UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD LEÓN

Expresión global de genes y predicción de rutas metabólicas involucradas en las respuestas de defensa del aguacate *Persea americana* Mill a la marchitez por Fusarium.

T E S I S

QUE PARA OBTENER EL TÍTULO DE:

LICENCIADA EN CIENCIAS AGROGENÓMICAS

P R E S E N T A:

Itzel Aislinn Aguirre Pérez

TUTOR EXTERNO:
Dr. Alfonso Méndez Bravo

TUTOR INTERNO:
Dr. Julio Vega Arreguín

ENESUNAM
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El presente trabajo fue realizado durante mi estancia de proyecto de investigación en el Laboratorio Nacional de Análisis y Síntesis Ecológica (LANASE) de la Escuela Nacional de Estudios Superiores Unidad Morelia, bajo la dirección y supervisión del Dr. Alfonso Méndez Bravo. Dicho trabajo fue financiado gracias a el proyecto IN214917 de PAPIIT por DGAPA y la beca de colaboración de UC MEXUS-CONACYT 2015.

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DEDICATORIA

Esta historia no inicio hace 4 años, esta historia es un hecho que fue dado por un sinfín de acontecimientos a lo largo de mi vida. Desde niña, siempre me interesó saber cómo funcionan las cosas y la razón de su existencia. Crecí rodeada de enseñanzas e inspiración para cumplir mis metas. Después de unos años empecé a darme cuenta que las ciencias podrían despejar muchas de mis dudas, pues son esenciales para saciar la curiosidad humana por la vida. Me preparé, esforcé y di todo de mí para poder lograr mi objetivo. Nunca pensé que ese objetivo me alejaría tanto de mis seres queridos, sin embargo, siempre conté con su apoyo. Para ellos no importaban los kilómetros mientras mis metas se cumplieran. Fue difícil, arduo y muchas veces la lejanía me hacía sentir sola y triste. Pero en cada mal momento, fueron sus palabras las que me hicieron superar esas etapas y seguir adelante esforzándome al máximo. En algún momento escribí para ingresar a esta licenciatura: "Estoy segura que puedo lograr que esta carrera se convierta para mí, en un gran descubrimiento, en un trabajo que gozare y en lo mejor de mi vida" y así ha sido, con altibajos como todo pero al final he aprendido, disfrutado y crecido extraordinariamente al tomar esta enorme decisión. Conocí personas maravillosas durante esta travesía, de quienes siempre recibí apoyo y cariño. En especial, quisiera destacar y agradecerle a mi madre, mi padre, mi hermana y mi pareja, quienes han sido mi principal motor para cumplir mis sueños. Y a todas las personas importantes en mi vida, mi familia y amigos más cercanos, que siempre estuvieron apoyándome en esta difícil pero grandiosa etapa. Definitivamente, todo esto jamás lo hubiera logrado de no ser por el amor, esfuerzo, dedicación y paciencia de todas estas personas hacia mí.

Los ama: Itzel Aislinn.

"Si una idea no parece absurda de entrada, pocas esperanzas hay de ella".

Albert Einstein.

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ABREVIATURAS

Abreviatura	Significado	Traducción al inglés
°C	Grados Celsius	Celsius degrees
ACCase	Acetil-CoA carboxilasa	Acetyl-CoA carboxylase
AsA	Ácido ascórbico	Ascorbic acid
C7	Siete Carbonos	Seven-Carbon
Cm	Centímetro	Centimeter
DGAT	Diacilglicerol aciltransferasa	Diacylglycerol acyltransferase
ET	Etileno	Ethylene
FA	Alcoholes Grasos	Fatty Alcohol
FAR	Fatty Acyl-CoA Reductasa	Fatty Acyl-CoA Reductase
FD	Marchitez por Fusarium	Fusarium Dieback
FPKM	Fragmentos por kilobase del modelo de exón por millón de lecturas mapeadas	Fragments per kilobase of exon model per million reads mapped
GB	Gigabyte	Gigabyte
GTS	Glutatión S-Transferasas	Glutathione S-Transferases
Ha	Hectárea	Hectare
HFA	Ácidos Grasos Hidroxilados	Hydroxylated Fatty Acids
JA	Ácido Jasmónico	Jasmonic Acid
KDO	3-desoxi-d-manno-octulosonato	3-deoxy-d-manno-octulosonate
KDOP synthase	3-desoxi-8-fosfato octulonato	3-deoxy-8-phosphooctulonate
KSHB	Euwallacea kuroshio	Kuroshio Shot Hole Borer
M	Metro	Meter
m.a.s.l	Metros sobre el nivel del mar	Meters above sea level
MIOX	myo-inositol oxigenasa	myo-inositol oxygenase
Mm	Milímetro	Millimeter
OPLS-DA	Proyecciones Ortogonales a Estructuras Latentes - Análisis Discriminante	Orthogonal Projections to Latent Structures - Discriminant Analysis
PCA	Análisis de Componentes Principales	Principal Component Analysis
PR	Relacionadas con la patogénesis	Pathogenesis Related
PSHB	Euwallacea sp. nr. fornicatus	Polyphagous Shot Hole Borer
RAMP	Proteína de membrana asociada al ribosoma	Ribosome associated membrane protein
RG-II	Rhamnogalacturonan II	Rhamnogalacturonan II
RNA	Ácido Ribonucleico	Ribonucleic Acid
SA	Ácido Salicílico	Salicylic Acid
TAG	Triacilglicerol	Triacylglycerol
UDPGlcA	Ácido UDP-glucurónico	UDP-glucuronic acid
UPLC-MS-QTOF	Cromatografía líquida de ultra rendimiento acoplada a un espectrómetro de masas	Ultra performance liquid chromatography coupled to a mass spectrometer

CAPÍTULO I

Presentación de la tesis

RESUMEN

El transporte y comercio de diversos productos en el mundo ha contribuido a la dispersión de una amplia diversidad de organismos a nuevas áreas. Los escarabajos barrenadores polífagos de tronco y ramas *Euwallacea* sp. nr. *fornicatus* (PSHB) y *Euwallacea kuroshio* (KSHB) son un ejemplo actual de plagas invasoras. Estos escarabajos son de origen asiático y poseen una alta capacidad de expansión ya que se asocian simbióticamente con algunos hongos, entre ellos dos del género *Fusarium*, provocando la enfermedad necrosante denominada marchitez por Fusarium (FD). Esta enfermedad afecta a más de 300 especies vegetales hospedantes; entre ellas al aguacate (*Persea americana* Mill., Lauraceae), que es especialmente susceptible al complejo KSHB/FD. Su expansión en California, el estado productor de aguacate más importante en los EUA, ha incrementado aceleradamente desde el 2014. A pesar de la alta susceptibilidad del cultivo de aguacate a la invasión y patogenicidad de los complejos PSHB/FD y KSHB/FD reportada en diferentes regiones productoras del mundo, se desconocen los mecanismos químicos y genéticos de defensa vegetal que pudieran generar resistencia contra la enfermedad. En este estudio se analizó el efecto ocasionado por esta enfermedad sobre la expresión de genes involucrados en la defensa vegetal y en la biosíntesis de metabolitos, a partir de datos transcriptómicos obtenidos por RNA-Seq de árboles de aguacate sanos y árboles infectados por *Fusarium kuroshium* provenientes de un huerto en California.

INTRODUCCIÓN

El comercio globalizado hoy en día es muy dinámico, diariamente se transporta una alta cantidad de productos en todo el mundo. Esta actividad ha contribuido a la dispersión de diversos organismos a áreas nuevas y en algunos casos ha sido el principio de desafíos ecológicos, económicos y ambientales importantes (Hulme, 2009). Tanto en México como en el mundo, se han introducido especies que causan impactos importantes en ecosistemas tanto agrícolas como forestales (Hulme, 2009). Recientemente, dos especies de escarabajos barrenadores polífagos invasivos de origen asiático, *Euwallacea* sp. nr. *fornicatus* (Polyphagous Shot Hole Borer, PSHB) *y Euwallacea kuroshio* (Kuroshio Shot Hole Borer, KSHB), que forman complejos simbióticos con hongos necrosantes del género *Fusarium*, han ocasionado importantes pérdidas de producción de aguacate e importantes daños en zonas forestales y parques urbanos en California al transmitir la enfermedad denominada marchitez por Fusarium (FD) (Mendel *et al.*, 2012; Eskalen *et al.*, 2012, 2013) y se ha extendido hacia la frontera mexicana llegando finalmente en 2016 a Tijuana, Baja California, México con posibles hospedantes presentes alrededor de la zona de detección como arce, aguacate, fresno, guayaba, ricino, naranja, olivo, palmera Washingtonia, pimiento brasileño y uvas (García-Avila & Trujillo-Arriaga, 2016).

La enfermedad FD afecta más de 300 especies vegetales; incluyendo el aguacate (*Persea americana Mill*., Lauraceae) (Eskalen *et al.*, 2013). Este cultivo es de alta importancia para México,

debido a que es el principal productor, exportador y consumidor mundial (FAO, 2018). El aguacate es una planta de cultivo con frutos oleaginosos (Scora & Bergh, 1989; Sánchez-Colín *et al.*, 1998) con grandes beneficios nutricionales (Schaffer *et al.*, 2013) que es sensible al frio y a la humedad ambiental y se adapta a precipitaciones de alrededor de 1200 mm y a una altitud de entre 800 a 2500 m (SAGARPA, 2017), muy parecido a las características que requiere *Euwallacea* spp. para sobrevivir en todas sus etapas (Walgama & Zalucki, 2007; Kumar *et al.*, 2011; Ministry-of-agriculture-food-and-environment, 2015). Al tener una gran variedad de hospedantes, estas plagas podrían expandirse eventualmente en México y afectar así áreas tanto forestales como productoras de aguacate, siendo un peligro potencial para este cultivo.

Existen diferentes prácticas para controlar parcialmente la expansión del escarabajo. Se suelen hacer monitoreos extensos con trampas e inspecciones visuales, eliminación de ramas infestadas delgadas y de ramas marchitas, tratamiento de cortes en la rama principal con Bifentrina (piretroide) y el área de la corteza alrededor de las lesiones, saneamiento usando solarización o triturado, o control directo con insecticidas de contacto o sistémicos, pero, a futuro se busca identificar agentes de control biológico y/o inducir resistencia a las plantas como una medida efectiva de control (Mendel & Freeman, 2015; Umeda *et al.*, 2016; Dunlap *et al.*, 2017; López-Buenfil *et al.*, 2017); por ello es importante estudiar y comprender las respuestas de la planta a la enfermedad a niveles específicos químicos y moleculares. Las plantas han desarrollado diversos mecanismos de defensa que les permiten responder favorablemente ante sus patógenos. Uno de estos mecanismos es la producción inducible de metabolitos de defensa a través del metabolismo secundario.

En este estudio, analizamos el efecto ocasionado por la enfermedad FD sobre la expresión de genes de defensa vegetal y de genes potencialmente involucrados en la producción de metabolitos involucrados en sus respuestas de defensa, estos datos fueron contrastados con un análisis metabolómico realizado previamente (Aguirre-Pérez, en preparación). Se realizó el análisis bioinformático comparativo a partir de datos transcriptómicos obtenidos por RNA Seq de árboles de aguacate asintomáticos y árboles con síntomas de infección por *Fusarium kuroshium*, ubicados en una huerta en el condado de San Diego, California; de este modo, se obtuvieron los perfiles de expresión de cada grupo de plantas para posteriormente ser comparados con el análisis metabolómico. Estudios comparativos entre plantas con y sin síntomas de alguna enfermedad permiten predecir cambios funcionales relevantes a partir de datos de expresión génica, la evidencia de expresión diferencial es la que nos permite obtener información de datos de secuencias anónimas (Nowak, 1995; Adams, 1996; Bains, 1996). El análisis transcriptómico junto con el análisis funcional dan pie a la obtención de nuevos biomarcadores y tratamientos asociadas con diversas enfermedades (Merdad *et al.*, 2015).

ANTECEDENTES

Persea americana Mill.

El aguacate (*Persea americana* Mill.) es una planta arbórea de cultivo con frutos oleaginosos que pertenece al clado Magnoliidae, un linaje basal de plantas con flores, y es miembro de la familia Lauraceae (Scora & Bergh, 1989; Sánchez-Colín *et al.*, 1998). Es sensible al frío y a la humedad ambiental, por lo cual se recomienda cultivar en zonas libres de heladas y de vientos calurosos y secos así como con una precipitación de alrededor de 1200 mm con una altitud de entre 800 a 2500 m (SAGARPA, 2017). El fruto del aguacate ha sido descrito como la fruta más nutritiva (Schaffer *et al.*, 2013) debido a que, en la madurez, su pulpa contiene aproximadamente 20% de ácidos grasos benéficos, 6% de carbohidratos, 2% de proteínas y precursores de vitaminas y antioxidantes como carotenoides y vitaminas E, C, B2, B12, B1, K y D (Schaffer *et al.*, 2013).

En la actualidad existen ocho variedades bien definidas de *Persea americana*, de las cuales sólo tres pertenecen al aguacate comercial (Bergh & Ellstrand, 1986; Rendón-Anaya *et al.*, 2019). La raza mexicana, *P. americana* var. *drymifolia* (Schltdl. & Cham) S.F. Blake, que se adapta a las tierras altas tropicales; la guatemalteca, *P. americana* var. *guatemalensis* (L.O. Williams) Scora, que se adapta a elevaciones medias en los trópicos; y la raza de las Indias Occidentales, *P. americana* var. *americana*, que está adaptada a los trópicos húmedos de las tierras bajas (Popenoe, 1941). La producción de aguacate se basa en seleccionar dentro de estas tres razas y en los híbridos entre ellos (Chanderbali *et al.*, 2008) e injertar cultivares en porta injertos por lo general de razas mexicanas y guatemaltecas (Scora & Bergh, 1989).

En México y América Central, el aguacate ha sido cultivado y consumido durante al menos 9,000 años (Smith, 1966, 1969). Actualmente, México es el principal productor (contribuye con alrededor del 33% de la producción mundial total), exportador (el 54.10% de la producción es exportada otros países) y consumidor en el mundo de aguacate (FAO, 2018). En 2016, fue cultivado en 180,536 ha a nivel nacional y produjo más de 1.89 millones de toneladas (FAO, 2018) de las cuales fueron exportadas aproximadamente 1.02 millones de toneladas con un valor de 2,227,25 millones de dólares (precios de 2016; SAGARPA, 2017). Al ser un cultivo importante en términos ecológicos, culturales y económicos para el país, incrementar el entendimiento sobre su capacidad de respuesta a sus enfermedades más destructivas podría contribuir a desarrollar estrategias para mantener su producción o incluso a incrementarla de manera ambientalmente más amigable.

Marchitez por Fusarium

Descripción de la enfermedad, origen y propagación

La marchitez por Fusarium (FD) es provocada por una asociación simbiótica específica, donde ambos organismos se benefician, entre los escarabajos barrenadores polífagos de tronco y ramas *Euwallacea kuroshio* (Kuroshio Shot Hole Borer, KSHB) y *Euwallacea* sp. nr. *fornicatus* (Polyphagous Shot Hole Borer, PSHB) (Figura 1) con diferentes hongos, entre ellos dos del género *Fusarium* (Eskalen *et al.*, 2013; Kasson *et al.*, 2013; O'Donnell *et al.*, 2015; Stouthamer *et al.*, 2017). Uno de estos hongos, *Fusarium euwallaceae* es uno de los simbiontes de PSHB (Freeman *et al.*, 2013), y los hongos *Fusarium kuroshium* y *Graphium kuroshium*, del escarabajo KSHB (Na *et al.*, 2018). Estos complejos ambrosiales son una plaga perenne, con una distribución en un rango de elevación de 200 a 1400 m (Walgama, 2008), están adaptados a temperaturas entre 15 - 32ºC para todas las etapas de su ciclo de vida y a una humedad relativa de 75 a 95% (Walgama & Zalucki, 2007; Kumar *et al.*, 2011; Ministry-of-agriculture-food-and-environment, 2015).

Figura 1. *Euwallacea* sp. nr. *fornicatus.* Del lado derecho se encuentra una hembra madura (2.5 mm) y del lado izquierdo un macho joven (Mendel *et al.*, 2012).

Esta enfermedad ataca árboles sanos de diversos ecosistemas, tanto agrícolas como urbanos, botánicos y silvestres (incluidos los bosques nacionales) (Eskalen *et al.*, 2014). Dos de las especies de escarabajos del género *Euwallacea*, PSHB y KSHB, se han reportado en California (E.U.), atacando a más de 300 especies vegetales pertenecientes a 58 familias de plantas; los cultivos agrícolas incluyen al aguacate (*Persea americana* Mill., Lauraceae), naranja (*Citrus sinensis* [L.] Osbeck; Rutaceae), níspero (*Eriobotrya japonica* Lindley; Rosaceae), macadamia (*Macadamia integrifolia* Maiden & Betche; Proteaceae), olivo (*Olea europaea* L., Oleaceae), pera (*Prunus persica* [L.] Stokes; Rosaceae) y uvas (*Vitis vinifera* L.; Vitaceae) (Eskalen *et al.*, 2013; Eskalen, 2018a). En aguacate, fue reportada por primera vez en este mismo estado en el año 2012 (Eskalen *et al.*, 2012).

El origen de los escarabajos PSHB y KSHB es asiático (Stouthamer *et al.*, 2017). PSHB ha sido registrado en Tailandia, Vietnam, China e Israel y ambos han sido localizados tanto en Taiwán como en Okinawa (Japón), propagándose hasta California (E.U.) (Stouthamer *et al.*, 2017). En este estudio nos enfocamos en KSHB, que tiene como simbionte a *Fusarium kuroshium*, hongo con el que se encuentran infectadas nuestras muestras. Recientemente KSHB migró hacía el sur de California (Stouthamer *et al.*, 2017) extendiéndose hacia la frontera mexicana (Eskalen *et al.*, 2013; Graham, 2016) (Figura 2) hasta que finalmente, en 2016, fue reportado en Tijuana, Baja California, México a 200 m de la frontera con los Estados Unidos en una zona turística (García-Avila & Trujillo-

Arriaga, 2016). Existen posibles hospedantes presentes en las 1,600 ha que rodean el sitio de detección, incluyendo arce, aguacate, fresno, guayaba, ricino, naranja, olivo, palmera Washingtonia, pimiento brasileño y uvas, pero ninguno ha mostrado evidencia de daño o síntomas de infestación (García-Avila & Trujillo-Arriaga, 2016).

Figura 2. Distribución de escarabajos Polyphagous Shot Hole Borer (PSHB, rojo) y Kuroshio Shot Hole Borer (KSHB, azul) en California. Marcado con una flecha se encuentra el primer caso en Tijuana, Baja California, México del 2016https://ucanr.edu/sites/pshb/pest-overview/ishb-fd-distribution-in-california/ (Eskalen, 2018a).

No se sabe cómo estos escarabajos de origen asiático han alcanzado nuevas áreas (Ministry-of-agriculture-food-and-environment, 2015), pero existen algunas teorías. Una de ellas y la más probable hasta ahora, es que fueron trasladados a través de embalajes de madera (Wood Packaging), al ser reubicadas en diferentes lugares por el comercio (Ministry-of-agriculture-food-and-environment, 2015). Otra posibilidad es a través de contenedores marítimos, ya que hay datos antiguos sobre detecciones de *Euwallacea* spp. en ellos (20 insectos en 3 contenedores) (Stanaway *et al.*, 2001), sin embargo, no está clara la detección de los escarabajos y habría que estudiarse más. Y, por último, la teoría de dispersión por viento que, aunque hay divergencias en la literatura sobre la capacidad de vuelo en ambos casos (el escarabajo y el hongo), sólo se considera local (Ministry-of-agriculture-food-and-environment, 2015).

Ciclo de infección de marchitez por Fusarium y síntomas

La marchitez por Fusarium (FD) inicia tras la invasión de escarabajos hembra adulto que lleva el hongo simbionte en estructuras bucales especializadas llamadas micangias o micetangias. Las hembras colonizadoras crean galerías desde la corteza del árbol, donde depositan sus huevos (Figura 3) e inoculan al hongo en el xilema, el cual servirá como alimento a larvas y adultos (Browne, 1961; Calnaido, 1965; Mendel *et al.*, 2012; Walgama, 2012; Kasson *et al.*, 2013). Al invadir el sistema vascular de la planta, el crecimiento del hongo no permite el paso de agua o nutrimentos de la raíz al resto del árbol (Eskalen *et al.*, 2013). Las hembras permanecen en las galerías durante varios días

después del nacimiento, el apareamiento se lleva a cabo dentro de las galerías entre los descendientes varones y hembras de las hembras progenitoras (endogamia políginas) (Walgama, 2012). Posteriormente, los escarabajos hembra maduros, que contienen micelio del hongo simbionte en sus micangias, emergen a través del túnel de entrada original y vuelan a nuevas plantas hospedantes para continuar con el ciclo invasivo (Eskalen *et al.*, 2013; CABI, 2019). Por otra parte, los machos adultos son escarabajos no voladores y nunca abandonan la galería (Browne, 1961; Walgama, 2012).

Figura 3. Diagrama del ciclo de infección de la Marchitez por Fusarium basado en (Browne, 1961; Calnaido, 1965; Mendel *et al.*, 2012; Walgama, 2012; Eskalen *et al.*, 2013; Kasson *et al.*, 2013; CABI, 2019).

La enfermedad FD suele afectar a árboles de 1 a 30 años de edad con tallos y ramas de 2 a 30 cm (Mendel *et al.*, 2012). Los síntomas más evidentes son la decoloración alrededor del área de la corteza en la zona de invasión y un exudado azucarado denominado perseitol (Liu *et al.*, 2002; Mendel *et al.*, 2012; Freeman *et al.*, 2013) (Figura 4). A la par, se observa la marchitez de las ramas y la decoloración de las hojas; otros síntomas menos visibles son la tinción parda del xilema y la necrosis causada por el hongo que puede desplazarse hasta 150 cm a través de las traqueidas; además, en la zona donde se crean las galerías es muy común que exista rompimiento de las ramas causando muerte tanto en árboles jóvenes como maduros (Mendel *et al.*, 2012; Eskalen *et al.*, 2012).

Figura 4. Exudado azucarado característico en una rama de aguacate, causado por la actividad de escarabajos Polyphagous Shot Hole Borer /marchitez por Fusarium (PSHB/FD) (Eskalen, 2018b).

Técnicas para controlar la expansión de la marchitez por Fusarium

Existen diferentes tipos de prácticas para controlar la expansión del escarabajo. Se suelen hacer monitoreos extensos con trampas e inspecciones visuales (López-Buenfil *et al.*, 2017). Al mes de junio de 2017, se habían realizado 26,624 revisiones de las trampas establecidas en el estado de Baja California y se habían inspeccionado 4,027 sitios en los cuales se caracterizaron 25,759 árboles hospedantes de *Euwallacea* spp*.*, de estos, 10,153 presentaron evidencias de daño causado por la plaga y síntomas de marchitez causado por la presencia de *Fusarium* (López-Buenfil *et al.*, 2017).

Según Mendel y Freeman (2015), el manejo recomendado después del monitoreo es la eliminación de ramas infestadas delgadas (<6 cm de diámetro) y de ramas marchitas, posteriormente tratar los cortes en la rama principal con Bifentrina (piretroide) y el área de la corteza alrededor de las lesiones en ramas de un diámetro mayor a 6.35 cm para evitar ataques posteriores (Mendel & Freeman, 2015). Además, se puede llevar a cabo el saneamiento usando solarización o triturado, o el control directo del agente patógeno con insecticidas de contacto o sistémicos. Sin embargo, a futuro se busca identificar agentes de control biológico y/o inducir resistencia a las plantas afectadas (Umeda *et al.*, 2016; Dunlap *et al.*, 2017; López-Buenfil *et al.*, 2017).

Una de las herramientas importantes al estudiar la resistencia y defensa de las plantas es la transcriptómica, la cual se define como el estudio de secuencias génicas transcritas (Goodman, 2002). El análisis del transcriptoma evalúa la expresión de las moléculas de ARN producidas por una célula en un conjunto de condiciones dado (Xiong, 2006). Este análisis nos da como resultado el alto rendimiento de todos los genes expresados, por lo cual, nos ayuda a comprender cómo los conjuntos de genes trabajan juntos para formar vías metabólicas, reguladoras y de señalización dentro de la célula (Xiong, 2006).

En ausencia de pistas funcionales en los genes, la evidencia de expresión diferencial en dos condiciones contrastantes es una herramienta importante que debe priorizarse para la explotación de datos de secuencias anónimas en investigaciones básicas y farmacéuticas (Nowak, 1995; Adams, 1996; Bains, 1996) El análisis transcriptómico muestra patrones genéticos coexpresados y co-regulados, y permite la determinación de funciones genéticas que no se caracterizaron antes (Xiong, 2006), por lo cual, podría conducir a una nueva visión de los biomarcadores y tratamientos asociados con diversas enfermedades (Merdad *et al.*, 2015).

En este estudio, nos enfocamos en predecir la expresión de genes de defensa vegetal y la producción de metabolitos potencialmente involucrados en la respuesta a FD a partir de datos transcriptómicos de árboles de aguacate sanos e infectados por *Fusarium kuroshium*, ubicados en una huerta en el condado de San Diego, California, para poder entender detalladamente la resistencia y defensa de las plantas a esta enfermedad.

Defensa de patógenos en plantas

Existen diversas moléculas de señalización endógena vegetal que están involucradas en la respuesta a patógenos, como las hormonas. Algunas de ellas son: el etileno (ET), el ácido jasmónico (JA) y el ácido salicílico (SA) (Cheong & Do-Choi, 2003; Thatcher *et al.*, 2005; Nakano *et al.*, 2006; Fan *et al.*, 2007; Loake & Grant, 2007). Tras la percepción de la señal, ocurre la activación sucesiva de componentes de transducción de señales en cascada que conduce eventualmente a la expresión de genes protectores, que conlleva a la síntesis de moléculas de defensa de la planta, como las proteínas relacionadas con la patogénesis (PR) (Thatcher *et al.*, 2005) (proteínas solubles que se acumulan en plantas infectadas por virus, viroides, bacterias y hongos (Loon, 1985)), las glutatión S-transferasas (GTS), las peroxidasas, los inhibidores de la proteinasa y la producción de metabolitos antimicrobianos secundarios (Thatcher *et al.*, 2005).

Metabolismo Secundario

Los compuestos producidos en el metabolismo secundario, funcionan de múltiples maneras interfiriendo en las interacciones planta-entorno, planta-insecto, planta-microorganismo e incluso planta-planta (Dixon, 2001; Harborne, 2001). En este estudio, nos concentramos en la interacción planta-microorganismo. En esta interacción, la producción de metabolitos secundarios por la planta tiene funciones importantes en el sistema de defensa vegetal, ya que algunos de estos compuestos son producidos ante el ataque de patógenos llevando a cabo la señalización (Verpoorte & Memelink, 2002).

Identificación de compuestos químicos en árboles asintomáticos y sintomáticos a la infección por *Fusarium kuroshium*.

En el mes de noviembre de 2015, nuestro grupo de trabajo, en colaboración con investigadores del Instituto de Ecología (INECOL) y de la Universidad de California Riverside, llevaron a cabo un muestreo en un huerto localizado en el Condado de Escondido, California EUA con reporte previo y verificado de la presencia de FD para posteriormente identificar los metabolitos acumulados a causa de la enfermedad. Para lograr esto, se obtuvieron los extractos orgánicos de la albura (donde suele atacar el escarabajo) de cinco árboles de aguacate asintomáticos (visualmente sin ninguna enfermedad, incluida la marchitez por Fusarium) y cinco árboles con signos del ataque por KSHB y síntomas evidentes de FD (Aguirre-Pérez *et al.,* en preparación). Todos los compuestos fueron identificados por espectrometría de masas, primero se realizó una agrupación por análisis de componentes principales (PCA) considerando la composición química en modo positivo y modo negativo, y se pudo observar que los compuestos encontrados en árboles asintomáticos se diferencian de los compuestos de árboles con síntomas de FD al agruparse en diferentes extremos, en ambos modos (Figuras 5 y 6). Después, se generaron gráficos de dispersión (S-Plot) de las Proyecciones Ortogonales a Estructuras Latentes - Análisis Discriminante (OPLS-DA), tanto de los

datos en modo positivo como en modo negativo (Figuras 7 y 8). En el grupo de extractos provenientes de árboles asintomáticos se identificaron diferencialmente la acumulación de los compuestos Avocadyne 2-acetato ($C_{19}H_{34}O_4$) (Figura 9) y diversos ácidos grasos hidroxilados (Hydroxylated Fatty Acids, HFA) (Cuadro 1). Por otra parte, en los extractos de árboles sintomáticos, la mayor acumulación identificada fue de carbohidratos de siete carbonos (Cuadro 1).

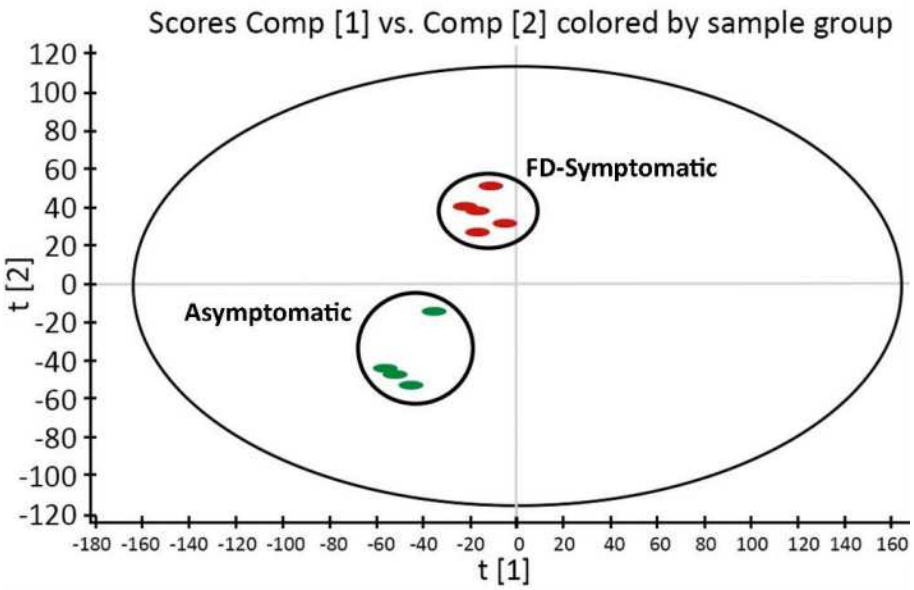

Figura 5. Agrupación de PCA considerando la composición química en modo positivo de árboles sanos y enfermos (Aguirre-Pérez *et al*., en preparación).

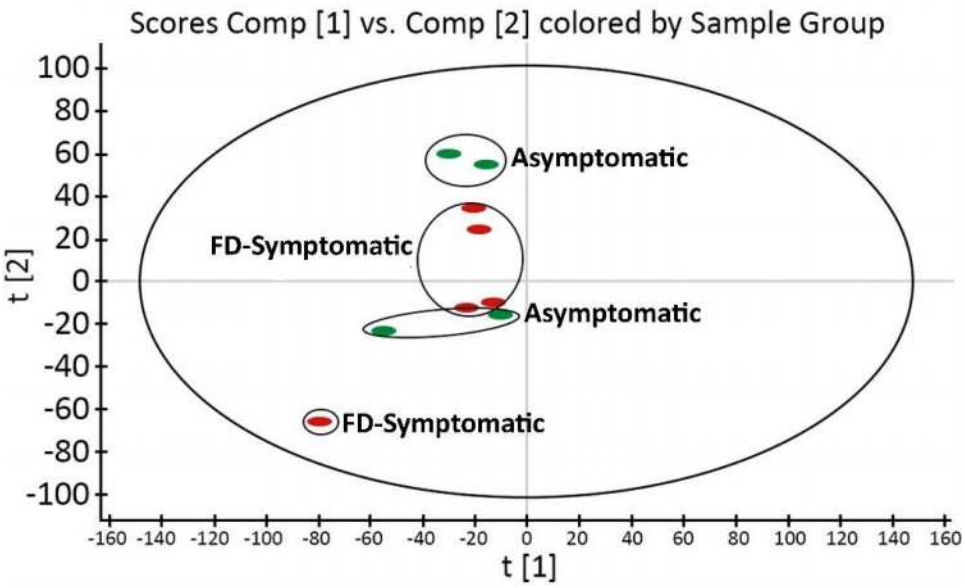

Figura 6. Agrupación de PCA considerando la composición química en modo negativo de árboles sanos y enfermos (Aguirre-Pérez et al., en preparación).

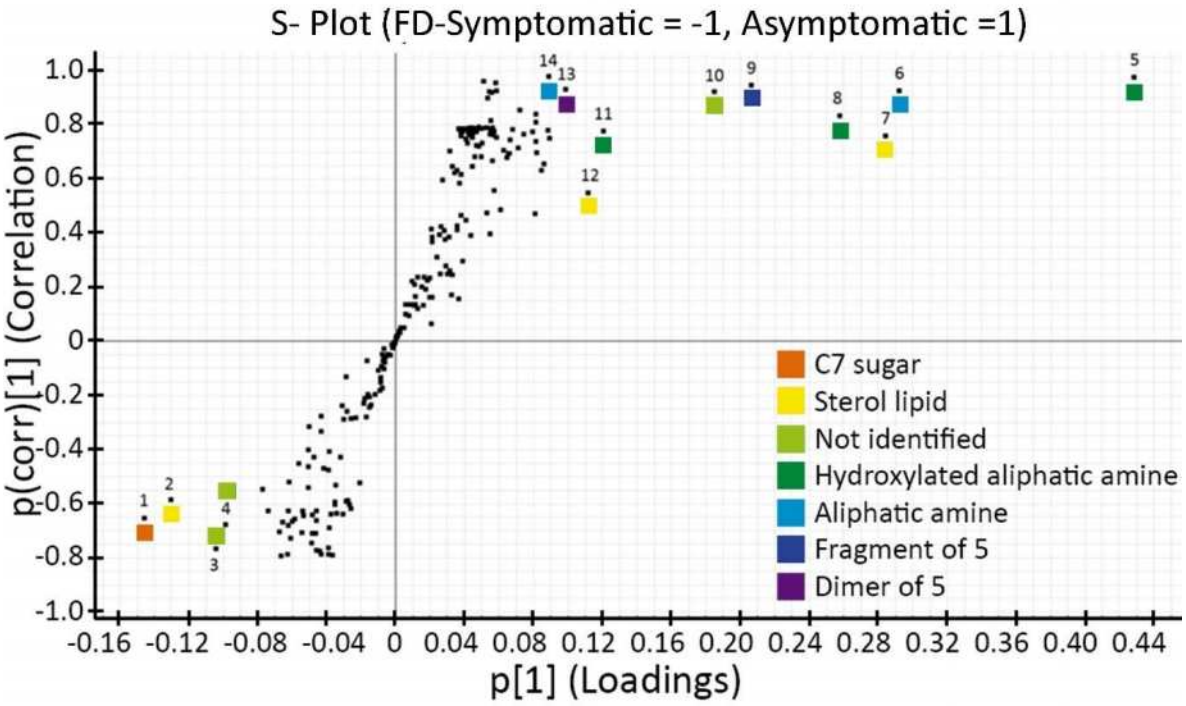

Figura 7. S-Plot del OPLS-DA realizado con los datos en modo positivo de árboles sanos y enfermos (Aguirre-Pérez *et al.,* en preparación).

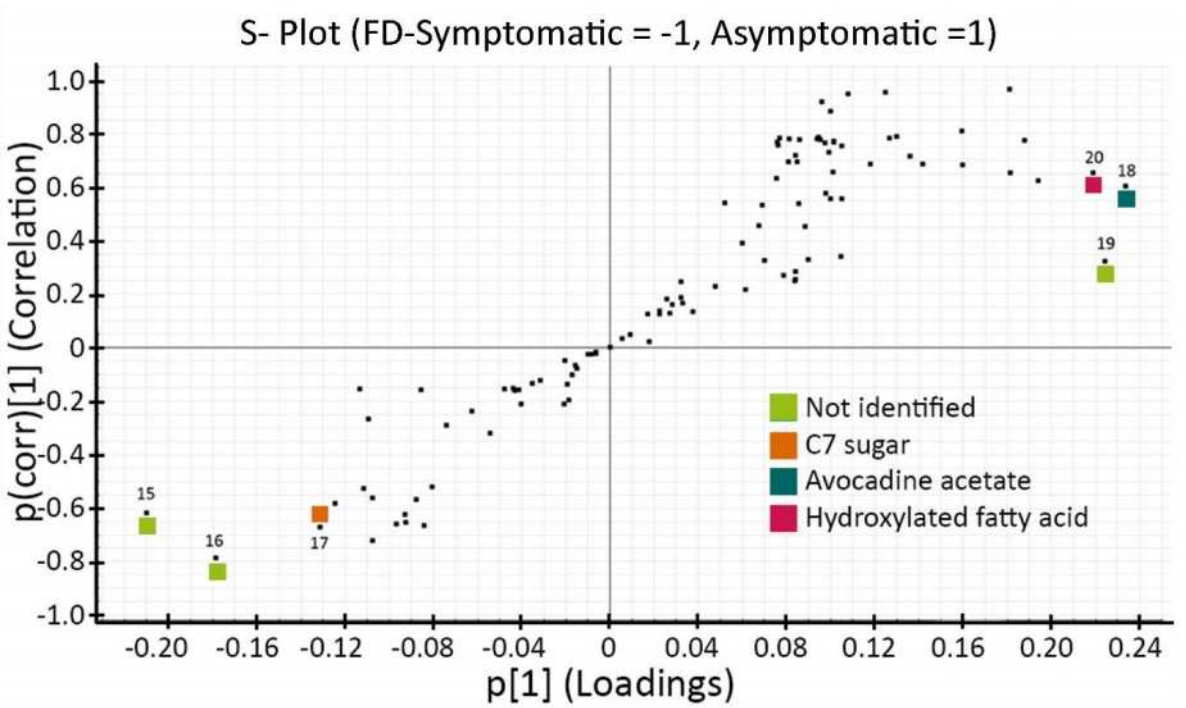

Figura 8. S-Plot del OPLS-DA realizado con los datos en modo negativo de árboles sanos y enfermos (Aguirre-Pérez *et al.,* en preparación).

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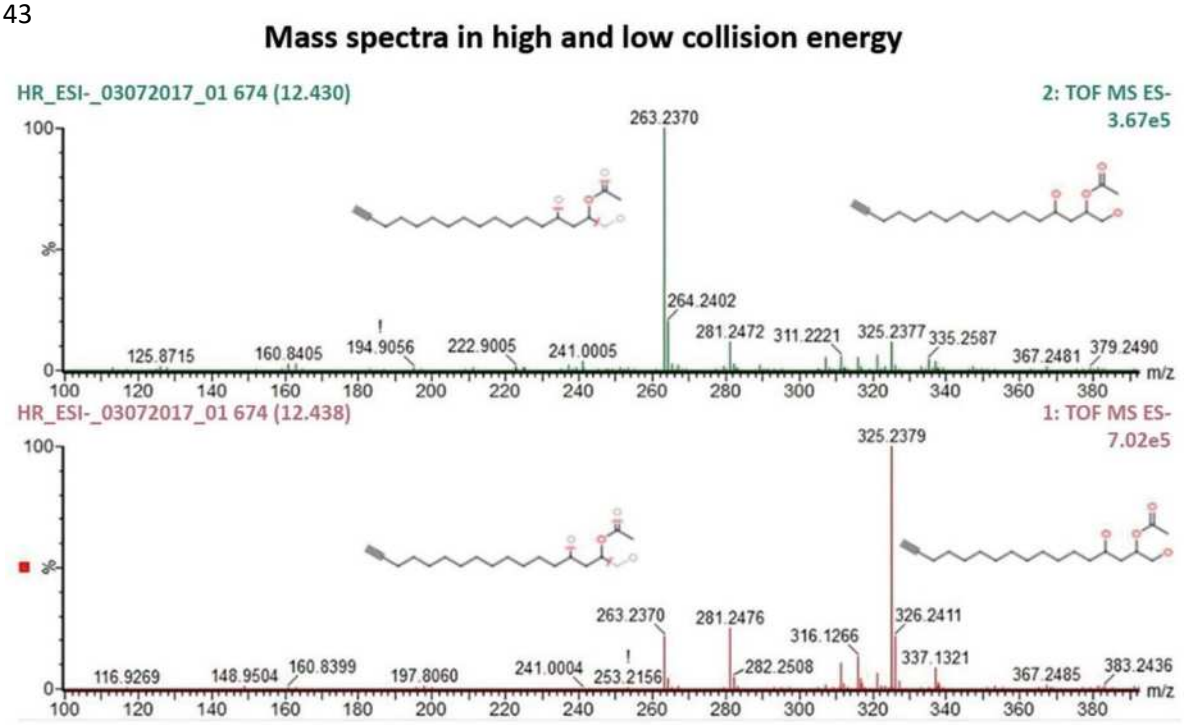

Figura 9. Forma química de Avocadyne 2-acetato, acetogenina y alcohol graso (FA) exclusivo de árboles sanos (Aguirre-Pérez *et al.,* en preparación).

Cuadro 1. Marcadores químicos encontrados en el OPLS-DA hecho entre árboles sanos y enfermos en modo positivo y negativo (Aguirre-Pérez *et al.,* en preparación).

Mode	Sample	#	Rt (min)	m/z	Formula	Ion	Error (ppm)	Candidates
Positive	FD-symptomatic	1	0.4	233.0631	$C_7H_{14}O_7Na^+$	$[M+Na]^+$	-2.6	C7 sugar
		2	14.77	413.3771	$C_{29}H_{49}O^+$	$[M+H]^+$	-2.9	Sterol lipid
		3	14.56	599.4648	$C_{36}H_{64}O_5Na^+$	$[M+Na]^+$	-0.5	Not identified
		4	13.73	309.2419	$C_{19}H_{33}O_3^+$	$[M+H]^+$	-3.6	Not identified
	Asymptomatic	5	9.78	230.2484	$C_{14}H_{32}NO^+$	$[M+H]^+$	0	Hydroxylated aliphatic amine
		6	9.58	200.2377	$C_{14}H_{30}N^+$	$[M+H]^+$	-0.5	Aliphatic amine
		7	12.99	393.315	$C_{28}H_{41}O^+$	$[M+H]^+$	-1.8	Sterol lipid
		8	12.94	397.415	$C_{25}H_{53}N_2O^+$	$[M+H]^+$	-2	Hydroxylated aliphatic amine

		9	9.7	214.2534	$C_{14}H_{32}N^+$	$[M+H]^+$	-0.5	Fragment of 5
		10	14.1	599.4647	$C_{36}H_{64}O_5Na^+$	$[M+Na]^+$	-0.7	Not identified
		11	13.39	453.4769	$C_{29}H_{61}N_2O^+$	$[M+H]^+$	-3.3	Hydroxylated aliphatic amine
		12	13.03	413.3773	$C_{29}H_{49}O^+$	$[M+H]^+$	-2.4	Sterol lipid
		13	9.77	459.4876	$C_{28}H_{63}N_2O_2^+$	$[M+H]^+$	-3	Dimer of 5
		14	13.65	256.2997	$C_{17}H_{38}N^+$	$[M+H]^+$	-2.7	Aliphatic amine
Negative	**FD-symptomatic**	15	14	633.4726	$C_{38}H_{65}O_7^-$	$[M-H]^-$	-0.6	Not identified
		16	12.93	321.2091	$C_{19}H_{29}O_4^-$	$[M-H]^-$	7.8	Not identified
		17	0.4	211.0805	$C_7H_{15}O_7^-$	$[M-H]^-$	-6.2	C7 sugar
	Asymptomatic	18	12.42	325.2374	$C_{19}H_{33}O_4^-$	$[M-H]^-$	-1.5	Avocadine acetate
		19	12.32	367.2483	$C_{21}H_{36}O_5^-$	$[M-H]^-$	-0.3	Not identified
		20	9.3	303.2165	$C_{16}H_{31}O_5^-$	$[M-H]^-$	-2	hydroxylated fatty acid

Acetogeninas: Avocadyne 2-acetato ($C_{19}H_{34}O_4$)

El compuesto Avocadyne 2-acetato ($C_{19}H_{34}O_4$) es una molécula insoluble (en agua) y relativamente neutra, es una acetoginina (Kashman *et al.*, 1969a, b) que pertenece al grupo de alcoholes grasos (Fatty Alcohol, FA) con una cola alifática de 13 a 21 átomos de carbono (TMIC The Metabolomics Innovation Centre) (Figura 10), su ruta biosintética no es bien conocida pero se sabe que la última reacción para que se forme un FA se realiza con ayuda de la enzima Fatty acyl-CoA reductasa (FAR) (Mudge *et al.*, 2018). Las acetogeninas son compuestos orgánicos que pertenecen a los alcoholes grasos de cadena larga y son derivados de ácidos grasos que típicamente contienen una cadena alifática de carbono impar (17, 19 o 21) y un grupo acetoxi que contribuye con dos carbonos adicionales (Kashman *et al*., 1969b). Estos compuestos han mostrado diversas bioactividades, como insecticidas (Kobiler *et al.*, 1993; Rodríguez-Saona & Trumble, 1996; Oberlies *et al.*, 1998; Rodríguez-Saona *et al.*, 2000), inhibición de la acetil-CoA carboxilasa (Hashimura *et al.*, 2001), producción de óxido nítrico y superóxido en las células (Kim *et al.*, 2000a, b), efectos proapoptóticos contra varias líneas celulares de cáncer (Oberlies *et al.*, 1998; Butt *et al.*, 2006; Yasir *et al.*, 2010; Brooke *et al.*, 2011), y recientemente, una actividad prometedora contra líneas celulares de Leucemia

mieloide aguda (Lee *et al.*, 2015), así como propiedades antimicrobianas, esporostáticas y bactericidas (de alto interés en la industria alimentaria, por su potencial como aditivos alimentarios) (Neeman *et al.*, 1970; Domergue *et al.*, 2000; Dharmaratne *et al.*, 2012; Hernandez-Brenes *et al.*, 2012; Rodríguez-Sánchez *et al.*, 2013a; Salinas-Salazar *et al.*, 2016), antioxidantes, antiplaquetarios y antitrombóticos (Hernandez-Brenes *et al.*, 2012; Rodríguez-Sánchez *et al.*, 2013a, b; Rodríguez-Sánchez *et al.*, 2015).

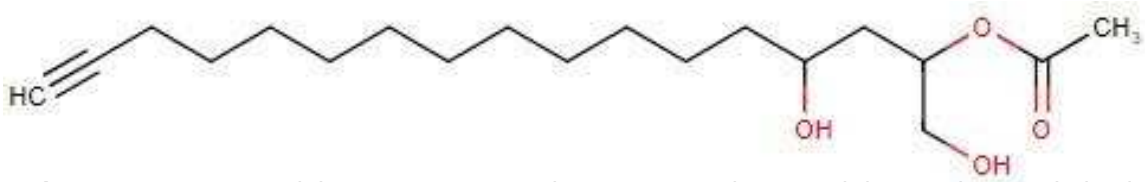

Figura 10. Estructura del compuesto Avocadyne 2-acetato ($C_{19}H_{34}O_4$) (TMIC The Metabolomics Innovation Centre).

Ácidos Grasos Hidroxilados

Los Ácidos Grasos Hidroxilados (Hydroxylated Fatty Acids, HFA) son moléculas que pueden ser saturadas o insaturadas, con grupos funcionales hidroxilo unidos a la cadena principal (Brondz, 2004), que parecen jugar un papel importante en la actividad contra hongos específicos (Hou & Forman-Iii, 2000). En cuanto a la biosíntesis de los HFAs, se conoce el gen LCR que codifica al citocromo P450 monooxigenasa, YP86A8, que hidroxila los ácidos grasos de C12 a C18 en el carbono-ω (Weber, 2002).

Azúcares de 7 carbonos

Los carbohidratos de siete carbonos (C7) son uno de los principales azúcares móviles del floema en el aguacate y es posible que estos compuestos sean parte del mecanismo que inhibe la maduración del fruto en asociación con la homeostasis hormonal y la señalización (Liu *et al.*, 2002). Su vía metabólica no es muy bien conocida pero hasta la fecha se conocen tres reacciones que intervienen en ella: a) Reacción de aldolasa: eritrosa-4-P + dihidroxiacetona-P ↔ sedoheptulosa-1,7-bis-P; b) Reacción de transcetolasa: xilulosa-5-P + ribosa-5-P ↔ sedoheptulosa-7-P + gliceraldehído-3-P; o c) una reacción transaldolasa: fructosa-6-P + eritrosa-4-P ↔ sedoheptulosa-7-P + gliceraldehído-3-P, que son parte de la ruta metabólica de los monosacáridos (Liu *et al.*, 2002).

PLANTEAMIENTO DEL PROBLEMA

La expansión de la marchitez por Fusarium ha incrementado rápidamente y el daño hacia el cultivo de aguacate en México es un riesgo potencial. *Euwallacea* spp*.* tiene una alta ubicuidad de hospedantes, por lo cual podría desplazarse hasta las regiones productoras en el país. El aguacate es un cultivo cultural, socialmente importante para la economía mexicana, por lo cual, es importante encontrar nuevas alternativas para el tratamiento y control de esta enfermedad. En este sentido, descubrir el efecto ocasionado por la marchitez por Fusarium sobre la expresión de genes de defensa vegetal y la producción de metabolitos potencialmente involucrados en la misma, podría ser un paso importante para entender a detalle cómo responde la planta al ser infectada y, por ende, a encontrar nuevos métodos de control del patógeno.

Hipótesis

Los genes asociados con la defensa vegetal y con la síntesis de lípidos y carbohidratos se expresan diferencialmente en árboles de aguacate sanos y en árboles con síntomas de marchitez por Fusarium (FD).

OBJETIVOS

Objetivo general

Predecir las rutas metabólicas del aguacate involucradas en la respuesta a marchitez por Fusarium a partir de datos transcriptómicos de árboles de California infectados por *Fusarium kuroshium.*

Objetivos específicos

- Conocer las rutas metabólicas que se expresan diferencialmente en árboles con o sin síntomas de FD.
- Comparar los perfiles de expresión global de ambas condiciones.
- Asociar los perfiles de expresión global de genes con algunos metabolitos diferencialmente acumulados con potencial actividad antifúngica.

Las secciones Material y Métodos, Resultados y Discusión se encuentran en los Capítulos II y III redactados en inglés. En este estudio se tomaron muestras de dos secciones del árbol, debajo de la corteza (albura) donde suele atacar el escarabajo (Subcapítulo I), y de los ápices de ramas con o sin síntomas de muerte regresiva (Subcapítulo II).

CHAPTHER II

Fusarium dieback induces the expression of genes related to defense responses and metabolic biosynthetic pathways in avocado

ABSTRACT

Transport and trade of diverse products in the world have contributed to dispersion of a wide organism variety to new areas. Polyphagous trunk and branch borer beetles like *Euwallaceae* sp. nr. (PSHB) and *Euwallacea* sp. nr *fornicatus* (PSHB) and *Euwallacea kuroshio* (KSHB) are a current example of invasive pests. These beetles are of Asian origin and have a high capacity for expansion since they are associated symbiotically with some fungi, including two from genus *Fusarium* whose cause a necrotic disease known as Fusarium Dieback (FD). This disease affects more than 300 host plant species; among them avocado (*Persea americana Mill*., Lauraceae), which is especially susceptible to KSHB/*Fusarium kuroshium* complex. Its expansion in California (the most important avocado producing state in the US) has increased rapidly since 2014. Despite the high susceptibility of avocado to invasion and pathogenicity to PSHB / FD and KSHB / FD complexes reported in different producing regions in the world, the chemical and genetic mechanisms of plant defense that could generate resistance against the disease are unknown. In this study, the effect of FD is analyzed at the gene expression level with special emphasis in plant defense and metabolite biosynthesis functions. Transcriptomic data were generated by RNA-Seq and comparing the expression profiles of asymptomatic and FD-symptomatic avocado trees from an orchard in Escondido California.

INTRODUCTION

Nowadays globalized trade is very dynamic, a large number of products are transported around the world. These activities have contributed to a wide expansion of a high diversity of invasive organisms to new areas and, in some cases, causing important ecological, economic and environmental challenges (Hulme, 2009). Both in Mexico and in the world, several species that cause important impacts on both agricultural and forestry ecosystems have been introduced (Hulme, 2009). Recently two species of invasive polyphagous borer beetles of Asian origin, *Euwallacea* sp. nr. *fornicatus* (Polyphagous Shot Hole Borer, PSHB) and *Euwallacea* sp. nr. *kuroshio* (Kuroshio Shot Hole Borer, KSHB), which form symbiotic complexes with necrotizing fungi from genus Fusarium, have caused significant losses of avocado production and significant damage in forest areas and urban parks in California by transmitting the disease called Fusarium Dieback (FD) (Mendel *et al.*, 2012; Eskalen *et al.*, 2012, 2013) Further, it has spread to the Mexican border, finally arriving in 2016 to Tijuana, Baja California, Mexico with potential hosts present around the detection zone such as maple, avocado, ash, guava, castor , orange, olive, Washingtonia palm, Brazilian pepper and grapes (García-Avila & Trujillo-Arriaga, 2016).

FD disease affects more than 300 plant species; including avocado (*Persea americana Mill*., Lauraceae) (Eskalen *et al.*, 2013). This crop is high important for Mexico, since currently, it is the main producer, exporter and world consumer (FAO, 2018). Avocado is a crop plant with oleaginous

fruits that have great nutritional benefits (Schaffer *et al.*, 2013). It is sensitive to cold and humidity and adapts to rainfall about 1200 mm and an altitude between 800 to 2500 m.a.s.l. (Schaffer *et al.*, 2013) that are very similar to the characteristics required by *Euwallacea* spp. to survive in all its stages (Walgama & Zalucki, 2007; Kumar *et al.*, 2011). Having a wide hosts variety, this kind of pests could eventually expand in Mexico and affect both forest areas and producing areas of avocado, emerging as a potential phytosanitary and ecological threat.

Different practices have developed to partially control the beetle expansion. Extensive monitoring is usually done with traps and visual inspections, thin infested branches and withered branches elimination, treatment of cuts in the main branch with Bifenthrin (pyrethroid) and in the area of the sapwood around the lesions in branches of certain diameter, sanitation using solarization or crushing, or direct control with contact or systemic insecticides, but in future it is sought to identify agents of biological control and/or induce resistance to plants as an effective control measure (Mendel & Freeman, 2015; Umeda *et al.*, 2016; Dunlap *et al.*, 2017; López-Buenfil *et al.*, 2017) therefore, it is important to study and understand the host responses to disease at specific chemical and molecular levels. Plants have developed defense mechanisms that allow them to respond favorably to their pathogens. One of these mechanisms is the metabolite inducible production through secondary metabolism. Some compounds produced in this process work in multiple ways, interfering in plant-environment, plant-insect, plant-microorganism and even plant-plant interactions. (Dixon, 2001; Harborne, 2001).

In this study, we analyze the effect caused by FD disease on the expression of plant defense genes and genes potentially involved in the production of metabolites involved in their defense responses, these data were contrasted with a previously performed metabolomic analysis. Comparative bioinformatics analysis was performed from transcriptome data obtained by RNA-Seq from asymptomatic avocado trees and trees with symptoms of infection by *Fusarium kuroshium*, located in an orchard in San Diego County, California; in this way, the expression profiles of each group of plants were obtained to later be compared with the metabolomic analysis. Comparative studies between plants with and without symptoms of any disease make it possible to predict relevant functional changes from gene expression data, the evidence of differential expression is what allows us to obtain information from anonymous sequence data (Nowak, 1995; Adams, 1996; Bains, 1996). Transcriptomic analysis together with functional analysis lead to obtaining new biomarkers and treatments associated with various diseases (Merdad et al., 2015).

SUBCHAPTER II.I

Transcriptional response in sapwood tissue of avocado to Fusarium dieback

MATERIALS AND METHODS

Plant material

Samples were collected in December 2015 from an avocado orchard located in Escondido, San Diego County, California. Five non-symptomatic avocado trees (visually without any disease, including FD) and five FD-symptomatic trees randomly selected within the same orchard to minimize the effect of soil or climate variation. Samples were taken from the sapwood area, where beetles usually attack. FD was verified by observing the entry points of the beetle, cutting the branch and looking for the presence of galleries and necrotic tissue under the cortex (sapwood).

Library preparation and sequencing

Preparation of four libraries was performed with the TruSeq RNA system of Illumina and sequenced in NextSeq 500 platform Illumina, 2x75 Medium yield. With this sequencing capacity, about 110 Million simple reads and 18 Gb are guaranteed; in total, two libraries for each condition (asymptomatic or FD-symptomatic trees) were constructed (Table 2).

Table 2. Relation of samples and libraries constructed. The sampling was carried out in December 2015 in Escondido, San Diego County, California.

Condition	Asymptomatic	FD-symptomatic
Number of sampled trees	5	5
Number of libraries	2	2

Data processing of Illumina RNA-Seq reads

Reads were quality-filtered using Trimmomatic (Bolger *et al.*, 2014). Four sequence files were generated in FASTQ format (sequence reading plus quality information in Phred format); two files corresponded to asymptomatic condition and two files to FD-symptomatic condition. Files of each condition were merged with Linux tools and one single file per condition was finally obtained.

Mapping of RNA-Seq reads using Bowtie2 and annotation.

Reads were processed and aligned to an avocado transcriptome reference (Ibarra-Laclette *et al.*, 2015) using Bowtie2 algorithm to perform the alignment (Langmead & Salzberg, 2012; Langmead *et al*., 2019). The mapping results (SAM files) were changed to BAM files and sorted with SAM tools and then used for the comparative transcriptomics analysis (Li *et al.*, 2009). Reference annotation was used (Ibarra-Laclette *et al.*, 2015).

Transcript assembly and abundance estimation using Cufflinks

The aligned read files were assembled with Cufflinks (Trapnell, 2014), the read abundance was estimated and differential expression and regulation tests were performed between samples with different conditions. Cufflinks uses the normalized RNA-Seq fragment counts to measure the relative abundance of transcripts, and is expressed in Fragments Per Kilobase of exon per Million fragments mapped (FPKM) (Trapnell, 2014). Two assemblies were obtained, one from asymptomatic condition and other from FD-symptomatic condition (Supplementary 1 and 2).

Comparison and differential expression testing using Cuffmerge and Cuffdiff

Expression level was acquired by comparing both assemblies in Cuffdiff. First, the transcriptomes were joined with Cuffmerge (Trapnell, 2014). Once all short-read sequences were assembled with Cufflinks, the output.GTF files were sent to Cuffmerge. Then, the obtained file (merged.gtf) and the assemblies from both conditions were sent to Cuffdiff.

Functional analysis and enrichment of transcriptomes

Functional classification of expressed genes was performed for each condition with PlantRegMap (http://plantregmap.cbi.pku.edu.cn/) (Supplementary 3 and 4) (Tian *et al.*, 2020). Those results were then uploaded to REVIGO, a Web server that summarizes lists of GO terms by finding a representative subset of the terms using a simple clustering algorithm that relies on semantic similarity measures (http://revigo.irb.hr/) (Supplementary 5) (Rudjer Boskovic Institute Croatia). The functional clustering tool was used to search functional enrichment for all genes and three categories were selected for this analysis (biological processes, molecular functions and cellular components).

KEGG enrichment of transcriptomes

SAM files were analyzed with Blast2GO, a tool for data mining analysis designed to enabling Gene Ontology (GO) for sequences for which no GO annotation is yet available (Supplementary 6). (Conesa *et al.*, 2005). In this way, all KEGG pathways and enzymes related to each condition studied were found (Supplementary 7). Furthermore, it was also used to find the annotation of those genes that had no annotations.

Visualization of mapped reads

Both, total expressed genes and differentially expressed genes for each condition were visualized by Venn diagram generator (http://bioinformatics.psb.ugent.be/webtools/Venn/) (VIB *et al.*) and the summary obtained in REVIGO and results from Cuffdiff were visualized by diverse R tools (Team R, 2015). Heatmaps were generated with R tools and the reference genes were obtained in TAIR (Swarbreck *et al.*, 2007). Only the 30 genes with the highest foldchange of each pathway were considered with the exception of monosaccharide catabolic process metabolic pathway of which less than 30 genes matched.

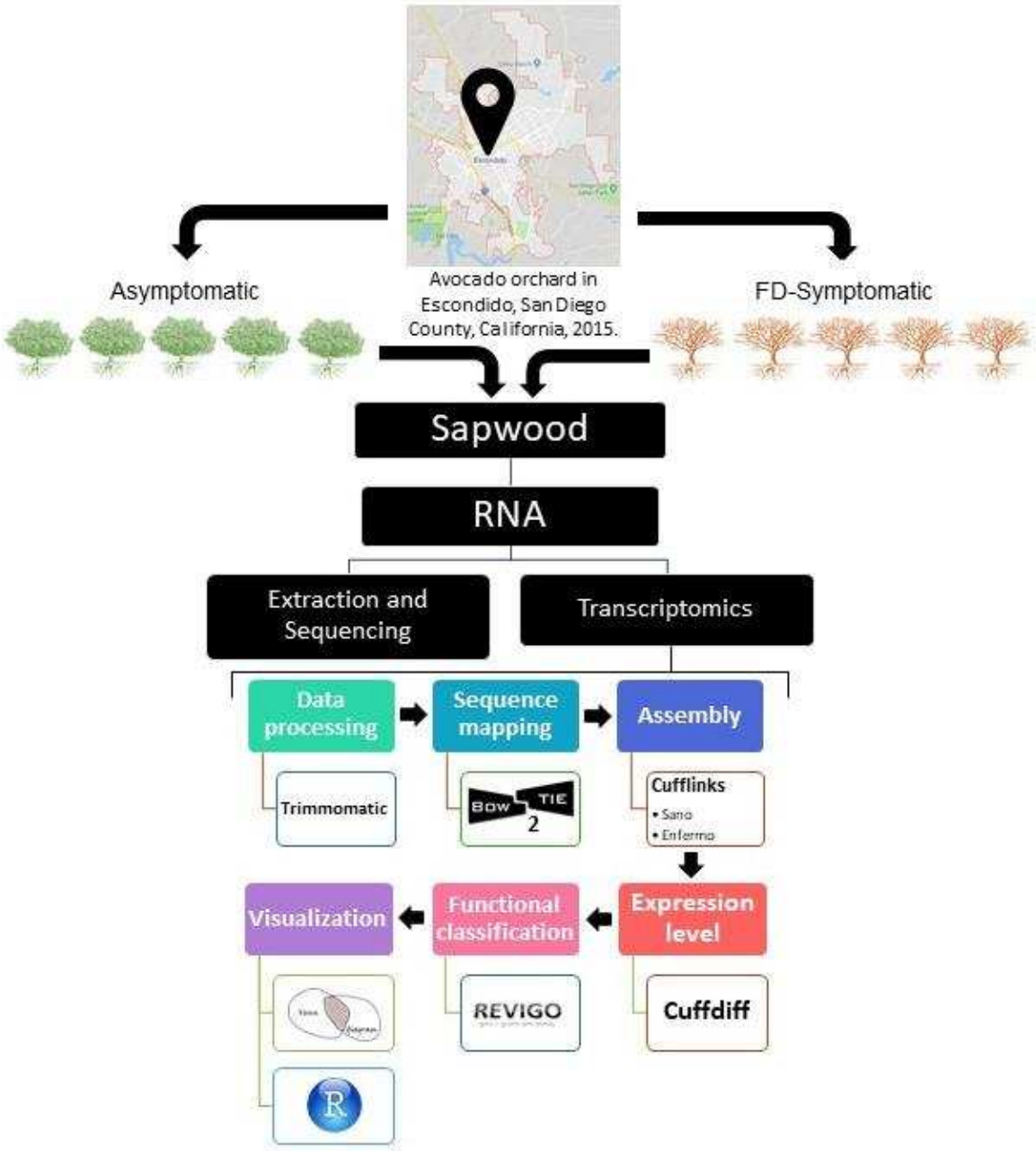

Figure 11. General workflow performed in this study.

RESULTS

Profiling expression of genes from asymptomatic and FD-symptomatic avocado trees

Distribution of detected transcripts was analyzed by PCA, first from the total of the expressed genes by considering the log2-Foldchange value (Figure 12), and second, only from the differentially expressed genes by considering the FPKM value (Figure 13). A clear segregation of data for both analyses was observed, whereby opposite tendencies between asymptomatic and FD-symptomatic condition were also observed (Figure 12 and 13).

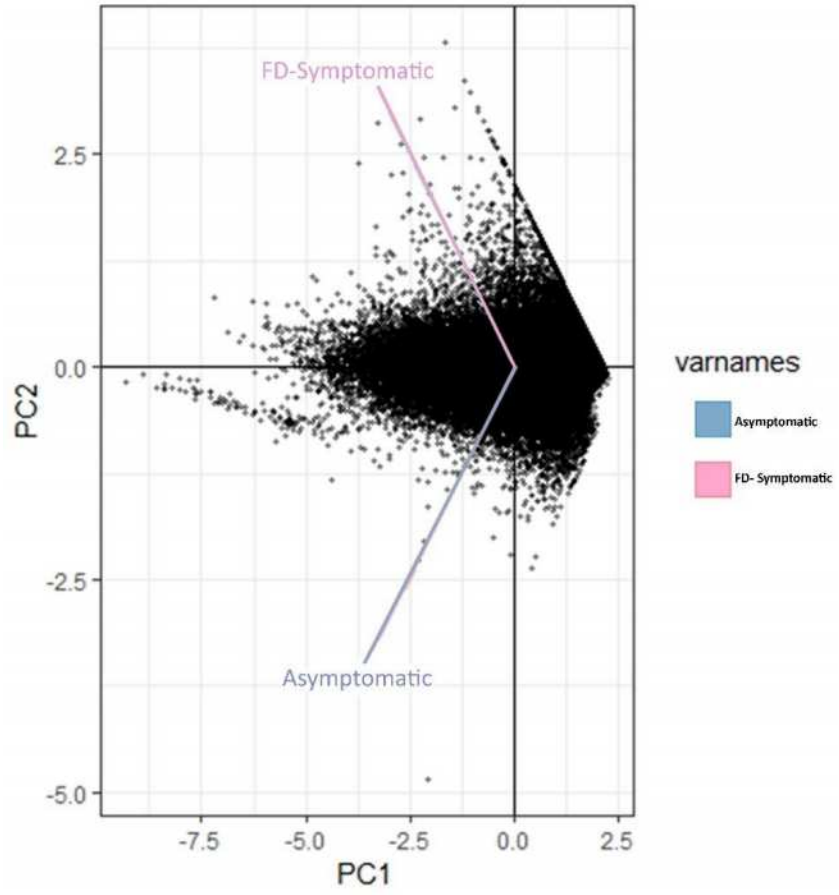

Figure 12. PCA grouping considering the log2-Foldchange value of sapwood tissue area. Asymptomatic condition is marked in blue and FD-symptomatic condition in pink color.

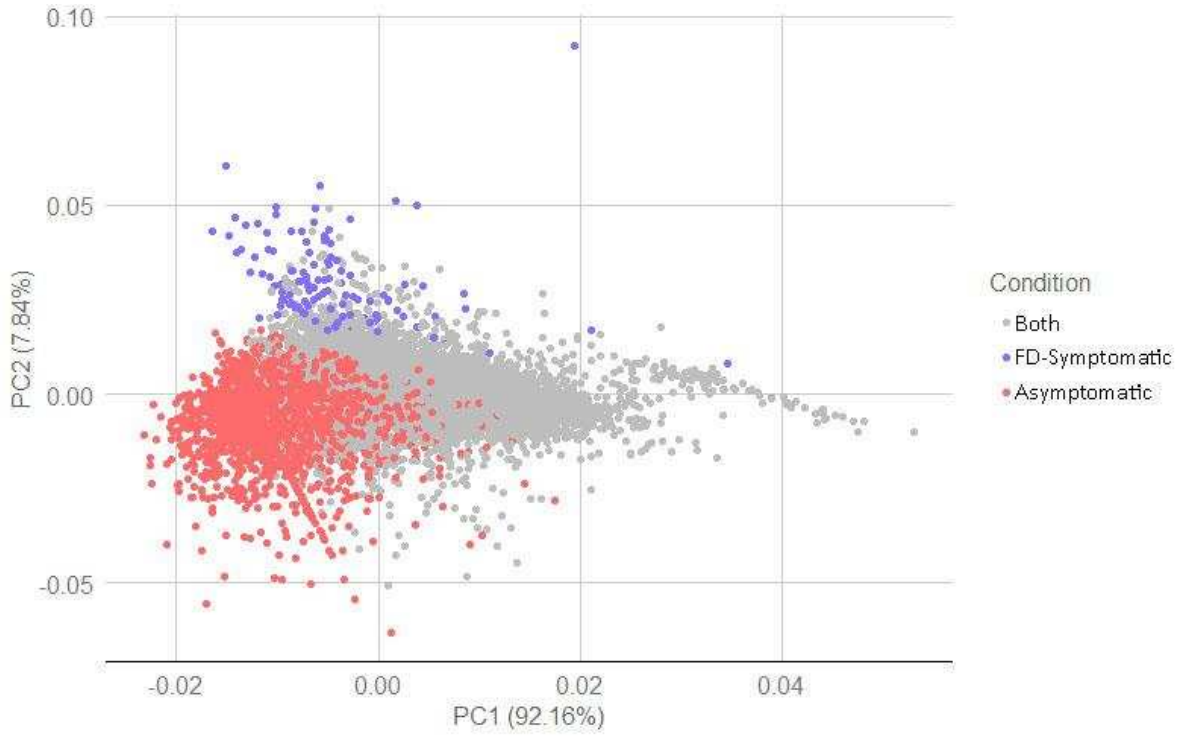

Figure 13. PCA grouping considering the FPKM value of sapwood tissue.

In addition, Venn diagrams were performed to analyze the abundance of the total significantly expressed genes in both conditions, by considering the log2-Foldchange value. In total, 14,773 expressed genes were detected, only 126 genes were exclusive of the asymptomatic group, and 2,532 were exclusive in FD-symptomatic group (Figure 14A). No exclusive upregulated genes were found in asymptomatic condition. Oppositely, 2,368 upregulated genes were exclusive to the FD-symptomatic, while 126 downregulated genes where exclusively found in asymptomatic condition and 164 only in FD-symptomatic condition (Figure 14B and C). Furthermore, from the total of genes expressed in both conditions, only 1,854 genes were differentially expressed in either condition, 114 of them were exclusive to asymptomatic condition and 1,063 to FD-symptomatic condition (Figure 15A). Of all the differentially expressed genes, there were none upregulated exclusive to asymptomatic condition; however, 1,063 upregulated genes were exclusively found FD-symptomatic condition (Figure 15B). Oppositely, 114 downregulated genes were exclusive to the asymptomatic condition, but no downregulated gene exclusive to the FD-symptomatic condition was found (Figure 15C). Moreover, it is important to note that there were more expressed genes in the FD-symptomatic condition than the asymptomatic.

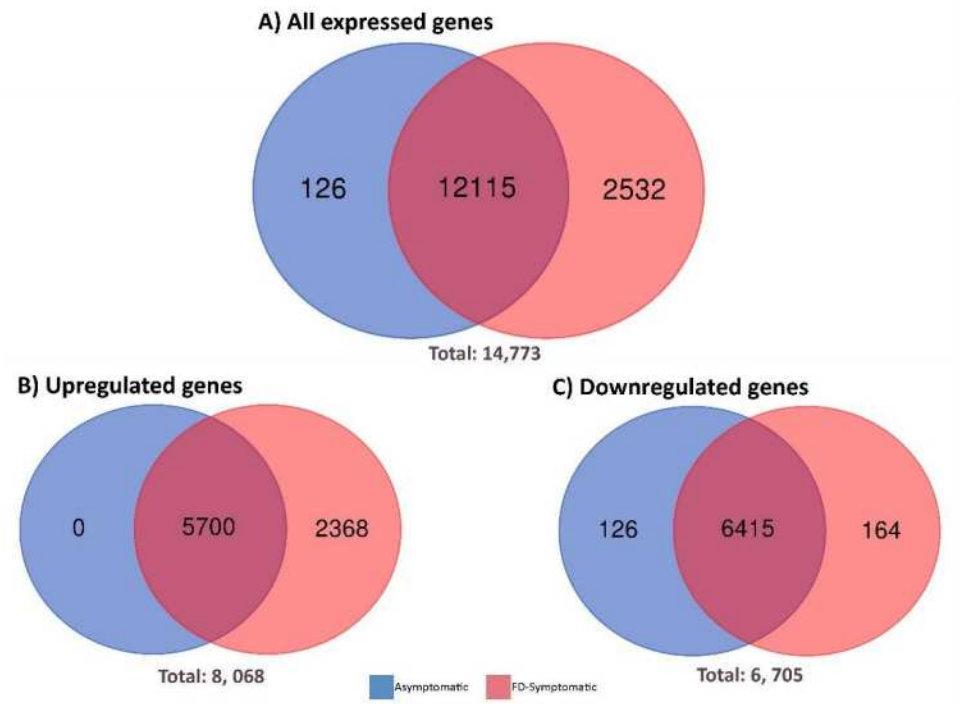

Figure 14. Venn Diagrams of all expressed genes from. A) shows the general interaction between asymptomatic and FD-symptomatic condition. B) shows the interaction between induced genes C) shows the interaction between repressed genes.

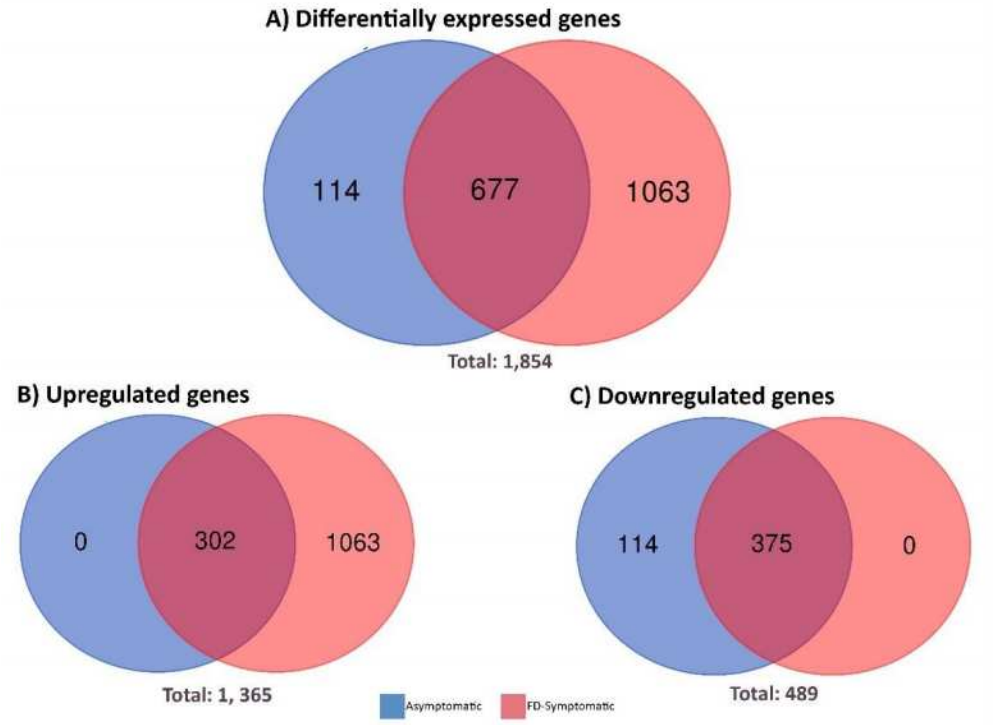

Figure 15. Venn Diagrams of differentially expressed genes from sapwood tissue. A) shows the general interaction between asymptomatic and FD-symptomatic condition. B) shows the interaction between induced genes C) shows the interaction between repressed genes.

In order to analyze the putative functions most affected by FD, a hierarchical clustering analysis was performed including the 30 annotated genes with the highest foldchange value for both, upregulated and downregulated genes (Supplementary 8). The annotation of the obtained transcripts was performed by searching sequence similarity to *Arabidopsis thaliana* proteins (http://www.arabidopsis.org/) and the top protein matches were assigned for each avocado unigene (Supplementary 8). The gene ontology (GO) functional classes and pathways for each avocado unigene were assigned based on Arabidopsis GO SLIM and pathway annotation. Within the group of upregulated genes, 11 processes were genes could be involved were found (biotic and abiotic stimulus, carbonate dehydratase, cellulose biosynthesis, defense response, development, lignan metabolism, pollen development, protein degradation, RNA degradation, transferase and transport) the most represented categories were transporter, biotic and abiotic stimulus, defense response, development and transporter (Figure 16). The group of the top 30 downregulated genes was classified in 11 processes (asparagine metabolism, biotic and abiotic stimulus, carbohydrate metabolism, development, elongation, flowering time, photosynthesis, RNA splicing, transcription factor, translation and transporter), being the primary metabolism and photosynthesis-related genes the most abundant (Figure 17). Interestingly, most of the upregulated genes were inducible in asymptomatic condition opposite to downregulated genes that were inducible in FD-symptomatic condition (Figure 16 & 17).

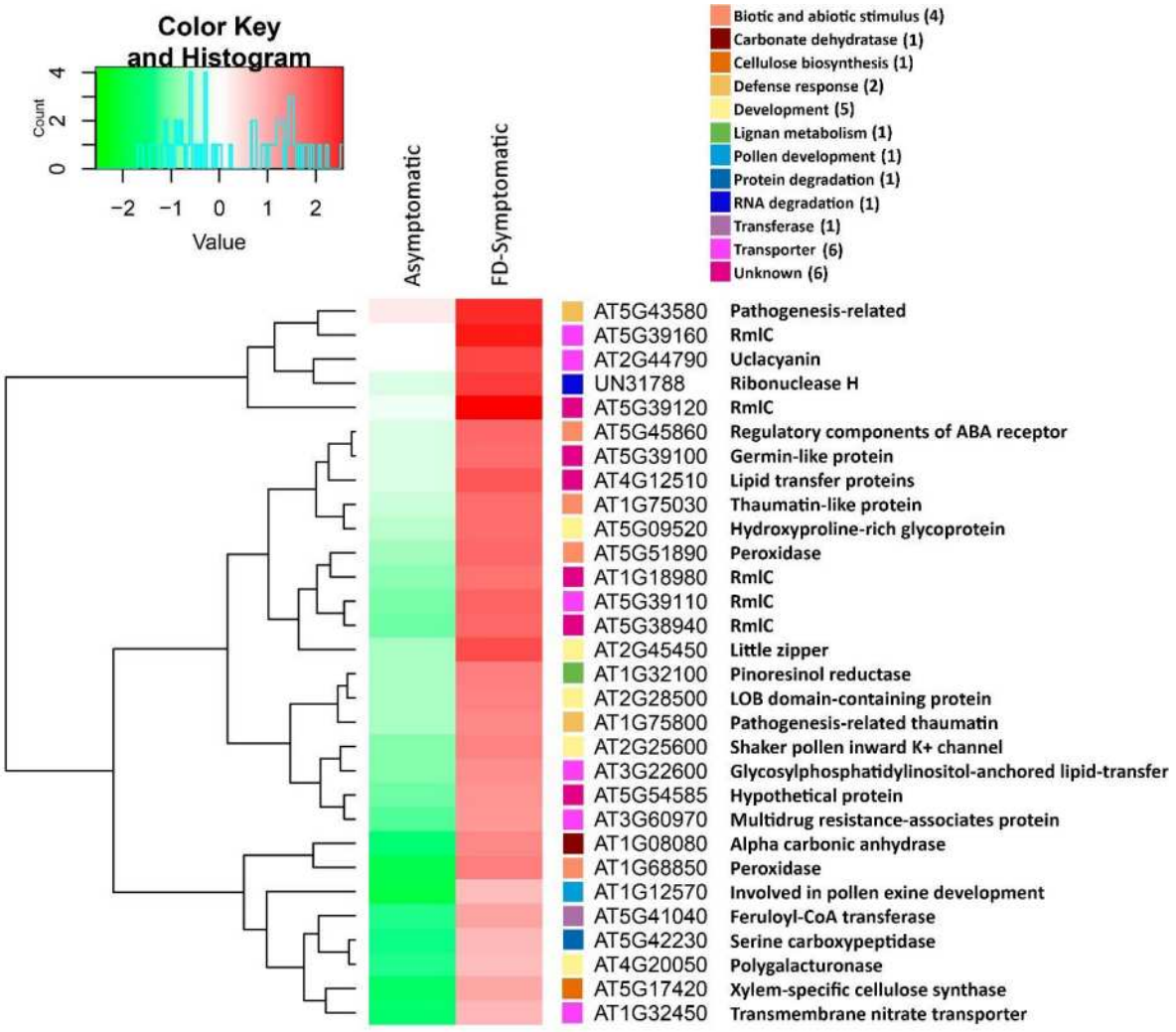

Figure 16. The top 30 upregulated genes with the highest foldchange from asymptomatic and FD-symptomatic avocado trees.

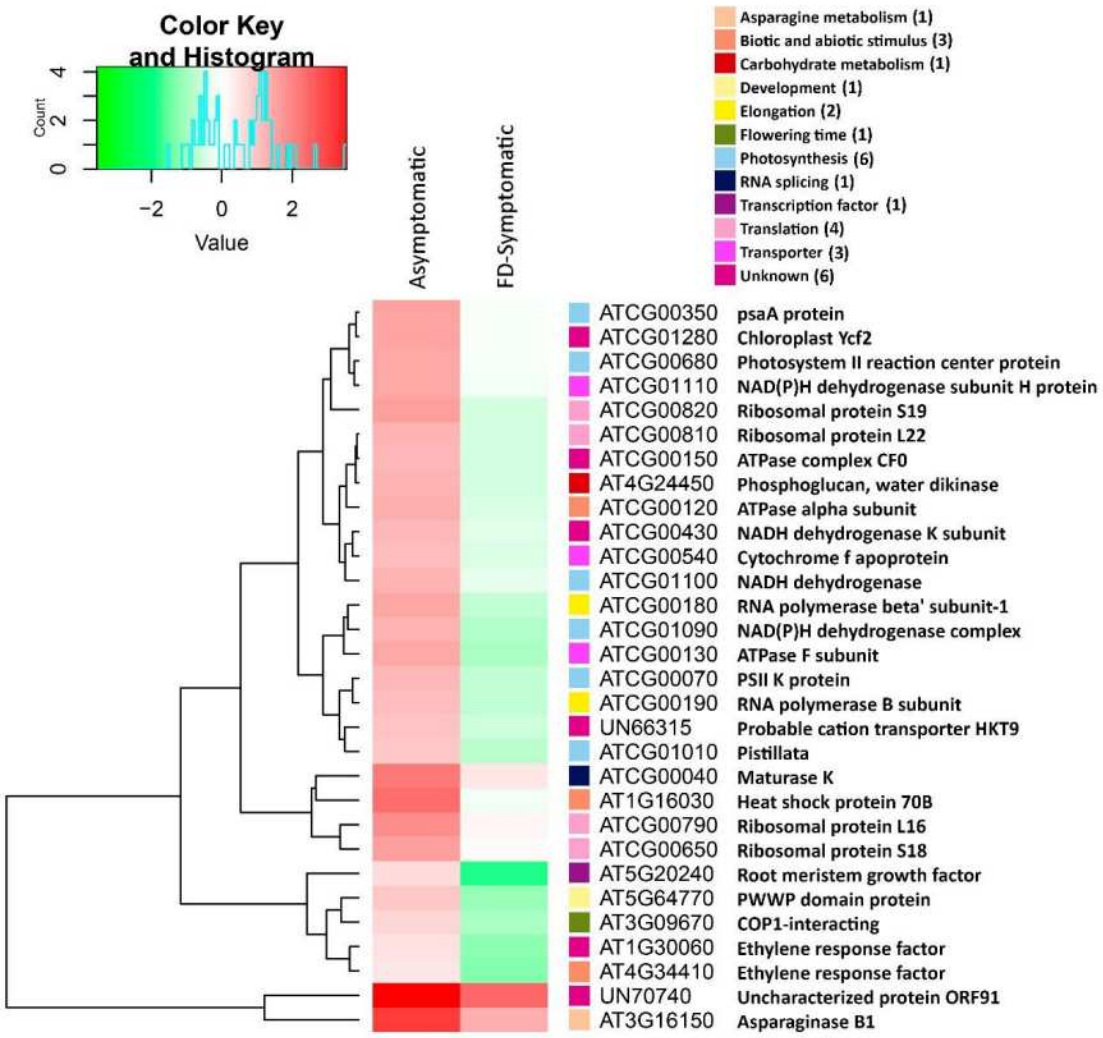

Figure 17. The top 30 downregulated genes with the highest foldchange from asymptomatic and FD-symptomatic avocado trees.

When the 30 genes with the highest expression level for both asymptomatic and FD-symptomatic conditions were listed (Supplementary 9), practically the same categories were represented (Figures 18 and 19). The category <<stabilization of membrane proteins>> was represented only in the asymptomatic category by one single gene (At1g27330), which encodes for a ribosomal protein (Ribosome associated membrane protein RAMP4) (Figure 18). We observe again more presence in the genes related to biotic and abiotic stimulus in the FD-symptomatic condition (Figures 19).

4.54811782	UN27328	TAR1 Protein	Biotic and abiotic stimulus (3)
4.53571934	UN18613	rDNA transcription protein regulator 15	Development (3)
4.53379242	AT3G18490	Aspartic protease in guard cell 1	Post-transcriptional regulation (1)
4.37195218	UN32262	rDNA transcription protein regulator 15	Regulator of ubiquitylation (1)
4.30971106	UN34153	rDNA transcription protein regulator 15	Stabilization of membrane proteins (1)
4.26947558	UN42298	rDNA transcription protein regulator 15	Transcription regulation (7)
4.18669082	UN42364	rDNA transcription protein regulator 15	Translation (1)
4.17454213	AT5G53330	Elongation factor 1B	Transporter (4)
4.09453161	ATCG00140	ATPase III subunit	Unknown (8)
4.08761107	UN36423	rDNA transcription protein regulator 15	Xyloglucan metabolism (1)
4.07600293	AT5G51440	HSP20-like chaperon	
4.01805523	UN38380	rDNA transcription protein regulator 15	
3.90575228	AT5G47080	Casein kinase II	
3.72161279	AT1G02060	Tetratricopeptide repeat	
3.70395788	AT5G57690	Diacylglycerol kinase 4	
3.65890683	AT4G29040	Regulatory particle AAA-ATPASE 2A	
3.51637401	AT1G47480	Hydrolase	
3.46717214	AT1G27330	Ribosome associated protein	
3.45341509	AT4G31890	ARM repeat	
3.44125653	AT3G28860	ATP-binding cassette B19	
3.36151725	AT5G36230	ARM repeat	
3.32557723	AT3G48140	B12D protein	
3.31032657	AT1G55060	Ubiquitin-like	
3.29724604	AT5G17860	Calcium exchanger 7	
3.29138031	AT2G42210	OEP16	
3.26656	AT4G03210	Endotransglucosylase/hydrolase 9	
3.19079784	AT2G18980	Peroxidase	
3.16232539	ATMG00030	Hypothetical protein	
3.11596482	AT1G12840	ATPase	
3.09873328	AT3G47470	Chlorophyll a/b-binding	

Figure 18. The 30 most expressed genes of asymptomatic condition.

3.63129465	AT3G18490	Aspartic protease in guard cell 1	Biotic and abiotic stimulus (6)
3.61520596	UN27328	TAR1 Protein	Development (3)
3.56513088	UN18613	rDNA transcription protein regulator 15	iron-sulfur assembly (1)
3.35380279	UN32262	rDNA transcription protein regulator 15	Modulator of photoinhibition (1)
3.31760583	UN34153	rDNA transcription protein regulator 15	Post-transcriptional regulation (1)
3.30230278	UN42298	rDNA transcription protein regulator 15	Regulator of ubiquitylation (1)
3.18564696	UN42364	rDNA transcription protein regulator 15	Transcription factor (1)
3.18270987	AT5G53330	Elongation factor 1B	Transcription regulation (6)
3.17047668	AT5G51440	HSP20-like chaperon	Translation (1)
3.1123972	AT1G55060	Ubiquitin-like	Transporter (2)
3.09784014	UN36423	rDNA transcription protein regulator 15	Unknown (7)
3.07512158	ATCG00140	ATPase III subunit	
3.04975281	AT3G48140	B12D protein	
2.93361111	AT2G18980	Peroxidase	
2.90394599	AT5G47080	Casein kinase II	
2.84284816	AT2G01930	Basic pentacysteine1	
2.80679199	AT4G29040	Regulatory particle AAA-ATPASE 2A	
2.79113598	AT5G52810	SAR-deficient4	
2.7605122	AT5G55910	AGC VIIIa Kinase	
2.75330026	AT2G47990	Slow walker1	
2.74167958	AT2G21620	Response to desiccation	
2.71987444	AT5G57690	Diacylglycerol kinase 4	
2.67467686	ATMG00030	Hypothetical protein	
2.66466078	AT1G02060	Tetratricopeptide repeat	
2.6581856	AT1G47480	Hydrolase	
2.64235262	AT1G77510	protein disulfide isomerase-like	
2.63358133	AT3G61470	Light harvesting antenna complex	
2.63175442	AT4G22220	ISU1	
2.59000748	AT2G21660	Glycine-rich RNA binding	
2.5882571	AT4G11800	Calcineurin-like	

Figure 19. The 30 most expressed of FD-symptomatic condition.

Functional classification of differentially expressed genes in response to FD

Functional classification of differentially expressed genes for both conditions (1,854 unigenes) was performed, and 27 functional subcategories of biological processes were represented (Figures 20 and 21). The most significant subcategories represented for both, asymptomatic and FD-symptomatic groups were: nucleotide metabolism, response to stimulus, organic substance transport and organic substance metabolism (Figures 20 and 21). Further, in asymptomatic condition, category <<nucleotide metabolism>> had a higher value than in FD-symptomatic condition; interestingly, the categories response to stimulus, organic substance metabolism and positive regulation of biological process had a higher value in FD-symptomatic condition and the catabolism, growth, and rhythmic process subcategories were only represented in this condition (Figures 20 and 21).

At the cellular component categorization, in total 11 subcategories were represented. For both tested conditions, cell and membrane were the most significantly represented subcategories (Figure 20). Further all represented subcategories had a higher significance in asymptomatic condition than FD-symptomatic condition (Figure 20). Categorization at the molecular function level showed that 18 subcategories were represented (Figure 20). For both conditions, binding and rRNA binding were the most significant subcategories; in asymptomatic condition, rRNA binding, catalytic activity, structural molecule activity and ligase activity had a higher value than in FD-symptomatic condition, whereas in FD-symptomatic condition binding, transferase activity, transporter activity, isomerase activity, hydrolase activity and carbon-carbon lyase activity had a higher value than in asymptomatic condition. Also, GTPase activity and photoreceptor activity were only found in asymptomatic condition whereas signaling receptor activity, oxidoreductase activity, electron carrier activity and ATPase activity subcategories were only found in FD-symptomatic condition (Figure 20).

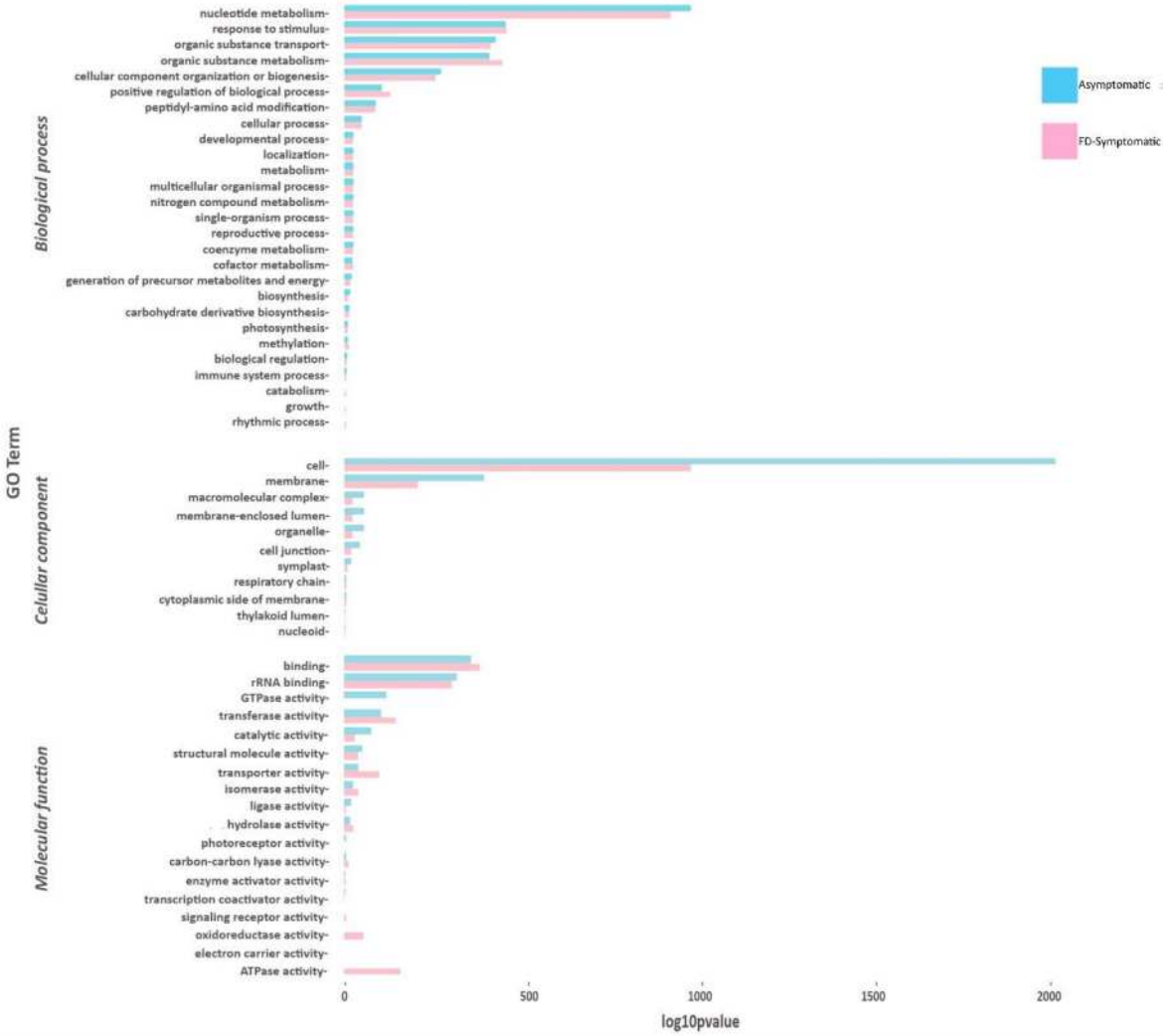

Figure 20. Functional classification summary of biological processes, obtained by REVIGO (Rudjer Boskovic Institute Croatia; Supek *et al.*). Blue bars represent asymptomatic condition and pink bars represent FD-symptomatic condition.

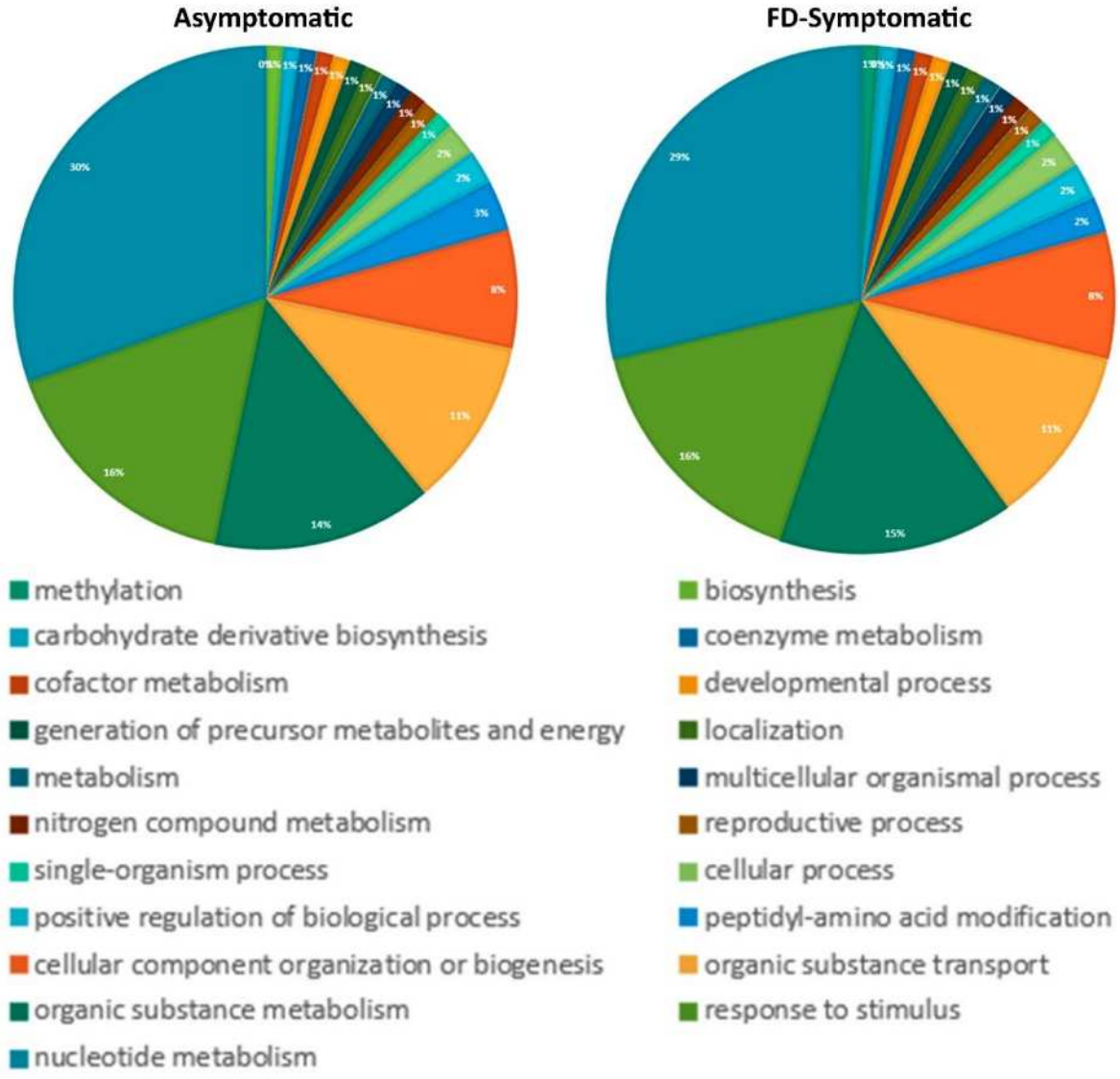

Figure 21. Pie-Plot of functional classification summary of biological processes, obtained by REVIGO (Rudjer Boskovic Institute Croatia; Supek *et al.*). On the left we can find asymptomatic condition represented and, on the right, we can find FD-symptomatic condition represented.

KEGG enrichment analysis

In order to determine which metabolic pathways were mostly expressed, Venn diagrams of the pathways obtained in KEGG enrichment analysis and maps of KEGG metabolic pathways of differentially expressed genes related to carbohydrate and lipid pathways were created. As a result, 106 KEGG pathways were obtained, whereof 17 were exclusive of asymptomatic condition and 18 of FD-symptomatic condition (Figure 22) and 111 KEGG enzymes were obtained, whereof 9 were exclusive of asymptomatic condition and 88 of FD-symptomatic condition (Figure 22). Furthermore, in both cases different steps of the studied pathways seem to be expressed in both conditions and a few only in FD-symptomatic condition (Figure 23 and 24).

Some of the upregulated genes (At1g68850, At1g75030, At5g51890 and At1g75800) that were associated with biotic and abiotic stimulus and defense response in the FD-symptomatic group were also represented in KEGG pathways, into phenylpropanoid biosynthesis pathway (Supplementary 7 and 8). In addition, one of the induced genes in FD-Symptomatic condition (At1g14520) related to monosaccharide pathway and all the induced genes related to lipids with the highest expression value in asymptomatic condition (At3g51520, At3g02630, Atcg00500 and At2g23800) were also represented into the Inositol phosphate metabolism and fatty acid biosynthesis-related pathways of the KEGG classification, respectively (Supplementary 7 and 10; Figure 23 and 24).

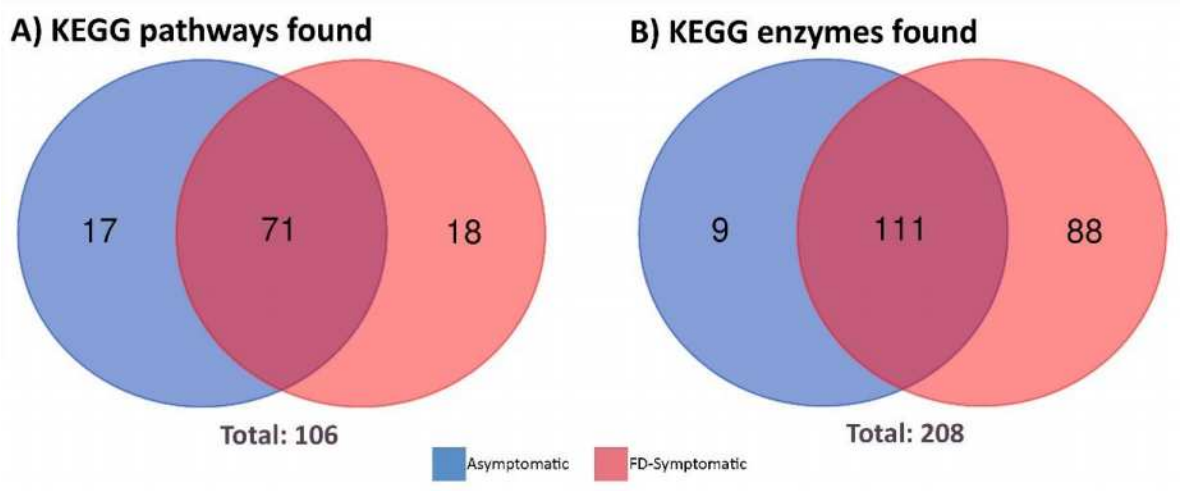

Figure 22. Venn Diagrams of pathway enrichment. A) shows the interaction between KEGG pathways in asymptomatic and FD-symptomatic condition. B) shows the interaction between KEGG enzymes found in asymptomatic and FD-symptomatic condition.

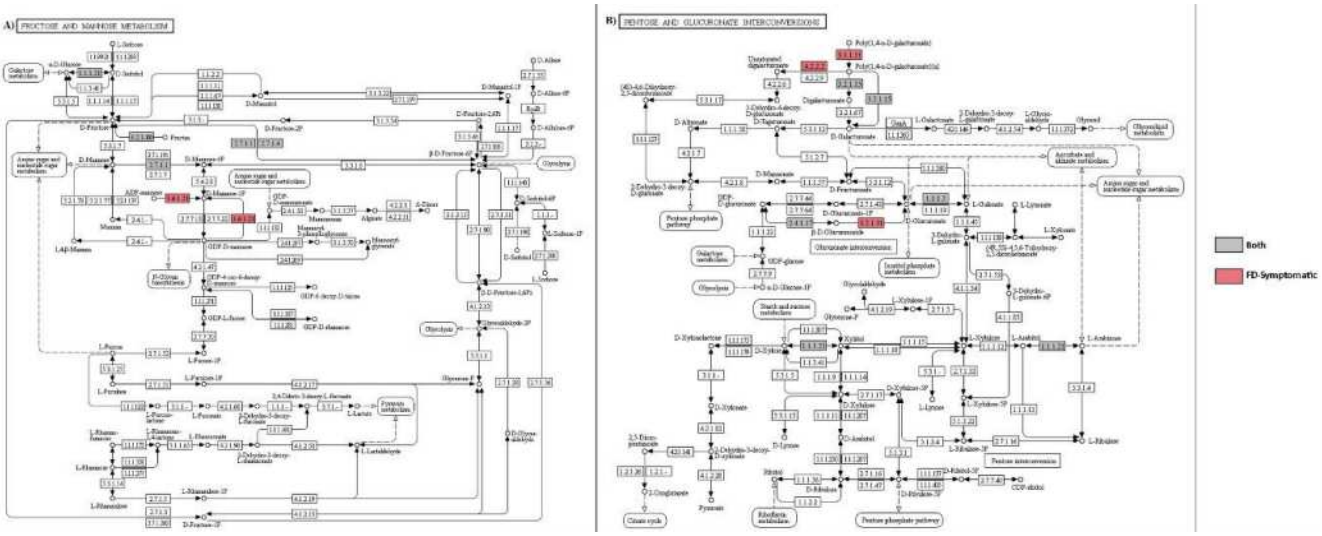

Figure 23. Maps of KEGG metabolic pathways of differentially expressed genes related to carbohydrate pathways. A) shows fructose and mannose metabolism pathway B) shows pentose and glucuronate interconversions pathway.

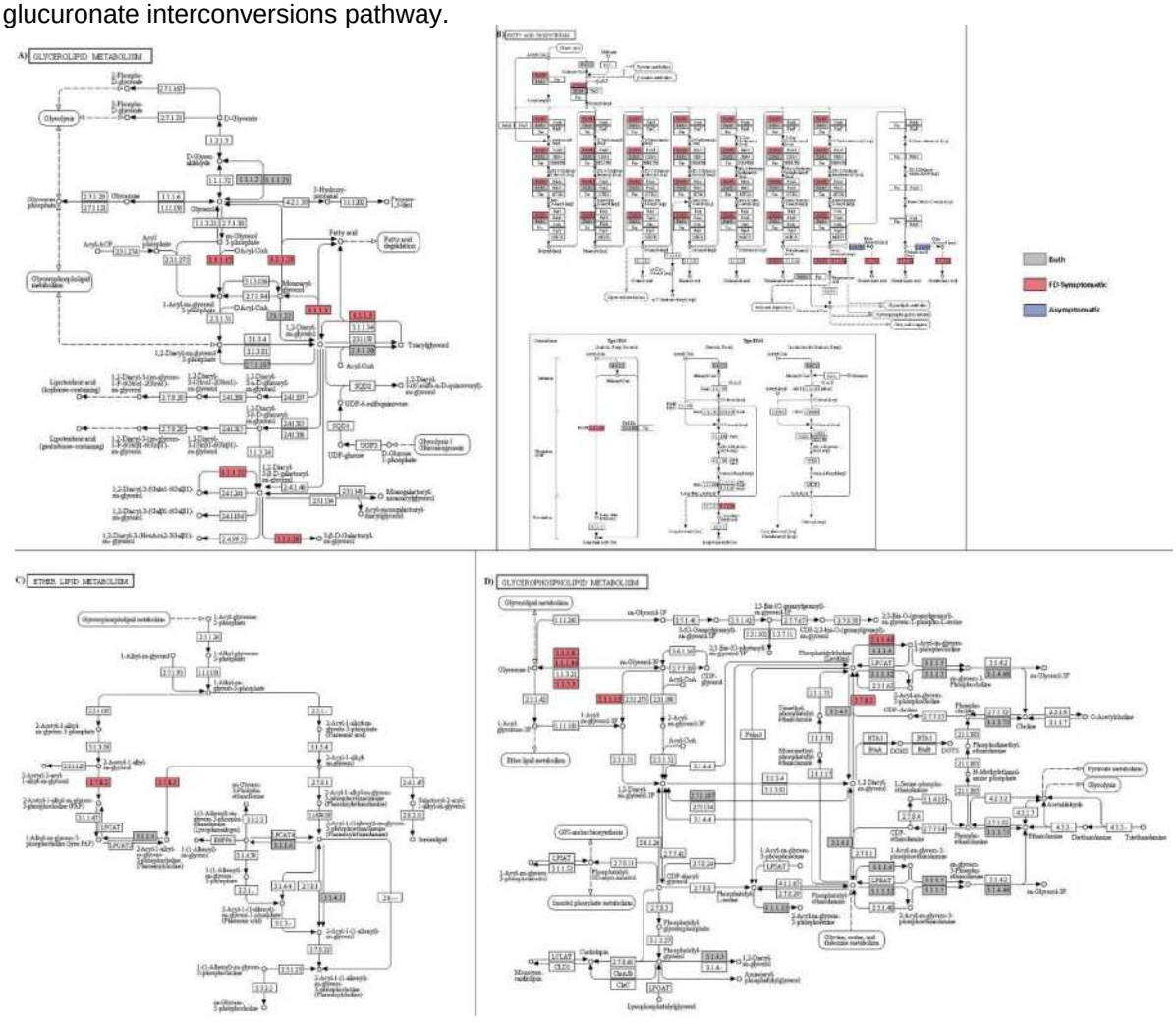

Figure 24. Maps of KEGG metabolic pathways of differentially expressed genes related to lipid pathways. A) shows glycerolipid metabolism pathway. B) shows fatty acid biosynthesis pathway. C) shows ether lipid metabolism pathway. D) shows glycerophospholipid metabolism pathway.

Expressed genes related to previously identified metabolites

Previous to our transcriptional analysis, an untargeted metabolomics profiling from the same asymptomatic and FD-symptomatic sampled trees was performed by ultra performance liquid chromatography coupled to a mass spectrometer (UPLC-MS-QTOF), identifying several acetogenins, aliphatic amines, fatty acid amines and two sterol lipids as the mainly chemical markers in the asymptomatic trees (Figures 5-10); while metabolic profile in FD-symptomatic trees was redirected to the carbohydrate mobilization. In this study, we analyzed transcriptional expression profiles of the 30 annotated genes with highest fold change value related to carbohydrate, monosaccharide and lipid pathways (Supplementary 10).

Genes related to carbohydrate metabolism (biosynthesis and catabolism) process had higher expression values in FD-asymptomatic trees (Figures 25). To assess specifically if monosaccharide metabolism was regulated at transcriptional level, we generated two heatmaps with annotated genes belonging to this category (Figures 26). Expression level in both groups, asymptomatic and FD-symptomatic differentially expressed genes was similar, except three avocado unigenes with induced expression in FD-symptomatic group, contrasting to asymptomatic condition (At1g79500, At2g45790 and At1g14520; Figure 26A). At1g79500 encodes a protein with 3-deoxy-8-phosphooctulonate synthase (KDOP synthase), At2g45790 encodes a cytoplasmic phosphomannomutase, At1g14520 encodes a myo-inositol oxygenase (MIOX1), all those genes contribute to the synthesis of different carbohydrates.

With respect to the 30 most expressed genes related to lipid biosynthesis, most of induced genes belonged to FD-symptomatic condition, although most of them encodes to proteins involved in specialized lipid production (hormones and secondary metabolites mainly), they do not seem being involved in primary lipid biosynthesis (At1g65690, that participates in ABA signaling and biosynthesis; At1g76680, in oxylipin biosynthetic process; At4g18780, in abscisic acid biosynthetic process; At2g34555, in gibberellin biosynthetic process; At3g45140, in jasmonic acid biosynthetic process; and At5g51810 in gibberellin biosynthetic process; Figure 27). Interestingly, overexpressed genes with the highest expression value in asymptomatic condition were genes that code for clue enzymes involved in the fatty acid biosynthesis (At3g51520, At3g02630, Atcg00500 and At2g23800; Figure 27A). At3g51520 encodes a functional acyl-CoA:diacylglycerol acyltransferase; At3g02630 one of seven acyl acyl carrier proteins; At2g23800 an endoplasmic reticulum-targeted geranyl geranyl

pyrophosphate synthase; and Atcg00500 encodes the carboxytransferase beta subunit of the Acetyl-CoA carboxylase (ACCase) complex in plastids. This complex catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA, the first committed step in fatty acid synthesis. In the opposite way, genes involved in lipid catabolism were more highly expressed in FD-symptomatic trees than asymptomatic (Figure 27B).

Although several genes were classified as part of lipid biosynthesis machinery (Figure 27A), some of them participates in hormonal biosynthetic pathways related to defense responses. Then, we analyzed the expression patterns of the 30 most expressed genes related to jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) responsive and biosynthetic routes (Figure 28). Clearly, these genes were overexpressed only in the FD-symptomatic condition, being represented the most important genes related to signaling, transcriptional regulation and biosynthesis of JA, ET and SA (Supplementary 11 and Figure 28).

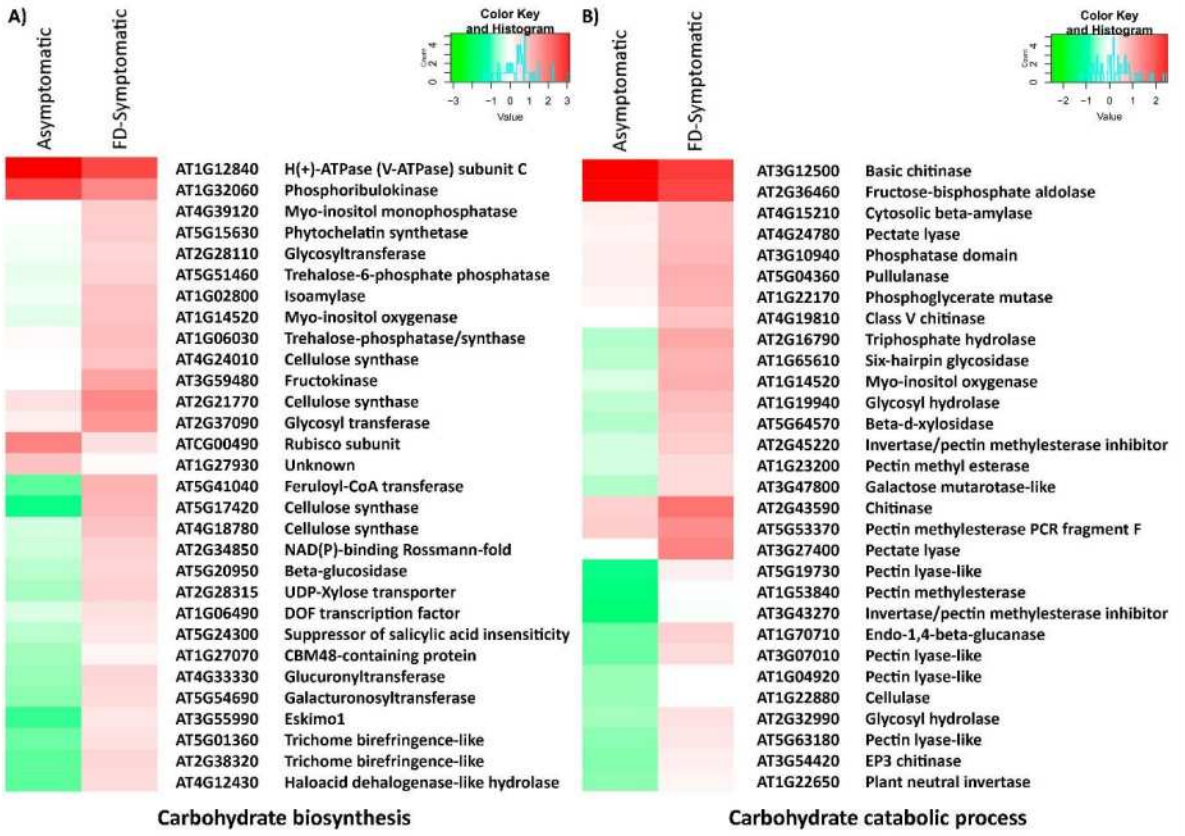

Figure 25. Heatmaps of the 30 genes with the highest fold change related to carbohydrate biosynthesis (left) and to carbohydrate catabolic process (right).

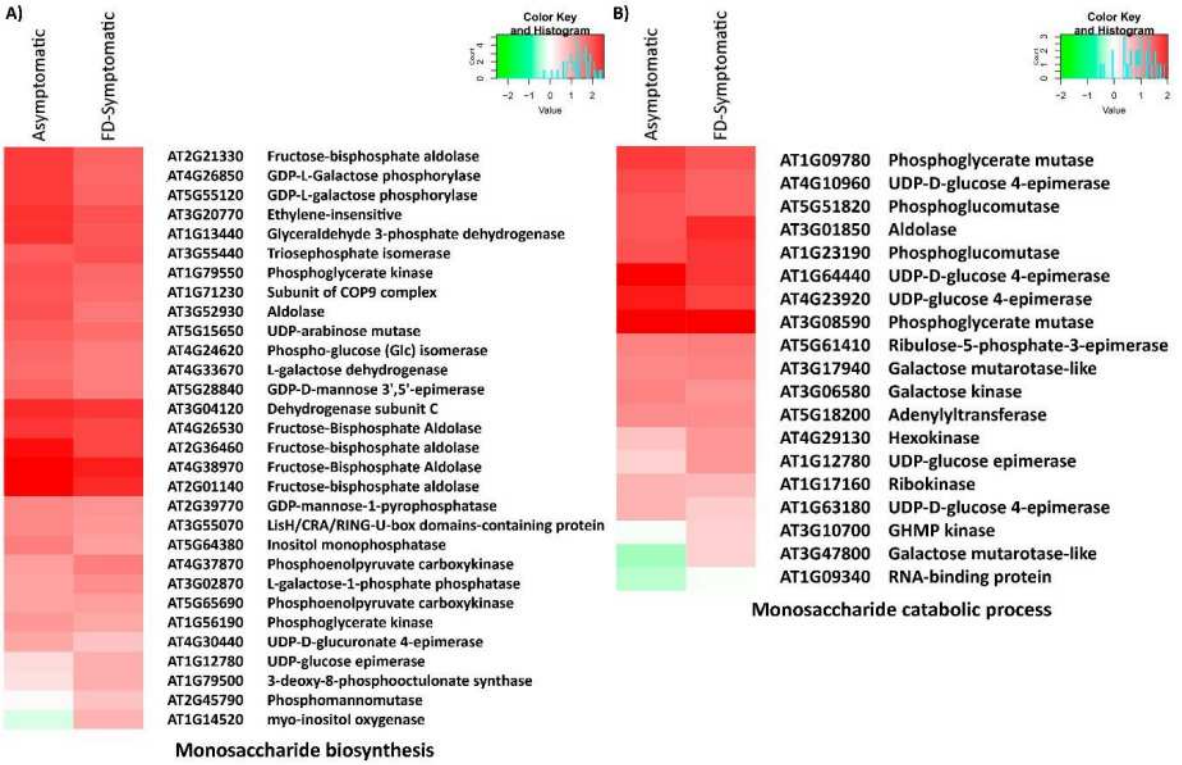

Figure 26. Heatmaps of the 30 genes with the highest fold change related to monosaccharide biosynthesis (left) and to monosaccharide catabolic process (right).

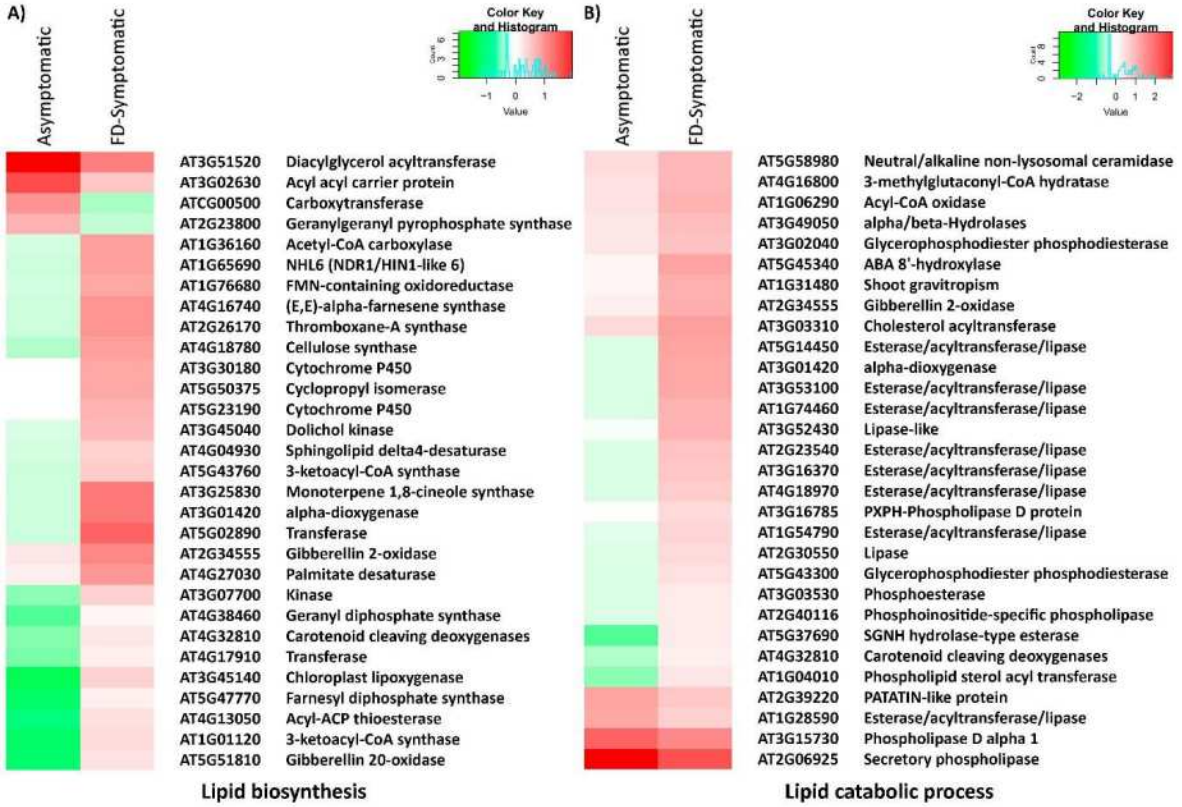

Figure 27. Heatmaps of the 30 genes with the highest fold change related to lipid biosynthesis (left) and to lipid catabolic process (right).

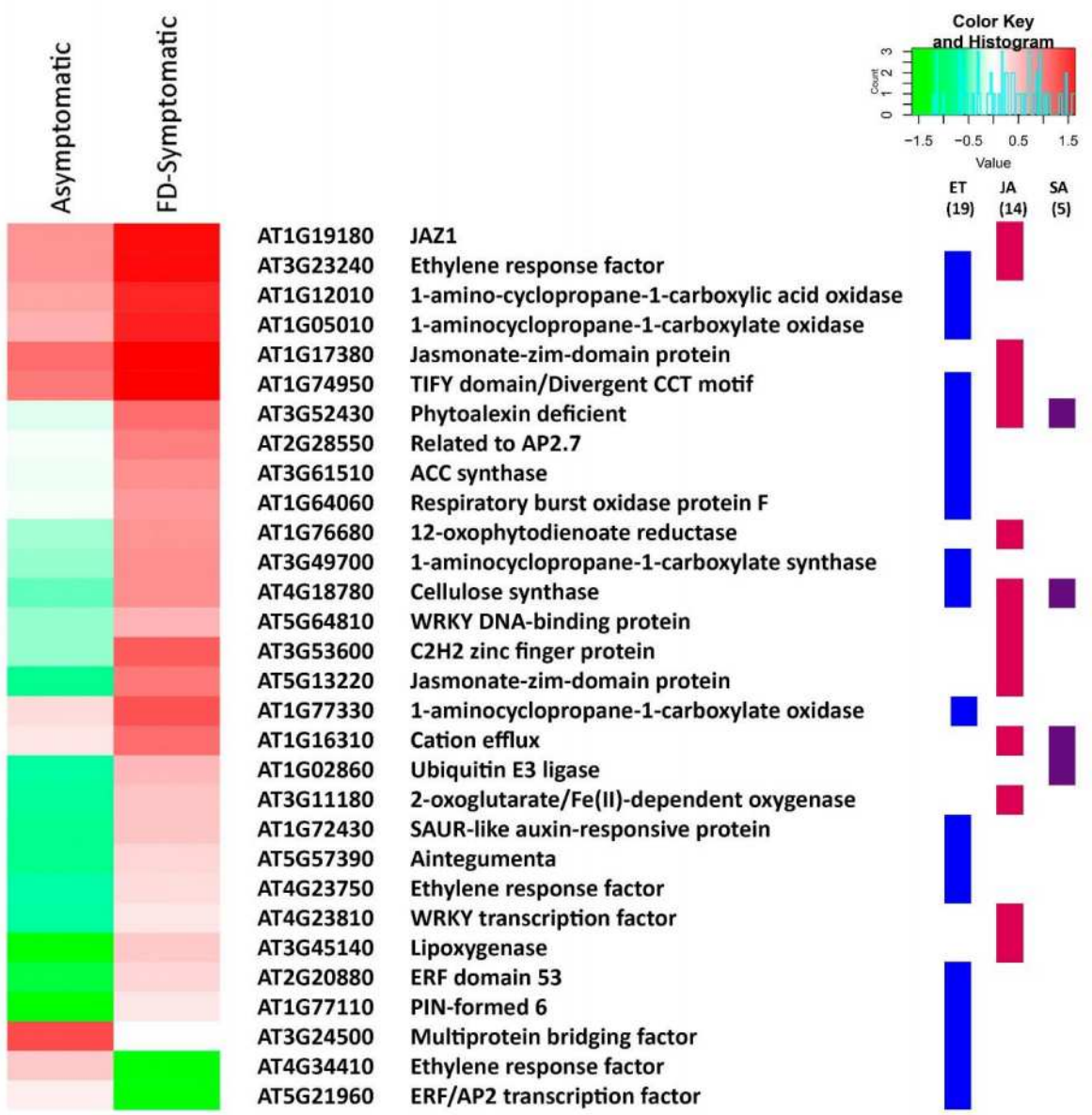

Figure 28. Heatmap of the 30 genes with the highest fold change related to hormone defense from sapwood tissue, ET means ethylene biosynthetic process and ethylene-activated signaling pathway, JA means jasmonic acid biosynthetic process and jasmonic acid-mediated signaling pathway, and SAB means salicylic acid biosynthetic process and salicylic acid-mediated signaling pathway.

SUBCHAPTER II.II

Transcriptional response in apical branches of avocado to Fusarium dieback

MATERIALS AND METHODS

Plant material

From the samples collected in December 2015 from an avocado orchard located in Escondido, San Diego County, California, samples were also taken from the apical branch tissue of the five asymptomatic trees and five FD-symptomatic trees (necrotic tissue). The samples were taken in the same way as the sapwood tissue samples.

Library preparation and sequencing

Libraries preparation was performed as with sapwood tissue samples, but just 2 libraries were obtained, 1 from the FD-symptomatic condition and one from the asymptomatic one (Table 3).

Table 3. Branch samples data taken in December 2015 in Escondido, San Diego County, California.

Condition	**Asymptomatic**	**FD-symptomatic**
Trees number	5	5
Libraries number	1	1

Bioinformatic Analysis

Data processing, mapping of reads, transcript assembly (Supplementary 12 and 13), differential expression, functional analysis (Supplementary 14 and 15), enrichment of transcriptomes (Supplementary 16, 17 and 18) and visualization of mapped reads was performed as the first analysis (sapwood tissue).

RESULTS

Gene expression profiling in branches from asymptomatic and FD-symptomatic avocado trees

Distribution of filtered transcripts from asymptomatic and FD-symptomatic trees was analyzed by PCA. Both the grouping from the total of the expressed genes by considering the log2-foldchange value (Figure 29), and that of the differentially expressed genes by considering the FPKM value (Figure 30), showed a clear segregation, whereby opposite tendencies between asymptomatic and FD-symptomatic condition were also observed (Figure 29 and 30).

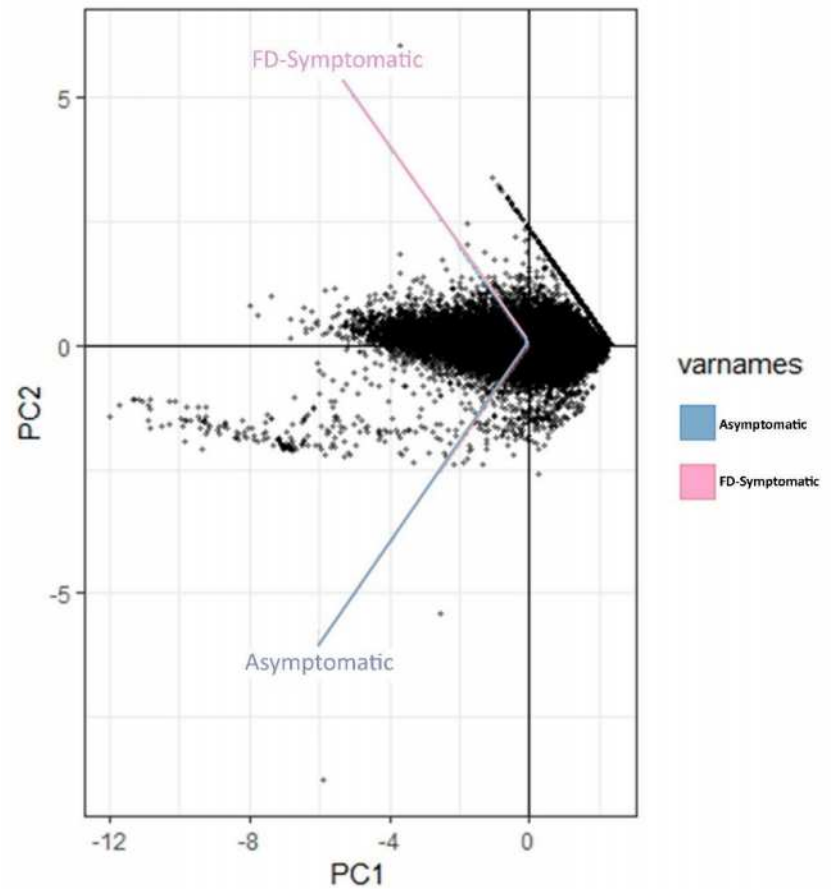

Figure 29. PCA grouping considering the log2-Foldchange value of apical branch tissue. Asymptomatic condition is marked in blue and FD-symptomatic condition in pink color.

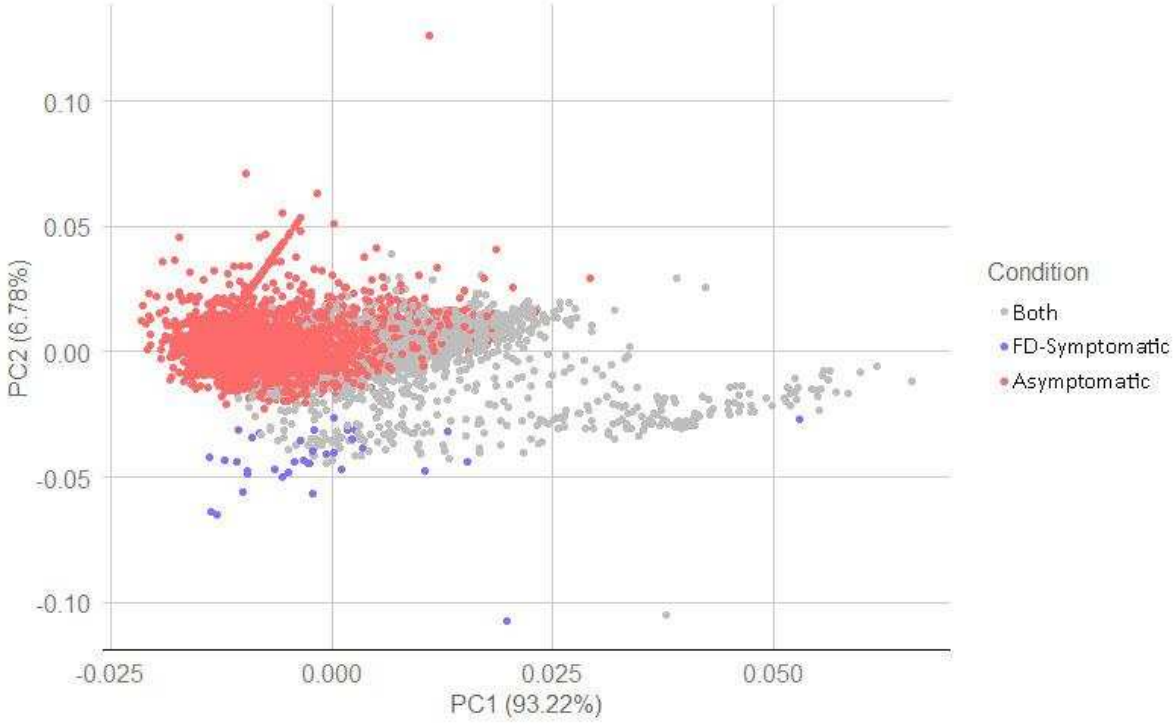

Figure 30. PCA grouping considering the FPKM value of apical branch tissue.

In total, 14,181 expressed genes were detected, 36 were exclusive from the asymptomatic condition and 4,088 from the FD-symptomatic group (Figure 31A). No exclusive upregulated genes were found in asymptomatic condition. Oppositely, 3,014 upregulated genes were exclusive to the FD-symptomatic, while 36 downregulated genes where exclusively found in asymptomatic condition and 1,074 in FD-symptomatic condition (Figure 31B and C). Besides, from total of genes expressed in both conditions, only 763 genes were differentially expressed in either condition, 36 of them were exclusive to asymptomatic condition and 399 to FD-symptomatic condition (Figure 32A). Within the differentially expressed total genes, no exclusive upregulated genes were found in asymptomatic condition, but 399 upregulated genes were exclusive to the FD-symptomatic (Figure 32B). There were 36 exclusive downregulated genes in asymptomatic condition, but no exclusive downregulated genes were found in FD-symptomatic condition (Figure 32C). And there were more expressed genes in FD-symptomatic condition than in asymptomatic condition in both totals (Figures 31 and 32).

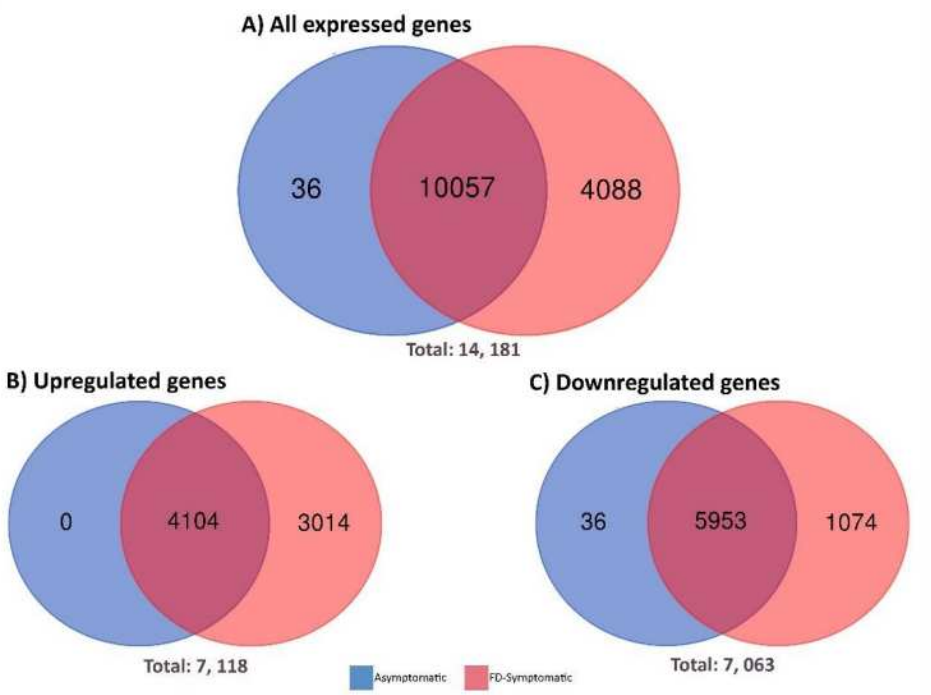

Figure 31. Venn Diagrams of all expressed genes from apical branch tissue. A) shows the general interaction between asymptomatic and FD-symptomatic condition. B) shows the interaction between induced genes and C) shows the interaction between repressed genes.

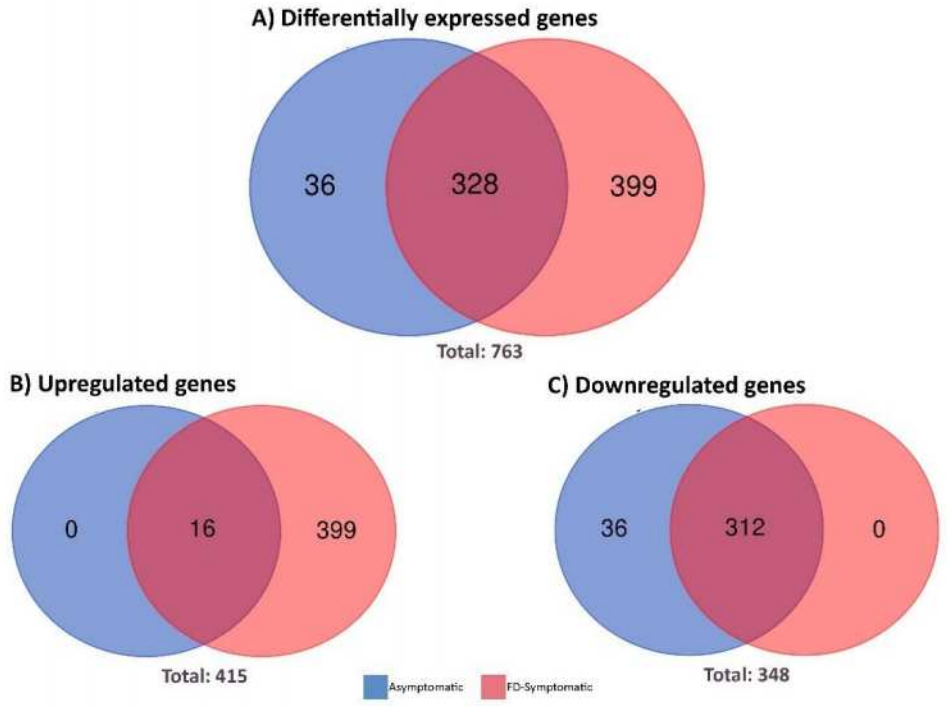

Figure 32. Venn Diagrams of differentially expressed genes from apical branch tissue. A) shows the general interaction between asymptomatic and FD-symptomatic condition. B) shows the interaction between induced genes C) shows the interaction between repressed genes.

The overview of this transcriptome studied, was similar to the first one (Supplementary 19). The annotation and the gene ontology (GO) functional classes and pathways for each avocado unigene of the obtained transcripts was performed like the first (Supplementary 19). Within the group of upregulated genes, 14 processes where genes could be involved were found (biotic and abiotic stimulus, coenzyme A biosynthetic process, defense response, development, energy, hormone response, lipid metabolism, metabolism, methylation, protein degradation, RNA degradation, transcription factor, transferase and transport) the most represented categories were biotic and abiotic stimulus, defense response, development, transcription factor and transporter (Figure 33). The group of the top 30 downregulated genes was classified in 8 processes (biotic and abiotic stimulus, development, lipid metabolism, photosynthesis, stabilization of membrane proteins, transcription factor, transporter and xyloglucan metabolism), being the primary development, photosynthesis and transporter-related genes the most abundant (Figure 34). Upregulated genes were more represented in FD-symptomatic condition (Figure 33), opposite to downregulated genes that are more represented in asymptomatic condition (Figure 34).

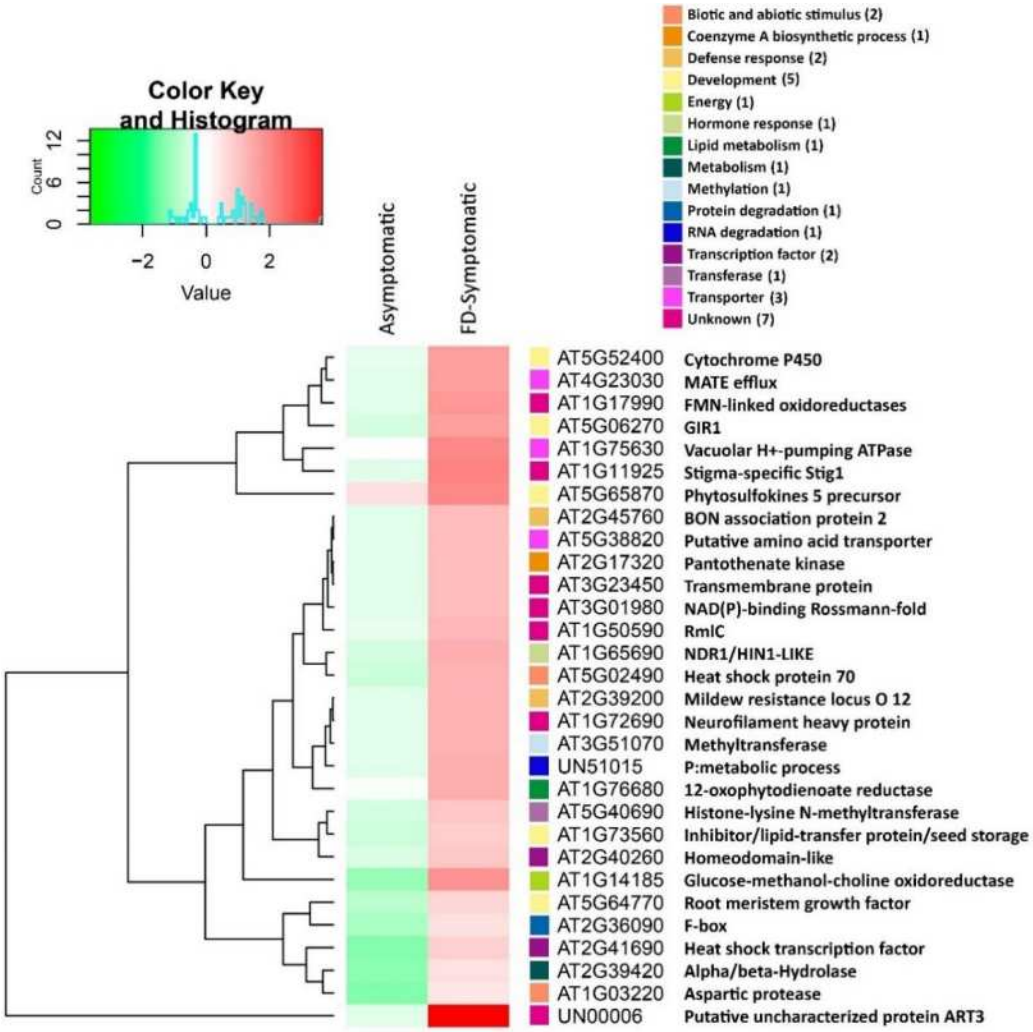

Figure 33. The top 30 upregulated genes with the highest foldchange from asymptomatic and FD-symptomatic avocado trees.

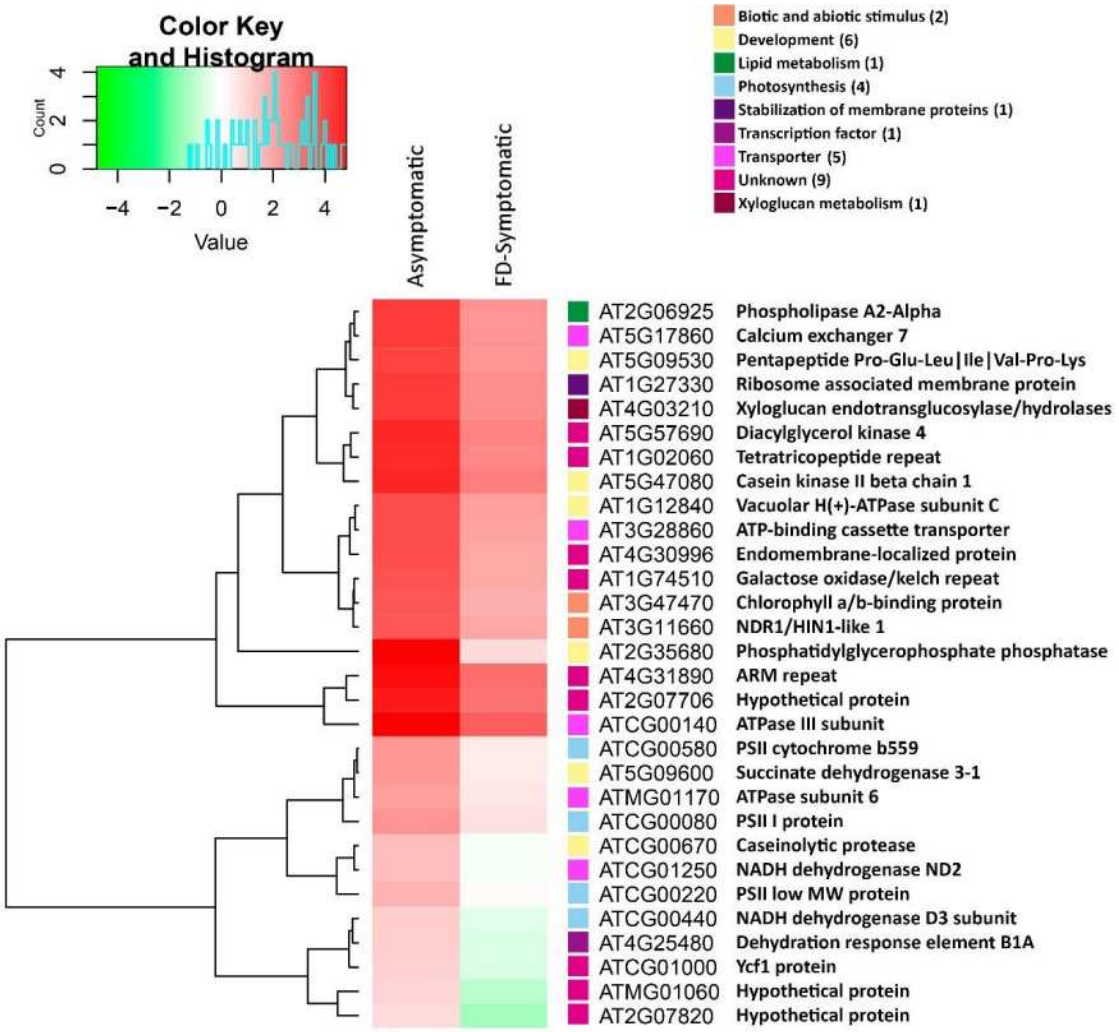

Figure 34. The top 30 downregulated genes with the highest foldchange from asymptomatic and FD-symptomatic avocado trees.

When the 30 genes with the highest expression level for both asymptomatic and FD-symptomatic conditions were listed (Supplementary 20), practically the same categories were represented (Figures 35 and 36). The category <<stabilization of membrane proteins>> was represented only in the asymptomatic category by one single gene, (At1g27330), which encodes for a ribosomal protein (Ribosome associated membrane protein RAMP4) (Figure 35). We also, observe again more presence in the genes related to biotic and abiotic stimulus in the FD-symptomatic condition (Figures 36).

4.81050297	AT2G35680	Tyrosine phosphatase	Biotic and abiotic stimulus (1)
4.79561366	AT3G18490	Aspartic protease in guard cell 1	Carbohydrate metabolism (1)
4.78209263	UN27328	TAR1 Protein	Development (5)
4.75222136	UN18613	rDNA transcription protein regulator 15	Lipid metabolism (1)
4.63209878	ATCG00140	ATPase III subunit	Post-transcriptional regulation (1)
4.58182114	UN32262	rDNA transcription protein regulator 15	Stabilization of membrane proteins (1)
4.55222153	UN34153	rDNA transcription protein regulator 15	Transcription regulation (5)
4.51014538	UN42298	rDNA transcription protein regulator 15	Translation (1)
4.40092462	UN42364	rDNA transcription protein regulator 15	Transporter (5)
4.36296287	AT4G31890	ARM repeat	Unknown (8)
4.23799013	AT5G53330	Elongation factor 1B	Xyloglucan metabolism (1)
4.1681998	AT2G07706	Hypothetical protein	
4.14725649	AT2G35800	S-Adenosyl methionine transporter	
4.0955065	AT1G47480	Hydrolase	
4.05579311	AT4G29040	Regulatory particle AAA-ATPASE 2A	
4.02838001	AT5G47080	Casein kinase II	
3.94979946	AT5G57690	Diacylglycerol kinase 4	
3.94425066	AT1G02060	Tetratricopeptide repeat	
3.88608726	AT5G51440	HSP20-like chaperon	
3.66700934	AT2G42210	OEP16	
3.64294732	AT2G06925	Phospholipase A2	
3.64162232	AT1G27330	Ribosome associated protein	
3.59456193	AT5G36230	ARM repeat	
3.59324731	AT3G61490	Pectin lyase-like	
3.59258677	AT4G03210	Endotransglucosylase/hydrolase 9	
3.58490886	AT5G17860	Calcium exchanger 7	
3.51344202	AT5G09530	Pentapeptide	
3.35960062	AT1G12840	ATPase	
3.33795874	AT3G28860	ATP-binding cassette B19	
3.32953172	AT4G30996	NA(+)- and K(+)-sensitive 1	

Figure 35. The 30 most expressed genes of asymptomatic condition.

3.6605904	UN00006	Putative uncharacterized protein ART3	Biotic and abiotic stimulus (3)
3.40729214	AT3G18490	Aspartic protease in guard cell 1	Carbohydrate metabolism (3)
3.30234175	UN27328	TAR1 Protein	Development (3)
3.26298947	UN18613	rDNA transcription protein regulator 15	Floral organ abscission (1)
3.10736941	UN42298	rDNA transcription protein regulator 15	iron-sulfur assembly (1)
3.10032574	UN34153	rDNA transcription protein regulator 15	Post-transcriptional regulation (1)
3.00213149	UN32262	rDNA transcription protein regulator 15	Transcription regulation (4)
2.99711847	ATCG00140	ATPase III subunit	Translation (1)
2.85722877	AT2G35800	S-Adenosyl methionine transporter-like	Transporter (3)
2.84585462	AT5G53330	Elongation factor 1B	Unknown (10)
2.82116142	AT1G47480	Hydrolase	
2.76971175	AT4G29040	Regulatory particle AAA-ATPASE 2A	
2.72075037	AT4G31890	ARM repeat	
2.68659606	AT2G07706	Hypothetical protein	
2.68049285	AT2G44010	Hypothetical protein	
2.54619089	AT5G51440	HSP20-like chaperon	
2.47970775	AT5G47080	Casein kinase II	
2.45462537	AT3G48140	B12D protein	
2.38722741	AT5G07290	Mei2-like	
2.37377917	AT5G57690	Diacylglycerol kinase 4	
2.37136119	AT2G42210	OEP16	
2.36474793	AT3G13784	Cell wall invertase 5	
2.31526483	AT2G18980	Peroxidase	
2.30337912	AT3G61490	Pectin lyase-like	
2.2764095	AT5G03690	Fructose-bisphosphate aldolase	
2.26514708	AT1G54410	Dehydrin	
2.25320954	AT1G02060	Tetratricopeptide repeat	
2.24394378	AT5G36230	ARM repeat	
2.24108629	AT4G22220	ISU1	
2.23745995	ATMG00030	Hypothetical protein	

Figure 36. The 30 most expressed genes of FD-symptomatic condition.

Functional classification of differentially expressed genes in response to FD

Functional classification of differentially expressed genes for both conditions (763 unigenes) was also performed, and 29 functional subcategories of biological processes were represented (Figures 37 and 38). The most significant subcategories represented for both, asymptomatic and FD-symptomatic groups were: nucleotide metabolism, response to stimulus, organic substance transport and organic substance metabolism (Figures 37 and 38). Further, in asymptomatic condition, category <<nucleotide metabolism>> had a higher value than in FD-symptomatic condition; interestingly, the categories response to stimulus, organic substance metabolism and organic substance metabolism had a higher value in FD-symptomatic condition and the methylation, organic hydroxy compound metabolism, peptidyl-amino acid modification, rhythmic process subcategories were only represented in this condition (Figures 37 and 38).

At the cellular component categorization, in total 9 subcategories were represented. For both tested conditions, cell and membrane were the most significantly represented subcategories (Figure 37). Further almost all subcategories had the same significance in both conditions (asymptomatic and FD-symptomatic) except cell junction that had a higher value in asymptomatic condition, membrane in FD-symptomatic condition and the thylakoid lumen was only represented in this condition (Figure 37). Categorization at the molecular function level showed that 16 subcategories were represented (Figure 37). For asymptomatic condition, binding was the most significant subcategory and that subcategory was only in this condition (Figure 37). For FD-symptomatic condition, rRNA binding was the most significant subcategory; in asymptomatic condition, structural molecule activity had a higher value than in FD-symptomatic condition, whereas in FD-symptomatic condition, transporter activity, ATPase activity, rRNA binding, transferase activity had a higher value than in asymptomatic condition. Also, CoA carboxylase activity, enzyme activator activity, isomerase activity, receptor activity and transcription cofactor activity subcategories were only found in FD-symptomatic condition (Figure 37).

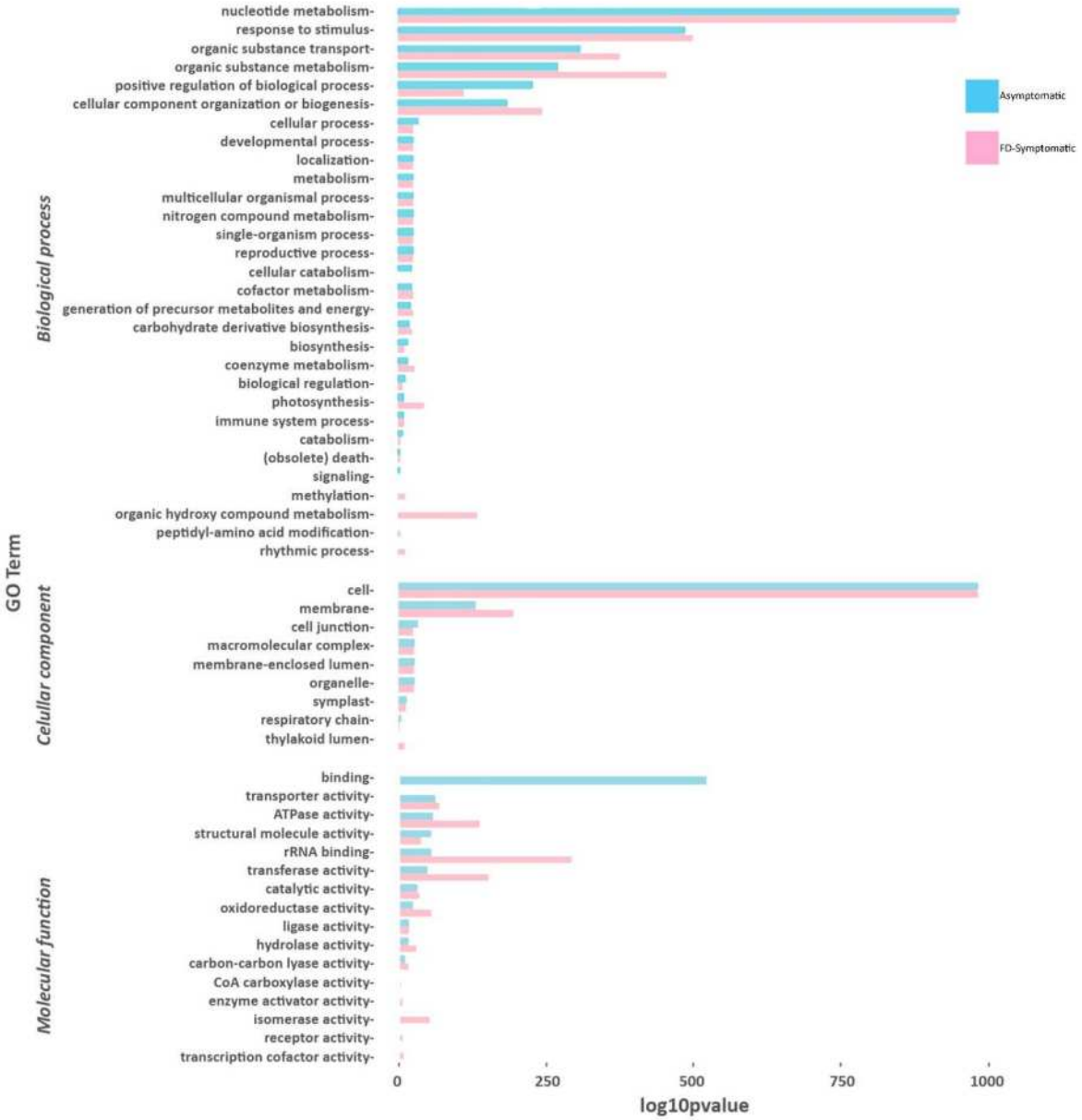

Figure 37. Functional classification summary, obtained by REVIGO (Rudjer Boskovic Institute Croatia; Supek *et al.*). Blue bars represent asymptomatic condition and pink bars represent FD-symptomatic condition.

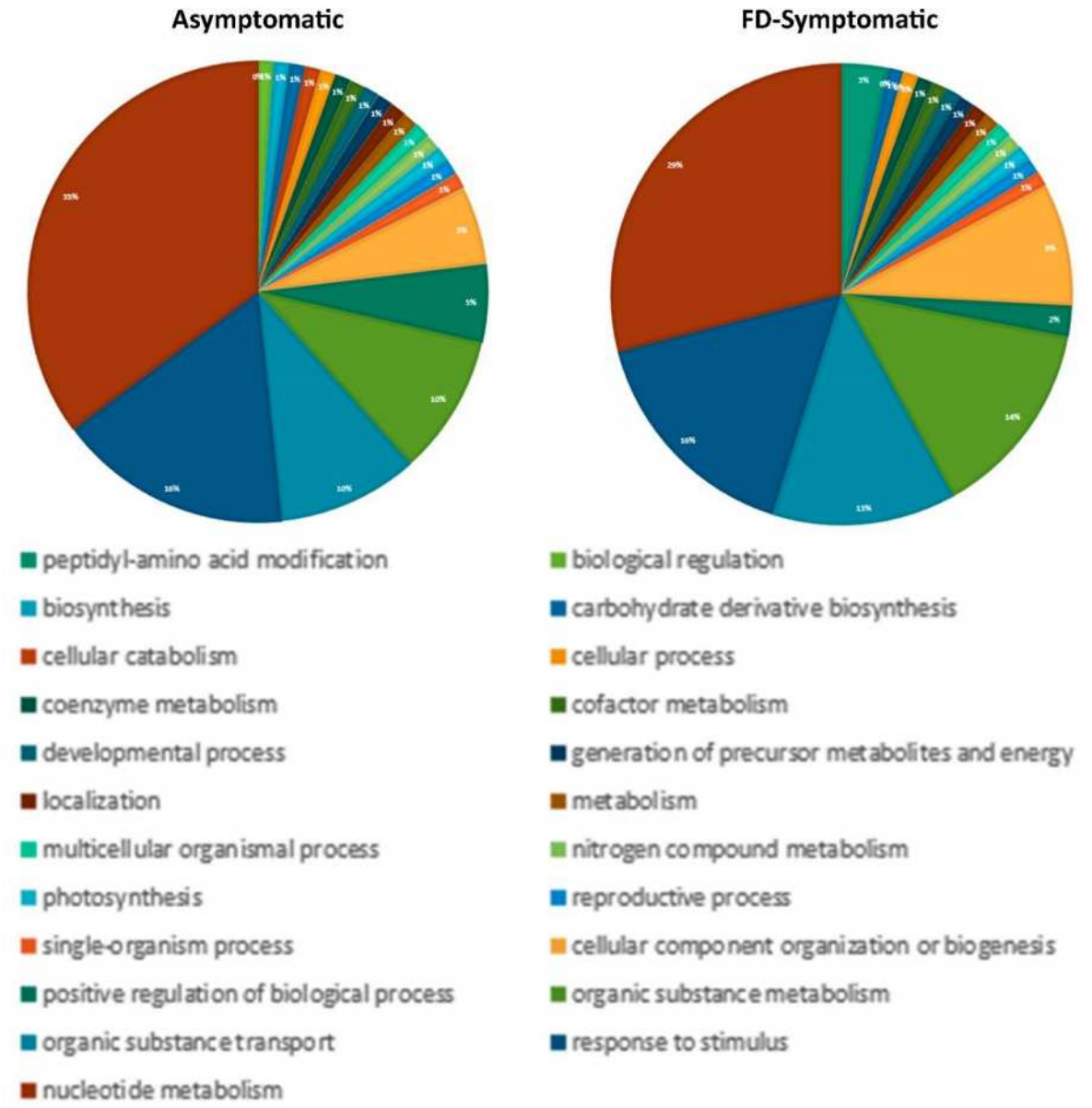

Figure 38. Pie-Plot of functional classification summary of biological processes, obtained by REVIGO (Rudjer Boskovic Institute Croatia; Supek *et al.*). On the left we can find asymptomatic condition represented and, on the right, we can find FD-symptomatic condition represented.

KEGG enrichment analysis

To know how what metabolic pathways were being expressed, Venn diagrams of all the pathways obtained in KEGG enrichment analysis and maps of KEGG metabolic pathways of differentially expressed genes related to carbohydrate and lipid pathways were created. As a result, 86 KEGG pathways were obtained and no exclusive KEGG pathways were found in asymptomatic condition, but 36 exclusive pathways were found in FD-symptomatic condition (Figure 39). 107 KEGG enzymes were obtained and no exclusive KEGG enzymes were found in asymptomatic condition but 66 of FD-symptomatic condition (Figure 39). Furthermore, in both cases different steps of the studied pathways seem to be expressed in both conditions and a few only in FD-symptomatic condition (Figure 40 and 41). In addition, two induced genes related to lipids with the highest expression value in asymptomatic condition (At3g51520 and Atcg00500) were found in the KEGG pathways for fatty acid biosynthesis-related pathways (Supplementary 21; Figure 41).

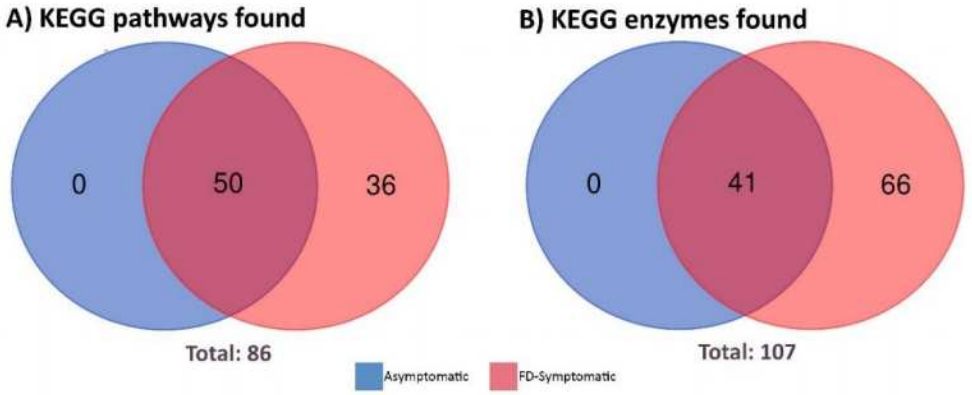

Figure 39. Venn Diagrams of pathway enrichment. A) shows the interaction between KEGG pathways in asymptomatic and FD-symptomatic condition. B) shows the interaction between KEGG enzymes found in asymptomatic and FD-symptomatic condition.

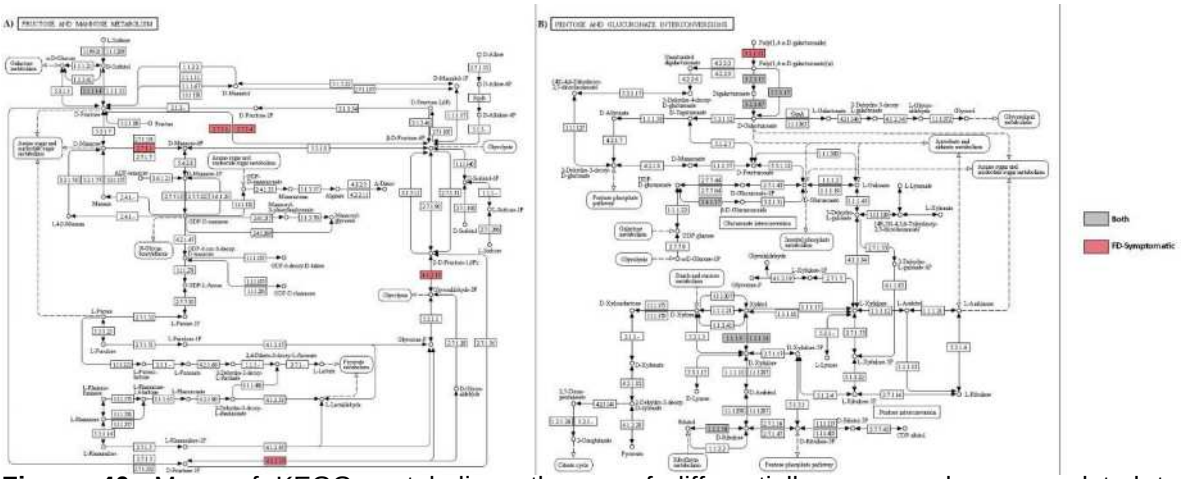

Figure 40. Maps of KEGG metabolic pathways of differentially expressed genes related to carbohydrate pathways. A) shows fructose and mannose metabolism pathway B) shows pentose and glucuronate interconversions pathway.

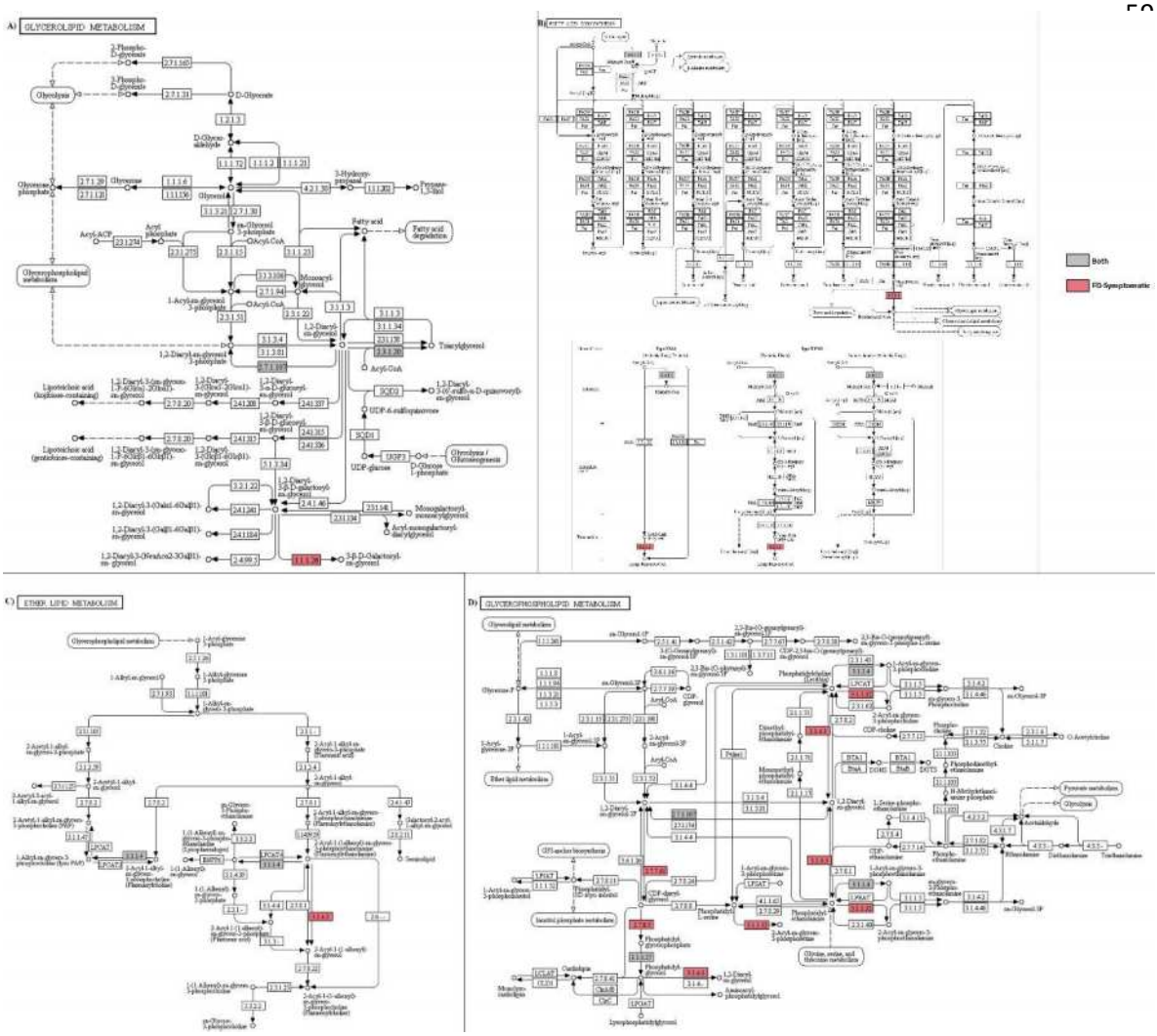

Figure 41. Maps of KEGG metabolic pathways of differentially expressed genes related to lipid pathways. A) shows glycerolipid metabolism pathway. B) shows fatty acid biosynthesis pathway. C) shows ether lipid metabolism pathway. D) shows glycerophospholipid metabolism pathway.

Expressed genes related to metabolic and defense pathways

As the first analysis, we analyzed transcriptional expression profiles of the 30 annotated genes with highest fold change value related to carbohydrate, monosaccharide and lipid pathways (Supplementary 22). Genes related to carbohydrate metabolism (biosynthesis and catabolism) process showed different patterns, most of them were inducible in FD-symptomatic condition but inducible genes were also found in the asymptomatic condition (Figure 42). About genes related to monosaccharide metabolism (biosynthesis and catabolism) process expression level in both groups, asymptomatic and FD-symptomatic differentially expressed genes was similar and biosynthesis activity seems to be low, but catabolism activity is high (Figures 43).

With respect to the 30 most expressed genes related to lipid biosynthesis, most of induced genes belonged to FD-symptomatic condition, although most of them encodes to proteins involved in

specialized lipid production (hormones and secondary metabolites mainly), they do not seem being involved in primary lipid biosynthesis (At1g76690, that participates in jasmonic acid biosynthetic process; At2g34555, in gibberellin biosynthetic process; At1g65690, in ABA signaling and biosynthesis; At1g76680, in oxylipin biosynthetic process; At2g36690, gibberellin metabolic process; At5g51810, in gibberellin biosynthetic process; and At4g02780, gibberellin biosynthetic process; Figure 44). Interestingly, some of the overexpressed genes with the highest expression value in asymptomatic condition were genes that code for clue enzymes involved in fatty acid biosynthesis (At3g51520, At3g10520 and Atcg00500; Figure 44A). At3g51520 encodes a functional acyl-CoA:diacylglycerol acyltransferase; At3g10520 encodes a class 2 non-symbiotic hemoglobin; and Atcg00500 encodes the carboxytransferase beta subunit of the Acetyl-CoA carboxylase (ACCase) complex in plastids. Oppositely, the expression level of genes involved in lipid catabolism in both groups, asymptomatic and FD-symptomatic differentially expressed genes was similar, but still being more inducible genes in the FD-symptomatic condition (Figure 44B).

Although several genes were classified as part of lipid biosynthesis machinery (Figure 44A), some of them participates in hormonal biosynthetic pathways related to defense responses. Then, we analyzed the expression patterns of the 30 most expressed genes related to jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) responsive and biosynthetic routes and a similar pattern was also observed in both groups (asymptomatic and FD-symptomatic) (Supplementary 23 and Figure 45).

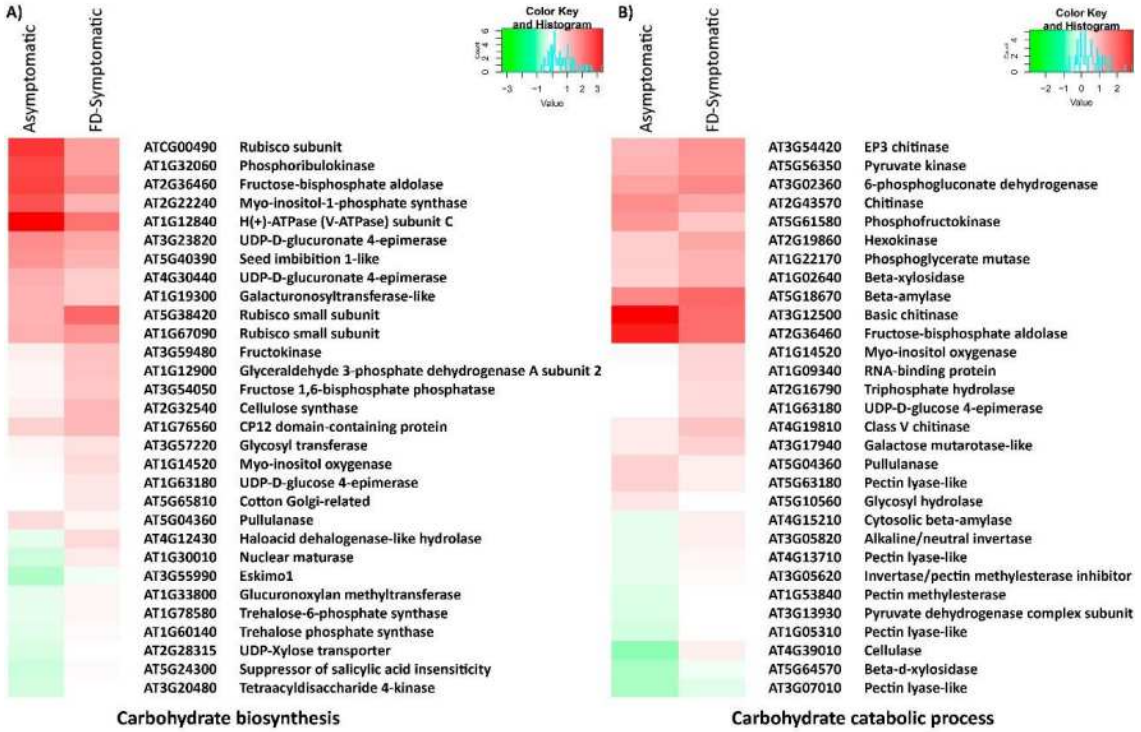

Figure 42. Heatmaps of the 30 genes with greater fold change related to carbohydrate biosynthesis (left) and to carbohydrate catabolic process (right).

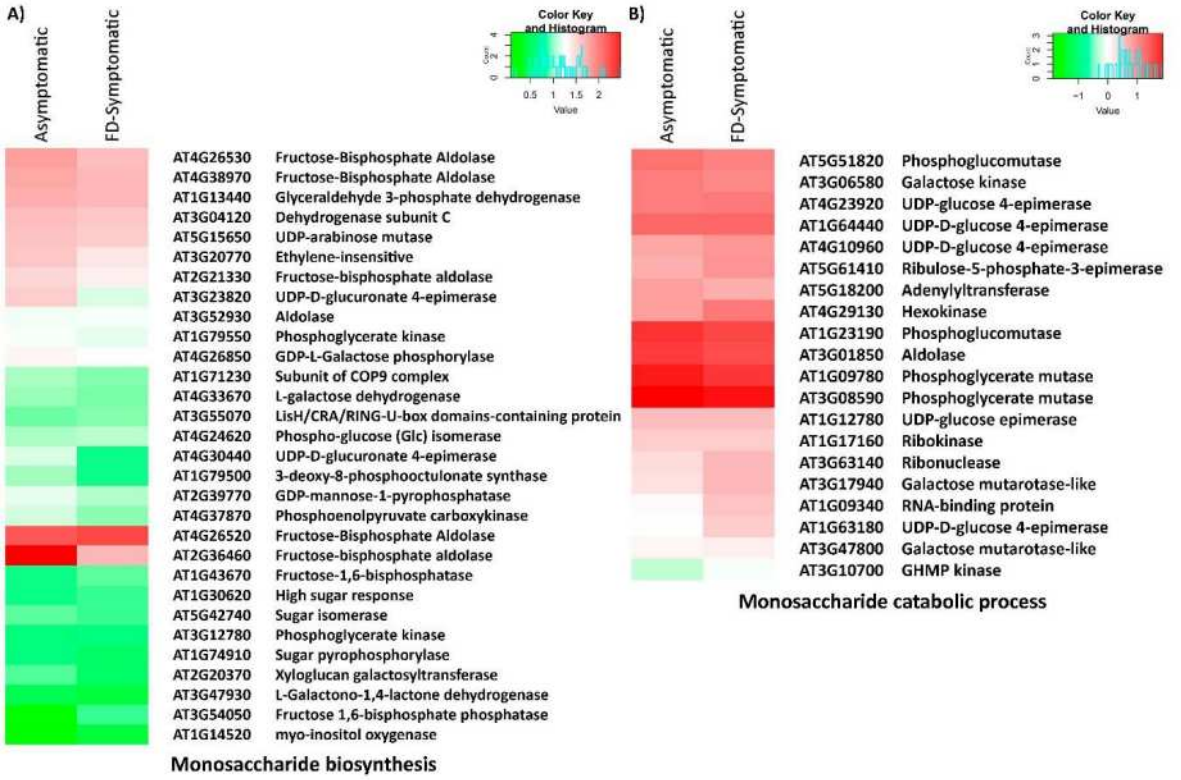

Figure 43. Heatmaps of the 30 genes with greater fold change related to monosaccharide biosynthesis (left) and to monosaccharide catabolic process (right).

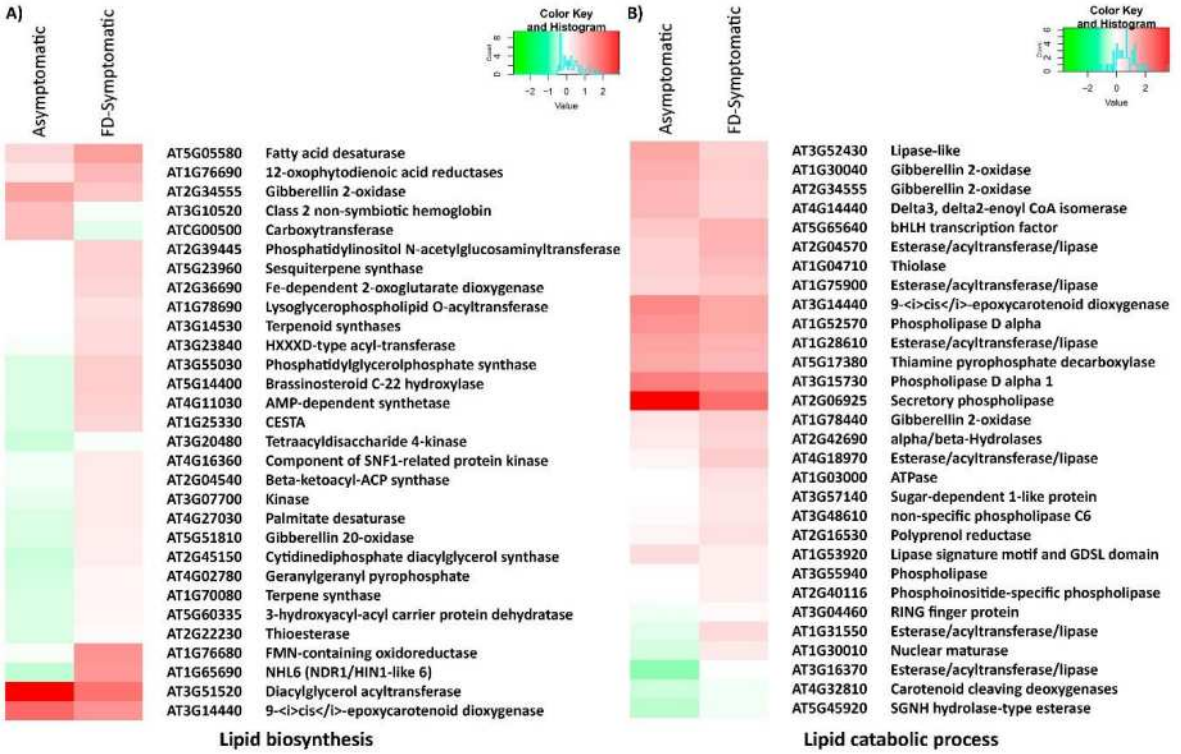

Figure 44. Heatmaps of the 30 genes with greater fold change related to lipid biosynthesis (left) and to lipid catabolic process (right).

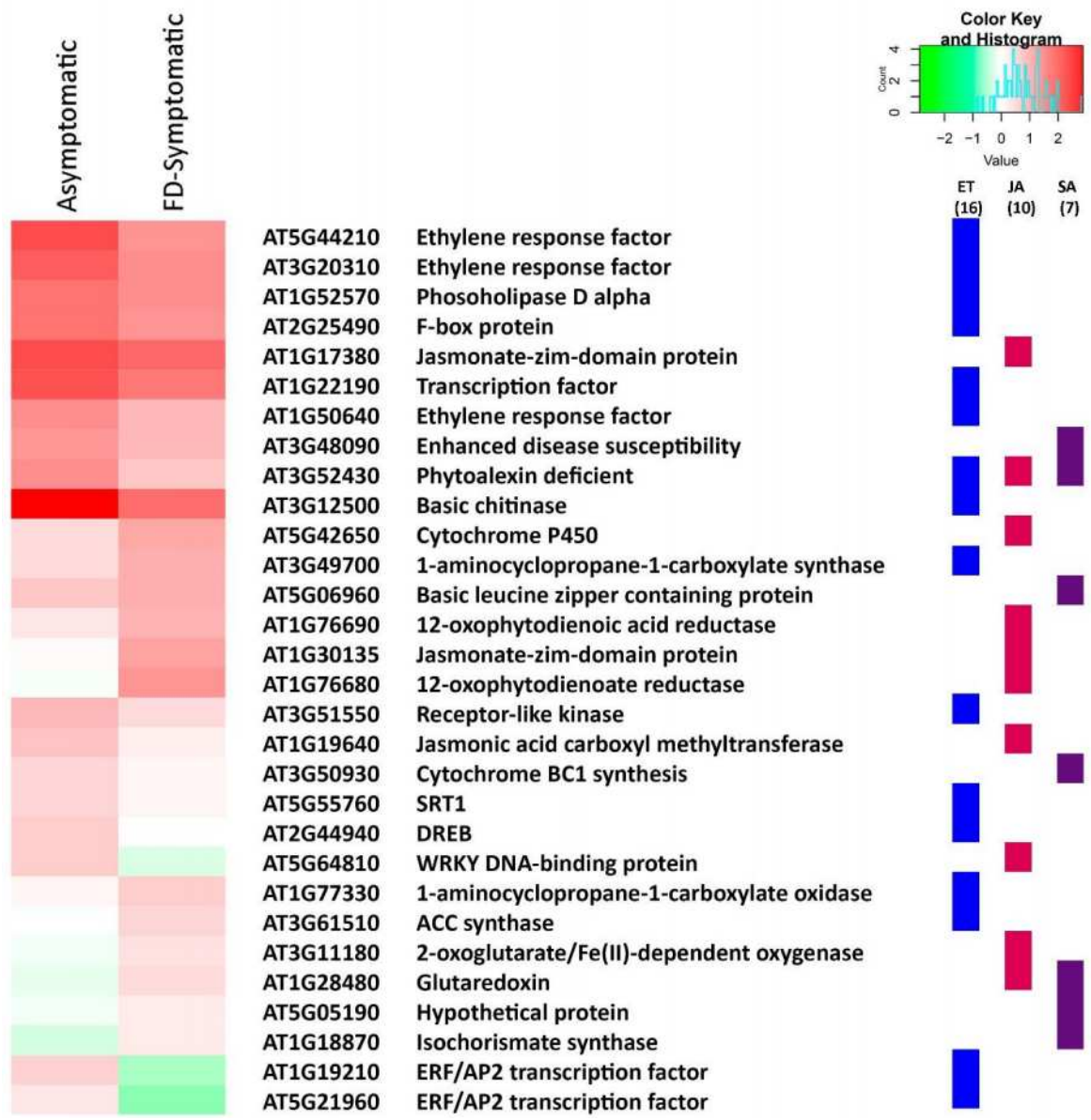

Figure 45. Heatmap of the 30 genes with the highest fold change related to hormone defense from apical branch tissue, ET means for ethylene biosynthetic process and ethylene-activated signaling pathway, JA means jasmonic acid biosynthetic process and jasmonic acid-mediated signaling pathway, and SA means salicylic acid biosynthetic process and salicylic acid-mediated signaling pathway.

SUBCHAPTHER II.III

DISCUSION

Expressed genes differentiation

In both analyzed tissues of the sampled trees (sapwood tissue and apical branch tissue) a clear segregation of data for both conditions was observed, hence, this information indicates that each condition is different from the other. In addition, of both total genes expressed (sapwood tissue: 14,773; and apical branch tissue: 14,181) and differentially expressed genes (sapwood tissue: 1,854; and apical branch tissue: 763) there were more genes expressed exclusively in a FD-symptomatic condition than in a asymptomatic one (total of genes expressed: In sapwood tissue 126 in asymptomatic condition and 2532 in FD-symptomatic condition and in apical branch tissue 36 in asymptomatic and 4088 in FD-symptomatic group; Total differentially expressed genes: In sapwood tissue 114 in asymptomatic and 1063 in FD-symptomatic and in apical branch tissue 36 in asymptomatic and 399 in FD-symptomatic). Perhaps because FD is causing the expression of several genes in the plant to fight it.

Also, if we look at the upregulated and downregulated genes of the total expressed genes there are more genes both induced and repressed in the FD-symptomatic condition in both areas of the tree (Upregulated: In sapwood tissue 0 in asymptomatic and 2368 in FD-symptomatic and in apical branch tissue 0 in asymptomatic and 3014 in FD-symptomatic; Downregulated: In sapwood tissue 126 in asymptomatic and 164 in FD-symptomatic and in apical branch tissue 36 in asymptomatic and 1074 in FD-symptomatic). Nevertheless, if we observe in the total differentially expressed genes there are no genes induced in asymptomatic condition nor are there repressed genes in FD-symptomatic condition (Upregulated: In sapwood tissue 0 in asymptomatic and 1063 in FD-symptomatic and in apical branch tissue 0 in asymptomatic and 399 in FD-symptomatic ; Downregulated: In sapwood tissue 114 in asymptomatic and 0 in FD-symptomatic and in apical branch tissue 36 in asymptomatic and 0 in FD-symptomatic). Therefore, if we focus only on differentially expressed genes in asymptomatic condition there are only repressed genes and in FD-symptomatic condition there are only induced genes.

About genes with the highest foldchange value for both, sapwood and apical branch tissue, upregulated genes are more represented in a FD-symptomatic condition, opposite to downregulated genes that are more represented in asymptomatic condition. In sapwood tissue the most represented categories in upregulated genes were transporter, biotic and abiotic stimulus and defense response, while in apical branch where biotic and abiotic stimulus, defense response, development, transcription factor and transporter. On the other hand, in sapwood tissue the most represented categories in downregulated genes were metabolism and development-related genes the most abundant while in apical branch where development, photosynthesis and transporter-related. The fact that the FD-symptomatic condition is more gene-induced follows the same pattern as the previous analyzes. Likewise, it makes sense that biotic and abiotic stimulus and defense response were

induced in the FD-symptomatic condition and that genes related to primary metabolism and development were induced in asymptomatic condition, since it seems to act as it normally would.

When we analyzed genes with the highest expression level, we found that they are mostly in asymptomatic condition in both, sapwood and apical branch tissue. For both areas, practically the same categories were represented and the category <<stabilization of membrane proteins; At1g27330>> was represented only in the asymptomatic condition and encodes a Ribosome associated membrane protein RAMP4 that responses to oxidative stress (Kobayashi *et al.*, 1996). Also, for both areas, we observe again more presence in the genes related to biotic and abiotic stimulus in the FD-symptomatic condition, which agrees with the above.

Expressed genes functional classification

There was no great difference in number of subcategories obtained between both, sapwood and apical branch tissue. Besides, there were several subcategories that were only found in FD-symptomatic condition, which supports the first analysis, where it is observed that possibly the disease is causing the expression of many genes to fight it. In this analysis genes also are more represented in FD-symptomatic condition in both areas. Moreover, response to stimulus is more represented in FD-symptomatic condition and genes related to primary metabolism and development were more represented in asymptomatic condition.

KEGG enrichment analysis

As the genes, the number of metabolic pathways and enzymes increases considerably in FD-symptomatic condition and in case of apical branch tissue, there are no pathways or enzymes exclusive to asymptomatic condition which also supports previous information. Furthermore, the upregulated genes that were associated with biotic and abiotic stimulus and defense response in the FD-symptomatic group (At1g68850, At1g75030, At5g51890 and At1g75800) were also found in KEGG pathways for phenylpropanoid biosynthesis pathway, important in plant defense. Moreover, some relevant pathways were found in the KEGG. In sapwood tissue At1g14520, one of the induced genes in FD-Symptomatic condition of the monosaccharide pathway was represented in KEGG inositol phosphate metabolism pathway. In addition, in both, sapwood and apical branch tissue, some of the genes related to lipid biosynthesis with the highest expression value in asymptomatic condition (At3g51520, At3g02630, Atcg00500 and At2g23800 for sapwood tissue and At3g51520 and Atcg00500) was represented in KEGG fatty acid biosynthesis-related pathways, which reinforces the following analysis.

Expressed genes in interest pathways

Differentially expressed genes related to carbohydrate metabolism (biosynthesis and catabolism) process showed a large difference in both conditions (asymptomatic and FD-symptomatic) and areas (sapwood tissue and apical branch tissue), most of them being inducible in FD-symptomatic condition, which indicates that carbohydrates are being produced in FD-symptomatic condition. This gives us a possibility to find possible disease genetic markers in this specific path. However, although carbohydrates seem to be produced mostly in FD-symptomatic condition, it also show degradation of them in this condition.

Carbohydrate metabolism can be difficult to figure out, since much of an organism's metabolism is within the carbohydrate metabolic pathways. Hence, a study of only monosaccharides was also carried out, considering that 7-carbon sugars are formed through this metabolic pathway. The expression level of both conditions was similar in genes related to biosynthesis and those related to catabolism in both areas (sapwood and apical branch tissue). In sapwood tissue it seems that both biosynthesis and catabolism are active while in apical branch tissue biosynthesis activity seems to be low, but catabolism activity is high. This result may be because monosaccharide pathway is also related to a series of important metabolic processes such as glycolysis, an important enzyme in this process is the Fructose-bisphosphate aldolase (Marsh & Lebherz, 1992) which is one of the enzymes that appears most active in the heatmaps.

In sapwood tissue three avocado unigenes with induced expression in FD-symptomatic group, contrasting to asymptomatic condition (At1g79500, At2g45790 and At1g14520). At1g79500 encodes a protein with 3-deoxy-8-phosphooctulonate synthase (KDOP synthase) activity which is involved in the biosynthesis of 3-deoxy-d-manno-octulosonate (KDO) (Matsuura *et al.*, 2003) that in higher plants, is one of the constituent sugars of rhamnogalacturonan II (RG-II) (York *et al.*, 1985) and provide the cross-linking site for boric acid along pectic polysaccharide chains (Kobayashi *et al.*, 1996). At2g45790 encodes a cytoplasmic phosphomannomutase, which is part of GDP-mannose biosynthesis, an activated sugar nucleotide (Luhua *et al.*, 2008) which contributes to the synthesis of different structural carbohydrates in plant cell walls and plays a key role in the biosynthesis of ascorbic acid (AsA) (Qian *et al.*, 2007) that participates in resistance to environmental stress and synthesis of ethylene, gibberellins, anthocyanins and hydroxyproline (Smirnoff & Wheeler, 2000). At1g14520 encodes a MIOX1 that belongs to myo-inositol oxygenase gene family. This enzyme participe on glucuronic acid biosythesis, which is activated to UDP-glucuronic acid (UDPGlcA) and serves as a precursor for plant cell wall polysaccharides (Wakabayashi *et al.*, 1989; Loewus & Murthy, 2000; Endres & Tenhaken, 2011) and also could be part of AsA biosynthesis (Lorence *et al.*, 2004).

Lipids seem to be in both conditions and both areas especially in FD-symptomatic condition but also seem to be degraded mostly in this condition. This gives us the possibility that the metabolic

pathway is being expressed and forms the antifungal molecules in both conditions, but due to their degradation in FD-symptomatic condition it cannot finish forming. Thus supporting the metabolic information obtained in the chemical profiles. Some genes with the highest expression value in asymptomatic condition were interest genes involved in fatty acid biosynthesis (At3g51520, At3g02630, Atcg00500 and At2g23800 for sapwood tissue; and At3g51520, At3g10520 and Atcg00500 for apical branch tissue), At3g51520 encodes a functional acyl-CoA:diacylglycerol acyltransferase that increases polyunsaturated fatty acid as Diacylglycerol acyltransferase (DGAT) is part of the triacylglycerol (TAG) biosynthesis and plays an important role in fatty acid storagen (Ichihara *et al.*, 1988; Liu *et al.*, 2012). At3g02630 is one of seven acyl acyl carrier proteins that primarily regulates the ratios of saturated to monounsaturated FAs (Kachroo *et al.*, 2007). At2g23800 encodes an endoplasmic reticulum-targeted geranyl geranyl pyrophosphate synthase which is an important enzyme for isoprenoid biosynthesis (Zhu *et al.*, 1997) and it is an essential precursor for carotenoid biosynthesis (Chappell, 1995). Atcg00500 encodes the carboxytransferase beta subunit of the Acetyl-CoA carboxylase (ACCase) complex in plastids. This complex catalyzes the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase, the first step in fatty acid biosynthesis (Kanai *et al.*, 2013). At3g10520 encodes a class 2 non-symbiotic hemoglobin that promote the accumulation of polyunsaturated fatty acids (Vigeolas *et al.*, 2011).

Although most of the induced genes of FD-symptomatic condition encodes to proteins involved in hormones and secondary metabolites (At1g65690, At1g76680, At4g18780, At2g34555, At3g45140 and At5g51810 for sapwood tissue; and At1g76690, At2g34555, At1g65690, At1g76680, At2g36690, At5g51810 and At4g02780 for apical branch tissue), we analyzed the expression patterns of genes related to jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) responsive and biosynthetic routes. In sapwood tissue, genes were mainly represented in FD-symptomatic condition, perhaps because the disease was causing its expression. Nevertheless, in apical branch tissue the expression was similar in both conditions, indicating that both the disease and health activate the response of defense hormones in this area.

Future perspectives

In future, we expect to refine the search for both, chemical and genetic markers specific to FD, in order to create an effective disease diagnosis. It is possible that molecular markers could be found in genes related to carbohydrate metabolic pathways but it could be difficult to find it in genes related to monosaccharide metabolic pathways, so it would be necessary to further study the specific metabolic pathway of sugars of 7 carbons and thus look for molecular markers related to these pathways. However, it is clear that the disease marks a different pattern that the health at the expression level, so it would be ideal to look for markers on routes with a differentially high expression level. By obtaining molecular markers, it will be easier to detect the disease without being an invasive

diagnosis. A question we ask is whether this will only work in avocado plant or if there are more plants families that act in a similar way with this disease. On the other side, in the case of compounds that were found accumulated in asymptomatic conditions, it is sought to test them directly against the disease or fungus and then create a pest biocontrol by inducing or applying these metabolites.

CONCLUSION

The two conditions studied are expressed differently in both areas (sapwood and apical branch tissue). There are more genes, pathways and enzymes represented in FD-symptomatic condition in both areas. In addition, the number of upregulated genes in response to fusarium dieback exceeded the number of downregulated genes in both areas. While in health responses the number of downregulated genes exceeded the number of upregulated genes in both areas. Hence, it is possible that the disease is causing the expression of many genes to fight it. About the functional classification, biotic and abiotic stimulus and defense response subcategories were induced in the FD-symptomatic condition and genes related to primary metabolism and development were induced in asymptomatic condition. Moreover, some relevant pathways related to monosaccharide and lipid biosynthesis were found in the KEGG enrichment analysis.

Genes associated with carbohydrate metabolic pathways showed a great difference in both areas, this gives us the possibility of locating genetic markers to use them, later, for the diagnosis of diseases. on the other hand, genes related to monosaccharide metabolic pathways were similar. Therefore, molecular markers are more likely to be found in genes related to carbohydrate metabolic pathways than in those of monosaccharides. It is necessary to study further the specific metabolic pathway of 7-carbon sugars in order to look for molecular markers in this metabolic pathway. Furthermore, in sapwood tissue we found three avocado induced unigenes in the FD-symptomatic group (At1g79500, At2g45790 and At1g14520).

Genes associated with lipid biosynthesis are expressed in both conditions and in both areas but there is degradation in FD-symptomatic condition, possibly because in both conditions the plant tries to defend itself by creating secondary metabolites, but, in FD-symptomatic condition due to the degradation the molecule is not formed. In addition, we found some interest induced genes in asymptomatic condition that are involved in fatty acid biosynthesis (At3g51520, At3g02630, Atcg00500 and At2g23800 for sapwood tissue; and At3g51520, At3g10520 and Atcg00500 for apical branch tissue). About the pathways related to defense hormones (ET, JA and SA), they were expressed mainly in FD-symptomatic condition, this perhaps because the disease was causing its expression. But, in apical branch tissue the expression was similar in both conditions, which indicates that in both disease and health the hormone defense is activated.

The results of this study showed that FD increases the expression of several defense-related genes in the host to fight infections, while the metabolic profile is redirected to carbohydrate mobilization. Results from this study showed that FD increases the expression of many defense-related genes in the host to battle the infection, while metabolic profile is redirected to the carbohydrate mobilization. These results contribute to the diagnosis of FD by identifying genetic markers. Additionally, the evaluation of the biological activity of some differentially produced metabolites in the asymptomatic trees to search for antifungal properties could be promising for FD management.

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ANNEXES

Sapwood tissue:

https://drive.google.com/file/d/1uzqwp4pROZfqMD9QUaYGhDRCKSwUiGRT/view?usp=sharing

Apical branch tissue:

https://drive.google.com/file/d/1aD3yPlboHPla9sMcVbwcy-Y1aQGbnWeB/view?usp=sharing

CONCLUSIONES GENERALES

Las dos condiciones estudiadas se expresan de manera diferente en ambas áreas (tejido de la albura y tejido de rama apical). Hay más genes, vías y enzimas representados en condiciones de enfermedad en ambas áreas. Además, el número de genes regulados al alza en respuesta a la marchitez por fusarium excedió el número de genes regulados negativamente en ambas áreas. Mientras que, en las respuestas a salud, el número de genes regulados negativamente excedió el número de genes regulados al alza en ambas áreas. Por lo tanto, es posible que la enfermedad esté causando la expresión de muchos genes para combatirla. En cuanto a la clasificación funcional, las subcategorías bióticos y abióticos y respuesta de defensa se relacionaban con genes inducidos en la condición FD-sintomática y en la condición asintomática los genes inducidos se relacionaban con el metabolismo primario y el desarrollo. Además, en el análisis de enriquecimiento de KEGG se encontraron algunas rutas relevantes relacionadas con la biosíntesis de monosacáridos y lípidos.

Los genes asociados con las vías metabólicas de los carbohidratos mostraron una gran diferencia en ambas áreas, esto nos da la posibilidad de localizar marcadores genéticos para usarlos, más adelante para el diagnóstico de la enfermedad. Por otro lado, los genes relacionados con las vías metabólicas del monosacárido fueron similares. Por lo cual, es más probable que se encuentren marcadores moleculares en los genes relacionados con las rutas metabólicas de los carbohidratos que en las de monosacáridos. Es necesario estudiar más a fondo la ruta metabólica específica de los azúcares de 7 carbonos para buscar marcadores moleculares en esta ruta. Además, en el tejido de albura encontramos tres unigenes inducidos por aguacate en el grupo sintomático de FD (At1g79500, At2g45790 y At1g14520).

Los genes asociados con la biosíntesis de lípidos se expresan en ambas condiciones y en ambas áreas, pero hay degradación en la condición enferma, posiblemente porque en ambas condiciones la planta intenta defenderse creando metabolitos secundarios, pero en condiciones de enfermedad, debido a la degradación, la molécula no se está formado. Además, encontramos algunos genes inducidos por el interés en condiciones asintomáticas que están involucrados en la biosíntesis de ácidos grasos (At3g51520, At3g02630, Atcg00500 y At2g23800 para tejido de albura;

y At3g51520, At3g10520 y Atcg00500 para tejido de rama apical). Sobre las vías relacionadas con las hormonas de defensa (ET, JA y SA), se expresaron principalmente en la condición de enfermedad, esto tal vez porque la enfermedad estaba causando su expresión. Pero, en el tejido de rama apical, la expresión fue similar en ambas condiciones, lo que indica que tanto en la enfermedad como en la salud se activa la defensa hormonal.

Los resultados de este estudio mostraron que FD aumenta la expresión de muchos genes relacionados con la defensa en el huésped para combatir la infección, mientras que el perfil metabólico se redirige a la movilización de carbohidratos. Estos resultados contribuyen al diagnóstico de FD mediante la identificación de marcadores genéticos. Además, la evaluación de la actividad biológica de algunos metabolitos producidos diferencialmente en los árboles asintomáticos para buscar propiedades antifúngicas podría ser prometedora para el manejo de la FD.