM and 1.0 mM) at 80 and 160 dpi (Fig. 2 & 3). S-methyl acibenzolar (ASM) also significantly reduced ($p<0.05$) severity of symptoms at both concentrations at 80 dpi and 160 dpi relative to the control. However, methyl-jasmonate outperformed ASM at 160 dpi. Incidence was not reduced significantly by ASM except for 0.1mM at 80 dpi, (Fig 2). This could be explained by the phytotoxicity of ASM at higher doses e.g. at 1mM. In a previous phytotoxicity experiment, ASM (1mM), formulated as Bion (Syngenta), induced strong toxicity symptoms (data not shown) when applied on leaves of young banana vitroplants in controlled conditions. Foc race 1 inoculations did not affect the less susceptible Cavendish.

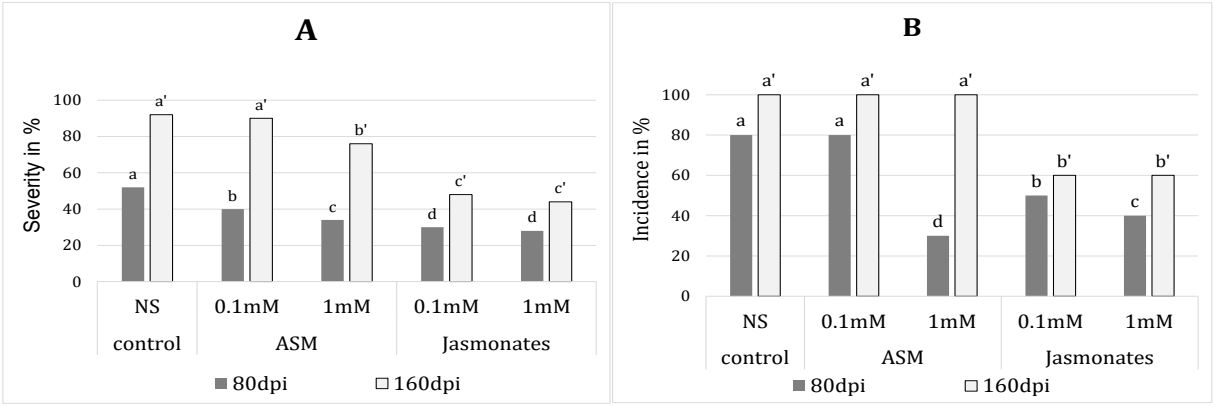


Figure 2. Severity % (A) and incidence % (B) of foliar symptoms compared in stimulated or unstimulated Manzano vitroplants inoculated with Fusarium oxysporum f. sp. cubense race 1. Different letters mean significant differences within each series 80 (a, b, c, d) and 160dpi (a', b' and c').

Internal observations in banana corms were consistent with the external symptoms (Fig. 3). Jasmonate at 0.1mM and 1mM, reduced the severity of symptoms in corms at both 80 (1) and 160 (2) dpi but it was not the case for ASM where symptoms were reduced only with 1mM at 80dpi (Fig. 3).

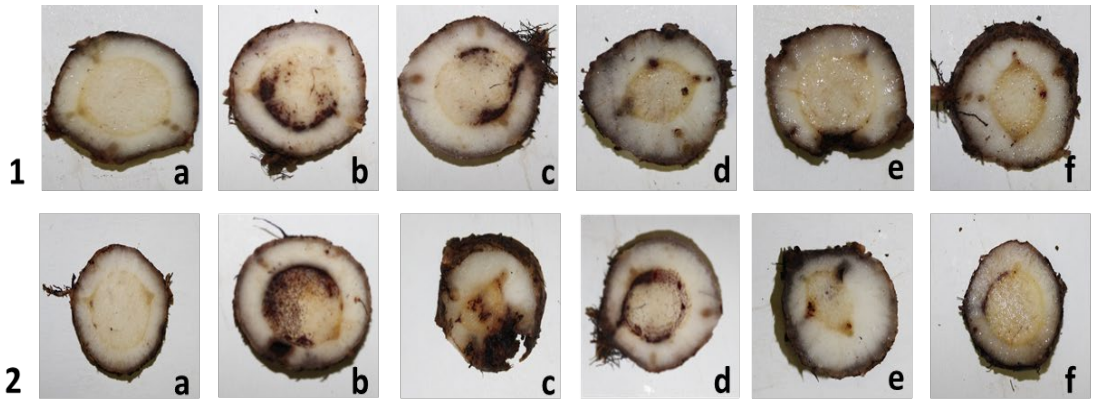


Figure 3. Internal Fusarium wilt symptoms in stimulated or unstimulated vitro Manzano banana plant corms 80 (line1) and 160 (line 2) days post inoculation after inoculation with *Fusarium oxysporum* f. sp. *cubense*. Respectively, a, b, c, d, e, and f denote uninoculated control, inoculated control, treatment with 0.1mM ASM, treatment with 1mM ASM, treatment with 0.1mM Methyl-jasmonate and treatment with 1 mM Methyl-jasmonate

Although ASM is considered an inducer of SAR, only a slight reduction was measured in the severity of external symptoms after inoculation. The results were inconclusive for the incidence of the symptoms. It would be interesting to test other inducers than ASM, in particular SA or MESA. We successfully used them in the study of the interaction pineapple (Ananas comosus)/mealybugs to evaluate the effectiveness of the defense induced and to identify potential molecular markers of the SAR priming (Soler et al., 2022).

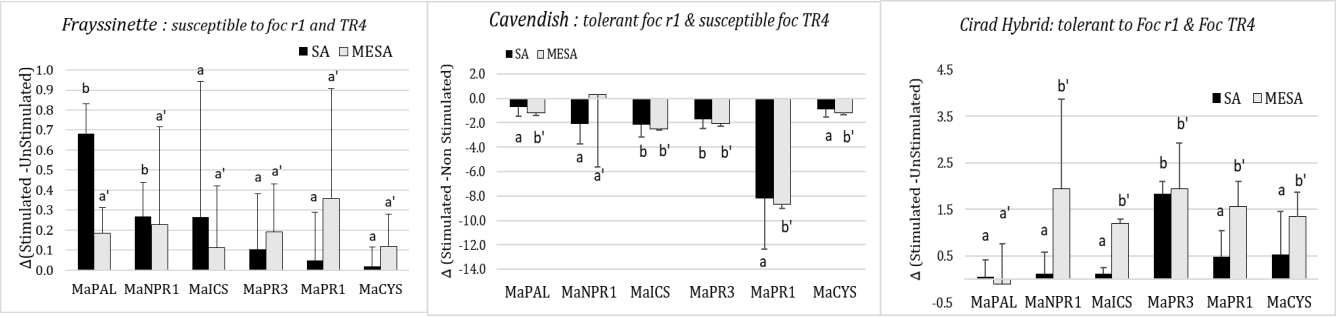
Soler et al. (2016) also tested with success methyl-jasmonate as a systemic resistance (RS) inducer in banana/nematode interaction. RS against nematodes was induced on 3 varieties of bananas. Two Cirad hybrids (one very tolerant and one semi-tolerant) compared to a nematode susceptible Cavendish (cv902) cultivar. When stimulated by a RS inducer, the Cavendish (cv902) showed the same tolerance to nematodes as for the naturally semi-tolerant hybrid. Moreover, RS inducer could not increase the natural tolerance of this semi-tolerant hybrid. The same authors obtained similar results comparing RS stimulation against nematodes in pineapple variety MD2 (stimulable) and smooth cayenne (not stimulable) (Soler et al., 2013).

Molecular markers

We hypothesized that *Foc* race 1 as a hemi-biotrophic pathogen, should exhibit a biotrophic behavior upon initial root penetration, and later a necrotrophic behavior in the corm and the rest of the plant. Consequently, in early stage of penetration in roots, *Foc* race 1 should be more susceptible to SAR induced by SA or MESA. Then later it should become more susceptible to ISR induced by jasmonates as shown by the reduced symptoms in leaf and corm at 80dpi and 160dpi.

To identify SAR molecular markers in this preliminary study, we hypothesized that *Foc* race 1 can penetrate faster in the roots of the susceptible variety Frayssinette, thus inducing the regulation of genes linked to systemic resistances (SR) faster than in resistant varieties. Based on a histological study (Dong et al., 2019), a different timing between inoculation and deep-freezing of samples has been used at 12h and 48h for the susceptible (Frayssinette) and the non-susceptible varieties (Cavendish cv902 and Cirad hybrid).

The molecular data (Fig. 4) showed that, in the susceptible variety Frayssinette, 12h after *Foc* race 1 inoculation the SA stimulation induced higher up-regulation of genes linked to synthesis of salicylic acid (PAL, NPR1 and ICS2), meanwhile the up-regulation of genes of defense (MaPR1, MaPR3 and MaCys) was higher with MESA stimulation.



Foc race 1 and TR4 susceptible Frayssinette (AA), Foc race 1 resistant and TR4 susceptible Cavendish (cv902, AAA) and Foc race 1 and TR4 resistant Cirad Hybrid (AAA) following stimulation of SAR with either salicylic acid (SA) or methyl-salicylate (MESA). Gene expression was measured 12h and 48h after inoculation of Foc race 1 respectively in Frayssinette, and in the 2 other varieties. Unstimulated plants served as controls. MaPAL, MaICS2 are genes related to salicylic acid synthesis, MaNPR1 is a gene coding for a transcription factor linked to SAR signaling pathways and level control of SA, MaPR1, MaPR3 are genes coding for two pathogenesis proteins linked to defense, and MaCYS gene coding for cystatin, a protease inhibitor linked to defense. Different letters mean significant differences within each gene expression, SA (a, b) and MESA (a', b').

Figure 4. Differential expression of genes in 3 banana varieties after SAR stimulation and Foc race 1 inoculation.

In the Foc race 1 and TR4 resistant Cirad hybrid, to the MESA stimulation induced a stronger up-regulation of most of the potential molecular markers/genes except PAL compared to unstimulated control at 48h after inoculation (Fig. 5). MESA induced a stronger up-regulation compared with SA. In the Foc race 1 resistant and TR4 susceptible cavendish, the unstimulated control had a stronger gene expression than for plants stimulated with SA or MESA for most of the genes at 48h after inoculation. It appears that in resistant varieties genes of defense are more up-regulated than those involved in SAR pathways and salicylic synthesis control after Foc race 1 inoculation. At this stage, the results on Cavendish do not necessarily mean down-regulation of SAR signaling and defense pathway genes but more likely, as seen in pineapple, they showed that the timing to compare expression of genes between stimulated and unstimulated plants needs to be determined more accurately. Nevertheless, the genes chosen as markers for SAR characterization, showed differentiated expression after inoculation of Foc race 1 with or without stimulation. The priming does not induce new defenses but rather allows the stimulated plants to mobilize existing basic defenses faster and eventually stronger compared to unstimulated plants. An initial experiment to determine for the interaction Foc race 1-banana the best timing(s) to compare the gene expressions with or without SAR stimulation for each variety could not be done at this stage (on going experiment on time-course gene expressions). Very short timings (few hours) to longer timings (few hours to 72h) may be necessary as shown for other Fusarium species (Ding et al., 2011).

The timing between the pathogen inoculation and the molecular markers analysis is a crucial data because the expression of the molecular markers may be shifted in time but also transient. Soler et al. (in submission) observed unstimulated pineapple plants the increase of the AcPAL expression after pathogen inoculation to be delayed by 24h compared to the stimulated plants, and the time-course expression showed a transient up-regulation (Fig. 5). This may induce incorrect evaluation of up or down regulation after inoculation if the correct timing is not used.

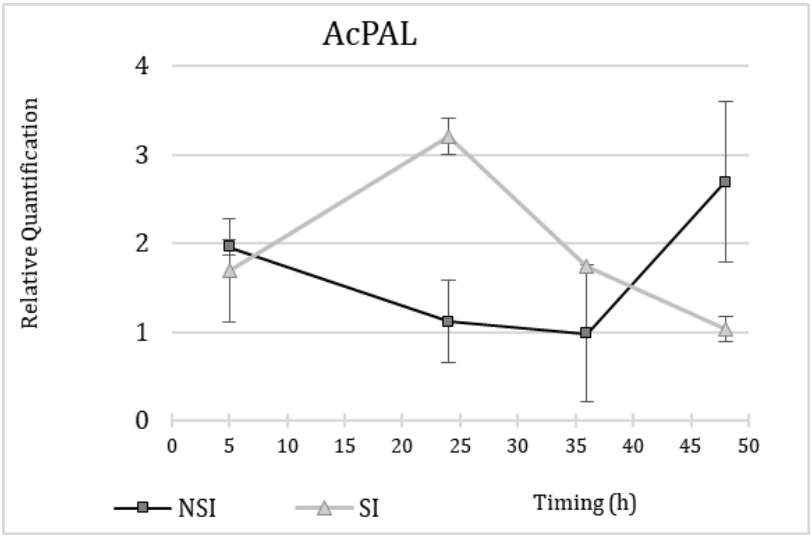


Figure 5. Evolution of relative expression of AcPAL on non-stimulated and stimulated Ananas comosus plants (SA, 1mM) after mealybug inoculation. Soler et al. (in submission).

In addition, other mechanisms than systemic resistances may be involved in naturally resistant varieties. Dong et al. (2019), in Brazilian cultivar of Cavendish (resistant to *Foc* race 1 and susceptible to TR4) observed that both *Foc* race 1 and TR4 hypha attached epidermal cells of roots 24h after inoculation and penetrated the cortex parenchyma in 48h. Then, *Foc* race 1 progression was reduced but not for TR4. They related this to a higher production of starch (particularly in the corm) with *Foc* race 1 infection than with TR4 infection. According to the authors the starch inhibited the toxic effect of fusaric acid in the plant, slowing down the progression of *Foc* race 1. Cheng et al. (2014) suggested also another mechanism where *Fusarium oxysporum* strains, due to their hemi-biotrophic behavior, are able to hijacks non defensive aspects of the JA signaling pathway used for non-pathogenic interactions, to enhance disease in early stage of infection in roots.

CONCLUSION

The Foc - banana interaction is not yet fully unraveled with different defense mechanisms developed by the plant over time and different responses of the fungus to this evolution. Some banana varieties developed the capacity of priming their basic defenses after appropriate stimulation. This priming with two different signaling pathways, SAR and ISR, which allows self-protection of the whole plant against pathogens after a first attack or after an appropriate stimulation, is considered as a plant immune system. Different inducers such as salicylate and methyl salicylate, and jasmonates, at very low concentrations (0.1 mM) were able to reduce severity and incidence of wilt symptoms, and to prime defenses in different banana varieties, up-regulating signaling pathway and defense genes in the different banana varieties tested. The behavior of Foc, biotrophic then necrotrophic, makes more complex the use of a priming of defenses that could contribute a pest management of Foc race 1 without pesticides. Finally, inducing SAR priming before root infection begins, appears to be a relevant strategy based on previous studies with other Fusarium species.

Literature cited

Boller, T., and Felix, G. (2009). A renaissance of elicitors: perception of Microbe–associated molecular patterns and danger signals by pattern-recognition. Ann Rev Plant Biol 60, 379-406.

Chen, Y.C., Kidd, B.N., Carvalhais, L.C., and Schenk, P.M. (2014). Molecular defense responses in roots and the rhizosphere against Fusarium oxysporum. Plant signaling & behavior 9, e977710.

Conrath, U. (2011). Molecular aspects of defense priming. Trends Plant Sci 16, 524-531.

Ding, L., H. Xu, H. Yi, L. Yang, Z. Kong, L. Zhang, S. Xue, H. Jia and Z. Ma. (2011). Resistance to hemi-biotrophic F. graminearum infection is associated with coordinated and ordered expression of diverse defense signaling pathways. PloS one 6:e19008

Dong, H., Fan, H., Lei, Z., Wu, C., Zhou, D., Li, H. (2019). Histological and Gene Expression Analyses in Banana Reveals the Pathogenic Differences between Races 1 and 4 of Banana Fusarium Wilt Pathogen. Phytopathology 109, 1029-1042.

Guo, L., L. Han, L. Yang, H. Zeng, D. Fan, Y. Zhu, Y. Feng, G. Wang, C. Peng and X. Jiang. (2014). Genome and transcriptome analysis of the fungal pathogen Fusarium oxysporum f. sp. cubense causing banana vascular wilt disease. PLoS One 9:e95543.

Klessig, D.F., Choi, H.W., and Dempsey, D.M.A. (2018). Systemic acquired resistance and salicylic acid: past, present, and future. Mol Plant-Microbe Interact 31, 871-888.

Lescot, T., and Moine, C. (2018). Diversite genetique des bananiers. In Fruitrop, pp. 92-96.

Mehari, Z.H., Elad, Y., Rav-David, D., Graber, E.R., and Harel, Y.M. (2015). Induced systemic resistance in tomato (Solanum lycopersicum) against Botrytis cinerea by biochar amendment involves jasmonic acid signaling. Plant Soil 395, 31-44.

Pai Li, Lu, Y.-J., Chen, H., and Day, B. (2020). The Lifecycle of the Plant Immune System. Crit Rev Plant Sci, 1-29.

Pérez Vicente, L., Dita, M., and Martinez De La Parte, E. (2014). Technical Manual: Prevention and diagnostic of Fusarium Wilt (Panama disease) of banana caused by Fusarium oxysporum f. sp. cubense Tropical Race 4 (TR4). Paper presented at: Proceedings of Regional Workshop on the Diagnosis of Fusarium Wilt (Panama disease) caused by Fusarium oxysporum f sp cubense Tropical Race 4: Mitigating the Threat and Preventing its Spread in the Caribbean, , 05-09/05/2014FAO (St. Augustine, Trinidad and Tobago.: FAO).

Sherwood, R., and Hagedorn, D. (1958). Determining the common root rot potential of pea plants. Wis Agric Stn Bull 531.

Soler, A., Pochat, C., Perrin, M., Mobarak, T., and N'Guessan, L. (2022). A summary of CIRAD research on pest control based on the stimulation of natural defenses of the MD2 pineapple in pesticide-free cropping system. Newsletter of the Workgroup Pineapple, International Society for Horticultural Science 28, 89-94.

Soler, A., Marie-Alphonsine, P.-A., Corbion, C., and Quénéhervé, P. (2013). Differential response of two pineapple cultivars (Ananas comosus (L.) Merr.) to SAR and ISR inducers against the nematode Rotylenchulus reniformis. Crop Prot 54, 48-54.

Soler, A., Marie-Alphonsine, P.-A., Quénéhervé, P., Prin, Y., Sanguin, H., Tisseyre, P., Daumur, R., Pochat, C., Dorey, E., and Rodriguez, R.G. (2021). Field management of Rotylenchulus reniformis on pineapple combining crop rotation, chemical-mediated induced resistance and endophytic bacterial inoculation. Crop Prot 141, 105446.

Soler, A., Quénéhervé, P., Portal González, N., and Marie Alphonsine, P.-A. (2016). Induced systemic resistance (ISR) and native tolerance to pathogens in banana: preliminary results. Poster presented at: X International Symposium on Banana / ISHS-ProMusa symposium Agroecological approaches to promote innovative banana produclion systems (Montpellier, France: ISHS-ProMusa).

Villena, J., Kitazawa, H., Van Wees, S.C.M., Pieterse, C.M.J., and Takahashi, H. (2018). Receptors and Signaling Pathways for Recognition of Bacteria in Livestock and Crops: Prospects for Beneficial Microbes in Healthy Growth Strategies. Front Immunol 9, 2223-2223.

Zipfel, C. (2014). Plant pattern-recognition receptors. Trends Immunol 35, 345-351.