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Molecular and physiological studies of avocado tolerance to *Rosellinia necatrix* and water stress

Memoria de tesis Doctoral por compendio de publicaciones
presentada por la licenciada en Biología

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Para optar al grado de

Doctor por la Universidad de Málaga

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Abbreviations

A	West Indian race (*raza antillana*)
ABA	Abscisic acid
AFD	1-acetoxy-2-hidroxiy-4-oxoheneicosa 12, 16 diene
$A_{\mathrm{N}}/C_{\mathrm{i}}$	Rubisco instantaneous carboxylation efficiency
A_{N}/E	Instantaneous water use efficiency
$A_{\mathrm{N}}/g_{\mathrm{s}}$	Intrinsic water use efficiency
A_{N}	Net CO_2 assimilation rates
ANOVA	Analysis of variance
ASBVd	Avocado Sunblotch Viroid
AsES	Extracellular protein of the fungus *Acremonium strictum*
AUDPC	Area under disease progress curve
BP	Biological processes
Ca	Calcium
CC	Cellular component
cDNA	Complementary DNA
CF	Culture filtrate (*filtrado crudo)*
ChNPs@Met	Encapsulated methionine
ChNPs@SA	Encapsulated salicylic acid
CHP	Chaperone
C_{i}	Intercellular CO_2 concentration
CTAB	Cetyltrimethylammonium bromide
CWO	Cell wall organization
DEGs	Differentially expressed genes
DI	Disease index
DNA	Desoxyribonucleic Acid
DNAse	Desoxyribonuclease
dpi	Days post inoculation
D_W	Dry weight
E	Transpiration rates
EEA	European Environmental Agency
E_{plant}	Plant transpiration rate
ERF	Ethylene-responsive transcription factors
ET_0	Potential evapotranspiration
ETI	Effector triggered immune responses
F_0	Minimal fluorescence
F_0'	Minimal fluorescence of a pre-illuminated sample

FAA	Formalin:acetic:alcohol
FAO	Food and Agriculture Organization
Fc	Field capacity
FC	Fold change
FDR	False discovery rate
F_m	Maximal fluorescence
F_m'	Maximal fluorescence of a pre-illuminated sample
F_t	Steady state fluorescence
F_v/F_m	Maximal photochemical efficiency of PSII
F_v'/F_m'	Maximum photochemical efficiency of the open reaction centres of PSII
F_W	Fresh weight
G	Guatemalan race (*raza Guatemalteca*)
GC content	Percentage of Guanine and Cytosine base numbers of total bases
GO	Gene ontology
g_s	Stomatal conductance
HCL	Hierarchical clustering
HDMS	Hexamethyldisilazane
HDPAF	High degree polymerized agave fructans
hpi	Hours post inoculation
HSD	Tukey’s honest significant difference
HSP	Heat shock protein
I	Inoculated
IFAPA	Institute of Agricultural Research and Training
JA	Jasmonic acid
K	Potassium
KEGG	Kyoto Encyclopedia of Genes and Genomes
K_h	Plant hydraulic conductance
K_s	Soil hydraulic conductivity
LMA	Specific leaf mass area
LOX	Lipoxygenase
LSD	Fisher’s least significant difference
M	Mexican race (*raza Mexicana*)
MeJA	Methyl jasmonate
MF	Molecular functions
Mg	Magnesium
MS	Murashige and Skoog medium
MSP	Murashige and Skoog medium supplemented with picloram

NA	Non annotated
NAC	NAM and ATAF1, ATAF2, and CUC2
NaCl	Sodium Chloride
NI	Noninoculated
NIR	Near infrared analyzer
NLR	Nucleotide Binding-Leucine rich repeat
NPQ	Non-photochemical quenching of fluorescence
NPR1	NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1
P	Phosphorus
PAL	Phenylalanine ammonia-lyase
PAM	Pulse Amplitude Modulation
PBR	*Podredumbre blanca radicular*
PDA	Potato dextrose agar
PDB	Potato dextrose broth
POD	Peroxidase
PPFD	Photosynthetic photon flux density
PPO	Polyphenol oxidase
PR	Pathogenesis-related
PRR	Phytophthora root rot (*podredumbre radicular por Phytophthora*)
PSII	Photosystem II
Q20	Phred values greater than 20 base number contain the percentage of total bases
Q30	Phred values greater than 30 base number contain the percentage of total bases
Q_A	Chloroplastic quinone A
*q*L	Fraction of PSII centres in open state
*q*N	Coefficient of non-photochemical quenching
qRT-PCR	Real time quantitative PCR
R/S	Root/shoot ratio
RH	Relative humidity
RNA	Ribonucleic Acid
RNA-Seq	RNA sequencing
ROS	Reactive oxygen species
RPKM	Reads per kilobase of exon model per million mapped reads
RT-PCR	Real time PCR
RWC	Relative leaf water content
SA	Salicylic acid
SAR	*Resistencia sistémica adquirida*

SCL	Susceptible callus line (AN-9) not exposed to fungal culture filtrate
SCLF	Susceptible callus line (AN-9) exposed to fungal culture filtrate
SD	Stomatal density
SE	Standard error
SEM	Scanning electron microscope
SOD	Superoxide dismutase
SPAD index	Relative chlorophyll content
SWC	Soil water content
T	Temperature
TCL	Resistant callus line (L3) not exposed to fungal culture filtrate
TCLF	Resistant callus line (L3) exposed to fungal culture filtrate
TF	Transcription factor
TLA	Total plant leaf area
T_m	Primer melting temperature
T_W	Turgid weight
VC	Vegetative clones
WAO	World Avocado Organization
WRR	White root rot
WS	Water stress
WW	Well-watered
Ψ_{MD}	Midday leaf water potential
Ψ_{PD}	Predawn leaf water potential
Ψ_w	Leaf water potential
ΦPSII	Relative quantum yield of PSII photochemistry

Resumen/Summary

El aguacate (*Persea americana* Mill.) es un cultivo de gran relevancia económica en todo el mundo. Existen evidencias que sitúan su origen en Mesoamérica hace más de 10.000 años. Durante todos estos años, el cultivo ha ido sufriendo domesticaciones con la intención de seleccionar caracteres deseados. Esto ha dado lugar a la aparición de tres razas distintas: *P. americana* var. *drymifolia* (raza mexicana, M), *P.* americana var. *americana* (raza antillana, A) y *P. americana* var. *guatemalensis* (raza guatemalteca, G). A pesar de las diferencias entre las tres razas, es posible la hibridación entre ellas dando lugar a ventajas como la adaptación a diferentes climas y la mejora de características agronómicas. Esto ha permitido que la especie sea capaz de crecer en regiones con climas tropicales, subtropicales y templados y estar presente en los cinco continentes.

El fruto del aguacate posee altas cantidades de ácidos grasos monoinsaturados, fibra, minerales como potasio, magnesio, fósforo, calcio o vitaminas A, B-6, C y E. Este alto contenido nutricional ha hecho que aumente el interés de los consumidores de todo el mundo en las últimas décadas.

En la última década (2012-2022), la producción mundial de aguacate se ha duplicado alcanzando valores de 8.978.275,2 t y una superficie cultivada de 884.035 ha en 2022. Entre los países productores, México ha ocupado el primer lugar durante años, así, en 2022 representó el 28% de la producción mundial con 2.529.581 t. Por su parte, España se encuentra entre los 20 primeros países con una producción de 105.930 t y una superficie cultivada de 19.520 ha, liderando el cultivo de aguacate en Europa con aproximadamente el 72% de la producción y el 77% de la superficie cultivada. El éxito de las exportaciones de aguacate español en Europa frente a las de países más lejanos, se debe a la alta calidad de la fruta española y a su proximidad a los mercados, lo que permite comercializar los aguacates en su punto óptimo de maduración de forma que el consumidor puede comprar la fruta y consumirla inmediatamente (concepto conocido como '*ready to eat*'). Además, desde el punto de vista medioambiental, la huella de carbono que se produce es menor. En España, la producción de aguacate se concentra en la denominada costa subtropical andaluza, principalmente en Málaga y Granada. En este sentido, Andalucía representa el 83% de la producción española y el 96% de sus exportaciones las recibe Europa.

Los árboles en plantaciones comerciales de aguacate están compuestos por el portainjerto, que proporciona el sistema radicular, y la variedad, que proporciona la parte aérea. Existen variedades y portainjertos comerciales que corresponden a una de las tres razas descritas anteriormente, pero la mayoría son híbridos entre razas. En cuanto a las variedades, 'Hass', un híbrido entre las razas MxG, se ha convertido en la variedad predominante en la mayoría de los países productores y prácticamente monopoliza el comercio mundial.

El portainjerto ejerce una fuerte modulación sobre el comportamiento agronómico de la variedad injertada. Esta interacción afecta a diferentes aspectos del árbol como el tamaño, el rendimiento, el grado de alternancia productiva, la floración, la absorción y acumulación de nutrientes, la postcosecha y la respuesta a estreses bióticos y abióticos.

Los primeros portainjertos utilizados para este cultivo procedían de semillas que, debido a la polinización cruzada, presentan una variabilidad considerable en sus características, sin embargo, en los cultivos comerciales, es deseable minimizar la variabilidad genética seleccionando plantas que sean genéticamente uniformes, asegurando así un rendimiento agronómico similar entre árboles de una misma plantación. En este sentido, el uso de plantas nodriza, procedentes de semillas vigorosas, sobre las que se injerta el material que se desea enraizar, seguido de la etiolación y posterior acodo del brote obtenido, ha facilitado la producción de portainjertos genéticamente idénticos, conocidos como portainjertos clonales.

Para el establecimiento de una plantación de aguacate son varios los factores que se deben tener en cuenta: la correcta selección del portainjerto y la variedad (factor genético), el conocimiento de las características del suelo y la disponibilidad de agua (factores agroclimáticos o abióticos), requisitos de mantenimiento del cultivo (factores humanos), y ser consciente de las enfermedades y plagas que podrían potencialmente afectar al cultivo (factores biológicos o bióticos). El impacto que un factor determinado tendrá sobre un cultivo dependerá de la región en la que se encuentre.

A nivel mundial, uno de los factores más limitantes en el cultivo de aguacate por su frecuencia, severidad y elevadas pérdidas económicas es la podredumbre radicular, causada por el oomiceto hemibiotrófico *Phytophthora cinnamomi* Rands (PRR), que puede afectar a más de 3.500 especies vegetales y está presente en más de 70 países. En España, entre los principales factores que afectan a la viabilidad de este cultivo, además de la PRR, encontramos la escasez de agua y la podredumbre blanca radicular (PBR) causada por el hongo necrotrofo *Rosellinia necatrix*.

En relación a la falta de agua, bien por la expansión del cultivo a regiones con condiciones climáticas diferentes a las de su hábitat nativo, bien por los cambios ambientales que han contribuido a una baja disponibilidad de agua derivada de intensos episodios de sequía y del impacto del calentamiento global en estas regiones, el aguacate se enfrenta a nuevos retos climáticos. Este es el caso de zonas de clima mediterráneo como California, Israel, Chile y España. En este sentido, un riego adecuado es un factor determinante para garantizar un óptimo desarrollo del fruto, especialmente durante las fases de floración y cuajado así como para minimizar la caída de frutos. Así pues, para hacer frente a la escasez de precipitaciones, las plantaciones comerciales necesitan suplir la escasez de agua de lluvia con un riego externo que complemente el agua disponible para

los árboles. Sin embargo, la ausencia de precipitaciones no solo ha afectado a los cultivos agrícolas sino también a la disponibilidad de agua para el abastecimiento urbano. Esto ha llevado a las autoridades locales a imponer limitaciones o incluso restricciones a la utilización del agua con fines agrícolas, como ha ocurrido en zonas de la Axarquía de Málaga (España). Estas restricciones han provocado una reducción significativa de las producciones de aguacate y un notable aumento de la mortalidad de los árboles en los huertos en los que no era posible el riego.

En relación a las enfermedades de suelo, los árboles afectados por PRR presentan raíces necróticas y una reducción de la masa radicular, lo que se traduce en una disminución de la capacidad de la planta para absorber agua y nutrientes. Esto provoca clorosis, caída de hojas y, finalmente, la muerte de la planta. El patógeno puede sobrevivir en condiciones adversas durante largos periodos de tiempo, por lo que es muy difícil erradicar la enfermedad por completo. La gestión de la PRR es una tarea compleja que requiere un enfoque integrado y para la que se dispone de portainjertos tolerantes, tales como 'Duke 7' (Universidad de California, Riverside) o 'Dusa' (Hans Merensky Foundation, Westfalia, Sudáfrica) entre otros, que están disponibles comercialmente.

Por otra parte, la PBR afecta a más de 335 plantas hospedadoras, incluidas plantas leñosas y herbáceas, muchas de ellas de interés económico, como el aguacate. A pesar de su prevalencia en los cinco continentes, el patógeno ha adquirido gran relevancia en la limitación del cultivo de aguacate en Israel, Sudáfrica y el sur de España. El hongo se dispersa por el suelo a través del micelio por el contacto de raíces sanas con raíces infectadas. Al entrar en contacto con una raíz, el micelio prolifera, cubriendo la superficie de la raíz y penetrando posteriormente a través de aberturas naturales como lenticelas o heridas, o directamente formando un agregado micelar. Las plantas infectadas por *R. necatrix* suelen manifestar síntomas aéreos y radiculares que surgen como consecuencia de los daños causados en las raíces y el posterior vertido de compuestos tóxicos al sistema vascular de la planta. Este patógeno produce diferentes metabolitos con efectos fitotóxicos, como rosellichalasin, diketopiperazines, ácido rosélico, rosnecatrone y cytochalasin E, que tiene un efecto directo sobre la fotosíntesis, aunque sus papeles en la patogenicidad requieren de un mayor estudio. A nivel radicular, puede observarse la presencia de micelio algodonoso blanco, además de filamentos miceliales blancos o negros, y a nivel aéreo, los árboles infectados pueden perder vigor, las hojas se marchitan y secan y, finalmente, los árboles mueren. El control de esta enfermedad es un reto debido a la capacidad del patógeno para tolerar la desecación y los suelos ácidos, su amplia gama de hospedadores, su capacidad para sobrevivir en el suelo y su resistencia a numerosos fungicidas utilizados habitualmente. Los principales métodos para controlar la enfermedad son las prácticas culturales, el control físico, químico, biológico y la resistencia genética. Entre las prácticas culturales se distinguen el uso de herramientas desinfectadas, aislamiento de árboles afectados y abono equilibrado. Otra práctica cultural es el manejo del agua de riego. En este sentido, estudios previos del grupo demostraron que en portainjertos de aguacate susceptibles a *R. necatrix*, un estrés hídrico moderado puede inducir un estado de '*priming*' que aumente la posterior tolerancia de la planta a *R. necatrix*. Por otra parte, el control físico como la solarización cada dos años, es una estrategia que suele ser bastante efectiva, así como los tratamientos con el fungicida fluazinam (control químico), aunque su uso no está permitido en plantaciones comerciales. Sin embargo, la solución más recomendada es el uso de un control integrado partiendo de material vegetal tolerante al hongo. Sin embargo, a diferencia de *P. cinnamomi*, actualmente no existen portainjertos tolerantes a PBR disponibles para los productores. Para hacer frente a este reto, el IFAPA-Málaga ha estado llevando a cabo un programa de mejora genética durante varias décadas para desarrollar portainjertos tolerantes a la PBR. Un número de selecciones están siendo evaluadas actualmente en condiciones de campo. Los estudios derivados de este programa, cuyo objetivo era determinar las bases moleculares y fisiológicas de la interacción aguacate/*R. necatrix*, han revelado que la resistencia a este patógeno parece estar relacionada con la capacidad de soportar el desequilibrio osmótico a través de la inducción de genes relacionados con el estrés salino y osmótico, así como la sobreexpresión de inhibidores de proteasas. Además, se observó que la respuesta de tolerancia a este hongo y al estrés hídrico comparten mecanismos y vías de defensa similares. En consecuencia, sería razonable plantear la hipótesis de que los portainjertos seleccionados que muestran tolerancia a *R. necatrix* también mostrarían cierto grado de tolerancia al estrés hídrico.

En el **Capítulo 2** de la presente tesis doctoral, se han utilizado dos selecciones avanzadas de portainjertos tolerantes a *R. necatrix* del programa de mejora del IFAPA (BG48 y BG181) injertadas con la variedad 'Hass' para estudiar las relaciones hídricas y la respuesta bajo dos niveles de estrés hídrico, medio y severo, correspondientes al 50% y 25% de la capacidad de campo, respectivamente. Los resultados muestran que, en ausencia de limitaciones hídricas, existe un fuerte efecto del portainjerto sobre la variedad 'Hass'. En este sentido, las plantas injertadas sobre BG48 mostraron un mayor consumo de agua y valores más altos de la tasa de transpiración y de las tasas fotosintéticas, que las injertadas sobre BG181, que exhibieron una estrategia de ahorro de agua (*water-saving*) basada en un compromiso entre la asignación de biomasa foliar y un estricto control estomático de la transpiración, no ligado a una reducción del crecimiento. Los resultados sugieren que las plantas injertadas sobre BG181 requieren menos agua, por lo que estas serían más eficientes desde el punto de vista hídrico. Sin embargo, tras la privación de agua, tanto las injertadas en BG48 como en BG181 mostraron un aumento de la masa foliar y disminuyeron la tasa de transpiración, la conductancia hidráulica y el potencial hídrico foliar, en concordancia con la severidad del estrés hídrico, mostrando ambos genotipos un comportamiento de evitación de la sequía (*avoidance*). Tras ambas situaciones de estrés hídrico, se repuso el agua en las plantas, que recuperaron las tasas fotosintéticas por completo, lo que sugiere cierta capacidad de estos portainjertos para recuperarse del estrés hídrico, lo que podría estar asociado a su tolerancia a la infección por *R. necatrix*. Los resultados obtenidos han contribuido a una mayor comprensión de la relación en el uso del agua entre el portainjerto y la variedad en árboles de aguacate, además de proporcionar información acerca de los mecanismos para gestionar la escasez de agua.

A pesar de los beneficios innegables del uso de portainjertos tolerantes frente a diversos factores, el desarrollo de programas de selección requiere periodos muy prolongados de tiempo. Sería de gran interés poder acortar estos márgenes para abastecer el mercado con portainjertos tolerantes a los distintos problemas que enfrenta el aguacate en cada región. En este sentido, los estudios moleculares de la interacción aguacate/patógeno para identificar marcadores empleando material *in vitro* en lugar de plantas adultas, podrían ayudar a acelerar el proceso de selección de genotipos de interés.

En el **Capítulo 3** de la presente tesis doctoral se llevó a cabo un estudio con una línea de callo embriogénico de aguacate de la variedad ‘Anaheim’ de la que posteriormente se obtuvo una línea celular resistente (L3) mediante exposiciones recurrentes a concentraciones progresivamente crecientes (del 60% al 80%) de filtrado de *R. necatrix* (CF). Posteriormente, se realizó un análisis de secuenciación de ARN (ARN-Seq) para comparar los perfiles transcriptómicos de la línea de callo embriogénico resistente L3 (capaz de sobrevivir en presencia de 80% de CF) y la línea control AN-9 (no expuesta a CF), tras 24 h de crecimiento en un medio suplementado con un 40% de CF. Esto puso de manifiesto una menor desregulación génica de la línea resistente frente a la línea susceptible. Además, mientras que ambas líneas expresaban genes enriquecidos en categorías tales como actividad catalítica, oxidorreductasa, hidrolasa, peroxidasa, antioxidante, respuesta al estímulo, actividad transportadora, respuesta de defensa y fotosíntesis, otros genes relacionados con actividad lipasa, quinasa y procesos metabólicos, solo se expresaron en la línea susceptible. La línea resistente mostró enriquecimiento en genes incluidos en la categoría de detoxificación, de gran interés dados los mecanismos de acción del hongo.

Por otra parte, la exposición de ambas líneas al filtrado del hongo, puso de manifiesto la inducción de genes previamente relacionados con la defensa del aguacate frente a enfermedades fúngicas tales como genes relacionados con la modificación de la pared celular, proteínas de detoxificación y resistencia a enfermedades, proteínas relacionadas con la patogénesis (PR), proteasas e inhibidores de proteasas, factores de transcripción, regulación hormonal y genes relacionados con la homeostasis redox. Entre los genes sobreexpresados, cabe destacar el inhibidor de proteinasa (Pag64949) que comparte la misma secuencia de codificación con uno previamente identificado en portainjertos de aguacate tolerantes, tras la inoculación con *R. necatrix* en condiciones de invernadero, lo que sugiere la importancia que podría jugar esta proteína en la defensa de los portainjertos frente a este hongo y apoyaría el uso de técnicas de cultivo celular para el estudio de la interacción aguacate-*R. necatrix*, ayudando a la selección de portainjertos tolerantes a este patógeno.

Sin embargo, es necesario tener en cuenta que la mayoría de las plantaciones de aguacate actuales están establecidas sobre portainjertos no tolerantes. Por ello, se hace necesario usar métodos alternativos en la lucha frente a la PBR. El uso de elicitores, capaces de inducir rutas de defensa relacionadas con la tolerancia de plantas frente a hongos patógenos, se plantea como una buena estrategia. Los elicitores presentan ciertas ventajas sobre otras metodologías alternativas, como es el hecho de que no son tóxicos ni perjudiciales para el medio ambiente, y que incluso pequeñas cantidades son suficientes para proporcionar protección a largo plazo contra una amplia gama de patógenos.

En el **Capítulo 4** de esta tesis doctoral se evaluó el efecto de la aplicación exógena de dos elicitores, el metil jasmonato (MeJA) y el ácido salicílico (SA) en la tolerancia del aguacate a *R. necatrix*. Ambos elicitores tienen gran importancia en la mediación de las respuestas de las plantas a estreses abióticos y bióticos, siendo capaces de desencadenar respuestas de defensa de la planta similares a las inducidas por patógenos e incluso pueden proporcionar protección a largo plazo frente a ellos.

Plantas de aguacate 'Dusa', susceptibles a *R. necatrix*, fueron tratadas con uno u otro elicitor. Además, algunas de las plantas elicitadas se inocularon con *R. necatrix*. Finalmente se realizaron mediciones fisiológicas en ambos grupos de plantas, inoculadas y no inoculadas y se evaluó la expresión de genes relacionados con la tolerancia al patógeno y la progresión de la enfermedad en las inoculadas vs no inoculadas.

La aplicación de MeJA y SA en las plantas de aguacate aumentó los mecanismos fotoprotectores y la expresión de la glutatión S-transferasa, lo que sugiere la activación de mecanismos estrechamente relacionados con atenuadores del estrés oxidativo y la eliminación de especies reactivas del oxígeno. Se observó que los efectos del MeJA fueron más pronunciados a nivel morfoanatómico, incluyendo elevada masa foliar, una alta densidad estomática y una elevada relación raíz/brote, estrechamente relacionados con estrategias para hacer frente a la escasez de agua y a la PBR.

Además, el MeJA incrementó un mayor número de genes relacionados con la defensa, incluyendo un inhibidor de la proteasa, un gen clave en la defensa del aguacate contra *R. necatrix*. Los efectos generales del MeJA incrementaron la tolerancia del aguacate 'Dusa' a *R. necatrix* al inducir un estado de '*priming*' que retrasó la aparición de los síntomas de la enfermedad. Estos resultados apuntan al uso de MeJA como una estrategia, respetuosa con el medio ambiente, para mitigar el impacto de esta enfermedad en plantaciones de aguacate infectadas por el patógeno.

Como conclusión general, esta tesis ha permitido conocer más acerca de los mecanismos fisiológicos que subyacen en portainjertos tolerantes de aguacate frente a *R. necatrix* al ser expuestos a estrés hídrico, uno de los principales problemas de la costa andaluza. Además, ha puesto de manifiesto la posibilidad del uso de técnicas de *cultivo in vitro* para ayudar en la búsqueda de genes diana que permitan acelerar los procesos de selección de genotipos en el programa de mejora frente a *R. necatrix*. Por último, ha mostrado que el uso de elicitores como MeJA, puede ser una estrategia de interés en el manejo de plantaciones infectadas con la PBR.

The avocado (*Persea americana* Mill.) is a crop of significant economic relevance across the world. There is evidence that places the origin of this tree in Mesoamerica more than 10,000 years ago. During this period, the species has undergone a process of domestication that involves the selection of desired quality traits. This has resulted in the emergence of three distinct avocado races: the race *P. americana* var. *drymifolia* (Mexican race, M), the race *P. americana* var. *americana* (West Indian or Antillean race, WI) and the race *P. americana* var. *guatemalensis* (Guatemalan race, G). Despite the differences between these three races, they are able to hybridise with one another, thus giving rise to advantageous characteristics that include adaptation to diverse climates and improved agronomic traits. This has allowed the species to grow in regions with tropical, subtropical and temperate climates and to be present across all five continents.

The avocado fruit is notable for present a high content of monounsaturated fatty acids, fibre, minerals such as potassium, magnesium, phosphorus, calcium or vitamins A, B-6, C and E. The high nutritional content of this fruit has increased the interest of consumers in avocado in recent decades all over the world.

In the last decade (2012-2022), global avocado production has had a two-fold increase in production, reaching values of 8,978,275.2 t and a harvested area of 884,035 ha in 2022. Among the producing countries, Mexico has been in first place for years, accounting for 28% of world production with 2,529,581 t in 2022. Spain is among the top 20 countries in terms of production with a production of 105,930 t and a cultivated area of 19,520 ha, leading avocado production in Europe with approximately 72% of the production and 77% of the total cultivated areas dedicated to avocado. The success of Spanish avocado exports in Europe when compared to those from more distant countries can be attributed to the high quality of the Spanish fruit and its proximity to the European markets, which allows avocados to be commercialized at their optimum point of ripeness so that the consumer can buy the fruit and consume it immediately (a concept known as ‘ready to eat’). Furthermore, from an environmental point of view, the carbon footprint produced is lower due to the short distance. In Spain, avocado production is concentrated on the so-called subtropical Andalusian coast, mainly in the provinces of Malaga and Granada. In this sense, Andalusia represents 83% of Spanish avocado production and 96% of its exports are received by Europe.

Avocado commercial crops are composed of two main components: the rootstock, which provides the root system, and the variety, which provides the aerial part. There are commercial varieties and rootstocks that correspond to one of the three races described above, but most of them are hybrids between races. Regarding varieties, 'Hass', a hybrid between the MxG races, has become the predominant variety in most producing countries and practically monopolises world trade.

Rootstock exerts a strong modulation on the agronomic behavior of the grafted variety. This interaction affects different aspects of the tree such as the tree size, yield and the degree of alternate bearing, the flowering, the nutrient uptake and accumulation, the post-harvest and the response of the plant to biotic and abiotic stresses.

The earliest rootstocks used for avocado crops were derived from seeds which, due to cross-pollination, exhibit considerable variability in characteristics. However, in commercial avocado crops, it is desirable to minimise genetic variability by selecting plants that are genetically uniform, thereby ensuring a similar agronomic performance between trees in the same orchard. In this sense, the use of nurse plants from vigorous seeds, on which the material to be rooted is grafted, followed by etiolation and subsequent layering of the shoot obtained, has facilitated the production of genetically identical rootstocks, known as clonal rootstocks.

The establishment of an avocado orchard is a process that requires the consideration of numerous factors, among which the following should be taken into account: the selection of appropriate rootstock and variety (i.e. the genetic factors), an understanding of soil characteristics and water availability (i.e. the agro-climatic or abiotic factors), the maintenance requirements of the crop (i.e. the human factors), and awareness of diseases and pests that could potentially affect the crop (i.e. the biological or biotic factors). The impact that a given factor will have on a crop is dependent on the region in which the crop is located.

On a worldwide scale, one of the most limiting factors in avocado crops due to its frequency, severity and high economic losses is root rot caused by the hemibiotrophic oomycete *Phytophthora cinnamomi* Rands (PRR), a fungus that has the capacity to affect more than 3,500 plant species and is present in more than 70 countries. In Spain, in addition to PRR, the main factors affecting the viability of avocado crops include water scarcity and white root rot (WRR), a fungal disease which is caused by the necrotrophic fungus *Rosellinia necatrix*.

In the context of water scarcity, avocado trees are facing new climatic challenges, either due to the expansion of their cultivation to regions with differing climatic conditions compared to their native habitat or due to environmental changes which has contributed to low water availability derived from intense drought episodes and the impact of global warming in these regions. This is indeed the case in zones with a Mediterranean climate-type, such as California, Israel, Chile and Spain. In this sense, adequate irrigation is a crucial factor in ensuring optimal fruit development, particularly during the flowering and fruit-set stages with the purpose of minimizing fruit drop from the tree. Therefore, in order to cope with the low rainfall, avocado commercial orchards need to supplement the insufficient rainwater with external irrigation to complement the water available to the trees. The absence of rainfall has had repercussions for more than just agricultural crops, it has also impacted on the availability of water for urban water supply. The present situation has prompted regional administrations to enforce constraints, and in certain cases, to prohibit the utilisation of water for agricultural purposes. An example of this phenomenon is illustrated by the circumstances observed in the Axarquia, a region of the province of Málaga (Andalusia, Spain), where water restrictions have been instituted, resulting in substantial reductions in avocado yields and notable increases in tree mortality within orchards where irrigation has been rendered unfeasible.

In relation to soil borne diseases, trees affected by PRR show signs of necrotic roots and a reduction of root mass, which results in a reduction of the capacity of the plant to absorb water and nutrients. This phenomenon leads to chlorosis, leaf drop and eventually, the death of the plant. The pathogen exhibits a capacity for survival in adverse conditions for long periods of time, making the eradication of this fungus a highly challenging task. The management of PRR is a complex issue that requires an integrated approach and for which different avocado breeding programs have development tolerant rootstocks, such as 'Duke 7' (University of California, Riverside) or 'Dusa' (Hans Merensky Foundation, Westfalia, South Africa) among others. These avocado rootstocks tolerant to *P. cinnamomi* are commercially available to growers.

On the other hand, *R. necatrix*, the fungal pathogen causing the WRR disease, has been identified as a threat to more than 335 host plants, including woody and herbaceous plants, among these, numerous economically significant plants such as olive trees, almond, apple, citrus, strawberry, mango, have been documented, including avocado trees. Despite its global distribution across five continents, the pathogen has become a significant limiting factor in avocado crops in regions such as Israel, South Africa and southern Spain. The fungus spreads through the soil via mycelium by contact of healthy roots with infected roots. Upon contact with a root, the mycelium proliferates, covering the root surface and subsequently penetrating avocado roots through natural openings such as lenticels or wounds, or directly forming a penetration sclerotium. The hyphae of *R. necatrix* invade and penetrate the vascular system of the root avoiding water and nutrient flow to upper parts of the plant. Plants infected by *R. necatrix* frequently exhibit symptoms in both, the aerial and root systems. These symptoms are often the result of root damage which subsequently leads to the release of toxic compounds into the vascular system of the plant. The pathogen in question produces a variety of metabolites with phytotoxic effects, including rosellichalasin, diketopiperazines, roselic acid, rosnecatrone and cytochalasin E, the latter of which exerts a direct effect on photosynthesis. However, further research is required to elucidate the precise roles of these metabolites in the pathogenesis process. At the root level, white cottony mycelium may be observed, along with white or black mycelial filaments, and at the aerial level, infected trees exhibit symptoms such as reduced vigour, leaves may wilt and dry out, and trees may eventually die. Control of this disease is challenging because of the ability of the pathogen to tolerate desiccation and acidic soils, its wide host range, its ability to survive in soil and its resistance to many commonly used fungicides. The main methods to control the disease are cultural practices, physical methods, chemical methods, biological methods and genetic resistance control. Cultural practices include the use of disinfected tools, the isolation of affected trees, and the use of balanced fertilisation. One further cultural practice is the management of irrigation water. In this regard, previous studies of the group demonstrated that in avocado rootstocks susceptible to *R. necatrix*, moderate water stress can induce a 'priming' state that increases the subsequent tolerance of the plant to *R. necatrix*. Moreover, the application of physical controls, such as solarisation, at two-year intervals, has proven to be a strategy of efficacy, along with treatments employing the fungicide fluazinam (chemical control), although it should be noted that the utilization of fluazinam is not authorized within the context of commercial orchards. Nevertheless, the most recommended solution is the adoption of integrated control measures, encompassing the use of fungus-tolerant plant material. However, in contrast to *P. cinnamomi*, there are currently no WRR-tolerant rootstocks commercially available to growers. To address this challenge, the investigation center IFAPA-Málaga has been carrying out a breeding programme for several decades with the aim of develop avocado rootstocks tolerant to WRR disease. A number of selections are currently being evaluated under field conditions. Studies derived from this programme, which aimed to determine the molecular and physiological basis of the avocado/*R. necatrix* interaction, have revealed that resistance to this pathogen appears to be related to the ability to withstand osmotic imbalance through the induction of genes related to salt and osmotic stress, as well as the overexpression of protease inhibitors. Furthermore, it was observed that the tolerance response to this fungus and to water stress share similar defence mechanisms and pathways. Consequently, it can be hypothesised that selected rootstocks showing tolerance to *R. necatrix* would also show some degree of tolerance to water stress.

In **Chapter 2** of the present PhD thesis, two advanced selections of *R. necatrix* tolerant rootstocks from the IFAPA-Málaga breeding programme (BG48 and BG181) grafted with the commercial avocado variety ‘Hass’, were used with the purpose of studying the water relations and response under two levels of water stress, medium and severe, corresponding to 50% and 25% of field capacity, respectively. The results of this experiment show that, in the absence of water constraints, there is a strong effect of the rootstock on the ‘Hass’ variety. In this sense, ‘Hass’ variety grafted on BG48 showed higher water consumption and higher values of transpiration rate and leaf photosynthetic rates than when is grafted on BG181, which exhibited a water-saving strategy based on a compromise between leaf biomass allocation and tight stomatal control of transpiration, not linked to a reduction in growth. The results suggest that avocado 'Hass' plants grafted on BG181 require less water, so plants are more water efficient. However, following a period of water deprivation, both BG48 and BG181 grafted plants showed an increase in leaf mass and a decrease in transpiration rate, hydraulic conductance and leaf water potential, in accordance with the severity of water stress, with both genotypes showing drought avoidance behaviour. After both water stress situations, water was replenished in the plants, which recovered photosynthetic rates completely. This suggests a certain capacity of these rootstocks to recover from water stress, which may be associated with their ability to withstand to *R. necatrix* infection. The results obtained have contributed to a better understanding of the relationship in water use between rootstock and variety in avocado trees, as well as providing information about mechanisms to manage water scarcity.

Despite the undeniable advantages inherent in the utilisation of rootstocks demonstrating tolerance to a range of factors, it is necessary to consider that the development of rootstocks selection programmes requires very long periods of time. It would be of great interest to be able to shorten these margins in order to supply the market with tolerant rootstocks to the different problems faced by avocado in each region. In this sense, molecular studies of the avocado/pathogen interaction to identify markers using in vitro material instead of adult plants could help to accelerate the selection process of genotypes of interest.

In **Chapter 3** of the present PhD thesis, a study was carried out with an 'Anaheim' avocado embryogenic callus line from which a resistant cell line (L3) was subsequently obtained by recurrent exposures to progressively increasing concentrations (from 60% to 80%) of *R. necatrix* fungal filtrate (CF). Later, a RNA sequencing analysis (RNA-Seq) was performed to compare the transcriptomic profiles of the resistant embryogenic callus line L3 (able to survive in the presence of 80% CF) and the control line AN-9 (not exposed to CF), after 24 h of growth in medium supplemented with 40% CF. RNA sequencing analysis revealed less gene deregulation of the resistant line compared to the susceptible line. Furthermore, while both lines expressed genes enriched in categories such as catalytic activity, oxidoreductase, hydrolase, peroxidase, antioxidant, stimulus response, transporter activity, defence response and photosynthesis, other genes related to lipase activity, kinase and metabolic processes were only expressed in the susceptible line. The resistant callus line showed enrichment in genes included in the detoxification category, of great interest given the mechanisms of action of the fungus.

Furthermore, exposure of both lines to fungal filtrate revealed the induction of genes previously related to avocado defence against fungal diseases such as genes related to cell wall modification, detoxification and disease resistance proteins, pathogenesis-related proteins (PR), proteases and protease inhibitors, transcription factors, hormone regulation and genes related to redox homeostasis. Among the overexpressed genes, it is noteworthy the proteinase inhibitor (Pag64949) which shares the same coding sequence with one previously identified in tolerant avocado rootstocks after inoculation with *R. necatrix* under greenhouse conditions, suggesting the importance that this protein could play in the defence of avocado rootstocks against this fungus and would support the use of cell culture techniques for the study of the avocado-*R. necatrix* interaction, helping in the selection of tolerant rootstocks to this pathogen.

However, it is necessary to take into account that most of the current avocado orchards are established on non-tolerant rootstocks. Therefore, there is a necessity to use alternative methods in the fight against WRR disease until tolerant rootstocks become commercially available. The use of elicitors, which are capable of inducing defence pathways related to plant tolerance against pathogenic fungi, is proposed as a very promising approach in this situation. Elicitors have certain advantages over the use of alternative methodologies, such as the fact that they are non-toxic and environmentally friendly, and that even small amounts of them are enough to provide long-term protection in plants against a wide range of pathogens.

In **Chapter 4** of the present PhD thesis, an experiment was carried out to evaluate the effect of exogenous application of two elicitors, methyl jasmonate (MeJA) and salicylic acid (SA) on avocado tolerance to *R. necatrix*. Both elicitors are of great importance in mediating plant responses to abiotic and biotic stresses, being able to trigger plant defence responses similar to those induced by pathogens and may even provide long term protection against them.

Avocado 'Dusa' plants susceptible to *R. necatrix* were treated with one of both elicitors (MeJA or SA). In addition, some of the elicited plants were inoculated with *R. necatrix*. Finally, physiological measurements were performed on both groups of inoculated and noninoculated plants and the expression of genes related to pathogen tolerance and disease progression was evaluated in the inoculated vs. noninoculated plants.

The application of MeJA and SA elicitors to avocado plants resulted in an increase of photoprotective mechanisms and the glutathione S-transferase gene expression, suggesting the activation of mechanisms closely related to oxidative stress attenuators and reactive oxygen species scavenging. The effects of MeJA were observed to be most pronounced at the morphoanatomical level than SA, including high leaf mass, high stomatal density and high root/shoot ratio, closely related to coping strategies for water scarcity and WRR disease.

In addition, MeJA application increased a greater number of defence-related genes, including a protease inhibitor, a key gene in avocado defence against *R. necatrix*. The overall effects of MeJA increased the tolerance of 'Dusa' avocado to *R. necatrix* by inducing a priming state that delayed the onset of disease symptoms. These results suggest that MeJA could be used as an environmentally friendly strategy to mitigate the impact of the WRR disease on avocado orchards infected by the pathogen *R. necatrix*.

As a general conclusion, this thesis has allowed us to learn more about the physiological mechanisms underlying avocado rootstocks tolerant to *R. necatrix* when exposed to water stress, one of the main problems of the Andalusian coast. In addition, it has shown the possibility of using in vitro culture techniques to help in the search for target genes to accelerate the selection processes of genotypes in the breeding programme against *R. necatrix*. Finally, it has been shown that the use of elicitors such as MeJA may be a strategy of interest in the management of plantations infected with WRR.

Chapter 1

Introduction

1.1. Avocado biology

The avocado tree (*Persea americana* Mill.) belongs to the Lauraceae family. The Spanish name '*aguacate*', comes from the Nahualt word '*ahuacatl*'. Its origin dates to more than 10,000 years ago in Mesoamerica (Smith, 1966).

The avocado is an evergreen tree which forms a dome canopy that could reach up to 30 m high (Scora et al., 2002) especially in their native rainforest environments. It shows a monopodial growth type with a trunk that grows rhythmically developing tiers of branches, morphogenetically identical to the trunk. During the annual crop cycle, there are two vegetative sprouting flows: the first in the spring season and the second in the summer. Shoot growth alternates with root flushes.

Roots do not spread much beyond the tree canopy (Bergh, 1992). Avocado anchoring roots can reach up to 3-4 m in deep soils; however, it has a secondary shallow root system which is found in the first 0.6 m that provides water and nutrients (Whiley and Schaffer, 1994; Rocha-Arroyo et al., 2011).

The leaves are alternate and variable in shape and size. The leaf blade can be elliptic to lanceolate, ovate or obovate in shape (Mandemaker, 2008). When they are young, they are pubescent and reddish and when they mature, leaves become smooth and leathery. They present a prominent ribbing on both faces of the leaf. The dark green upper waxy surface and the light green hairy lower surface (Scora et al., 2002) where stomata can be found (Mickelbart et al., 2000).

Flowers appear as compound inflorescences also known as panicles developed from lateral buds. A single tree may have hundreds of panicles each with about 100 flowers so may develop up to a million flowers (Chanderbali et al., 2013). Inflorescences have been divided into two types, determinate inflorescences in which the main meristem forms a terminal flower and indeterminate inflorescences in which the apex of the primary axis ends in a vegetative bud that will give rise to the growth of a shoot. The latter tend to be more abundant, however, determinate inflorescences tend to be more productive (Rebolledo and Romero, 2011).

The avocado floral cycle shows a unique behavior described as synchronous protogynous dichogamy, i.e. the trees have hermaphrodite flowers with female (pistil) and male (stamens) organs (**Figure 1.1**) that mature and are functional at different times (Davenport, 1986). Each flower opens twice, the first time as a female flower, after which it closes until the following day when it opens again as a male flower and then closes definitively (Davenport, 1986; Rebollero and Romero, 2011). Avocado cultivars are classified into two flower groups, A and B, which complement each other. Type A cultivars open their female flowers in the morning, close and open again the next day in the afternoon as male. Type B cultivars open their flowers in the afternoon as female, close and open the next morning as male (Stout, 1923; Rebollero and Romero, 2011).

Figure 1.1. Female **(A)** and male **(B)** avocado flower stages (Modified from Alcaraz et al., 2013).

Despite high flowering, during two months (May/June) there is a massive drop of flowers and small fruit, resulting in a very low fruit set percentage and usually less than 0.1% of the flowers set fruit (Whiley and Schaffer, 1994; Garner and Lovatt, 2008). Flower drop and low fruit set have been related to various factors such as extreme temperatures, nutritional deficiencies and genetic factors (Rebollero and Romero, 2011). Low fruit set has also been associated with the efficiency of pollination of avocado flowers, which is mostly entomophilous, mediated mainly by bees and, to a lesser extent, by wasps, flies and beetles (de la Peña et al., 2018).

Moreover, even if adequate pollination occurs, temperature and humidity conditions may affect the germination of pollen grains and thus fertilisation. Furthermore, not all pollinated and fertilised flowers will eventually develop fruit (Alcaraz and Hormaza, 2021). Since in avocado, reproductive and vegetative growth occur simultaneously in spring and summer, both compete for the plant's resources. (Biran, 1979; Scholefield et al., 1985; Buchholz, 1986; Cutting and Bower, 1990; Wardlaw, 1990). Therefore, any episode or stress factor that reduces the photosynthetic capacity and nutritional balance of the tree during this period will result in a loss of crop productivity. During its development, the fruit has two stages, one of cell division (taking place in the first eight weeks after flowering), and another of cell elongation (present during the whole life cycle of the fruit) (Hernández Valdés, 2020). Therefore, the size of the fruit lies in the number of cells and not in their size (Cowan et al., 1997). In this sense, any stress event that occurs during the initial stage of cell division is crucial and this phase will greatly condition the final size of the fruit (Cowan et al., 2008).

The avocado tree has a reproductive phenology characterized by the alternation of harvests, with a production cycle or 'on' year with an intense flowering that culminates in a high percentage of fruit set and a high crop yield; and a production cycle or 'off' year that, on the contrary, presents low flowering, low percentage of fruit set and low yield (Dixon et al., 2007; Paz-Vega, 1997; Rebollero and Romero, 2011).

Finally, the result is a berry-like fruit with a single large seed surrounded by a buttery pulp. Avocado fruit varies in size (from few grams to more than two kilograms); shape (usually pyriform to oval and round); pulp (from pale to rich-yellow when is mature); skin (with different thickness and texture); and seed (oblate, round, conical or ovoid) (Rebollero and Romero, 2011; Ayala Silva and Ledesma, 2014). Although avocado fruit composition depends on diverse factors as the variety, grade of ripening, climate, or soil composition and fertilizers (Alvarez et al., 2012), avocado fruit is known to be high in nutritional content (Dreher and Davenport, 2013) due to the presence of high amounts of monounsaturated fatty acids, fiber, minerals such as potassium (K), magnesium (Mg), phosphorus (P), calcium (Ca), or vitamins A, B-6, C, E. (Araújo et al., 2018; USDA; https://www.mapa.gob.es/es/ministerio/servicios/informacion/aguacate_tcm30-103002.pdf).

The time between flowering and harvesting fruit depends on the race. Furthermore, avocado fruit does not fully ripen on the tree and must be harvested at physiological maturity to achieve adequate flavor and firmness characteristics after harvest (Gamble et al., 2010).

1.2. Avocado crop relevance

Although Mesoamerican origin and archaeological evidence indicate that avocado has been used in Mexico for a period of 10,000 years, during all this time, a domestication has taken place, which is linked to a progressive selection for desired qualities of the fruit (Smith, 1966;1969). Nowadays, avocado fruit has a high nutritional content, which increased global interest in this crop in the last decades (Araújo et al., 2018; **Figure 1.2**). Avocado is present in five continents growing in regions with tropical, subtropical and temperate climates (Alcaraz et al., 2013; **Figure 1.3**). The global avocado harvested area and production in 2022, 884,035 ha and 8,978,275.2 t, respectively, with the Americas accounting for the largest share of production (71.9 %) (FAOSTAT, 2022). In the last ten years, from 2012 to 2022, avocado has had a two-fold increase in production (FAOSTAT; **Figure 1.2**).

At the country level, Mexico has been the largest producer of avocados for years, with a production of 2,529,581 t in 2022, representing 28% of world production, followed by

Colombia (1,090,664 t), Perú (866,457 t), Dominican Republic (737,201 t) and Kenya (458,439 t) (FAOSTAT, 2022).

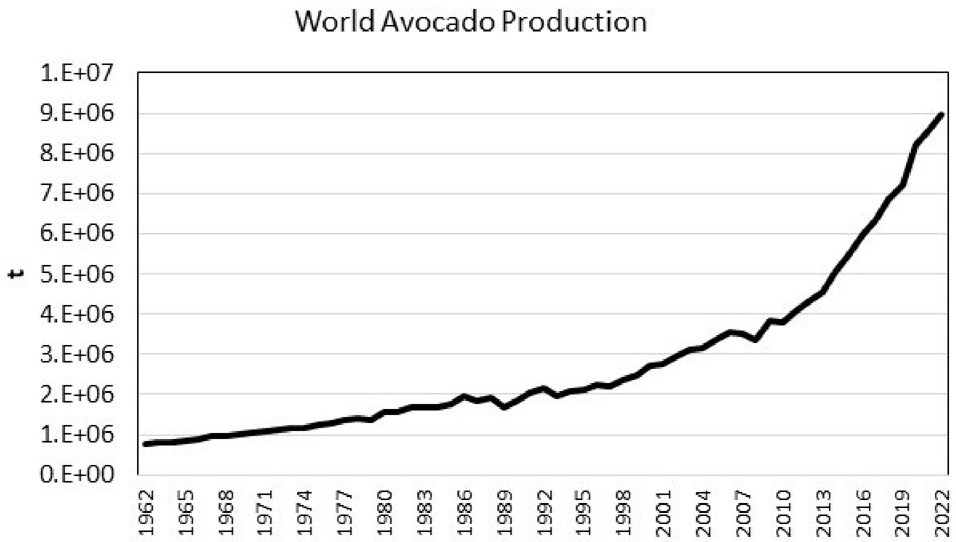

Figure 1.2. World avocado production (t) (FAOSTAT, 2022).

Avocado imports and exports have increased to cover the high market demand throughout the year. 'Hass' is the most traded variety. In 2022, the United States was the main importer of avocados (1,132,799.53 t) and Mexico the main exporter (1,041,787 t).

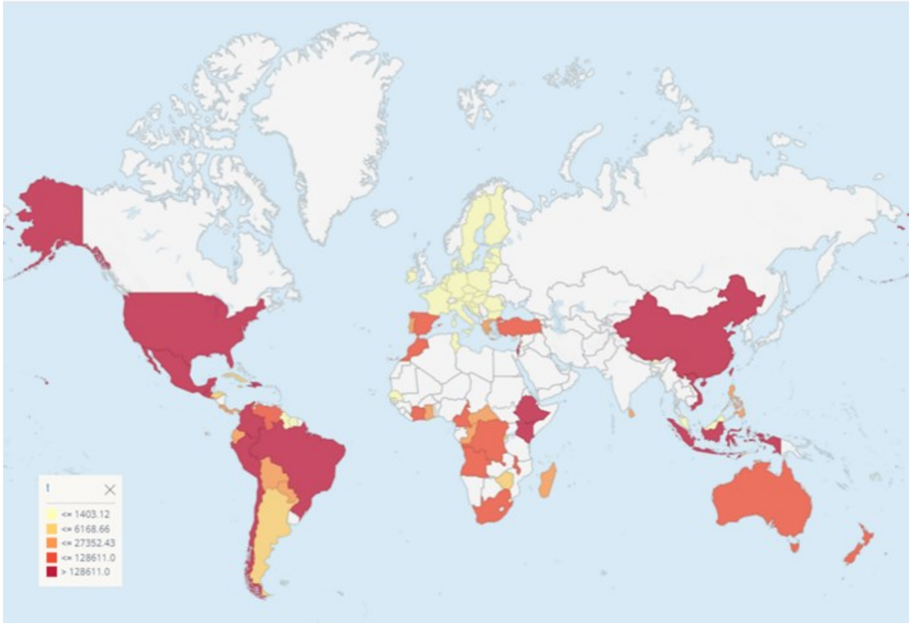

Figure 1.3. Map of the main avocado producers worldwide in 2022 (FAOSTAT, 2022).

Spain was the third country in terms of imports (208.57 t) and the fourth in terms of exports (149,917 t). This is possible because Spain was among the top 20 avocado producing countries in 2022 with a production of 105,930 t and a cultivated area of 19,520 ha. Spain is the leading producer country in Europe with approximately 72% of the total

European production and 77% of the total cultivated areas dedicated to avocado in 2022 (FAOSTAT, 2022).

In 2021, Andalusia represented approximately 76% of the cultivated area and 83% of the Spanish avocado production (**Table 1.1**) with Málaga the main producing province with 59,592 t and an area of 7,449 ha. Europe received 96% of Andalusian exports. These exports represent an economic value of 355.1 million € for Andalusia (Observatorio de precios y mercados, Junta de Andalucía).

Table 1.1. Cultivated area, production and yield of avocado in 2021 at the province level (Source: Ministerio de Agricultura, Pesca y Alimentación España)

Provinces and Autonomus Communities	Cultivated area (ha)		Production (t)	Kilograms/tree (kg/tree)
	Total	In production		
A Coruña	1	1	3	-
Lugo	-	-	-	-
Ourense	-	-	-	-
Pontevedra	2	2	1,400	3
Galicia	**3**	**3**	**1,967**	**6**
Barcelona	-	-	-	-
Girona	-	-	-	-
Lleida	-	-	-	-
Tarragona	2	-	-	-
Cataluña	**2**	**-**	**-**	**-**
Baleares	**9**	**8**	**49**	**6,075**
Alicante	363	243	2,187	9,000
Castellón	575	190	2,100	11,050
Valencia	1,155	444	4,706	10,600
C. Valenciana	**2,093**	**877**	**8,993**	**10,254**
R. de Murcia	**10**	**6**	**48**	**8**
Almería	6	5	52	10,382
Cádiz	1,569	781	6,150	12,190
Córdoba	-	-	-	-
Granada	2,793	2,705	28,227	10,435
Huelva	1,400	700	2,590	3,700
Jaén	-	-	-	-
Málaga	7,657	7,449	59,592	8,000
Sevilla	235	25	88	3,500
Andalucía	**13,651**	**11,665**	**96,699**	**12,311**
Las Palmas	314	295	1,972	4,990
S.C. de Tenerife	1,941	1,732	9,002	5,098
Canarias	**2,255**	**2,027**	**10,974**	**5,062**
España	**18,061**	**14,586**	**116,769**	**11,879**

Málaga and Granada are located on the so-called subtropical Andalusian coast, an area with conditions that allow the cultivation of subtropical species such as avocado and mango. The success of the Spanish avocado in the European market is due to the high quality of the fruit and its proximity, which allows avocado to be traded at their optimum ripeness, enabling the consumer to buy the fruit and consume it immediately (a concept known as 'ready to eat'). Moreover, from an ecological point of view, it has a lower carbon footprint. These advantages have made the Andalusian subtropical coast the protagonist of the European avocado market and have generated an associated industry (farmers, cooperatives, transport...) that grows year after year and has become the socio-economic engine of this part of Andalusia.

1.3. Main factors affecting avocado crops

The yield of avocado orchards could depend on a number of factors and how these factors impact growth and yield of the crop is dependent on the specific region and conditions under which avocado is cultivated:

- *Agro-climatic or abiotic factors*: are associated with the region where the orchard is located. These include factors such as radiation, temperature, rainfall, the availability of water and nutrients, and the soil type.
- *Human factors*: are those related to the management of the orchard, including the use of fertilisers, the level of technical expertise required for the orchard, the planting framework, and the irrigation system, among other factors.
- *Genetic factors*: The choice of an appropriate rootstock and variety that is well-suited to the prevailing growing conditions is of paramount importance in determining the success of a plantation.
- *Biological or biotic factors:* may be defined as the presence or absence of pollinators, such as bees, as well as the presence of pests and diseases caused by bacteria, viruses, oomycetes and fungi, occurring with varying incidence in the crop-producing areas of the world.

The most significant pests affecting the growth and yield of avocado crops are thrips (e.g.: Frankiniella *spp.*, *Heliothrips haemorrhoidalis*), mealybugs, aphids, mites such as Tetranychids (e.g. *Oligonychus punicae* causal agent of avocado brown mite of great importance in Mexico), stem, fruit and avocado seed borers (e.g. *Copturus aguacatae*, *Heilipus* spp.) and plant-parasitic nematodes (e.g. *Meloidogyne* spp., *Rotylenchulus reniformis*) (Peña et al., 2013; Carrillo-Fasio and Báez-Sañudo, 2020). On the other hand, economically significant diseases affecting avocado orchards include Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* (Zentmyer, 1978), anthracnose caused by *Colletotrichum gloesporioides*, *Glomerella cingulata* and *Colletotrichum acutatum* (López-Herrera, 2020); stem-end rot caused by various fungi (Dann et al., 2013); Cercospora spot caused by *Pseudocercospora purpurea* (Dann et al., 2013); scab caused by *Sphaceloma perseae* (Jenkins, 1934); sunblotch caused by *Avocado sunblotch viroid* (ASBVd); white root rot (Demathophora or Rosellinia root rot) caused by the fungus *Rosellinia necatrix* Prill. (Sztejnberg, 1994); brown root rot caused by *Phellinus noxius* (Dann et al., 2013); laurel wilt caused by the ascomycete *Harringtonia lauricola* (Dann et al., 2013); Armillaria root rot caused by species of Armillaria fungus (Dann and Gazis, 2024); Verticillium wilt, caused by the soil-borne fungus, *Verticillium dahliae* (Dann et al., 2013); branch canker caused by fungi of the *Botryosphaeriaceae* family, *Neofusicoccum parvum*, *Neofusicoccum luteum*, *Lasiodiplodia theobromae* (López-Herrera, 2020); bacterial canker caused by *Pseudomonas syringae* in Australia and South Africa and *Xanthomonas campestris* in California (Dann et al., 2013); and black streak which causal agent is unknown (Menge and Ploetz, 2003).

1.4. Main problems affecting avocado orchards in Southern Spain and control methods

1.4.1. Water scarcity

Currently, the avocado tree is facing new climatic challenges, either due to the expansion of its cultivation to regions with differing climatic conditions compared to its native habitat or due to environmental changes which has contributed to low water availability, derived from intense drought episodes and the impact of global warming in these regions (Lahav et al., 2013; Rondon et al., 2024).

This is indeed the case in zones with a Mediterranean climate, such as California, Israel, Chile and Spain (IPCC, 2022). Particularly in Spain, where avocados are cultivated in the so-called subtropical coast of Andalusian, which has been identified as a climate change hotspot (EEA, 2024).

Adequate irrigation is a crucial factor in ensuring optimal fruit development, particularly during the flowering and fruit-set stages. This practice not only promotes optimal fruit conditions but also helps to minimise fruit drop from the tree (Carr, 2013; Lahav et al., 2013). In this regard, field studies were carried out at IFAPA-Malaga in collaboration with the research team of TROPS SAT 2803, to determine the response of mature avocados to different water treatments and to what extent it is possible improve water use without compromising fruit production and quality (Moreno-Ortega et al., 2019). The results obtained, in addition to shedding light on the irrigation needs of the crop in the area (~8000 m^3 ha^{-1} yr^{-1}), provided the first insights on the use of sustained deficit irrigation strategies for saving water and their impact on yield and fruit quality.

At present, absence of precipitation has prompted local authorities to impose limitations or even restrictions on the utilization of urban water for agricultural purposes in certain regions, including the Axarquia (Málaga, Spain) which resulted in a significant reduction in avocado yields and a notable increase in tree mortality in orchards where irrigation is not possible. In this scenario, it is suggested that the success of avocado cultivation in the region is linked to good management of reduced irrigation allocations, making better use of irrigation water and with proper management of water levels in the soil and plant.

In addition, the choice and/or use of different rootstock/variety combinations with low water requirements and high-water productivity, and/or with greater tolerance to water stress, represents a method to cope with the water scarcity we are facing and guarantee the long-term sustainability of the crop.

1.4.2. Soil-borne diseases

Main soil-borne diseases affecting avocado crops in Spain, are Phytophthora root rot (PRR) and white root rot (WRR), caused by *P. cinnamomi* and *R. necatrix*, respectively.

- *Phytophthora root rot*

PRR is considered the most limiting phytopathological problem of the crop worldwide, due to its frequency, severity and high economic losses (Coffey, 1987; Ramírez et al., 2014). Although it is now known to affect more than 3,500 plant species from over 70 countries, the first report on avocado was in Puerto Rico in 1929 (Tucker, 1929). The causal agent is the hemibiotrophic oomycete *P. cinnamomi* Rands (Rands, 1922; Zentmyer, 1980; Pegg et al., 2002). Affected trees exhibit necrotic roots and a reduction in root mass, which results in a decrease in the ability of the plant to absorb water and nutrients. This leads to chlorosis, leaf drop and eventually, the death of the tree (Dann et al., 2013; **Figure 1.4**).

Figure 1.4. Defoliation of an avocado tree infected with *P. cinnamomi* (López-Herrera, 2020)

P. cinnamomi produces different spore forms, zoospores, chlamydospores and oospores, which play a role in disease development and survival (Zentmyer, 1980) allowing the pathogen to survive in adverse conditions for long periods of time, making it very difficult to eradicate the disease completely. The management of PRR requires an integrated approach, encompassing mulching, irrigation management, phosphite treatments and the utilisation of tolerant rootstocks (Pegg et al., 1985). In this regard, a number of *P. cinnamomi* tolerant rootstock are commercially available such as 'Dusa' and 'Duke 7' and other ones will be available in the near future (Spann, 2020)

- *White root rot*

This disease is caused by the soil-borne fungus *R. necatrix* Prilleux (anamorph: *Dematophora necatrix* Hartig), a pathogen of increasing importance, which has been associated with 335 plant hosts (https://fungi.ars.usda.gov/, USDA) including woody and herbaceous species of economic interest such as grapevines, olives, almonds, apples, pears, citrus fruits, coffee, potatoes, strawberries, mangoes and avocado. Despite its distribution across five continents, the pathogen has become a significant constraint on avocado production in Israel and southern Spain. Its prevalence in these regions has been increasing steadily, being the predominant cause of endemic avocado root rot (López-Herrera, 1998; López-Herrera and Zea-Bonilla, 2007).

This fungus is categorized within the phylum Ascomycota, family Xylariaceae (Prillieux E., 1904). When cultivated on a potato dextrose agar growth medium, the young mycelium of *R. necatrix* is initially observed to be white and cottony, before acquiring a dark appearance. A distinctive feature of this fungus is the formation of pear-shaped swellings in the hyphae, closed to the septum (**Figure 1.5**). This has been used as a diagnostic indicator for the species.

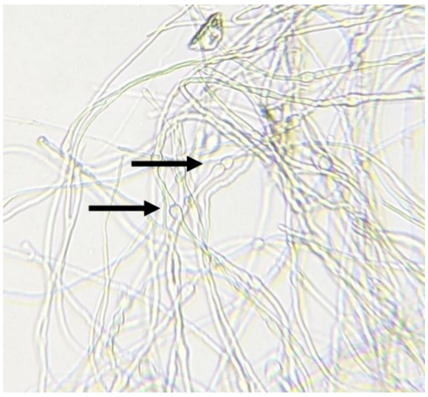

Figure 1.5. Characteristic pear-shaped swellings in *R. necatrix* hyphae.

The life cycle of *R. necatrix* has an asexual phase, during which chlamydospores and conidiospores are produced, and a sexual phase, with ascospores as the reproductive structure (Pérez-Jiménez et al., 2003). The roles of these three spore types in the epidemiology of the fungus remain unclear, with evidence suggesting that their involvement may vary across different countries.

The fungus is dispersed throughout the soil by the mycelium and mycelial strands or along the infected roots. Upon contact with a healthy root, the mycelia network proliferates, covering the root surface and subsequently penetrating avocado roots through natural openings, such as lenticels, wounds, or directly by forming a penetration sclerotium. The hyphae of *R. necatrix* invade and penetrate the vascular system of the root avoiding water and nutrient flow to upper parts of the plant (Pliego et al., 2009).

Plants infected by *R. necatrix* typically manifest both aerial and root symptoms. These arise as a consequence of damage to roots and the subsequent delivery of toxic compounds into the plant's vascular system (López-Herera and Zea-Bonilla, 2007). This pathogen produces different metabolites with phytotoxic effects such as rosellichalasin (Kimura et al., 1989; Edwards et al., 2001) diketopiperazines, rosellic acid and rosnecatrone (Edwards et al., 2001), as well as cytochalasin E, which has a direct effect on photosynthesis (Kshirsagar et al., 2001), although their roles in pathogenicity need to be clarified (Kanematsu et al., 1997).

At the root level, the presence of white cottony mycelium, in addition to white or black mycelial strands, can be observed (**Figure 1.6**). The fungus is located between the bark and the wood in woody plants, where it forms characteristic white mycelial fans which lately turns to greenish-grey or black. The pathogen invades the entire root system, leading to a generalized rotting effect, and subsequently, the roots show a dark brown coloration.

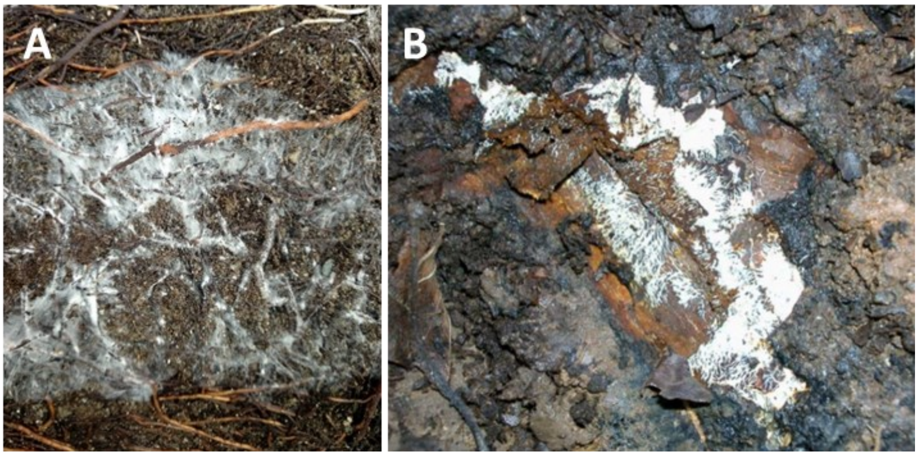

Figure 1.6. (A) *R. necatrix* mycelia growing on avocado root surface. **(B)** Typical fan-shaped mycelium of *R. necatrix* on wood under the bark of an avocado root (López-Herrera, 2020).

At the aerial level, infected trees may decline in vigor, leaves wilt and trees eventually die (**Figure 1.7**).

Figure 1.7. Aerial symptoms of an avocado tree infected by *R. necatrix*.

However, Infected trees do not always show aerial symptoms, making the diagnosis of this fungus extremely difficult (Pliego et al., 2012). Furthermore, the fungus is capable of surviving in the soil in a dormant state for extended periods of time and, when environmental conditions become favorable for its propagation, become active and initiate an infectious process in new host plants (López-Herrera, 2020).

Control of this disease is challenging due to the pathogen's ability to tolerate desiccation and acidic soils, its extensive host range, its ability to survive deep within the soil, and its resistance to numerous fungicides commonly used to manage plant pathogens (Khan, 1959).

In this regard, to reduce WRR incidence an integrated approach involving cultural practices, physical, chemical and biological control as well as genetic resistance must be carried out. Cultural practices may be conducted to avoid the use of infected plants and in orchards where the pathogen is present, to prevent the dispersion of the fungus to non-infested soil by removing and burning affected material. In addition, irrigation water management can induce tolerance in avocado plants, as mild water stress can induce cross-factor priming that increases the expression of avocado tolerance genes to *R. necatrix* from a susceptible rootstock (Martinez-Ferri et al., 2019).

Physical control such as soil solarization every two years keep *R. necatrix* inoculum in soil at low levels preventing disease development (López-Herrera et al., 1998). Different fungicides have been tested for chemical control of WRR disease with Fluazinam being the most effective, although it has not been registered to date in Spain for use in avocado. Biological control represents an alternative to the overuse of pesticides. Isolates of rhizobacteria species such as *Pseudomonas chlororaphis*, *Bacillus subtilis* and *Pseudomonas pseudoalcalígenes* (Pliego et al., 2007) and fungi isolates belonging to the genera *Thrichoderma* spp. have been successfully employed as a bio-control agent against *R. necatrix* in avocado (Cazorla et al., 2006; Cazorla et al., 2007; Pliego et al., 2008; Ruano-Rosa et al., 2010). Ruano-Rosa et al. (2014) showed that the combination of antagonistic microorganisms, specifically *Trichoderma* spp. along with strains of rhizobacteria (such as *B. subtilis* and *Pseudomonas* spp.), reduced the *in vitro* growth of *R. necatrix*. Additionally, the combination resulted in a significant improvement in the management of white root rot on avocado plants; e.g., utilization of *Trichoderma* spp. isolates in conjunction with Fluazinam has reduced the concentration of this fungicide to combat WRR in the field (Ruano-Rosa et al., 2018). However, none of the previously mentioned approaches has proven to be fully effective. In this sense, the utilization of tolerant/resistant rootstocks may represent an effective alternative to lessen the impact of this soil-borne pathogen. To date, there are no commercial tolerant avocado rootstocks to *R. necatrix*, and tolerant selections to *P. cinnamomi*, such as 'Dusa' or 'Duke 7', have been shown to be highly susceptible to this pathogen under artificial inoculation (Pérez-Jimenez, 2006). To overcome this problem, the biotechnology group at IFAPA-Málaga has been involved since 1995 in a breeding program aimed at obtaining material with tolerance/resistance to WRR.

1.5. Facing avocado problems

1.5.1. Avocado rootstock breeding

Avocado rootstock breeding is generally aimed at searching for resistance to specific traits, depending upon the area in which avocados are cultivated. In general, researchers look for attributes such as resistance to soil-borne pathogens, salt tolerance and adaptation to soils with high calcium content; in addition, dwarfing rootstocks are also desirable (Ben-Ya´acov and Michelson, 1995; Crane et al., 2013; Celis et al., 2018). The amount of P in the leaf (Salazar-García et al., 2016) and fruit quality are also affected by the rootstock (Reyes-Herrera et al., 2020; Hernández et al., 2023). These observations lead Reyes-Herrera et al. (2020) to recommend the inclusion of fruit quality as a target in avocado rootstock breeding programs.

Probably, the major objective in rootstock breeding has been finding resistance to the oomycete *P. cinnamomi*, a devastating disease of California avocados since 1942 (Crane et al., 2013). The pioneer work of Dr. Zentmyer at University of California, Riverside (UCR) resulted in the selection of the 'Duke 7' genotype, a rootstock which has been widely used in infected areas of California, South Africa and other countries (Menge et al., 2001). Avocados are very difficult to propagate vegetatively, however, the development of the Frolich method (Frolich and Platt, 1972), involving the grafting of scion to be rooted in a nurse seedling followed by incubation under dark conditions, has allowed for the propagation of selected rootstocks at commercial level. The ‘Dusa’ selection from Westfalia, South Africa, was released later and it is currently the most resistant rootstock available for growers; in addition, it is fairly tolerant to cold winters as well as saline conditions (Kremer-Köhne and Köhne, 2007; Crowley and Arpaia, 2002). ‘Bounty’, another rootstock selected in South Africa, ranks third in popularity, after ‘Dusa’ and ‘Duke 7’, for nurserymen of this country (Crane et al., 2013). Other selections from the UCR, such as ‘Steddom’, ‘Uzi’ and ‘Zentmyer’, among others, are under evaluation in field trials (Crane et al., 2013). In South Africa the breeding program also continuous with emphasis on superior crop yields besides resistance to *P. cinnamomi* (Kremer-Köhne et al., 2011; van Rooyen, 2011). More recently, other selections from UCR harboring resistance to *P. cinnamomi* and differing in their response to salinity are being evaluated in the field (Celis et al., 2018; Spann, 2020) while in South Africa, the new selection ‘Leola’, shows high resistance to *P. cinnamomi* while ‘Zerala’, besides resistance to the oomycete, is highly productive and tolerant to salinity (Spann, 2020).

Salinity and high calcium content in the soil drastically affect avocado production in areas where water is scarce. There is great variability in salinity tolerance within avocado races, with West Indian genotypes showing a better performance. These genotypes also perform well in calcareous soils while those of Guatemalan race are highly sensitive (Ben-Ya´acov and Michelson, 1995). In Israel, the rootstock breeding program has focused on the search for high productive combinations based on the scion/rootstock interaction as well as tolerance to abiotic stress in the soil. Numerous selections were released, all with the VC prefix (Vegetative clones from the Volcani Center Research Programme) and evaluated in the field to provide recommendations to growers in different areas (Ben-Ya´acov and Zilberstaine, 1999; Crane et al., 2013).

In Australia, a rootstock breeding program is also underway searching for *P. cinnamomi* resistance and improved crop yields (Smith et al., 2011). ‘Velvick’ (West Indian x Guatemalan hybrid) is a vigorous Australian selection that performs well in *P. cinnamomi* infested soils. Moreover, this rootstock significantly reduces the incidence of anthracnose in the scion (Willingham et al., 2001; 2006). According to Whiley and Whiley (2011), ‘Velvick’ seedlings sometimes outyielded equivalent clonal rootstocks; however, ‘Velvick’ seedlings had to be derived from isolated (self-pollinated) trees. These authors recommend the use of proven seedlings on new plantings and clonal rootstocks with good tolerance to *P. cinnamomi* for replanting.

In Spain, there is a breeding program focused on finding resistance to the necrotrophic fungus, *R. necatrix*, causal agent of the WRR, and several promising selections, (BG48, BG83, BG181, among others) were obtained (Barceló et al., 2007). Currently, evaluation of agronomical traits of selected genotypes is being carried out at La Mayora Experimental Station (Barceló-Muñoz, A., personal communication, September, 2024). In this thesis, some of these genotypes have been used in experiments to evaluate their performance under water shortage.

1.5.2. Biotechnological approaches

- *Induced mutations*

Induced mutations have been a common strategy in plant breeding for a long time (Joint FAO/IAEA, 2010). In avocado, Coto et al. (2014) attempted to obtain resistance to stress (salinity and *P. cinnamomi*) through irradiation of 'Duke 7' zygotic embryos cultured *in vitro*. A mutagenic dose of LD_{50}= 28 Gy was used while selective doses for salinity were (LD_{20} = 157 mM of NaCl, LD_{50} = 6% PEG-6000). Several M_1 lines were obtained and are currently under evaluation.

Resistance to *P. cinnamomi* appears to operate at cellular level; Phillips et al. (1991) established callus from different avocado cultivars that differed in their susceptibility to *P. cinnamomi*, 'Topa-Topa' (susceptible), 'Duke 7' (fairly resistant) and 'Martin Grande' (moderately resistant). Following inoculation with the oomycete, obtained responses were similar to that at the plant level with the two resistant cultivars showing a hypersensitive reaction type. These results led Witjaksono et al. (2009) and Avenido et al. (2009) to attempt the obtainment of avocado plants resistant to *P. cinnamomi* by using embryogenic cultures of Indonesian selections cultured in the presence of culture filtrate of this oomycete; however, no results regarding plant regeneration and performance following pathogen inoculation were reported. A similar approach was attempted in this thesis, with the obtainment of embryogenic cultures resistant to crude culture filtrate of *R. necatrix* and evaluation of gene expression response following a new exposure to the fungal filtrate.

- *Genetic transformation*

Genetic transformation can be used in plant breeding to address specific traits, generally under the control of single genes. In any case, prior to development of a transformation protocol for a given species, efficient regeneration systems need to be established. In the case of avocado, plants can be regenerated through the somatic embryogenesis pathway; however, initial explants are of juvenile origin (immature zygotic embryos) and germination rates of somatic embryos are very low (Palomo-Ríos et al., 2013; Pliego-Alfaro et al., 2020). In any case, transformation protocols via *Agrobacterium* *tumefaciens*, using embryogenic cultures as explants, have been established (Cruz-Hernandez et al., 1998; Palomo-Ríos et al., 2012; 2017). Targets addressed are disease resistance and increased shelf life; e.g., Palomo-Ríos et al. (2010; 2011) obtained 4 independent transgenic lines with the *At*NPR1 gene, known to play a key role in the salicylic acid mediated plant immune response (Cao et al., 1997). This gene has also been used in other species to induce resistance to fungal pathogens (Lin et al., 2004; Wally et al., 2009). In the case of avocado, the obtained transgenic lines showed very different *in vitro* behavior, hence they are being propagated by the Frolich method (Frolich and Platt, 1972) to evaluate their response after inoculation with *R. necatrix* (Pliego-Alfaro, F., University of Málaga, personal communication, October, 2024). Regarding the Avocado Sunblotch Viroid (ASBVd), Litz et al. (2010) attempted to eliminate it through transformation of somatic embryos with the pac1 ribonuclease gene from *Schizosaccharomyces pombe.* Preliminary analysis of resulting plants through RT-PCR indicated that they were viroid-free. There have also been attempts to increase avocado shelf-life by blocking ethylene biosynthesis (Litz et al., 2007); however, obtained plants did not flower (Pliego-Alfaro et al., 2020).

- *Genes related to soil-borne-pathogens resistance*

In depth knowledge of the avocado/*P. cinnamomi* interaction is a critical step prior to developing efficient biotech tools to control this oomycete (van den Berg et al., 2021). In a transcriptomic study, Mahomed and van den Berg (2011) were able to identify specific (*thaumatin*, *PR-10*) and general defence genes such as *metallothionein*, following inoculation of avocado roots with a *P. cinnamomi* mycelial preparation. Subsequently (Engelbrecht and van den Berg, 2013) identified higher expression of *phenylalanine ammonia-lyase (PAL*) and *lipoxygenase (LOX*) genes in avocado rootstocks showing tolerance to *P. cinnamomi*, in relation to the susceptible controls.

Flooding conditions increase susceptibility of avocado to *P. cinnamomi*. In a massive sequencing study, Reeksting et al. (2014) found that flood responsive genes such as sucrose synthase showed increased expression in infected plants; however, flooding causes so many metabolic changes that probably mask the response to the oomycete. In any case, it appears that most genes related to plant defence are down regulated under flooding and this could explain the higher avocado susceptibility to the oomycete. Moreover, the observed decrease in water uptake linked to flooding could be explained by the observed down-regulation of aquaporins (Reeksting et al., 2016). The role played by salicylic acid (SA) and jasmonic acid (JA) in the avocado/*P. cinnamomi* interaction has been addressed by van den Berg et al. (2018); initially the SA response takes place (6 h) while after 24 h, an increased expression of genes related to the JA pathway took place. At this time, phenolics accumulation could also be observed. At intermediate times (18 h) signaling overlapping of the two pathways was reported. In resistant rootstocks, suppression of SA pathway takes place earlier allowing a more rapid initiation of JA signaling (van den Berg et al., 2021). Trying to decipher the role played by the *NPR1* gene and NPR1-like genes family in avocado defence against *P. cinnamomi*, Backer et al. (2015; 2019) identified five NPR1-like genes in avocado plants treated with SA, JA or infected with the oomycete; these authors found that three of them were possibly related to plant defence while another two could be associated to plant development. Moreover, in resistant rootstocks, *PR-1* expression (linked to SA pathway) was induced earlier than in susceptible material with a drastic downregulation at 24 hours post inoculation (hpi).

Fick et al. (2022) studied the role of the Nucleotide Binding-Leucine rich repeat (NLR) proteins in the avocado/*P. cinnamomi* pathosystem. NLR are components of the effector triggered immune responses (ETI) in plant pathogen interactions. These authors indicated that 84 *Pa*NLR showed expression at 24 hpi in avocado genotypes showing some resistance to the pathogen while in susceptible material, expression took place at 6 hpi; according to these authors, these differences in temporal expression could account for the diverse behavior against the oomycete.

Regarding the avocado/*R. necatrix* pathosystem, another soil-borne pathogen causing the white root rot disease, Zumaquero et al. (2019b), working with genotypes showing different response to the fungus, reported that *protease inhibitors* played a key role in disease tolerance. In addition, upregulation was also associated with genes related to salt and osmotic stress, which seems to indicate that capacity to withstand osmotic imbalance could be associated to tolerance to this disease.

1.5.3. Use of elicitors for disease control

Another biological approach to improve avocado health is the use of elicitors (molecules and compounds such as carbohydrates, proteins, peptides, lipids, glycoproteins, plant hormones, and others) that can induce physiological changes or stimulate certain defense mechanisms in plants (Baenas et al., 2014; Jamiołkowska, 2020). According to Zheng et al. (2020), the use of elicitors in crops is safe, nontoxic, and environmentally friendly, and small amounts are enough to provide the plants with long-term protection against a wide range of pathogens.

Plant-elicitor interaction is a rather complex phenomenon, and its success will depend upon a number of interrelated factors (Vasconsuelo and Boland, 2007; Narayani and Srivastava, 2017). A plant may exhibit positive responses to different elicitors, while a given elicitor may induce defence reactions in different plants, in this regard, Jesús et al. (2015) observed that foliar treatments with SA (0.75-5 mM) enhanced drought tolerance in *Eucalyptus globulus*, with the effect being dependent on the dosage applied. The time and frequency of application are also crucial factors to be considered when optimising elicitor effects on plant response. The application frequency is linked to the persistence of the response, while the culture type and growing conditions determine the most appropriate application method. For example, in accordance with Rohwer and Erwin, (2008), methyl jasmonate (MeJA) can be applied as a gas (Farmer and Ryan, 1990), mixed with lanolin paste (Saniewski et al., 1998), in liquid form (Baldwin, 1996) or, most commonly, as a spray with a surfactant agent (Janoudi and Flore, 2003).

Following the elicitation, physiological changes are induced in such a way that the plant will exhibit a faster and stronger response when subsequently exposed to additional stress conditions (priming). Elicitor treatments generally induce modifications in histone proteins at defence related genes, suggesting an epigenetic base of the priming mechanism (Molinier et al., 2006; Pastor et al., 2013). Hence, use of elicitors has become an important tool to gain knowledge on defense responses in plants and at the same time, they can be used to improve plant response to stress situations in an environmentally friendly way.

In the avocado, the fruit/fungi pathosystem has been the most widely used for elicitation studies; e.g., Chitosan, essential oils, silicon, SA and JA, among others, have been known to induce defence genes and reduce fungi viability, hence, having a positive effect on increasing fruit shelf life (Munhuweyi et al., 2020; Herrera-González et al., 2021). MeJA, a derivative from jasmonic acid and SA, are the most widely used elicitors for disease control (Awang et al., 2013; Thakur and Sohal, 2013). To date, numerous studies have shown that exogenous application of MeJA and SA increases tolerance to pathogens by inducing plant defense-related genes (Awang et al., 2013; Cervantes-Landaverde et al., 2009; Laredo Alcalá et al., 2017), and by triggering several signaling pathways, for example, ion flux and the synthesis of signal molecules resulting in the reinforcement of cell walls, accumulation of antimicrobial compounds and synthesis of proteins that inhibit pathogen growth and proliferation (Thakur and Sohal, 2013; van den Berg et al., 2018; Yu et al., 2018). SA and JA have been applied as vapours on harvested fruit, giving rise to enhanced activity of defence related genes such as *chitinase*, *β-1,3-glucanase* resulting in a drastic reduction of anthracnose incidence (Glowacz et al., 2017). Chitosan, a chitin derived polymer of N-acetyl-D-glucosamine units, has been associated with defence responses in plants, triggering phytoalexins production and increasing chitinases, protease inhibitors, PAL, peroxidase (POD), and polyphenol oxidase (PPO) activities (Terry and Joyce, 2004; Amborabé et al., 2008; Meng et al., 2010). When applied to the avocado fruit, an increased activity of genes related with phenylpropanoids pathway as well as higher levels of the antifungal diene AFD (1-acetoxy-2-hidroxiy-4-oxoheneicosa 12, 16 diene) were found, leading to decreased damage of the fungus *C. gloeosporioides* (Xoca-Orozco et al., 2017;

2019). Positive effects of chitosan and cinnamon essential oil were reported by Herrera-González et al. (2024) to control growth of *N. parvum* in the avocado fruit; e.g., mycelial development and sporulation were inhibited and this correlated with increased PAL, POD, and PPO activities. Interestingly, Chitosan nanoparticles (ChNPs) have also been used as biodegradable substances to induce accumulation of metabolites in avocado callus. Encapsulated methionine (ChNPs@Met) gave rise to fresh weight and glutathione accumulation while encapsulated SA (ChNPs@SA), induced biosynthesis of polyphenolic compounds and fatty bioactive components; moreover, antioxidant activity was also higher in treated callus (Abo El-Fad et al., 2022).

High degree polymerized agave fructans (HDPAF) have also been used to extend avocado fruit shelf life; following application of these compounds, genes related to phenylpropanoid biosynthesis, calcium ion signal decoding, and pathogenesis-related proteins, among others, were induced while peak of ethylene biosynthesis was delayed (Cuellar-Torres et al., 2023). However, the extracellular protein of the fungus *Acremonium strictum* (AsES), known to induce the SAR response in strawberry (Chalfoun et al., 2013), increases ethylene biosynthesis in avocado fruit, accelerating ripening although it also enhances defence against opportunistic pathogens resulting in improved fruit shelf life (Perato et al., 2018).

Silicon has been associated with disease resistance in plants, playing a role in cell wall strengthening (Fawe et al., 2001) and increasing production of defence related proteins such as chitinases and glucanases (Dann and Muir et al., 2002). Postharvest application of silicon in soluble form or as potassium silicate to the avocado showed positive effects on fruit quality and this was linked to accumulation of antioxidants and polyphenols, and a decreased respiration rate (Tesfay et al., 2011; Herrera-González et al., 2021). When injections were applied to the trunk, a drastic reduction in anthracnose incidence was found in the harvested fruit (Anderson et al., 2005).

Silicon (potassium silicate solution) has also been applied in the irrigation water to improve the response of avocado plants to the oomycete *P. cinnamomi.* Leaf analysis on silicon treated plants following inoculation with the pathogen (3-12 h) indicated an increase of enzymatic pathways related to oxidative stress as well as the synthesis of structural components. These results led the authors to conclude that silicon could be used as a strategy for managing this disease under field conditions (Álvarez et al., 2023).

Curdlan (Curd), *β*-1,3-glucan, is a water insoluble microbial exopolysacharide known to activate defence responses in tobacco, pepper and potato. Sprays on avocado prior to infection with *P. cinnamomi* induced plant defence through increasing POD, PPO, superoxide dismutase (SOD) and PAL activities. In addition, it increased cholophyll and carotenoids contents (Guarnizo et al., 2022).

In this thesis, the role of the two most important elicitors will be evaluated to improve the avocado response to the necrotrophic pathogen *R. necatrix*.

Objectives

The sustainability of avocado cultivation on the Andalusian coast is particularly important for the Andalusian economy and depends on the resolution of priority problems for the sector that affect its profitability, such as limited water availability and the root rot caused by *Rosellinia necatrix*. The use of tolerant rootstocks has been particularly successful for the control of the root rot caused by *Phytophthora cinnamomi* and it is also the most recommended approach for the control of *R. necatrix* (Barceló et al., 2007; Pliego et al., 2012). The avocado breeding program being carried out at IFAPA-Malaga for more than a decade has selected 8 *R. necatrix* tolerant rootstocks, which are being evaluated under field conditions. Information on the water requirements and water stress tolerance of the selected rootstocks is of great importance for the avocado sector. In addition, further attempts are needed to elucidate the avocado defence mechanisms against *R. necatrix* to help in the selection of new tolerant avocado rootstocks, as well as to improve performance of susceptible rootstocks currently used in commercial orchards. In this research, the following objectives have been addressed as a first step to counteract these problems.

1. Evaluation of tolerance to water stress of avocado rootstocks selected for their tolerance to the pathogen *R. necatrix* **(Chapter 2)**.
2. Identification of defence genes in avocado cells tolerant/susceptible to the crude filtrate of *R. necatrix* through transcriptomic analysis **(Chapter 3)**.
3. Evaluation of the effect of exogenous application of commercial elicitors on the induction of tolerance to *R. necatrix* in the susceptible commercial avocado rootstock 'Dusa' (**Chapter 4)**.

Chapter 2

Water relations and physiological response to water deficit of 'Hass' avocado grafted on two rootstocks tolerant to *Rosellinia necatrix*

Moreno-Pérez, A.; Barceló, A.; Pliego, C.; Martínez-Ferri, E. Water Relations and Physiological Response to Water Deficit of 'Hass' Avocado Grafted on Two Rootstocks Tolerant to *R. necatrix*. Agronomy 2024, 14, 9, 1959. https://doi.org/10.3390/agronomy14091959

2.1. Abstract

Avocado (*Persea americana* Mill.) cultivation has spread to many countries from the tropics to the Mediterranean region, where avocado crops commonly face water shortages and diseases, such as white root rot (WRR) caused by *Rosellinia necatrix*. The use of drought- and WRR-tolerant rootstocks represents a potential solution to these constraints. In this research, water relations and the morpho-physiological response of avocado 'Hass' grafted on two selections of *R. necatrix*-tolerant rootstocks (BG48 and BG181) were evaluated under well-watered (WW) and at two soil-water-availability conditions (WS, ~50% and ~25% field capacity). Under WW, scion water use was markedly affected by the rootstock, with BG48 displaying a *water-spender* behavior, showing higher water consumption (~20%), plant transpiration rates (~30%; E_{plant}) and leaf photosynthetic rates (~30%; A_N) than BG181, which exhibited a *water-saving* strategy based upon a trade-off between leaf-biomass allocation and tight stomatal control of transpiration. This strategy did not reduce biomass, with BG181 plants being more water use efficient. Under WS, BG48 and BG181 exhibited a *drought-avoidance* behavior based on distinct underlying mechanisms, but increases in leaf mass area (~18-12%; LMA), and decreases in E_{plant} (~50-65%), plant hydraulic conductance (~44-86%; K_h) and leaf water potential (~48-73%; Ψ_w) were observed in both rootstocks, which aligned with water stress severity. After rewatering, photosynthetic rates fully recovered, suggesting some ability of these rootstocks to withstand water stress, enabling the 'Hass' variety to adapt to region-specific constraints.

Chapter 3

A comparative transcriptome analysis of avocado embryogenic lines susceptible or resistant to *Rosellinia necatrix* exudate

Moreno-Pérez, A.; Zumaquero, A.; Martínez-Ferri, E.; López-Herrera, C.; Pliego-Alfaro, F.; Palomo-Ríos, E.; Pliego, C. A Comparative Transcriptome Analysis of Avocado Embryogenic Lines Susceptible or Resistant to *Rosellinia necatrix* Exudate. Agronomy 2023, 13, 1354. https://doi.org/10.3390/agronomy13051354.

3.1. Abstract

Avocado embryogenic cultures were selected for resistance to the culture filtrate (CF) of *Rosellinia necatrix*, the causal agent of white root rot disease. A resistant callus line was obtained through recurrent selections in progressively increasing concentrations of fungal CF (from 60% to 80%). RNA sequencing (RNA-Seq) technology was used to compare the transcriptomic profiles of the avocado embryogenic-callus-resistant line L3 (capable to survive in the presence of 80% CF) and control line AN-9 (not exposed to CF), after 24 h of growth in a medium containing 40% CF. A total of 25,211 transcripts were obtained, of which 4,918 and 5,716 were differentially expressed in the resistant and control line, respectively. Interestingly, exposure of embryogenic callus lines to 40% of *R. necatrix* exudates induced genes previously reported to be related to avocado defense against fungal diseases (lignin biosynthesis, Pathogenesis Related (PR) proteins, WRKY (WRKYGQK) Transcription Factor (TF), NAC (NAM, ATAF1/2, and CUC2) TF, proteinase inhibitors and Ethylene Response Transcription Factor (ERF), among others), which were accumulated in greater amounts in the resistant line in comparison to the susceptible one. This research will contribute to the understanding of avocado defense against this pathogen, thereby aiding in the selection of resistant avocado rootstocks.

Chapter 4

Effects of exogenous application of methyl jasmonate and salicylic acid on the physiological and molecular response of ‘Dusa’ avocado to *Rosellinia necatrix*

Moreno-Pérez, A.; Martínez-Ferri, E.; van den Berg, N.; Pliego, C. Effects of Exogenous Application of Methyl Jasmonate and Salicylic Acid on the Physiological and Molecular Response of ‘Dusa’ Avocado to *Rosellinia necatrix*. Plant Dis. 2024. https://doi.org/10.1094/PDIS-11-23-2316-RE.

4.1. Abstract

Methyl jasmonate (MeJA) and salicylic acid (SA) are important in mediating plant responses to abiotic and biotic stresses. MeJA and SA can act as elicitors by triggering plant defense responses similar to those induced by pathogens and may even provide long-term protection against them. Thus, exogenous application of MeJA and SA could protect susceptible avocado plants against white root rot (WRR) disease caused by the necrotrophic fungus *Rosellinia necatrix*, one of the main diseases affecting avocado orchards. This work evaluates the effects of MeJA or SA on the physiological and molecular response of susceptible ‘Dusa’ avocado rootstock and their ability to provide some protection against WRR. The application of MeJA and SA in avocado increased photoprotective mechanisms (nonphotochemical chlorophyll fluorescence quenching) and upregulated the *glutathione S-transferase*, suggesting the triggering of mechanisms closely related to oxidative stress relief and reactive oxygen species scavenging. In contrast to SA, MeJA’s effects were more pronounced at the morphoanatomical level, including functional traits such as high leaf mass area, high stomatal density, and high root/shoot ratio, closely related to strategies to cope with water scarcity and WRR disease. Moreover, MeJA upregulated a greater number of defense-related genes than SA, including a *glu protease inhibitor*, a key gene in avocado defense against *R. necatrix*. The overall effects of MeJA increased ‘Dusa’ avocado tolerance to *R. necatrix* by inducing a primed state that delayed WRR disease symptoms. These findings point toward the use of MeJA application as an environmentally friendly strategy to mitigate the impact of this disease on susceptible avocado orchards.

Summary of results

The main results obtained in this doctoral thesis are presented in accordance with the objectives proposed:

Regarding the first objective **(Chapter 2)**, avocado plants cv. 'Hass', grafted on BG48 and BG181 (two rootstock selections tolerant to *R. necatrix)*, were evaluated under different conditions of water supply; e.g., well-watered (WW) and two water stress levels, mild-WS and severe-WS (~50% and ~25% of field capacity, respectively). In the absence of soil water limitations, a strong effect of rootstock on 'Hass' scion was found. Plants grafted on BG48 showed a water-spender behavior as they exhibited higher water consumption as well as higher values of plant transpiration rate (E_{plant}) and leaf photosynthetic rates than those grafted on BG181, which exhibited a water-saving strategy based on a compromise between leaf biomass allocation and tight stomatal control of transpiration not linked to a reduction in growth. These results suggest that 'Hass' avocado plants grafted onto BG181 require less water, so plants are more water efficient.

Following water deprivation, BG48 and BG181 plants increased leaf mass area (LMA) and decreased E_{plant}, plant hydraulic conductance (K_h) and leaf water potential (Ψ_w) in agreement with the severity of water stress, showing both genotypes, drought avoidance behavior based on different underlying mechanisms.

After mild- and severe-water stress conditions, photosynthetic rates of rewatered plants were fully recovered, suggesting some ability of these rootstocks to recover from water stress which may be associated with their ability to with-stand *R. necatrix* infection.

These results contribute to our understanding of the relationship between the rootstock and scion of avocado trees proving that water use of 'Hass' avocado is strongly affected by rootstocks and provides insights into the mechanisms that enable them to withstand water scarcity. Consequently, the choice of a suitable rootstock can be decisive for the success or failure of a plantation. Further studies are needed to assess the influence of rootstocks on yield and water use efficiency under field conditions.

Regarding the second objective **(Chapter 3)**, an avocado embryogenic callus line (AN-9, derived from an immature zygotic embryo of cv. 'Anaheim') was exposed to progressively increasing concentrations of fungal filtrate (CF; from 60% to 80%). After the last exposure, line L3, resistant to 80% CF was obtained. The transcriptome analysis of the resistant (L3) and the susceptible control (AN-9, not exposed to CF) callus lines after 24 h of growth in medium containing 40% of CF or in medium without CF, revealed that both lines showed more up-regulated differentially expressed genes (DEGs) than down-regulated, however, L3 showed a lower gene deregulation than the susceptible one.

Gene ontology enrichment revealed categories common to both callus lines (catalytic activity, oxidoreductase activity, binding, hydrolase activity, peroxidase activity, antioxidant activity, response to stimulus, transporter activity, defense response and photosynthesis) as well as specific categories for resistant (detoxification) and susceptible (lipase activity, kinase activity and metabolic processes) callus lines.

The exposure of callus lines to CF, induced the expression of genes previously related to avocado defense against fungal diseases such as genes related to cell wall modification (e.g. lignin biosynthesis), detoxification and disease-resistance proteins, pathogenesis related (PR) proteins, protease and protease inhibitor activity (e.g. proteinase inhibitor, subtilisin-like protein), transcription factor (e.g. WRKY transcription factor, NAC transcription factor), hormonal regulation (e.g. ethylene responsive) and redox homeostasis (e.g. peroxidase).

It is noteworthy the overexpression of the proteinase inhibitor (Pag64949) which share the same coding sequence with the one previously identified in tolerant avocado rootstocks after infection with *R. necatrix* under greenhouse conditions which suggest the importance that could play this protein in the defense of avocado rootstocks against *this pathogen* and could support the use of *in vitro* cell culture techniques for studying avocado-*R. necatrix* interaction. These results could help to understand the defense mechanisms of avocado against this necrotrophic fungus aiding in the selection of tolerant avocado rootstocks.

Regarding the third objective **(Chapter 4)**, the exogenous application of MeJA or SA elicitors in 'Dusa' avocado plants susceptible to *R. necatrix*, caused physiological changes at leaf level, decreased maximum photochemical efficiency of the open reaction centers of PSII (F_v'/F_m') and activated photoprotective mechanisms by increasing nonphotochemical chlorophyll fluorescence quenching (NPQ). At molecular level, the expression of the glutathione S-transferase gene on roots after MeJA application was upregulated, which suggests the triggering of mechanisms closely related to oxidative stress relief and reactive oxygen species scavenging. MeJA application produced effects more pronounced at the morphoanatomical level, including functional traits such as high leaf mass area (LMA), high stomatal density, high percentage of root dry weight and high root/shoot ratio, closely related to strategies to cope with water scarcity and white root rot (WRR) disease. MeJA also upregulated a greater number of defense-related genes than SA, including a glu protease inhibitor, a key gene in avocado defense against *R. necatrix*.

After MeJA and SA applications, a subset of control and elicited plants were inoculated with *R. necatrix*. Inoculated plants showed significantly higher values of NPQ than noninoculated control plants, which were accompanied by significantly lower F_v'/F_m' values, indicating the operation of energy-dissipating mechanisms regardless of the elicitation treatment. A delay in WRR disease symptoms was observed in plants treated with MeJA, which could be an indication that MeJA increased ‘Dusa’ avocado tolerance to *R. necatrix* by inducing a primed state.

These findings could support the use of MeJA elicitor as an environmentally friendly strategy to mitigate the impact of white root rot on susceptible avocado orchards.

Discusión general

España es el único país europeo con una producción comercial significativa de frutas subtropicales, entre los que destaca el aguacate, permitiéndole acceder al mercado europeo en pocas horas con un producto de alta calidad. La rentabilidad de este cultivo especialmente importante para la economía andaluza pivota sobre la disponibilidad de agua para riego y el control de enfermedades como las podredumbres radiculares causadas por *Phytophthora cinnamomi* y *Rosellinia necatrix*. Estos problemas pueden verse agravados por la mayor incertidumbre climática (escasez de precipitaciones y temperaturas extremas) y por el incremento (~20%) en la demanda hídrica de los cultivos, como consecuencia del efecto del cambio climático en Andalucía. Este efecto empieza a ser especialmente notable en la principal región productora de la costa andaluza, en la que se vive actualmente una situación de sequía extrema que ha conllevado restricciones en la dotación de agua para riego en los últimos años, y que implica estrés hídrico en los cultivos (Moreno-Ortega et al., 2019). Este mayor estrés en las plantas podría incrementar su susceptibilidad a plagas y enfermedades por lo que es necesario aportar soluciones viables integrales para estos problemas.

En referencia a las enfermedades, es necesario el desarrollo de estrategias de control, ecológicamente sostenibles, ante la eliminación progresiva de algunos fungicidas por su efecto medioambiental negativo, según el Real Decreto 1311/2012. Como se ha mencionado anteriormente, dos de las principales enfermedades que afectan al sector aguacatero en el litoral andaluz son las podredumbres radiculares, causadas fundamentalmente por *P. cinnamomi* y *R. necatrix*. En relación con *P. cinnamomi*, California y Sudáfrica desarrollan, desde hace aproximadamente 25 años, programas de selección de material tolerante a este oomiceto, existiendo en el mercado una serie de portainjertos comerciales con diversos grados de tolerancia (‘Duke 7’, ‘Toro Canyon’ y ‘Dusa’) (Coffey, 1987; Gabor y Coffey, 1990; Kremer-Köhne et al., 2001). Respecto a *R. necatrix*, solo España (IFAPA Málaga) ha iniciado un programa de obtención de portainjertos resistentes a este patógeno, aunque en los últimos años han comenzado a aparecer focos de infección en California, norte de Israel, México, Corea y Sudáfrica. El control de esta enfermedad es difícil porque cuando aparecen los primeros síntomas, el patógeno ya está bien establecido en el suelo y las raíces y, pese a haberse desarrollado técnicas de detección precoz (Ruano-Rosa et al., 2007), para un control efectivo de la enfermedad han de utilizarse medidas preventivas más que curativas. Hoy día, el uso de portainjertos de aguacate tolerantes a *R. necatrix* se propone, sin duda, como uno de los métodos más efectivos para el control de esta enfermedad en las plantaciones del litoral andaluz. En el IFAPA Málaga, en colaboración con el IAS-CSIC e IHSM-UMA-CSIC, se inició hace más de una década un programa de selección de material tolerante a este patógeno, en el que se ha evaluado la respuesta frente a la infección de material vegetal de distintas procedencias, en su mayoría híbridos de las razas guatemalteca y mexicana. Actualmente, se cuenta con 8 selecciones, con distinto grado de tolerancia a este hongo necrotrofo.

Es conocido que las razas de aguacate muestran diferencias en tolerancia/susceptibilidad a estreses ambientales (salinidad, baja temperatura) derivadas de su origen (raza mexicana,M: templado subtropical; raza guatemalteca, G: subtropical; raza antillana, A: tropical), por lo que las selecciones avanzadas del programa pueden presentar diferentes necesidades hídricas, así como distinto comportamiento frente a estrés hídrico. En esta línea y dada la actual escasez de agua, se ha incorporado como objetivo del programa el estudio de la tolerancia a estrés hídrico de estas selecciones.

En la presente tesis doctoral se ha llevado a cabo el primer estudio para determinar el consumo de agua y tolerancia a estrés hídrico de dos de las selecciones que muestran tolerancia a *R. necatrix* con la finalidad de proporcionar a los agricultores portainjertos con bajo consumo de agua y tolerancia a estrés. Los resultados obtenidos han puesto de manifiesto que el portainjerto ejerce una modulación estrecha de las relaciones hídricas de la variedad injertada 'Hass', observándose que el consumo de agua puede diferir substancialmente dependiendo del patrón en condiciones de no limitación hídrica, y por tanto, la elección de portainjerto puede representar una estrategia para ahorrar agua (~20%). Estos resultados son coherentes con estudios previos, en los que se observó que las necesidades hídricas del aguacate pueden variar en función de la combinación patrón-variedad injertada (Fassio et al., 2009). Las diferentes necesidades hídricas de ambos portainjertos estuvieron relacionadas con rasgos asociados a dos estrategias de uso del agua: gastadora de agua (BG48) y conservadora (BG181). En esta última, se observó que el portainjerto ejerce una modulación estrecha de la apertura estomática, controlando la transpiración sin repercutir negativamente en el crecimiento de la planta, lo que conlleva una regulación de la relación fuente-sumidero que condiciona la eficiencia del uso del agua.

Esta estrategia puede representar una ventaja para hacer frente a la escasez de recursos hídricos, ya que además de aumentar la productividad del agua, en este portainjerto, el efecto del estrés hídrico fue menos acusado. Si bien estos resultados pueden contribuir a la sostenibilidad del cultivo a largo plazo, sería necesario dar un paso más para evaluar la respuesta de estos portainjertos a la salinidad, ya que la utilización de fuentes de agua alternativas, de calidad marginal (regeneradas y desaladas), es inevitable para garantizar la viabilidad del cultivo del aguacate en el litoral andaluz. En este sentido, hay que tener en cuenta que las respuestas tempranas a la sequía y la salinidad son muy similares (Munns, 2002), ya que ambas causan una disminución en el potencial hídrico del suelo (generalmente asociado a un aumento del potencial osmótico), lo que conduce a una disminución del crecimiento de las raíces que conlleva una menor absorción de agua y nutrientes, y que tiene un efecto negativo en el crecimiento de las plantas y el rendimiento de los cultivos (Munns y Tester, 2008; Khan et al., 2015). Por lo tanto, podría esperarse que los portainjertos con mayor tolerancia a estrés hídrico posean cierta habilidad para hacer frente al estrés salino, tal y como se ha observado en el portainjerto 'Dusa' (Acosta-Rangel et al., 2019; Moreno-Ortega et al., 2021). En este sentido, la evaluación en un futuro próximo de la respuesta a estrés hídrico y salino en el resto de las selecciones avanzadas del programa de mejora de portainjertos del IFAPA, IAS-CSIC, IHSM-UMA-CSIC, será de gran interés para obtener patrones con características mejoradas ante los principales retos a los que se enfrenta el cultivo.

Recientemente, se ha establecido una parcela de cruzamientos de polinización abierta en la Estación Experimental La Mayora, que contiene los 8 portainjertos con tolerancia a *R. necatrix*, así como otros portainjertos, comerciales o de otros programas de mejora, de gran interés por su tolerancia a estrés hídrico, salino u otros patógenos como *P. cinnamomi*. Las semillas procedentes de esta parcela se testarán, en primer lugar frente a *R. necatrix* y posteriormente, frente a estrés hídrico o salino.

Los programas de mejora genética en aguacate son muy largos en el tiempo debido al largo periodo intergeneracional de esta especie y a su heterocigosidad, de tal manera que la obtención de un genotipo de interés puede tardar entre 20-30 años. Es por ello que el desarrollo de herramientas biotecnológicas que ayuden a la obtención de una variedad o patrón de interés, así como a acortar los procesos de selección de individuos en los programas de mejora, son de gran relevancia. Se han realizado estudios moleculares, fisiológicos e histológicos de la interacción de diferentes portainjertos de aguacate con *P. cinnamomi* permitiendo la identificación de distintos mecanismos de tolerancia al patógeno (en van den Berg et al., 2021). En un sentido amplio, un genotipo de alta tolerancia, como 'Dusa' es capaz de reducir la infección por el oomiceto mediante la limitación de la penetración de este en la raíz y la posterior colonización (en van den Berg et al., 2021); sin embargo, otros patrones como 'Duke 7', también pueden mitigar los efectos del patógeno mediante mecanismos alternativos, como el aumento de la absorción de nutrientes o la capacidad de regeneración radicular sin tener un efecto directo sobre la presencia del patógeno (en van den Berg et al., 2021). En el caso de 'Dusa', el portainjerto más utilizado en suelos infectados por *P. cinnamomi*, la cantidad de patógeno disminuye en las raíces tras la activación de la resistencia sistémica adquirida (SAR) y la deposición de callosa en los sitios de penetración del patógeno en la raíz (van den Berg et al., 2018). Van den Berg et al. (2021) hacen hincapié en la importancia de estos hallazgos para la correcta identificación de genotipos valiosos en programas de mejora genética. Además, la posibilidad de seleccionar portainjertos con diferentes mecanismos de tolerancia, parece ser una herramienta útil para una comprensión más profunda de la interacción aguacate-patógeno.

Por otra parte, estos estudios han dado lugar a la identificación de genes implicados en la tolerancia a éste patógeno, que codifican defensinas, proteínas relacionadas con la patogénesis y ribonucleasas. Estos genes, si en un futuro se desarrollan protocolos de transformación de aguacate eficientes y estables, podrían utilizarse para incrementar la resistencia a la enfermedad mediante transformación genética (Pliego-Alfaro et al., 2020; van den Berg et al., 2021).

En cuanto al patosistema aguacate-*R. necatrix*, los estudios realizados en una de estas selecciones (BG83), han revelado que la tolerancia a este patógeno parece estar relacionada con la capacidad para soportar el desequilibrio osmótico a través de la inducción de genes relacionados con el estrés salino y osmótico, así como la sobreexpresión de inhibidores de proteasas (Zumaquero et al., 2019b). En la presente tesis doctoral, el uso del cultivo *in vitro* se propone como una alternativa en la búsqueda de soluciones frente a la podredumbre blanca radicular (PBR), permitiendo identificar genes diana que, junto con herramientas biotecnológicas, podrían ser usados para acelerar los programas de mejora de portainjertos. Así, a partir de callo embriogénico de la variedad de aguacate 'Anaheim', se ha conseguido obtener una línea celular resistente al 80% de filtrado de *R. necatrix* mediante exposiciones sucesivas a dosis crecientes de filtrado crudo (CF). Los filtrados de cultivos fúngicos suelen contener toxinas no específicas del huésped que causan efectos nocivos en las células vegetales (Abbas et al., 1995), por ejemplo, se sabe que *R. necatrix* exuda al menos dos toxinas, citocalasina E y rosnecatrona (Edwards et al., 2001; Edwards et al., 1989), ambas se cree que están implicadas en la aparición de síntomas en plantas infectadas por este patógeno (Whalley, 1996). Varios estudios han demostrado que los exudados liberados inducen reacciones de defensa en las células vegetales (Knogge, 1996). La línea resistente podría haber surgido como consecuencia de un evento de variación somaclonal, como se ha demostrado en otros sistemas celulares tras el cultivo en presencia de filtrados fúngicos (Svabova y Lebeda, 2005; Rai et al., 2011).

El análisis del transcriptoma reveló un menor número de genes, con expresión significativamente alterada en la línea de callo resistente L3, en comparación con la susceptible AN-9, lo que podría atribuirse a un mejor comportamiento de la línea L3 durante la exposición a CF. Estos resultados concuerdan con estudios moleculares realizados en condiciones de invernadero, en plantas de aguacate tolerantes y susceptibles a la infección con *R. necatrix*, en los que se encontró un menor número de DEGs desregulados en el genotipo tolerante BG83 en comparación con el portainjerto susceptible 'Dusa' (Zumaquero et al., 2019b).

La exposición de líneas de callo embriogénico al exudado de *R. necatrix* indujo genes que, según se ha informado anteriormente, están relacionados con la defensa de la planta frente a enfermedades fúngicas. Curiosamente, los transcritos de estos genes se acumularon en mayor cantidad en la línea resistente en comparación con la susceptible. La inducción de genes como la cinamoil-CoA reductasa y el gen que codifica la peroxidasa aniónica formadora de lignina, que son dos enzimas clave en la biosíntesis de lignina (Lagrimini et al., 1993; Kawasaki et al., 2006), destaca la importancia de la producción de lignina en la protección frente a *R. necatrix*.

También se han detectado enzimas fúngicas degradadoras de la pared celular, como endoquitinasas y glucanasas, en el portainjerto tolerante de aguacate 'Dusa' tras la infección con *P. cinnamomi* (van den Berg et al., 2018). En esta investigación, se pudieron identificar proteínas β-1,3- glucosidasas y proteínas similares a las endoquitinasas. Las actividades de estas proteínas se detectaron previamente en cultivos embriogénicos de mango expuestos a CF de *Colletotrichum gloeosporioides* (Jayasankar y Litz, 1998), mientras que la β-glucanasa aumentó en una línea celular de *Cicer arietinum* expuesta a CF de *Fusarium oxysporum* (Singh et al., 2003).

Se observaron otras proteínas PR en los callos embriogénicos de aguacate expuestos a *R. necatrix* CF. En concreto, los transcritos que codifican PR-4 (Pag289080), la proteína STH-21 relacionada con la patogénesis (Pag168264) y las proteínas similares a la taumatina (PR-5) (Pag154162, Pag154170) se indujeron 1,5 veces más en la línea resistente en comparación con la línea susceptible. La presencia de proteínas PR-5 en el medio de células de uva elicitadas con filtrado fúngico de *Elsinoe ampelina* se relacionó con la inhibición del crecimiento fúngico en un sistema de cultivo dual (Jayasankar et al., 2000). En el caso del aguacate, no se ha descrito que las proteínas PR-4 y taumatina estén relacionadas con la tolerancia a *R. necatrix* o *P. cinnamomi*; sin embargo, se cree que su inducción temprana después de un evento de "*preacondicionamiento*" podría representar un beneficio para las plantas de aguacate en la superación de la infección por *R. necatrix* (Martínez-Ferri et al., 2019).

La inducción de inhibidores de proteasas en la defensa de las plantas contra patógenos fúngicos ha sido ampliamente reportada (Jashni et al., 2015). Un hallazgo importante de este trabajo fue la sobreexpresión de inhibidores de proteinasas en la línea L3 con respecto a la línea AN-9. El mismo resultado se obtuvo en condiciones de invernadero, donde la expresión de estos genes fue al menos 2,6 veces mayor en el portainjerto tolerante de aguacate BG83 frente al susceptible 'Dusa' tras la infección con *R. necatrix* (Zumaquero et al., 2019b; Martínez-Ferri et al., 2019). Curiosamente, el inhibidor de proteinasas (Pag64949), identificado en el estudio de callo expuesto a CF, compartía la misma secuencia de codificación con el identificado por Zumaquero et al. (2019b) en experimentos de invernadero realizados para estudiar la interacción del portainjerto tolerante BG83/*R. necatrix* (Pa_Contig05213), apoyando el uso de este gen como potencial marcador para acelerar el programa de mejora genética que actualmente se está llevando a cabo.

Esta investigación contribuirá a la comprensión de la defensa del aguacate frente a este patógeno. Además, estos resultados apoyan los obtenidos recientemente en olivo (IHSM-UMA-CSIC e IFAPA), donde se ha seleccionado una línea, regenerada a partir de callo embriogénico de una semilla de la variedad Picual, tolerante al 60% del filtrado de *R. necatrix*. Las plantas de esta línea muestran un desarrollo de síntomas de la PBR significativamente menor respecto a plantas control tras la inoculación con *R. necatrix*; además, esta respuesta va ligada a la expresión de genes previamente relacionados, en aguacate, con la tolerancia a este patógeno (i.e., inhibidores de proteasas) (Santos-Casanova et al., 2024).

Llegados a este punto, el análisis funcional de la maquinaria de defensa del aguacate es un importante objetivo de investigación, con el fin de seleccionar potenciales dianas moleculares para su uso en los programas de mejora genética.

Finalmente, y hasta que estos nuevos materiales estén disponibles para los agricultores, es necesario buscar métodos que mitiguen el efecto negativo de este patógeno en plantaciones ya establecidas. En este sentido, los elicitores han sido propuestos como una herramienta inocua para el manejo integrado de plagas en agricultura, entre los cuales el metil jasmonato (MeJA) y el ácido salicílico (SA) son los más utilizados. En aguacate, el efecto de la aplicación exógena de MeJA y SA en la respuesta molecular de plantas jóvenes de aguacate 'Dusa', tolerantes al oomiceto hemibiotrofo *P. cinnamomi*, reveló que, entre otros, los genes relacionados con la defensa de la planta se sobreexpresaban antes de las 24 horas siguientes a la aplicación de ambos elicitores, sugiriendo la inducción de un *“estado preacondicionado”* que podría conferir cierta protección frente a nuevos ataques del patógeno (van den Berg et al., 2018). En esta tesis se ha estudiado el efecto de dichos elicitores sobre la respuesta fisiológica y molecular de plantas de aguacate 'Dusa' antes y después de la inoculación con el necrótrofo *R. necatrix*, así como su impacto en la progresión de la enfermedad.

La aplicación exógena de MeJA y SA en este portainjerto aumentó los mecanismos fotoprotectores (es decir, los valores de NPQ), en consonancia con la disminución observada en la eficiencia fotoquímica intrínseca de los centros de reacción abiertos del PSII (F_v'/F_m') (Murchie y Lawson, 2013). Sin embargo, aunque ambos elicitores indujeron una respuesta similar a nivel fotoquímico, los parámetros fotosintéticos de intercambio de gases solo mejoraron con SA. Esta mejora de la capacidad fotosintética se asoció con un mayor A_N/C_i, lo que sugiere que SA estimuló la actividad de rubisco en lugar de un aumento en el contenido de pigmentos (Khan et al., 2013; Khodary, 2004). Sin embargo, la mayor apertura estomática de las plantas tratadas con SA dio lugar a mayores tasas de transpiración, lo que implicó una mayor pérdida de agua consistente con los menores valores de RWC, así como una menor eficiencia intrínseca en el uso del agua (A_N/g_s). Estos resultados implicarían una menor capacidad de las plantas tratadas con SA para soportar el estrés hídrico (Hayat et al., 2008; Martínez-Ferri et al., 2019); probablemente porque el modo de acción del SA exógeno depende en gran medida de varios factores, como la especie vegetal (Khan et al., 2003), la concentración aplicada (Lotfi et al., 2020), las condiciones ambientales (Khan et al., 2013) y el tiempo transcurrido tras la aplicación del elicitor (Gonçalves et al., 2020; Khan et al., 2003).

A diferencia de SA, el rendimiento fotosintético de las plantas de aguacate 'Dusa' no se vio afectado por MeJA, probablemente porque el efecto de MeJA en la fotosíntesis es dependiente de la dosis y de la especie en cuestión (Fatma et al., 2021; Hanaka et al., 2015; Qiu et al., 2020). A nivel de planta, MeJA mostró una tendencia a reducir el peso seco de la hoja y aumentar significativamente el peso seco de la raíz, lo que resulta en una relación raíz/brote significativamente mayor en comparación con las plantas control y tratadas con SA. Consistentemente, las plantas tratadas con MeJA mostraron una menor superficie foliar total que en los otros tratamientos, lo que sugiere que un menor tamaño de la hoja puede estar asociado con menores pérdidas de agua foliar y, en consecuencia, una menor transpiración de la planta (Wang et al., 2019). Estas características, se han relacionado con la adaptabilidad de la planta al estrés hídrico (Anjum et al., 2011; Kou et al., 2022; Poorter et al., 2012; Vadez et al., 2007) pero también con la tolerancia del aguacate a *R. necatrix* (Magagula et al., 2021; Martínez-Ferri et al., 2016) y *P. cinnamomi* (Coffey, 1987; van den Berg et al., 2021).

Del mismo modo, se observó una respuesta molecular diferencial de las raíces de aguacate 'Dusa' tras el tratamiento con MeJA o SA; así, la regulación al alza del inhibidor de la proteasa glu solo se observó tras el tratamiento con MeJA. Este gen codifica proteínas vinculadas a diferentes aspectos de la defensa de la planta, como el estrés oxidativo (Srinivasan y Kirti, 2012), lo que puede englobar una mayor capacidad de las plantas para resistir la infección por *R. necatrix*, en consonancia con su importante papel en la tolerancia del aguacate a este patógeno (Moreno-Pérez et al., 2023; Zumaquero et al., 2019b). Del mismo modo, la mayor expresión de endoquitinasas, PR4 y PR5 inducida por MeJA también podría representar un beneficio para superar la enfermedad de la PBR (Martínez-Ferri et al., 2019) al minimizar la carga del patógeno o la aparición de la enfermedad en órganos de la planta no infectados (Ali et al., 2018).

Nuestros resultados muestran que los efectos del tratamiento con MeJA en aguacate se evidencian por la expresión de genes relacionados con la defensa y a través de cambios a nivel morfoanatómico más que por modificaciones a nivel fotosintético. Estas características también se han asociado con la resistencia a infecciones por patógenos del suelo (Magagula et al., 2021; Martínez-Ferri et al., 2016; van den Berg et al., 2021).

La inoculación de plantas de aguacate 'Dusa' con *R. necatrix* afectó los parámetros fisiológicos de las plantas tratadas y no tratadas antes de la aparición de cualquier síntoma visible, en comparación con las plantas control no inoculadas. Sin embargo, se observó un retraso en la progresión de la enfermedad en las plantas tratadas con MeJA en comparación con los tratamientos control y SA. Esto sugiere que la mejora de la capacidad de las plantas de aguacate para hacer frente a la infección por *R. necatrix* después del tratamiento con MeJA podría estar asociada con los cambios morfofisiológicos inducidos por MeJA y con la activación de diferentes vías (Ali et al., 2018) que implican la expresión diferencial de genes específicos relacionados con la defensa mencionados anteriormente. En conclusión, los resultados del presente estudio postulan el uso de MeJA como una estrategia ambientalmente amigable para aumentar la tolerancia del aguacate al hongo patógeno *R. necatrix* que podría mitigar el impacto de esta enfermedad en portainjertos susceptibles utilizados en las plantaciones comerciales.

En su conjunto, la presente tesis doctoral contribuye al conocimiento de la respuesta del aguacate ante dos de los principales factores que amenazan a la viabilidad de las plantaciones de este cultivo en el litoral andaluz: la escasez de agua y la PBR. Asimismo, proporciona algunas herramientas que, junto con otras estrategias para mejorar la eficiencia en el uso del agua y el control de la enfermedad, contribuyen a minimizar o erradicar el impacto de ambos en las plantaciones ya establecidas. No obstante, se necesitan más estudios para evaluar la tolerancia relativa de las selecciones avanzadas de los portainjertos frente a distintos tipos de estrés, así como su comportamiento agronómico en distintas condiciones de campo.

Conclusions

1. A strongly effect of rootstock on 'Hass' scion is observed at soil field capacity conditions. Avocado 'Hass' grafted on tolerant BG48 rootstock showed a water-saving behavior as they presented higher water consumption and higher values of plant transpiration (E_{plant}) and leaf photosynthetic rates than those grafted on BG181, which exhibited a water-saving strategy based on a compromise between leaf biomass allocation and tight stomatal control of transpiration not linked to a reduction in growth. Avocado 'Hass' plants grafted onto tolerant BG181 require less water, so plants are more water efficient.

2. The imposition of two levels of water stress (mild and severe) by substrate desiccation induced a physiological response increasing leaf mass area (LMA) and decreasing plant transpiration rate (E_{plant}), plant hydraulic conductance (K_h) and leaf water potential (Ψ_w) in agreement with the severity of water stress, which correspond with a drought avoidance behavior of both genotypes based on different underlying mechanisms. The replenishment of water-stressed plants through irrigation led to a recovery in photosynthetic rates, suggesting some ability of these rootstocks to recovery from water stress which may be associated with their ability to withstand *R. necatrix* infection.

3. A set of general defense-related transcripts, known to be induced in the tolerant response of avocado rootstocks to *R. necatrix*, were also induced in embryogenic callus lines capable to survive in the presence of 80% *R. necatrix* crude filtrate (CF; 80%), when embryogenic cells were newly exposed to the fungal filtrate (40%).

4. Gene encoding the avocado proteinase inhibitor (Pag64949), induced in tolerant callus lines and tolerant rootstocks after exposure to *R. necatrix* CF and hyphae respectively, can be used as a potential marker to accelerate selection of tolerant genotypes in the avocado rootstock breeding program against white root rot (WRR) disease.

5. The exogenous application of methyl jasmonate (MeJA) and salicylic acid (SA) in susceptible avocado 'Dusa' increased photoprotective mechanisms and upregulated the glutathione S-transferase gene expression, suggesting the triggering of mechanisms closely related to oxidative stress relief and reactive oxygen species scavenging.

6. The exogenous application of MeJA has a more pronounced effect than SA at morphoanatomical level in susceptible avocado 'Dusa' plants, including functional traits such as high leaf mass area, high stomatal density, and high root/shoot ratio, closely related to strategies to cope with water scarcity and WRR disease. Moreover, a greater number of defense-related genes, including a glu protease inhibitor, a key gene in avocado defense against *R. necatrix* were upregulated.

7. MeJA increased ‘Dusa’ avocado tolerance to *R. necatrix* by inducing a primed state that delayed WRR disease symptoms.

8. MeJA can be used as an environmentally friendly control strategy to increase avocado tolerance to *R. necatrix* mitigating the impact of this disease on susceptible rootstocks in avocado orchards.

References

Abbas, H.K.; Boyette, C.D.; Hoagland, R.E. Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid and moniliformin to jimsonweed. Phytoprotection 1995, 76, 17-25. https://doi.org/10.7202/706081ar.

Abo El-Fadl, R.; Mahdi, A.; Abdelhamid, A.E.; Abdel Magid, M.R.; Hassan, A.H. Enhancing the biochemical constituents in avocado callus using encapsulated chitosan nanoparticles. Egypt. J. Chem. 2022, 65, 12, 769-782. https://doi.org/10.21608/ejchem.2022.113494.5176.

Acosta-Rangel, A.M.; Li, R.; Celis, N.; Suarez, D.L.; Santiago, L.S.; Arpaia, M.L.; Mauk, P.A. The physiological response of 'Hass' avocado to salinity as influenced by rootstock. Sci. Hortic. 2019, 256, 108629. https://doi.org/10.1016/j.scienta.2019.108629.

Ahmad, P.; Rasool, S.; Gul, A.; Sheikh, S.A.; Akram, N.A.; Ashraf, M.; Kazi, A.M.; Gucel, S. Jasmonates: Multifunctional roles in stress tolerance. Front. Plant Sci. 2016, 7, 813. https://doi.org/10.3389/fpls.2016.00813.

Alam, P.; Balawi, T.A.; Faizan, M. Salicylic acid's impact on growth, photosynthesis, and antioxidant enzyme activity of *Triticum aestivum* when exposed to salt. Molecules 2023, 28, 100. https://doi.org/10.3390/molecules28010100.

Albacete, A.; Martínez-Andújar, C.; Martínez-Pérez, A.; Thompson, A.J.; Dodd, I.C.; Pérez-Alfocea, F. Unravelling rootstock × scion interactions to improve food security. J. Exp. Bot. 2015, 66, 2211-2226. https://doi.org/10.1093/jxb/erv027.

Alcaraz, M.L.; Hormaza, J.I. Fruit set in avocado: Pollen limitation, pollen load size, and selective fruit abortion. Agronomy 2021, 11, 1603. https://doi.org/10.3390/agronomy11081603.

Alcaraz, M.L.; Thorp, T.G.; Hormaza, J.I. Phenological growth stages of avocado (*Persea americana*) according to the BBCH scale. Sci. Hortic. 2013, 164, 434-439. https://doi.org/10.1016/j.scienta.2013.09.051.

Ali, S.; Ganai, B.A.; Kamili, A.N.; Bhat, A.A.; Mir, Z.A.; Bhat, J.A.; Tyagi, A.; Islam, S.T.; Mushtaq, M.; Yadav, P.; Rawat, S.; Grover, A. Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. Microbiol. Res. 2018, 212-213, 29-37. https://doi.org/10.3390/molecules28010100.

Alon, A.; Cohen, S.; Burlett, R.; Hochberg, U.; Lukyanov, V.; Rog, I.; Klein, T.; Cochard, H.; Delzon, S.; David-Schwartz, R. Acclimation limits for embolism resistance and osmotic adjustment accompany the geographical dry edge of Mediterranean species. Funct. Eco. 2023, 37, 1421-1435. https://doi.org/10.1111/1365-2435.14289.

Álvarez, A.; Oliveros, D.; Ávila, Y.C.; Sabogal Palma, A.C.; Murillo, W.; Joli, J.E.; Bermúdez-Cardona, M.B.; Guarnizo, N. Resistance induction with silicon in Hass avocado plants inoculated with *Phytophthora cinnamomi* Rands. Plant Signaling & Behavior 2023, 18, 2178362. https://doi.org/10.1080/15592324.2023.2178362.

Amborabé, B.E.; Bonmort, J.; Fleurat-Lessard, P.; Roblin, G. Early events induced by chitosan on plant cells. J. Exp. Bot. 2008, 59, 2317-2324. https://doi.org/10.1093/jxb/ern096.

Anderson, J.M.; Pegg, K.G.; Dann, E.K.; Cooke, A.W.; Smith, L.A.; Willingham, S.L.; Giblin, F.R.; Dean, J.R.; Coates, L.M. 2005. New strategies for the integrated control of avocado fruit diseases. New Zealand and Australia Avocado Grower's Conference. Tauranga, New Zealand. http://www.avocadosource.com/Journals/AUSNZ/AUSNZ_2005/AndersonJM2005.pdf. (accessed 10 September 2024)

Anil, V.S.; Bennur, S.; Lobo, S. Somaclonal variations for crop improvement: Selection for disease resistant variants *in vitro*. Plant Sci. Today 2018, 5, 44-54. https://doi.org/10.14719/pst.2018.5.2.382.

Anjum, S.A.; Xie, X.Y.; Wang, L.C.; Saleem, M.F.; Man, C.; Lei, W. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 2011, 6, 2026-2032.

Araújo, R.G.; Rodríguez-Jasso, R.M.; Ruiz, H.A.; Pintado, M.M.E.; Aguilar, C.N. Avocado by-products: Nutritional and functional properties. Trends Food Sci. Tech. 2018, 80, 51-60. https://doi.org/10.1016/j.tifs.2018.07.027.

Arif, Y.; Sami, F.; Siddiqui, H.; Bajguz, A.; Hayat, S. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. Environ. Exp. Bot. 2020, 175, 104040. http://doi.org/10.1016/j.envexpbot.2020.104040.

Arjona-Girona, I.; López-Herrera, C.J. First report of *Rosellinia necatrix* causing white root rot in mango trees in Spain. Plant Dis. 2018, 102, 2639. https://doi.org/10.1094/PDIS-01-18-0133-PDN.

Atucha, A.; Emmett, B.; Bauerle, T.L. Growth rate of fine root systems influences rootstock tolerance to replant disease. Plant Soil 2014, 376, 337-346. https://doi.org/10.1007/s11104-013-1977-5.

Avenido, R.A.; Galvez, H.F.; Dimaculangan, J.G.; Welgas, J.N.; Frankie, R.B.; Damasco, O.P. 2009. Somatic embryogenesis and embryo culture coupled with gamma irradiation for generating avocado (*Persea americana* Miller) mutants in the Philippines. Pages 47-69 in: Induced Mutation in Tropical Fruit Trees, IAEA-TECDOC-1615. International Atomic Energy Agency, Vienna, Austria.

Awang, N.A.; Islam, M.R.; Ismail, M.R.; Zulkarami, B.; Omar, D. Effectiveness of different elicitors in inducing resistance in chilli (*Capsicum annuum* L.) against pathogen infection. Sci. Hortic. 2013, 164, 461-465. https://doi.org/10.1016/j.scienta.2013.08.038.

Ayala Silva, T.; Ledesma, N. 2014. Avocado history, biodiversity and production. Pages 157-205 in: Sustainable horticultural systems: Issues, technology and innovation. D. Nandwani, eds. Springer, Cham. https://doi.org/10.1007/978-3-319-06904-3_8.

Backer, R.; Mahomed, W.; Reeksting, B.J.; Engelbrecht, J.; Ibarra-Laclette, E.; van den Berg, N. Phylogenetic and expression analysis of the NPR1-like gene family from *Persea americana* (Mill.). Front. Plant Sci. 2015, 6, 300. https://doi.org/10.3389/fpls.2015.00300.

Backer, R.; Naidoo, S.; van den Berg, N. The NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) and related family: mechanistic insights in plant disease resistance. Front. Plant Sci. 2019, 10, 102. https://doi.org/10.3389/fpls.2019.00102.

Baenas, N.; García-Viguera, C.; Moreno, D.A. Elicitation: A tool for enriching the bioactive composition of foods. Molecules 2014, 19, 13541-13563. https://doi.org/10.3390/molecules190913541.

Baker, N.R.; Harbinson, J.; Kramer, D.M. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell Environ. 2007, 30, 1107-1125. https://doi.org/10.1111/j.1365-3040.2007.01680.x.

Baldwin, I.T. 1996. Methyl jasmonate-induced nicotine production in *Nicotiana attenuata*: inducing defenses in the field without wounding. Pages 213-220 in: Proceedings of the 9th International Symposium on Insect-Plant Relationships. Series Entomologica, vol 80. E. Städler, M. Rowell-Rahier, R. Baur, eds. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-1720-0_49.

Barceló-Muñoz, A.; Zea-Bonilla, T.; Jurado Valle, I.; Imbroda-Solano, I.; Vidoy-Mercado, I.; Pliego-Alfaro, F.; López-Herrera, C.J. Selection program for avocado rootstocks tolerant to white rot caused by *Rosellinia necatrix* in southern Spain (1995-2007). www.avocadosource.com/WAC6/en/Extenso/1a-9.pdf. (accessed 20 December 2023).

Barrientos-Priego, A.F. Presente y futuro de los portainjertos y variedades de aguacate en el mundo y México. In Memorias del V Congreso Latinoamericano del Aguacate; Asociación de Productores y Exportadores de Jalisco (APEAJAL): Ciudad Guzmán, México, 2017; pp. 2-15.

Barrientos-Priego, A.F.; Borys, M.W.; Trejo, C.; López-López, L. Índice y densidad estomática foliar en plántulas de tres razas de aguacatero. Rev. Fitotec. Mex. 2003, 26, 285-285. https://doi.org/10.35196/rfm.2003.4.285.

Barrientos-Priego, A.F.; Sánchez-Colín, S.; Aguilar-Melchor, J.J.; López-Jimenez, A. Selection of Avocado Dwarfing Rootstocks. Proc. Second World Avocado Congr. 1992, 1, 515-520.

Barz, W.; Daniel, S.; Hinderer, W.; Jaques, U.; Kessmann, H.; Köster, J.K.; Tiemann, K. Elicitation and metabolism of phytoalexins in plant cell cultures. In Plant Cell Biotechnology; NATO ASI Series; Pais, M.S.S., Mavituna, F., Novais, J.M., Eds.; Springer: Berlin/Heidelberg, Germany, 1988; Volume 18. https://doi.org/10.1007/978-3-642-73157-0_20.

Bénard-Gellon, M.; Farine, S.; Goddard, M.L.; Schmitt, M.; Stempien, E.; Pensec, F.; Laloue, H.; Mazet-Kieffer, F.; Fontaine, F.; Larignon, P.; et al. Toxicity of extracellular proteins from *Diplodia seriata* and *Neofusicoccum parvum* involved in grapevine *Botryosphaeria* dieback. Protoplasma 2015, 252, 679-687. https://doi.org/10.1007/s00709-014-0716-y.

Ben-Ya'acov, A. Recommended rootstocks for new planting of avocado-Ettinger cultivar. Alon Hanotea 1992, 46, 919-926.

Ben-Ya'acov, A.; Michelson, E. Avocado rootstocks. Hortic. Rev. 1995, 381-429. Available online: http://avocadosource.com/Journals/HorticulturalReviews/HortRev_1995_PG_381-429.pdf. (accessed on 28 April 2024).

Ben-Ya'acov, A.; Michelson, E.; Sela, I. Rootstock effect on avocado vigor and productivity. In Proceedings of the 1992 V International Symposium on Orchard and Plantation Systems, Tel Aviv, Israel, 1 October 1993; Volume 349, 191-196. https://doi.org/10.17660/ActaHortic.1993.349.30.

Ben-Ya'acov, A.; Zilberstaine, M. Clonal avocado (*Persea americana* Mill.) rootstocks in Israel. Rev. Chapingo Ser. Hortic. 1999, 5, 39-42.

Berger, S.; Sinha, A.K.; Roitsch, T. Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. J. Exp. Bot. 2007, 58, 4019-4026. https://doi.org/10.1093/jxb/erm298.

Bergh, B. The origin, nature, and genetic improvement of the avocado. California Avocado Society Yearbook 1992, 76, 61-75.

Bergmann, D. Stomatal development: From neighborly to global communication. Curr. Opin. Plant Biol. 2006, 9, 478-483. https://doi.org/10.1016/j.pbi.2006.07.001.

Bertolino, L.T.; Caine, R.S.; Gray, J.E. Impact of stomatal density and morphology on water-use efficiency in a changing world. Front. Plant Sci. 2019, 10, 225. https://doi.org/10.3389/fpls.2019.00225.

Beyer, C.P.; Barrientos-Sanhueza, C.; Ponce, E.; Pedreschi, R.; Cuneo, I.F.; Álvaro, J.E. Differential hydraulic properties and primary metabolism in fine root of avocado trees rootstocks. Plants 2022, 11, 1059. https://doi.org/10.3390/plants11081059.

Beyer, C.P.; Cuneo, I.F.; Álvaro, J.E.; Pedreschi, R. Evaluation of aerial and root plant growth behavior, water and nutrient use efficiency and carbohydrate dynamics for Hass avocado grown in a soilless and protected growing system. Sci. Hortic. 2021, 277, 109830. https://doi.org/10.1016/j.scienta.2020.109830.

Bianchi, D.; Ricciardi, V.; Pozzoli, C.; Grossi, D.; Caramanico, L.; Pindo, M.; Steffani, E.; Cestaro, A.; Brancadoro, L.; De Lorenzis, G. Physiological and transcriptomic evaluation of drought effect on own-rooted and grafted grapevine rootstock (1103P and 101-14MGt). Plants 2023, 12, 1080. https://doi.org/10.3390/plants12051080.

Biran, D. Fruitlet abscission and spring growth retardation their influence on avocado productivity. MSc. Thesis, The Hebrew University of Jerusalem, Rehovot, Israel. 1979.

Boughalleb, F.; Hajlaoui, H. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol. Plant. 2011, 33, 53-65. https://doi.org/10.1007/s11738-010-0516-8.

Brodribb, T.J.; Buckley, T.N. Leaf water transport: A core system in the evolution and physiology of photosynthesis. In The Leaf: A Platform for Performing Photosynthesis; Springer: Cham, Switzerland, 2018; pp. 81-96.

Brodribb, T.J.; Holbrook, N.M. Diurnal depression of leaf hydraulic conductance in a tropical tree species. Plant Cell Environ. 2004, 27, 820-827. https://doi.org/10.1111/j.1365-3040.2004.01188.x.

Brunner, I.; Herzog, C.; Dawes, M.A.; Arend, M.; Sperisen, C. How tree roots respond to drought. Front. Plant Sci. 2015, 6, 547. https://doi.org/10.3389/fpls.2015.00547.

Buchholz, A. Young vegetative growth as a possible factor involved with fruitlet abscission in avocado. MSc. Thesis, The Hebrew University of Jerusalem, Rehovot, Israel. 1986.

Camargo, M.A.B.; Marenco, R.A. Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. Acta Amaz. 2011, 41, 205-212. https://doi.org/10.1590/S0044-59672011000200004.

Campbell, C.L.; Madden, L.V. 1990. Temporal analysis of epidemics. I: Descriptions and comparisons of disease progress curves. Pages 161-162 in: Introduction to Plant Disease Epidemiology. C.L. Campbell and L.V. Madden, eds. Wiley, Hoboken, NY.

Campos, M.L.; Kang, J.H.; Howe, G.A. Jasmonate-triggered plant immunity. J. Chem. Ecol. 2014, 40, 657-675. https://doi.org/10.1007/s10886-014-0468-3.

Cao, H.; Glazebrook, J.; Clarke, J.D.; Volko, S.; Dong, X. The *Arabidopsis* NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 1997, 88, 57-63. https://doi.org/10.1016/S0092-8674(00)81858-9.

Carr, M.K.V. The water relations and irrigation requirements of avocado (*Persea americana* Mill.): A review. Exp. Agric. 2013, 49, 256-278. https://doi.org/10.1017/S0014479712001317.

Carrillo-Fasio, J.A.; Báez-Sañudo, M.A. 2020. Plagas y nemátodos fitoparásitos. Pages: 159-197 in: Cultivo, poscosecha y procesado del aguacate. A. Namesny, C. Conesa, I. Hormaza, G. Lobo, eds. SPE3, Valencia, España.

Castillo-Argaez, R.; Konkol, J.L.; Vargas, A.I.; Ploetz, R.C.; Schaffer, B. Disease severity and ecophysiology of rootstock/scion combinations of different avocado (*Persea americana* Mill.) genotypes in response to laurel wilt. Sci. Hortic. 2021, 287, 110250. https://doi.org/10.1016/j.scienta.2021.110250.

Cazorla, F.M.; Duckett, S.B.; Bergström, E.T.; Noreen, S.; Odijk, R.; Lugtenberg, B.J.J.; Thomas-Oates, J.E.; Bloemberg, G.V. Biocontrol of avocado dematophora root rot by antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. Mol. Plant. Microbe In. 2006, 19, 418-428. https://doi.org/10.1094/MPMI-19-0418.

Cazorla, F.M.; Romero, D.; Pérez-García, A.; Lugtenberg, B.J.J.; de Vicente, A. Bloemberg, G. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. J. Appl. Microbiol. 2007, 103, 1950-1959. https://doi.org/10.1111/j.1365-2672.2007.03433.x.

Celis, N.; Suarez, D.L.; Wu, L.; Li, R.; Arpaia, M.L.; Mauk, P. Salt tolerance and growth of 13 avocado rootstocks related best to chloride uptake. HortScience 2018, 53, 1737-1745. https://doi.org/10.21273/HORTSCI13198-18.

Cervantes-Landaverde, J.J.; Torres-Pacheco, I.; Olvera-Luna, A.; Bárcena-Romero, A.R.; Quijas-Martínez, J.; Rosales-Cueto, L.; Gómez-Renauld, V.M. 2009. Aplicación de elicitores como medios de activación de defensa en *Capsicum annuum* contra el patógeno *Phytophthora capsici*.

https://www.uaq.mx/investigacion/difusion/veranos/memorias-2009/OctavoVerano_38/5_Cervantes_Landaverde.pdf. (accessed 21 Jul 2023).

Chalfoun, N.R.; Grellet-Bournonville, C.F.; Martínez-Zamora, M.G.; Díaz-Perales, A.; Castagnaro, A.P.; Díaz-Ricci, J.C. Purification and characterization of AsES protein: a subtilisin secreted by *Acremonium strictum* is a novel plant defense elicitor. J. Biol. Chem. 2013, 288, 14098-14113. https://doi.org/10.1074/jbc.M112.429423.

Chanderbali, A.S.; Soltis, D.E.; Soltis, P.S.; Wolstenholme, B.N. 2013. Taxonomy and Botany. Pages: 31-50 in: The Avocado: Botany, Production and Uses. B. Schaffer, A.W. Whiley, B.N. Wolstenholme, eds. CAB International, Wallingford, Oxon, UK.

Chang, S.; Puryear, J.; Cairney, J. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. 1993, 11, 113-116. https://doi.org/10.1007/BF02670468.

Chartzoulakis, K.; Patakas, A.; Bosabalidis, A.M. Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. Environ. Exp. Bot. 1999, 42, 113-120. https://doi.org/10.1016/S0098-8472(99)00024-6.

Chartzoulakis, K.; Patakas, A.; Kofidis, G.; Bosabalidis, A.; Nastou, A. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Sci. Hortic. 2002, 95, 39-50. https://doi.org/10.1016/S0304-4238(02)00016-X.

Chen, Y.E.; Mao, H.T.; Wu, N.; Mohi Ud Din, A.; Khan, A.; Zhang, H.Y.; Yuan, S. Salicylic acid protects photosystem II by alleviating photoinhibition in *Arabidopsis thaliana* under high light. Int. J. Mol. Sci. 2020, 21, 1229. https://doi.org/10.3390/ijms21041229.

Chittem, K.; Yajima, W.R.; Goswami, R.S.; del Río-Mendoza, L.E. Transcriptome analysis of the plant pathogen *Sclerotinia sclerotiorum* interaction with resistant and susceptible canola (*Brassica napus*) lines. PLoS ONE 2020, 15, e0229844. https://doi.org/10.1371/journal.pone.0229844.

Coffey, M.D. Phytophthora root rot of avocado: An integrated approach to control in California. Plant Dis. 1987, 71, 1046-1052. https://doi.org/10.1094/PD-71-1046.

Coto, O.; Rodríguez, N.N.; Fuentes, J.L.; Alvarez, A.; Machado, M.; Santiago, L.; Zamora, V.; Ramos-Leal, M. 2014. Mutation-based breeding of avocado in Cuba: state of the art. Pages: 265-282 in: Mutagenesis: Exploring Genetic Diversity of Crops. N.B. Tomiekova, M.I. Kozgar, M.R. Wani, eds. Wageningen Academic Publishers, Wageningen.

Cowan, A.K.; Cripps, R.F.; Richings, E.W.; Taylor, N.J. Fruit size: towards an understanding of the metabolic control of fruit growth using avocado as a model system. Physiol. Plantarum 2008, 111, 127-136. https://doi.org/10.1034/j.1399-3054.2001.1110201.x.

Cowan, A.K.; Moore-Gordon, C.S.; Bertling, I.; Wolstenholme, B.N. Metabolic control of avocado fruit growth (Isoprenoid growth regulators and the reaction catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase). Plant Physiol. 1997, 114, 511-518. https://doi.org/10.1104/pp.114.2.511.

Crane, J.H.; Douhan, G; Faber, B.A.; Arpaia, M.L.; Bender, G.S.; Balerdi, C.F. Barrientos-Priego, A.F. 2013. Cultivars and Rootstocks. Pages: 200-233 in: The Avocado: Botany, Production and Uses, B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Crowley, D. Salinity management in avocado orchards. Calif. Avo. Soc. Yrbk. 2008, 91, 83-104.

Crowley, D.; Smith, W.; Arpaia, M.L. Rootstock selections for improved salinity tolerance of avocado. Proceedings of the California Avocado Research Symposium. 2002, 83-86.

Cruz-Hernández, A.; Witjaksono; Litz, R.E.; Gómez Lim, M.A. *Agrobacterium tumefaciens* mediated transformation of embryogenic avocado cultures and regeneration of somatic embryos. Plant Cell Rep. 1998, 17, 493-503. https://doi.org/10.1007/s002990050431.

Cuéllar-Torres, E.A.; Aguilera-Aguirre, S.; López-García, U.M.; Hernández-Oñate, M.Á.; Montalvo-González, E.; Ortiz-Basurto, R.I.; Vega-Arreguín, J.; Chacón-López, A. Transcriptomic data exploring the effect of agave fructans on the induction of the defense system in avocado fruit. PLoS ONE 2023, 18, e0293396. https://doi.org/10.1371/journal.pone.0293396.

Cutting, J.G.M.; Bower, J.P. Spring vegetative flush removal: the effect on yield, size, fruit mineral composition and quality. South African Avocado Growers´ Association Yearbook. 1990, 13, 33-34.

Dai, L.; Wang, D.; Xie, X.; Zhang, C.; Wang, X.; Xu, Y.; Wang, Y.; Zhang, J. The novel gene VpPR4-1 from *Vitis pseudoreticulata* increases powdery mildew resistance in transgenic *Vitis vinifera* L. Front. Plant Sci. 2016, 7, 695. https://doi.org/10.3389/fpls.2016.00695.

Daniel, S.; Barz, W. Elicitor-induced metabolic changes in cell cultures of chickpea (*Cicer arietinum* L.) cultivars resistant and susceptible to *Ascochyta rabiei*. Planta 1990, 182, 279-286. https://doi.org/10.1007/BF00197122.

Dann, E.K.; Gazis, R. 2024. Foliar, fruit and soilborne diseases. Pages 481-547 in: The Avocado: Botany, Production and Uses, 3nd Edition. D. Carrillo, B. Schaffer, A.W. Whiley, B.N. Wolstenholme, eds. CAB International, Wallingford, Oxon, UK

Dann, E.K.; Muir, S. Peas grown in media with elevated plant-available silicon levels have higher activities of chitinase and β-1,3-glucanase, are less susceptible to a fungal leaf spot pathogen and accumulate more foliar silicon. Australas. Plant Path. 2002, 31, 9-13. https://doi.org/10.1071/AP01047.

Dann, E.K.; Ploetz, R.C.; Coates, L.M.; Pegg, K.G. 2013. Foliar, fruit and soilborne diseases. Pages 380-422 in: The Avocado: Botany, Production and Uses, 2nd Edition. B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Davenport, T.L. 1986. Avocado flowering. Pages: 257-289 in: Horticultural Reviews, vol. 8. J. Janick, ed. AVI Publishing Co., Westport, CT, EEUU.

De la Peña, E.; Pérez, V.; Alcaráz, L.; Lora, J.; Larrañaga, N.; Hormaza, J.I. Pollinators and pollination in subtropical fruit crops: management and implications for conservation and food-security. Ecosistemas 2018, 27, 91-101. https://doi.org/10.7818/ECOS.1480.

de la Riva, E.G.; Olmo, M.; Poorter, H.; Ubera, J.L.; Villar, R. Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. PLoS ONE 2016, 11, e0148788. https://doi.org/10.1371/journal.pone.0148788.

Delzon, S. New insight into leaf drought tolerance. Funct. Ecol. 2015, 29, 1247-1249. https://doi.org/10.1111/1365-2435.12500.

Ding, L.N.; Li, Y.T.; Wu, Y.Z.; Li, T.; Geng, R.; Cao, J.; Zhang, W.; Tan, X.L. Plant disease resistance-related signaling pathways: Recent progress and future prospects. Int. J. Mol. Sci. 2022, 23, 16200. https://doi.org/10.3390/ijms232416200.

Dixon, J. Shoot growth of ‘Hass’ avocado trees in ‘on’ and ‘off’ flowering years in the western bay of plenty. NZ Avocado Growers Association Ann. Res. Report 2007, 7, 41-48.

Dorantes-Álvarez, L.; Ortiz-Moreno, A.; García-Ochoa, F. 2012. Avocado. Pages 435-454 in: Tropical and subtropical fruits: Postharvest physiology, processing and packaging. M. Siddiq, eds. Oxford: Wiley-Blackwell. https://doi.org/10.1002/9781118324097.ch23.

Douhan, G.W.; Fuller, E.; McKee, B.; Pond, E. Genetic diversity analysis of avocado (*Persea americana* Miller) rootstocks selected under greenhouse conditions for tolerance to Phytophthora root rot caused by *Phytophthora cinnamomi*. Euphytica 2011, 182, 209-217. https://doi.org/10.1007/s10681-011-0433-y.

Dreher, M.L.; Davenport, A.J. Hass avocado composition and potential health effects. Crit. Rev. Food Sci. 2013, 53, 738-750. https://doi.org/10.1080/10408398.2011.556759.

Edreva, A. Pathogenesis-related proteins: Research progress in the last 15 years. Gen. Appl. Plant Physiol. 2005, 31, 105-124.

Edwards, R.L.; Maitland, D.J.; Scowen, I.J.; De Sousa, A.J.T.; Whalley, A.J.S. Metabolites of the higher fungi. Part 32. A phytotoxic bicyclo[4.1.0]hept-3-en-2-one from the fungus *Rosellinia necatrix* Prill. J. Chem. Soc., Perkin Trans. 2001, 1, 537-542. https://doi.org/10.1039/B008195G.

Edwards, R.L.; Maitland, D.J.; Whalley, A.J.S. Metabolites of the higher fungi. Part 24. Cytochalasin N, O, P, Q, and R. New cytochalasins from the fungus *Hypoxylon terricola* Mill. J. Chem. Soc. Perkin Trans. 1989, 1, 57-65. https://doi.org/10.1039/p19890000057.

EEA. European Climate Risk Assessment. European Environmental Agency (EEA). Report No 1/2024. 2024. Available online: https://www.eea.europa.eu/publications/european-climate-risk-assessment (accessed on 28 April 2024).

Engelbrecht, J.; van den Berg, N. Expression of defence-related genes against *Phytophthora cinnamomi* in five avocado rootstocks. S. Afri. J. Sci. 2013, 109, 1-8. https://doi.org/10.1590/sajs.2013/20120058.

Engelbrecht, J.; van den Berg, N. Expression of defence-related genes against *Phytophthora cinnamomi* in five avocado rootstocks. S. Afr. J. Sci. 2013, 109, 1-8. https://doi.org/10.1590/sajs.2013/20120058.

FAOSTAT. Data. 2023. Available online: https://www.fao.org/faostat/es/#data (accessed on 21 February 2023).

FAOSTAT. http://www.fao.org/faostat/en/ (accessed 04 august 2024).

Farmer E.E.; Ryan, C.A. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. P. Natl. Acad. Sci. 1990, 87, 7713-7716. https://doi.org/10.1073/pnas.87.19.7713.

Fassio, C.; Heath, R.; Arpaia, M.L.; Castro, M. Sap flow in ‘Hass’ avocado trees on two clonal rootstocks in relation to xylem anatomy. Sci. Hortic. 2009, 120, 8-13. https://doi.org/10.1016/j.scienta.2008.09.012.

Fatma, M.; Iqbal, N.; Sehar, Z.; Alyemeni, M.N.; Kaushik, P.; Khan, N.A.; Ahmad, P. Methyl jasmonate protects the PSII system by maintaining the stability of chloroplast D1 protein and accelerating enzymatic antioxidants in heat-stressed wheat plants. Antioxidants 2021, 10, 1216. https://doi.org/10.3390/antiox10081216.

Fawe, A.; Menzies, J.G.; Chérif, M.; Bélanger, R.R. Silicon and disease resistance in dicotyledons. Stud. Plan. S. 2001, 8, 159-169. https://doi.org/10.1016/S0928-3420(01)80013-6.

Fick, A.; Swar, V.; Backer, R.; Bombarely, A.; Engelbrecht, J.; van den Berg, N. Partially resistant avocado rootstock Dusa® shows prolonged upregulation of nucleotide binding-Leucine rich repeat genes in response to *Phytophthora cinnamomi* infection. Front. Plant Sci. 2022, 13, 793644. https://doi.org/10.3389/fpls.2022.793644.

Fleischmann, F.; Koehl, J.; Portz, R.; Beltrame, A.B.; Oßwald, W. Physiological changes of *Fagus sylvatica* seedlings infected with *Phytophthora citricola* and the contribution of its elicitin 'citricolin' to pathogenesis. Plant Biol. 2005, 7, 650-658. https://doi.org/10.1055/s-2005-872891.

Flexas, J.; Baron, M.; Bota, J.; Ducruet, J.M.; Gallé, A.; Galmés, J.; Jiménez, M.; Pou, A.; Ribas-Carbó, M.; Sajnani, C.; et al. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri* × *V. rupestris*). J. Exp. Bot. 2009, 60, 2361-2377. https://doi.org/10.1093/jxb/erp069.

Frolich, E.F.; Platt, R.G. 1972. Use of the etiolation technique in rooting avocado cuttings. California Avocado Society 1971-72 Yearbook 55, 97-109.

Gabor, B.K.; Coffey, M.D. Quantitative analysis of the resistance to *Phytophthora cinnamomi* in five avocado rootstocks under greenhouse conditions. Plant Dis. 1990, 74, 882-885. https://doi.org/10.1094/PD-74-0882.

Gago, J.; Daloso, D.M.; Carriquí, M.; Nadal, M.; Morales, M.; Araújo, W.L.; Nunes-Nesi, A.; Perea-Castro, A.V.; Clemente-Moreno, M.J.; Flexas, J. The photosynthesis game is in the "inter-play": Mechanisms underlying CO_2 diffusion in leaves. Environ. Exp. Bot. 2020, 178, 104174. https://doi.org/10.1016/j.envexpbot.2020.104174.

Galmés, J.; Abadía, A.; Cifre, J.; Medrano, H.; Flexas, J. Photoprotection processes under water stress and recovery in Mediterranean plants with different growth forms and leaf habits. Physiol. Plant. 2007, 130, 495-510. https://doi.org/10.1111/j.1399-3054.2007.00919.x.

Gamble, J; Roger, F.; Jaeger, S.; White, A.; Bava, C.; Beresford, M.; Stubbings, B.; Wohlers, M.; Hofman, P.J.; Marques, R.; Woolf, A. The impact of dry matter, ripeness and internal defects on consumer perceptions of avocado quality and intentions to purchase. Postharvest Biol. Tech. 2010, 57, 35-43. https://doi.org/10.1016/j.postharvbio.2010.01.001.

Garner, L.C.; Lovatt, C.J. The relationship between flower and fruit abscission and alternate bearing of 'Hass' avocado. J. Am. Soc. Hortic. Sci. 2008, 133, 3-10. https://doi.org/10.21273/JASHS.133.1.3.

Geng, X.; Gao, Z.; Zhao, L.; Zhang, S.; Wu, J.; Yang, Q.; Liu, S.; Chen, X. Comparative transcriptome analysis of resistant and susceptible wheat in response to *Rhizoctonia cerealis*. BMC Plant Biol. 2022, 22, 235. https://doi.org/10.1186/s12870-022-03584-y.

Giorgi, F. Climate change hot-spots. Geophys. Res. Lett. 2006, 33. L08707. https://doi.org/10.1029/2006GL025734.

Glowacka, K.; Kromdijk, J.; Kucera, K.; Xie, J.Y.; Cavanagh, A.P.; Leonelli, L.; Leakey, A.D.B.; Ort, D.R.; Niyogi, K.K.; Long, S.P. Photosystem II subunit S overexpression increases the efficiency of water use in a field-grown crop. Nature Commun. 2018, 9, 868. https://doi.org/10.1038/s41467-018-03231-x.

Glowacz, M.; Bill, M.; Tinyane, P.; Sivakumar, D. Maintaining postharvest quality of cold stored 'Hass' avocados by altering the fatty acids content and composition with the use of natural volatile compounds- methyl jasmonate and methyl salicylate. J. Sci. Food Agric. 2017, 97, 5186-5193. https://doi.org/10.1002/jsfa.8400.

Gokbayrak, Z.; Dardeniz, A.; Bal, M. Stomatal density adaptation of grapevine to windy conditions. Trakia J. Sci. 2008, 6, 18-22.

Gonçalves, F.C.d.M.; Parreiras, N.d.S.; Campos, F.G.; Mantoan, L.P.B.; Boaro, C.S.F. Exogenous salicylic acid modifies gas exchange and biomass production of *Mentha* × *piperita* L. Aust. J. Crop Sci. 2020, 14, 98-107. https://doi.org/10.21475/ajcs.20.14.01.p1900.

Grimmer, M.K.; John Foulkes, M.; Paveley, N.D. Foliar pathogenesis and plant water relations: A review. J. Exp. Bot. 2012, 63, 4321-4331. https://doi.org/10.1093/jxb/ers143.

Guarnizo, N.; Álvarez, A.; Oliveros, D.; Barbosa, O.; Joli, J.E.; Bermúdez-Cardona, M.B.; Murillo-Arango, W. Elicitor activity of curdlan and its potential application in protection of Hass avocado plants against *Phytophthora cinnamomi* rands. Horticulturae 2022, 8, 646. https://doi.org/10.3390/horticulturae8070646.

Gullner, G.; Komives, T.; Király, L.; Schröder, P. Glutathione S-transferase enzymes in plant-pathogen interactions. Front. Plant Sci. 2018, 9, 1836. https://doi.org/10.3389/fpls.2018.01836.

Haberman, A.; Tsror, L.; Lazare, S.; Hazanovsky, M.; Lebiush, S.; Zipori, I.; Busatn, A.; Simenski, E.; Dag, A. Management of verticillium wilt of avocado using tolerant rootstocks. Plants 2020, 9, 531. https://doi.org/10.3390/plants9040531.

Hakim; Ullah, A.; Hussain, A.; Shaban, M.; Khan, A.H.; Alariqi, M.; Gul, S.; Jun, Z.; Lin, S.; Li, J.; Jin, S.; Munis, M.F.H. Osmotin: A plant defense tool against biotic and abiotic stresses. Plant Physiol. Biochem. 2018, 123, 149-159. https://doi.org/10.1016/j.plaphy.2017.12.012.

Hanaka, A.; Maksymiec, W.; Bednarek, W. The effect of methyl jasmonate on selected physiological parameters of copper-treated *Phaseolus coccineus* plants. Plant Growth Regul. 2015, 77, 167-177. https://doi.org/10.1007/s10725-015-0048-8.

Havko, N.E.; Major, I.T.; Jewell, J.B.; Attaran, E.; Browse, J.; Howe, G.A. Control of carbon assimilation and partitioning by jasmonate: An accounting of growth-defense tradeoffs. Plants. 2016, 5, 7. https://doi.org/10.3390/plants5010007.

Hayat, Q.; Hayat, S.; Irfan, M.; Ahmad, A. Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot. 2010, 68, 14-25. https://doi.org/10.1016/j.envexpbot.2009.08.005.

Hayat, S.; Hasan, S.A.; Fariduddin, Q.; Ahmad, A. Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. J. Plant Interact. 2008, 3, 297-304. https://doi.org/10.1080/17429140802320797.

Hernández Valdés, E.F. 2020. Nutrición y fertilización. Pages: 149-157 in: Cultivo, poscosecha y procesado del aguacate. A. Namesny, C. Conesa, I. Hormaza, G. Lobo, eds. SPE3, Valencia, España.

Hernández, I.; Molina, V.; Fuentealba, C.; Álvaro, J.E.; Defilippi, B.G.; Pedreschi, R. Do rootstocks influence global fruit quality, postharvest performance and metabolite profiles of *Persea americana* cv. Hass? Horticulturae 2023, 9, 184. https://doi.org/10.3390/horticulturae9020184.

Herrera-González, J.A.; Bautista-Baños, S.; Serrano, M.; Romanazzi, G.; Gutiérrez-Martínez, P. Non-chemical treatments for the pre- and post-harvest elicitation of defense mechanisms in the fungi-avocado pathosystem. Molecules 2021, 26, 6819. https://doi.org/10.3390/molecules26226819.

Herrera-González, J.A.; Ramos-Bell, S.; Bautista-Baños, S.; Velázquez-Estrada, R.M.; Rayón-Díaz, E.; Martínez-Batista, E.; Gutiérrez-Martínez, P. Chitosan and GRAS substances: an alternative for the control of *Neofusicoccum parvum in vitro*, elicitor and maintenance of the postharvest quality of avocado fruits. Horticulturae 2024, 10, 687. https://doi.org/10.3390/horticulturae10070687.

Hsiao, T.C. 1990. Measurements of plant water status. Pages 243-279 in: Irrigation of Agricultural Crops. Monograph No. 30. B. A. Stewart D.R. Nielsen, eds. American Society of Agronomy, Madison, WI.

Hsiao, T.C. Measurements of plant water status. In Irrigation of Agricultural Crops; Stewart, B.A., Nielsen, D.R., Eds.; Monograph No. 30; American Society of Agronomy: Madison, WI, USA, 1990, pp. 243-279.

Huang, H.; Liu, B.; Liu, L.; Song, S. Jasmonate action in plant growth and development. J. Exp. Bot. 2017, 68, 1349-1359. https://doi.org/10.1093/jxb/erw495.

Hubbard, R.M.; Ryan, M.G.; Stiller, V.; Sperry, J.S. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ. 2001, 24, 113-121. https://doi.org/10.1046/j.1365-3040.2001.00660.x.

IPCC. Climate Change 2022: Impacts, Adaptation and Vulnerability; Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; Pörtner, H.O., Roberts, D.C., Tignor, M., Poloczanska, E.S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2022; p. 3056. https://doi.org/10.1017/9781009325844.

Jain, S.M. Tissue culture-derived variation in crop improvement. Euphytica 2001, 118, 153-166. https://doi.org/10.1023/A:1004124519479.

Jalil, S.U.; Mishra, M.; Ansari, M.I. Current view on chitinase for plant defence. Trends Biosci. 2015, 8, 6733-6743.

Jamiołkowska, A. Natural compounds as elicitors of plant resistance against diseases and new biocontrol strategies. Agronomy 2020, 10, 173. https://doi.org/10.3390/agronomy10020173.

Janda, T.; Gondor, O.K.; Yordanova, R.; Szalai, G.; Pál, M. Salicylic acid and photosynthesis: Signalling and effects. Acta Physiol. Plant. 2014, 36, 2537-2546. https://doi.org/10.1007/s11738-014-1620-y.

Janoudi, A.; Flore, J.A. Effects of multiple applications of methyl jasmonate on fruit ripening, leaf gas exchange and vegetative growth in fruit trees. J. Hortic. Sci. Biotech. 2003, 78 793-797. https://doi.org/10.1080/14620316.2003.11511701.

Jashni, M.K.; Mehrabi, R.; Collemare, J.; Mesarich, C.H.; de Wit, P.J.G.M. The battle in the apoplast: Further insights into the roles of proteases and their inhibitors in plant-pathogen interactions. Front. Plant Sci. 2015, 6, 584. https://doi.org/10.3389/fpls.2015.00584.

Jayasankar, S.; Li, Z.; Gray, D.J. Constitutive expression of *Vitis vinifera* thaumatin-like protein after *in vitro* selection and its role in anthracnose resistance. Funct. Plant Biol. 2003, 30, 1105-1115. https://doi.org/10.1071/FP03066.

Jayasankar, S.; Li, Z.; Gray, D.J. *In vitro* selection of *Vitis vinifera* 'Chardonnay' with *Elsinoe ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta 2000, 211, 200-208. https://doi.org/10.1007/S004250000285.

Jayasankar, S.; Litz, R.E. Characterization of embryogenic mango cultures selected for resistance to *Colletotrichum gloeosporoides* culture filtrate and phytotoxin. Theor. Appl. Genet. 1998, 96, 823-831. https://doi.org/10.1007/s001220050808.

Jenkins, A.E. *Sphaceloma perseae* the cause of avocado scab. J. Agric. Res. 1934, 49, 859-869.

Jesus, C.; Meijón, M.; Monteiro, P.; Correia, B.; Amaral, J.; Escandón, M.; Cañal, M.J.; Pinto, G. Salicylic acid application modulates physiological and hormonal changes in *Eucalyptus globulus* under water deficit. Environ. Exp. Bot. 2015, 118, 56-66. https://doi.org/10.1016/j.envexpbot.2015.06.004.

Jiao, L.; Bian, L.; Luo, Z.; Li, Z.; Xiu, C.; Fu, N.; Cai, X.; Chen, Z. Enhanced volatile emissions and anti-herbivore functions mediated by the synergism between jasmonic acid and salicylic acid pathways in tea plants. Hortic. Res. 2022, 9, uhac144. https://doi.org/10.1093/hr/uhac144.

Joint FAO/IAEA Mutant Variety and Genetic Stock Database. Available at: http://mvgs.iaea.org/ (accessed 7 October 2024).

Jung, Y.J.; Melencion, S.M.B.; Lee, E.S.; Park, J.H.; Alinapon, C.V.; Oh, H.T.; Yun, D.J.; Chi, Y.H.; Lee, S.Y. Universal stress protein exhibits a redox-dependent chaperone function in *Arabidopsis* and enhances plant tolerance to heat shock and oxidative stress. Front. Plant Sci. 2015, 6, 1141. https://doi.org/10.3389/fpls.2015.01141.

Kanematsu, S.; Hayashi, T.; Kudo, A. Isolation of *Rosellinia necatrix* mutants with impaired cytochalasin E production and its pathogenicity. Jpn. J. Phytopathol. 1997, 63, 425-431. https://doi.org/10.3186/jjphytopath.63.425.

Kawasaki, T.; Koita, H.; Nakatsubo, T.; Hasegawa, K.; Wakabayashi, K.; Takahashi, H.; Umemura, K.; Umezawa, T.; Shimamoto, K. Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. Proc. Natl. Acad. Sci. USA 2006, 103, 230-235. https://doi.org/10.1073/pnas.0509875103.

Kepczynska, E.; Król, P. The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. J. Plant Interact. 2012, 7, 307-315. https://doi.org/10.1080/17429145.2011.645169.

Khan, A.H. Biology and pathogenicity of *Rosellinia necatrix* (Hart.) Berl. Biología 1959, 5, 199-425.

Khan, M.I.R.; Fatma, M.; Per, T.S.; Anjum, N.A.; Khan, N.A. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 2015, 6, 462. https://doi.org/10.3389/fpls.2015.00462.

Khan, M.I.R.; Iqbal, N.; Masood, A.; Per, T.S.; Khan, N.A. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal. Behav. 2013, 8, e26374. https://doi.org/10.4161/psb.26374.

Khan, W.; Prithiviraj, B.; Smith, D.L. Photosynthetic responses of corn and soybean to foliar application of salicylates. J. Plant Physiol. 2003, 160, 485-492. https://doi.org/10.1078/0176-1617-00865.

Khandagale, K.; Roylawar, P.; Kulkarni, O.; Khambalkar, P.; Ade, A.; Kulkarni, A.; Singh, M.; Gawande, S. Comparative Transcriptome Analysis of Onion in Response to Infection by *Alternaria porri* (Ellis) Cifferi. Front. Plant Sci. 2022, 13, 857306. https://doi.org/10.3389/fpls.2022.857306.

Khodary, S.E.A. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J. Agric. Biol. 2004, 6, 5-8.

Khokon, M.A.R.; Okuma, E.; Hossain, M.A.; Munemasa, S.; Uraji, M.; Nakamura, Y.; Mori, I.C.; Murata, Y. Involvement of extracellular oxidative burst in salicylic acid-induced stomatal closure in *Arabidopsis*. Plant Cell Environ. 2011, 34, 434-443. https://doi.org/10.1111/j.1365-3040.2010.02253.x.

Kimura, Y.; Nakajima, H.; Hamasaki, T. Structure of rosellichalasin, a new metabolite produced by *Rosellinia necatrix*. Agric. Biol. Chem. 1989, 53, 1699-1701. https://doi.org/10.1271/bbb1961.53.1699.

Knogge, W. Molecular basis of specificity in host/fungus interactions. Eur. J. Plant Pathol. 1996, 102, 807-816. https://doi.org/10.1007/BF01877049.

Kofidis, G.; Bosabalidis, A.M.; Chartzoulakis, K. Leaf anatomical alterations induced by drought stress in two avocado cultivars. J. Biol. Res. 2004, 1, 115-120.

Kõressaar, T.; Lepamets, M.; Kaplinski, L.; Raime, K.; Andreson, R.; Remm, M. Primer3_masker: Integrating masking of template sequence with primer design software. Bioinformatics 2018, 34, 1937-1938. https://doi.org/10.1093/bioinformatics/bty036.

Koressaar, T.; Remm, M. Enhancements and modifications of primer design program Primer3. Bioinformatics 2007, 23, 1289-1291. https://doi.org/10.1093/bioinformatics/btm091.

Kou, X.; Han, W.; Kang, J. Responses of root system architecture to water stress at multiple levels: A meta-analysis of trials under controlled conditions. Front. Plant Sci. 2022, 13, 1085409. https://doi.org/10.3389/fpls.2022.1085409.

Kremer-Köhne, S.; Duvenhage, J.A.; Mailula, S.M. Breeding and field testing of new avocado rootstocks for increased 'Hass' yields and resistance to root rot-progress report. South Afr. Avocado Grow. Assoc. Year. 2001, 25, 17-19.

Kremer-Köhne, S.; Köhne, J.S. 25 years of avocado rootstock development in South Africa. Proceedings of the VI World Avocado Congress, Viña del Mar. Chile 2007. https://www.avocadosource.com/WAC6/en/Extenso/1a-8.pdf (accessed October 2024).

Kremer-Köhne, S.; van Rooyen, Z.; Köhne, J.S. Avocado rootstock evaluation techniques over the last 30 years. Proceedings of the VII World Avocado Congress, Cairns, Aust. 2011. https://www.avocadosource.com/wac7/Section_14/KremerKohneS2011.pdf (accessed October 2024).

Krishna, H.; Alizadeh, M.; Singh, D.; Singh, U.; Chauhan, N.; Eftekhari, M.; Sadh, R.K. Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech 2016, 6, 54. https://doi.org/10.1007/s13205-016-0389-7.

Krokene, P.; Nagy, N.E.; Solheim, H. Methyl jasmonate and oxalic acid treatment of Norway spruce: Anatomically based defense responses and increased resistance against fungal infection. Tree Physiol. 2008, 28, 29-35. https://doi.org/10.1093/treephys/28.1.29.

Kshirsagar, A.; Reid, A.J.; McColl, S.M.; Saunders, V.A.; Whalley, A.J.S.; Evans, E.H. The effect of fungal metabolites on leaves as detected by chlorophyll fluorescence. New Phytol. 2001, 151, 451-457. https://doi.org/10.1046/j.0028-646x.2001.00192.x.

Kuhn, D.N.; Groh, A.; Rahaman, J.; Freeman, B.; Arpaia, M.L.; van den Berg, N.; Abeysekara, N.; Manosalva, P.; Chambers, A.H. Creation of an avocado unambiguous genotype SNP database for germplasm curation and as an aid to breeders. Tree Genet. Genomes 2019, 15, 71. https://doi.org/10.1007/s11295-019-1374-1.

Kulshrestha, S.; Seth, C.A.; Sharma, M.; Sharma, A.; Mahajan, R.; Chauhan, A. Biology and control of *Rosellinia necatrix* causing white root rot disease: A review. J. Pure Appl. Microbiol. 2014, 8, 1803-1814.

Kumar, K.A.; Singh, S.K.; Kumar, B.S.; Doble, M. Synthesis, anti-fungal activity evaluation and QSAR studies on podophyllotoxin derivatives. Open Chem. 2007, 5, 880-897. https://doi.org/10.2478/s11532-007-0036-6.

Labarga, D.; Mairata, A.; Puelles, M.; Martín, I.; Albacete, A.; García-Escudero, E.; Pou, A. The rootstock genotypes determine drought tolerance by regulating aquaporin expression at the transcript level and phytohormone balance. Plants 2023, 12, 718. https://doi.org/10.3390/plants12040718.

Lagrimini, L.M.; Vaughn, J.; Erb, W.A.; Miller, S.A. Peroxidase overproduction in tomato: Wound-induced polyphenol deposition and disease resistance. HortScience 1993, 28, 218-221. https://doi.org/10.21273/HORTSCI.28.3.218.

Lahav, E.; Whiley, A.W.; Turner, D.W. 2013. Irrigation and mineral nutrition. Pages: 301-341 in: The Avocado: Botany, Production and Uses, 2nd Edition. B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Lake, J.A.; Woodward, F.I. Response of stomatal numbers to CO_2 and humidity: Control by transpiration rate and abscisic acid. New Phytol. 2008, 179, 397-404. https://doi.org/10.1111/j.1469-8137.2008.02485.x.

Laredo Alcalá, E.I.; Martínez Hernández, J.L.; Iliná, A.; Guillen Cisneros, L.; Hernández Castillo, F.D. Aplicación de ácido jasmónico como inductor de resistencia vegetal frente a patógenos. Rev. Mex. Cienc. Agríc. 2017, 8, 673-683. https://doi.org/10.29312/remexca.v8i3.40.

Larkin, P.J.; Scowcroft, W.R. Somaclonal variation-A novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 1981, 60, 197-214. https://doi.org/10.1007/BF02342540.

Lavrnić, S.; Zapater-Pereyra, M.; Mancini, M.L. Water scarcity and wastewater reuse standards in Southern Europe: Focus on agriculture. Water Air Soil Pollut. 2017, 228, 251. https://doi.org/10.1007/s11270-017-3425-2.

Lazare, S.; Cohen, Y.; Goldshtein, E.; Yermiyahu, U.; Ben-Gal, A.; Dag, A. Rootstock-dependent response of Hass avocado to salt stress. Plants 2021a, 10, 1672. https://doi.org/10.3390/plants10081672.

Lazare, S.; Haberman, A.; Yermiyahu, U.; Erel, R.; Simenski, E.; Dag, A. Avocado rootstock influences scion leaf mineral content. Arch. Agron. Soil Sci. 2020, 66, 1399-1409. https://doi.org/10.1080/03650340.2019.1672163.

Lazare, S.; Yasuor, H.; Yermiyahu, U.; Kuhalskaya, A.; Brotman, Y.; Ben-Gal, A.; Dag, A. It takes two: Reciprocal scion-rootstock relationships enable salt tolerance in 'Hass' avocado. Plant Sci. 2021b, 312, 111048. https://doi.org/10.1016/j.plantsci.2021.111048.

Le Lagadec, D. Field evaluation of superior avocado rootstocks with 'Hass' as scions. In Proceedings of the 2011 VII World Avocado Congress (Actas VII Congreso Mundial del Aguacate 2011), Cairns, Australia, 5-9 September 2011.

Lestari, E.G. *In vitro* selection and somaclonal variation for biotic and abiotic stress tolerance. Biodiversitas 2006, 7, 297-301. https://doi.org/10.13057/biodiv/d070320.

Li, C.; Wang, P.; Menzies, N.W.; Lombi, E.; Kopittke, P.M. Effects of methyl jasmonate on plant growth and leaf properties. J. Plant Nutr. Soil Sci. 2018, 181, 409-418. https://doi.org/10.1002/jpln.201700373.

Lima, M.R.M.; Ferreres, F.; Dias, A.C.P. Response of *Vitis vinifera* cell cultures to *Phaeomoniella chlamydospora*: Changes in phenolic production, oxidative state and expression of defence-related genes. Eur. J. Plant Pathol. 2012, 132, 133-146. https://doi.org/10.1007/s10658-011-9857-4.

Lin, W.C.; Lu, C.F.; Wu, J.W.; Cheng, M.L.; Lin., Y.M.; Yang, N.S.; Black, L.; Green, S.K.; Wang, J.F.; Cheng, C.P. Transgenic tomato plants expressing the *Arabidopsis* NPR1 gene display enhanced resistance to a spectrum of fungal and bacterial diseases. Transgenic Res. 2004, 13, 567-581. https://doi.org/10.1007/s11248-004-2375-9.

Litz, R.E.; Efendi, D.; Raharjo, S.H.T.; Padilla, G.; Moon, P.A. Genetic transformation of avocado with S-adenosylmethionine hydrolase (samase) and evaluation of transformants after three years. Proceedings VI World Avocado Congress, Viña del Mar. Chile. 2007. https://www.avocadosource.com/wac6/en/Extenso/1c-24.pdf (accessed 15 July 2024).

Litz, R.E.; Perea-Arango, I.; Suárez Padron, I. A comparison of biotech methods for controlling infections by the avocado sunblotch viroid (ASBVD). 28th International Horticultural Congress Vol. II. Lisbon, Portugal. 2010, 280 (Abstract).

Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001, 25, 402-408. https://doi.org/10.1006/meth.2001.1262.

Lobato, A.K.D.S.; Barbosa, M.A.M.; Alsahli, A.A.; Lima, E.J.A.; Silva, B.R.S.D. Exogenous salicylic acid alleviates the negative impacts on production components, biomass and gas exchange in tomato plants under water deficit improving redox status and anatomical responses. Physiol. Plant. 2021, 172, 869-884. https://doi.org/10.1111/ppl.13329.

López-Herrera, C. 2020. Enfermedades de origen biológico. Pages: 199-248 in: Cultivo, poscosecha y procesado del aguacate. A. Namesny, C. Conesa, I. Hormaza, G. Lobo, eds. SPE3, Valencia, España.

López-Herrera, C.J. Hongos de suelo en el cultivo del aguacate (*Persea americana* Mill) del litoral andaluz. V Jornadas Andaluzas de Frutos Tropicales. Congresos y Jornadas 1998, 47, 139-152.

López-Herrera, C.J.; Pérez-Jiménez, R.M.; Basallote-Ureba, M.J.; Zea-Bonilla, T.; Melero-Vara, J.M. Loss of viability of *Dematophora necatrix* in solarized soils. Eur. J. Plant Pathol. 1999, 105, 571-576. https://doi.org/10.1023/A:1008755017575.

López-Herrera, C.J.; Pérez-Jiménez, R.M.; Zea-Bonilla, T.; Basallote-Ureba, M.J.; Melero-Vara, J.M. Soil solarization in established avocado trees for control of *Dematophora necatrix*. Plant Dis. 1998, 82, 1088-1092. https://doi.org/10.1094/PDIS.1998.82.10.1088.

López-Herrera, C.J.; Zea-Bonilla, T. Effects of benomyl, carbendazim, fluazinam and thiophanate methyl on white root rot of avocado. Crop Prot. 2007, 26, 1186-1192. https://doi.org/10.1016/j.cropro.2006.10.015.

Lotfi, R.; Ghassemi-Golezani, K.; Pessarakli, M. Salicylic acid regulates photosynthetic electron transfer and stomatal conductance of mung bean (*Vigna radiata* L.) under salinity stress. Biocatal. Agric. Biotechnol. 2020, 26, 101635. https://doi.org/10.1016/j.bcab.2020.101635.

Lovisolo, C.; Hartung, W.; Schubert, A. Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines. Funct. Plant Biol. 2002, 29, 1349-1356. https://doi.org/10.1071/FP02079.

Magagula, P.; Taylor, N.; Swart, V.; van den Berg, N. Efficacy of potential control agents against *Rosellinia necatrix* and their physiological impact on avocado. Plant Dis. 2021, 105, 3385-3396. https://doi.org/10.1094/pdis-08-20-1751-re.

Mahomed, W.; van den Berg, N. EST sequencing and gene expression profiling of defence-related genes from *Persea americana* infected with *Phytophthora cinnamomi*. BMC Plant Biol. 2011, 11, 167. https://doi.org/10.1186/1471-2229-11-167.

Major, I.T.; Constabel, C. P. Functional analysis of the Kunitz trypsin inhibitor family in poplar reveals biochemical diversity and multiplicity in defense against herbivores. Plant Physiol. 2008, 146, 888-903. https://doi.org/10.1104/pp.107.106229.

Makandar, R.; Nalam, V.; Chaturvedi, R.; Jeannotte, R.; Sparks, A.A.; Shah, J. Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis* interaction with *Fusarium graminearum*. Mol. Plant-Microbe Interact. 2010, 23, 861-870. https://doi.org/10.1094/mpmi-23-7-0861.

Malefo, M.B.; Mathibela, E.O.; Crampton, B.G.; Makgopa, M.E. Investigating the role of Bowman-Birk serine protease inhibitor in Arabidopsis plants under drought stress. Plant Physiol. Bioch. 2020, 149, 286-293. https://doi.org/10.1016/j.plaphy.2020.02.007.

Mandemaker, A.J. Photosynthesis of avocado. New Zealand Avocado Growers' Association Annual Research Report 2008, 7, 1-10.

Marguerit, E.; Brendel, O.; Lebon, E.; van Leeuwen, C.; Ollat, N. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. New Phytol. 2012, 194, 416-429. https://doi.org/10.1111/j.1469-8137.2012.04059.x.

Marrs, K.A. The functions and regulation of glutathione S-transferases in plants. Annu. Rev. Plant Biol. 1996, 47, 127-158. https://doi.org/10.1146/annurev.arplant.47.1.127.

Martínez-Ferri, E.; Balaguer, L.; Valladares, F.; Chico, J.M.; Manrique, E. Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. Tree Physiol. 2000, 20, 131-138. https://doi.org/10.1093/treephys/20.2.131.

Martínez-Ferri, E.; Moreno-Ortega, G.; van den Berg, N.; Pliego, C. Mild water stress-induced priming enhance tolerance to *Rosellinia necatrix* in susceptible avocado rootstocks. BMC Plant Biol. 2019, 19, 458. https://doi.org/10.1186/s12870-019-2016-3.

Martínez-Ferri, E.; Zumaquero, A.; Ariza, M.T.; Barceló, A.; Pliego, C. Nondestructive detection of white root rot disease in avocado rootstocks by leaf chlorophyll fluorescence. Plant Dis. 2016, 100, 49-58. https://doi.org/10.1094/PDIS-01-15-0062-RE.

Mendoza García, R.A.; ten Hoopen, G.M.; Kass, D.C.J.; Sánchez Garita, V.A.; Krauss, U. Evaluation of mycoparasites as biocontrol agents of *Rosellinia* root rot in cocoa. Biol. Control 2003, 27, 210-227. https://doi.org/10.1016/S1049-9644(03)00014-8.

Meng, X.; Yang, L.; Kennedy, J.F.; Tian, S. Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. Carbohyd. Polym. 2010. 81, 70-75. https://doi.org/10.1016/j.carbpol.2010.01.057.

Menge, J.A. Non-fungicidal control strategies for *Phytophthora cinnamomi* root rot of avocado. Proceedings of the Australian and New Zealand Avocado Growers' Conference 'Vision 2020'. Australian Avocado Growers' Federation, Brisbane. 2001, 1-10.

Menge, J.A.; Ploetz, R.C. 2003. Diseases of Avocado. Pages: 35-71 in: Diseases of Tropical Fruit Crops. R.C. Ploetz, ed. CAB International, Wallingford, UK. https://doi.org/10.1079/9780851993904.0035.

Mickelbart, M.V.; Bender, G.S.; Witney, G.W.; Adams, C.; Arpaia, M.L. Effects of clonal rootstocks on 'Hass' avocado yield components, alternate bearing, and nutrition. J.

Hortic. Sci. Biotech. 2007, 82, 460-466. https://doi.org/10.1080/14620316.2007.11512259.

Mickelbart, M.V.; Miller, R.; Parry, S.; Arpaia, M.L.; Heath, R. Avocado leaf surface morphology. California Avocado Society Yearbook 2000, 84, 139-150.

Ministerio de Agricultura, Pesca y Alimentación https://www.mapa.gob.es/estadistica/pags/anuario/2022/FORMATO%20PDF/AE22-C07/CAP%C3%8DTULO%2007.pdf (accessed 05 august 2024).

Molinier, J.; Ries, G.; Zipfel, C.; Hohn, B. Transgeneration memory of stress in plants. Nature 2006, 442, 1046-1049. https://doi.org/10.1038/nature05022.

Moloi, S.J.; Ngara, R. The roles of plant proteases and protease inhibitors in drought response: A review. Front. Plant Sci. 2023, 14, 1165845. https://doi.org/10.3389/fpls.2023.1165845.

Moreno-Ortega, G.; Pliego, C.; Sarmiento, D.; Barceló, A.; Martínez-Ferri, E. Yield and fruit quality of avocado trees under different regimes of water supply in the subtropical coast of Spain. Agric. Water Manag. 2019, 221, 192-201. https://doi.org/10.1016/j.agwat.2019.05.001.

Moreno-Ortega, G.; Zumaquero, A.; Matas, A.M.; Olivier, N.; van den Berg, N.; Palomo-Ríos, E.; Martínez-Ferri, E.; Pliego, C. Physiological and molecular responses of 'Dusa' avocado rootstock to water stress: Insights for drought adaptation. Plants 2021, 10, 2077. https://doi.org/10.3390/plants10102077.

Moreno-Pérez, A.; Martínez-Ferri, E.; Pliego-Alfaro, F.; Pliego, C. Elicitors and plant defence. JOJ Hortic. Arboric. 2020, 2, 555600. http://doi.org/10.19080/JOJHA.2020.19.555600.

Moreno-Pérez, A.; Zumaquero, A.; Martínez-Ferri, E.; López-Herrera, C.; Pliego-Alfaro, F.; Palomo-Ríos, E.; Pliego, C. A comparative transcriptome analysis of avocado embryogenic lines susceptible or resistant to *Rosellinia necatrix* exudate. Agronomy 2023, 13, 1354. https://doi.org/10.3390/agronomy13051354.

Mori, I. C.; Pinontoan, R.; Kawano, T.; Muto, S. Involvement of superoxide generation in salicylic acid-induced stomatal closure in *Vicia faba*. Plant Cell Physiol. 2001, 42, 1383-1388. https://doi.org/10.1093/pcp/pce176.

Moustaka, J.; Tanou, G.; Adamakis, I.D.; Eleftheriou, E.P.; Moustakas, M. Leaf age-dependent photoprotective and antioxidative response mechanisms to paraquat-induced oxidative stress in *Arabidopsis thaliana*. Int. J. Mol. Sci. 2015, 16, 13989-14006. https://doi.org/10.3390/ijms160613989.

Muir, C.D.; Hangarter, R.P.; Moyle, L.C.; Davis, P.A. Morphological and anatomical determinants of mesophyll conductance in wild relatives of tomato (*Solanum* sect. *Lycopersicon*, sect. *Lycopersicoides*; Solanaceae). Plant Cell Environ. 2014, 37, 1415-1426. https://doi.org/10.1111/pce.12245.

Müller, M.; Munné-Bosch, S. Ethylene response factors: A key regulatory hub in hormone and stress signaling. Plant Physiol. 2015, 169, 32-41. https://doi.org/10.1104/pp.15.00677.

Munhuweyi, K.; Mpai, S.; Sivakumar, D. Extension of avocado fruit postharvest quality using non-chemical treatments. Agronomy 2020, 10, 212. https://doi.org/10.3390/agronomy10020212.

Munns, R. 2002. Salinity, growth and phytohormones. Pages: 271-290 in: Salinity: environment-plants-molecules, André Läuchli, Ulrich Lüttge, eds. Dordrecht: Springer Netherlands. https://doi.org/10.1007/0-306-48155-3.

Munns, R.; Tester, M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008, 59, 1, 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 1962, 15, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. J. Exp. Bot. 2013, 64, 3983-3998. https://doi.org/10.1093/jxb/ert208.

Narayani, M.; Srivastava, S. Elicitation: a stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production. Phytochem. Rev. 2017, 16, 1227-1252. https://doi.org/10.1007/s11101-017-9534-0.

Nardini, A.; lo Gullo, M.A.; Trifilò, P.; Salleo, S. The challenge of the Mediterranean climate to plant hydraulics: Responses and adaptations. Environ. Exp. Bot. 2014, 103, 68-79. https://doi.org/10.1016/j.envexpbot.2013.09.018.

Nardini, A.; Salleo, S. Limitation of stomatal conductance by hydraulic traits: Sensing or preventing xylem cavitation? Trees 2000, 15, 14-24. https://doi.org/10.1007/s004680000071.

Naveed, Z.A.; Ali, G.S. Comparative transcriptome analysis between a resistant and a susceptible wild tomato accession in response to *Phytophthora parasitica*. Int. J. Mol. Sci. 2018, 19, 3735. https://doi.org/10.3390/ijms19123735.

Nazar, R.; Umar, S.; Khan, N.A.; Sareer, O. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. S. Afr. J. Bot. 2015, 98, 84-94. https://doi.org/10.1016/j.sajb.2015.02.005.

Niki, T.; Mitsuhara, I.; Seo, S.; Ohtsubo, N.; Ohashi, Y. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol. 1998, 39, 500-507. https://doi.org/10.1093/oxfordjournals.pcp.a029397.

Núñez-Lillo, G.; Ponce, E.; Beyer, C.P.; Álvaro, J.E.; Meneses, C.; Pedreschi, R. A First Omics Data Integration Approach in Hass Avocados to Evaluate Rootstock-Scion Interactions: From Aerial and Root Plant Growth to Fruit Development. Plants 2024, 13, 603. https://doi.org/10.3390/plants13050603.

Observatorio de precios y mercados, Junta de Andalucía https://www.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/FrontController?action=Static&subsector=34&url=subsector.jsp (accessed 05 august 2024).

Oliveira, M.B.; Junior, M.L.; Grossi-de-Sá, M.F.; Petrofeza, S. Exogenous application of methyl jasmonate induces a defense response and resistance against *Sclerotinia sclerotiorum* in dry bean plants. J. Plant Physiol. 2015, 182, 13-22. https://doi.org/10.1016/j.jplph.2015.04.006.

Opazo, I.; Pimentel, P.; Salvatierra, A.; Ortiz, M.; Toro, G.; Garrido-Salinas, M. Water stress tolerance is coordinated with water use capacity and growth under water deficit across six fruit tree species. Irrig. Sci. 2024, 42, 493-507. https://doi.org/10.1007/s00271-024-00915-9.

Opazo, I.; Toro, G.; Salvatierra, A.; Pastenes, C.; Pimentel, P. Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. Agric. Water Manag. 2020, 228, 105897. https://doi.org/10.1016/j.agwat.2019.105897.

Palomo Ríos, E.; Pérez, C.; Mercado, J.A.; Pliego-Alfaro, F. Enhancing frequency of regeneration of somatic embryos of avocado (*Persea americana* Mill.) using semi-permeable cellulose acetate membranes. Plant Cell Tiss. Org. 2013, 115, 199-207. https://doi.org/10.1007/s11240-013-0352-9.

Palomo-Ríos, E.; Barceló, M.; Barceló-Muñoz, A.; Pliego, C.; Blanco-Portales, R.; Caballero-Repullo, J.L.; Ruano-Rosa, D.; López-Herrera, C.; Mercado-Carmona, J.A.; Pliego-Alfaro, F. *Agrobacterium*-mediated transformation to induce tolerance to avocado white root rot. Proceedings of the VII World Avocado Congress, Cairns. Australia. 2011 http://www.avocadosource.com (accessed 01 October 2024, in Spanish).

Palomo-Ríos, E.; Barceló-Muñoz, A.; Mercado, J.A.; Pliego-Alfaro, F. Evaluation of key factors influencing *Agrobacterium*-mediated transformation of somatic embryos of avocado (*Persea americana*, Mill). Plant Cell Tiss. Org. 2012, 109, 201-211. https://doi.org/10.1007/s11240-011-0086-5.

Palomo-Ríos, E.; Cerezo, S.; Mercado, J.A.; Pliego-Alfaro, F. *Agrobacterium*-mediated transformation of avocado (*Persea americana* Mill.) somatic embryos with fluorescent marker genes and optimization of transgenic plant recovery. Plant Cell Tiss. Org. 2017, 128, 447-455. https://doi.org/10.1007/s11240-016-1122-2.

Palomo-Ríos, E.; Pliego, C.; Muñoz-Blanco, J.; Mercado, J.A.; Pliego-Alfaro, F. 2010. *Agrobacterium*-mediated transformation of avocado (*Persea americana* Mill.) with *At*NPR1 gene. Proceedings of the 28th International Horticultural Congress Vol. II, Lisbon. 2010.286 (Abstract).

Pastor, V.; Luna, E.; Mauch-Mani, B.; Ton, J.; Flors, V. Primed plants do not forget. Environ. Exp. Bot. 2013, 94, 46-56. https://doi.org/10.1016/j.envexpbot.2012.02.013.

Patakas, A.; Nortsakis, B. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. J. Plant Physiol. 1999, 154, 767-774. https://doi.org/10.1016/S0176-1617(99)80256-9.

Paz-Vega, S. Alternate bearing in the avocado (*Persea americana* Mill.). California Avocado Society Yearbook 1997, 81, 117-148.

Peel, J.R.; Mandujano Sánchez, M.C.; López Portillo, J.; Golubov, J. Stomatal density, leaf area and plant size variation of *Rhizophora mangle* (Malpighiales: Rhizophoraceae) along a salinity gradient in the Mexican Caribbean. Rev. Biol. Trop. 2017, 65, 701-712. http://doi.org/10.15517/rbt.v65i2.24372.

Pegg, K.G.; Coates, L.M.; Korsten, L.; Harding, R.M. 2002. Root, foliar and soilborne diseases. Pages: 299-338 in: The Avocado: Botany, Production and Uses, 2nd Edition. B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Pegg, K.G.; Whiley, A.W.; Saranah, J.B.; Glass, R.J. Control of Phytophthora root rot of avocado with phosphorus acid. Australas. Plant Path. 1985, 14, 25-29. https://doi.org/10.1071/APP9850025.

Peguero-Pina, J.J.; Vilagrosa, A.; Alonso-Forn, D.; Ferrio, J.P.; Sancho-Knapik, D.; Gil-Pelegrín, E. Living in drylands: Functional adaptations of trees and shrubs to cope with high temperatures and water scarcity. Forests 2020, 11, 1028. https://doi.org/10.3390/f11101028.

Peña, J.E.; Hoddle, M.S.; Aluja, M.; Palevsky, E.; Ripa, R.; Wysoki, M. 2013. Insect and mite pests. Pages: 423-488 in: The Avocado: Botany, Production and Uses, 2nd Edition. B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Perato, S.M.; Martínez-Zamora, M.G.; Salazar, S.M.; Díaz-Ricci, J.C. The elicitor AsES stimulates ethylene synthesis, induce ripening and enhance protection against disease naturally produced in avocado fruit. Sci. Hortic. 2018, 240, 288-292 https://doi.org/10.1016/j.scienta.2018.06.030.

Pérez-Jiménez, R.M. A review of the biology and pathogenicity of *Rosellinia necatrix*-the cause of white root rot disease of fruit trees and other plants. J. Phytopathol. 2006, 154, 257-266. https://doi.org/10.1111/j.1439-0434.2006.01101.x.

Pérez-Jiménez, R.M.; Zea-Bonilla, T.; López-Herrera, C.J. Studies of *Rosellinia necatrix* perithecia found in nature on avocado roots. Journal of Phytopathology 2003, 151, 660-664. https://doi.org/10.1046/j.0931-1785.2003.00782.x.

Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001, 29, e45. https://doi.org/10.1093/nar/29.9.e45.

Phillips, D.; Weste, G.; Hinch, J.M. Resistance to *Phytophthora cinnamomi* in callus derived from three avocado cultivars. Can. J. Bot. 1991, 69, 2026-2032. https://doi.org/10.1139/b91-255.

Pieterse, C.M.J.; Van der Does, D.; Zamioudis, C.; Leon-Reyes, A.; Van Wees, S.C.M. Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 2012, 28, 489-521. https://doi.org/10.1146/annurev-cellbio-092910-154055.

Pimentel, D.; Burgess, M. 2014. Environmental and economic costs of the application of pesticides primarily in the United States. Pages 47-71 in: Integrated Pest Management. D. Pimentel and R. Peshin, eds. Springer, Dordrecht, Germany.

Pliego, C.; De Weert, S.; Lamers, G.; De Vicente, A.; Bloemberg, G.; Cazorla, F.M.; Ramos, C. Two similar enhanced root-colonizing *Pseudomonas* strains differ largely in their colonization strategies of avocado roots and *Rosellinia necatrix* hyphae. Environ. Microbiol. 2008, 10, 3295-3304. https://doi.org/10.1111/j.1462-2920.2008.01721.x.

Pliego, C.; Kanematsu, S.; Ruano-Rosa, D.; de Vicente, A.; López-Herrera, C.; Cazorla, F.M.; Ramos, C. GFP sheds light on the infection process of avocado roots by *Rosellinia necatrix*. Fungal Genet. Biol. 2009, 46, 137-145. https://doi.org/10.1016/j.fgb.2008.11.009.

Pliego, C.; López-Herrera, C.; Ramos, C.; Cazorla, F.M. Developing tools to unravel the biological secrets of *Rosellinia necatrix*, an emergent threat to woody crops. Mol. Plant Pathol. 2012, 13, 226-239. https://doi.org/10.1111/j.1364-3703.2011.00753.x.

Pliego-Alfaro, F.; Murashige, T. Somatic embryogenesis in avocado (*Persea americana* Mill.) *in vitro*. Plant Cell Tissue Organ Cult. 1988, 112, 61-66. https://doi.org/10.1007/BF00043108.

Pliego-Alfaro, F.; Palomo-Ríos, E.; Mercado, J.A.; Pliego, C.; Barceló-Muñoz, A.; López-Gómez, R.; Hormaza, J.I.; Litz, R.E. 2020. *Persea americana* Avocado. Pages: 258-281 in: Biotechnology of Fruit and Nut Crops, 2nd Edition. R.E. Litz, F. Pliego-Alfaro, J.I. Hormaza, eds. CABI International.

Ploetz, R.; Schnell, R.J.; Haynes, J. Variable response of open-pollinated seedling progeny of avocado to Phytophthora root rot. Phytoparasitica 2002, 30, 262-268. https://doi.org/10.1007/BF03039994.

Poorter, H.; Niinemets, U.; Poorter, L.; Wright, I.J.; Villar, R. Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. New Phytol. 2009, 182, 565-588. https://doi.org/10.1111/j.1469-8137.2009.02830.x.

Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. New Phytol. 2012, 193, 30-50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.

Pospisilová, J. Participation of phytohormones in the stomatal regulation of gas exchange during water stress. Biol. Plant. 2003, 46, 491-506. https://doi.org/10.1023/A:1024894923865.

Prillieux, E. Sur la dehiscence des peritheces du *Rosellinia necatrix* (R. Hart.). Berleze. Bull. Soc. Myc. France 1904, *20*, 34-38.

Qi, X.; Torii, K.U. Hormonal and environmental signals guiding stomatal development. BMC Biol. 2018, 16, 21. https://doi.org/10.1186/s12915-018-0488-5.

Qiu, X.; Xu, Y.; Xiong, B.; Dai, L.; Huang, S.; Dong, T.; Sun, G.; Liao, L.; Deng, Q.; Wang, X.; Zhu, J.; Wang, Z. Effects of exogenous methyl jasmonate on the synthesis of endogenous jasmonates and the regulation of photosynthesis in citrus. Physiol. Plantarum. 2020, 170, 398-414. https://doi.org/10.1111/ppl.13170.

Rai, M.K.; Kalia, R.K.; Singh, R.; Gangola, M.P.; Dhawan, A.K. Developing stress tolerant plants through *in vitro* selection-An overview of the recent progress. Environ. Exp. Bot. 2011, 71, 89-98. https://doi.org/10.1016/j.envexpbot.2010.10.021.

Ramírez, J.G.; Castañeda, D.A.; Morales, J.G. Estudios etiológicos de la marchitez del aguacate en Antioquia-Colombia. Revista Ceres 2014, 61, 50-61. https://doi.org/10.1590/S0034-737X2014000100007.

Ramírez-Gil, J.G.; Castañeda-Sánchez, D.A.; Morales-Osorio, J.G. Production of avocado trees infected with *Phytophthora cinnamomi* under different management regimes. Plant Pathol. 2017, 66, 623-632. https://doi.org/10.1111/ppa.12620.

Ramirez-Suero, M.; Bénard-Gellon, M.; Chong, J.; Laloue, H.; Stempien, E.; Abou-Mansour, E.; Fontaine, F.; Larignon, P.; Mazet-Kieffer, F.; Farine, S.; et al. Extracellular compounds produced by fungi associated with *Botryosphaeria* dieback induce differential defence gene expression patterns and necrosis in *Vitis vinifera* cv. Chardonnay cells. Protoplasma 2014, 251, 1417-1426. https://doi.org/10.1007/s00709-014-0643-y.

Ramírez-Tejero, J.A.; Jiménez-Ruiz, J.; Serrano, A.; Belaj, A.; León, L.; de la Rosa, R.; Mercado-Blanco, J.; Luque, F. Verticillium wilt resistant and susceptible olive cultivars express a very different basal set of genes in roots. BMC Genom. 2021, 22, 229. https://doi.org/10.1186/s12864-021-07545-x.

Rands, R.D. Stripe canker of Cinnamon caused by *Phytophthora cinnamomi* n. sp. Mededelingen van het Instituut voor Plantenziekten 1922, 54, 1-53.

Rani, L.; Thapa, K.; Kanojia, N.; Sharma, N.; Singh, S.; Grewal, A.S.; Srivastav, A.L.; Kaushal, J. An extensive review on the consequences of chemical pesticides on human health and environment. J. Clean. Prod. 2021, 283, 124657. https://doi.org/10.1016/j.jclepro.2020.124657.

Rebolledo, A.; Romero, M.A. Avances en investigación sobre el comportamiento productivo del aguacate (*Persea americana* Mill.) bajo condiciones subtropicales. Cienc. Tecnol. Agropecu. 2011, 12, 113-120. https://doi.org/10.21930/rcta.vol12_num2_art:220.

Reeksting, B.J.; Coetzer, N.; Mahomed, W.; Engelbrecht, J.; van den Berg, N. De novo sequencing, assembly, and analysis of the root transcriptome of *Persea americana* (Mill.) in response to *Phytophthora cinnamomi* and flooding. PLoS ONE 2014, 9, e86399. https://doi.org/10.1371/journal.pone.0086399.

Reeksting, B.J.; Olivier, N.A.; van den Berg, N. Transcriptome responses of an ungrafted Phytophthora root rot tolerant avocado (*Persea americana*) rootstock to flooding and *Phytophthora cinnamomi.* BMC Plant Biol. 2016, 16, 205. https://doi.org/10.1186/s12870-016-0893-2.

Reeksting, B.J.; Taylor, N.J.; van den Berg, N. Flooding and *Phytophthora cinnamomi*: Effects on photosynthesis and chlorophyll fluorescence in shoots of non-grafted *Persea* *americana* (Mill.) rootstocks differing in tolerance to Phytophthora root rot. S. Afri. J. Bot. 2014, 95, 40-53. https://doi.org/10.1016/j.sajb.2014.08.004.

Reyes-Herrera, P.H.; Muñoz-Baena, L.; Velásquez-Zapata, V.; Patiño, L.; Delgado-Paz, O.A.; Díaz-Diez, C.A.; Navas-Arboleda, A.A.; Cortés, A.J. Inheritance of Rootstock Effects in Avocado (*Persea americana* Mill.) cv. Hass. Front. Plant Sci. 2020, 11, 555071. https://doi.org/10.3389/fpls.2020.555071.

Reyes-Santamaría, I.; Terrazas, T.; Barrientos-Priego, A.F.; Trejo, C. Xylem conductivity and vulnerability in cultivars and races of avocado. Sci. Hortic. 2002, 92, 97-105. https://doi.org/10.1016/S0304-4238(01)00284-9.

Rocha-Arroyo, J.L.; Salazar-García, S.; Bárcenas-Ortega, A.E.; González-Durán, I.J.L., Cossio-Vargas, L.E. Fenología del aguacate 'Hass' en Michoacán. Revista mexicana de ciencias agrícolas 2011, 2, 303-316.

Rohwer, C.L.; Erwin, J.E. Horticultural applications of jasmonates. J. Hortic. Sci. Biotech. 2008, 83, 283-304. https://doi.org/10.1080/14620316.2008.11512381.

Rondon, T.; Guzmán-Hernández, M.; Torres-Madronero, M.C.; Casamitjana, M.; Cano, L.; Galeano, J.; Goez, M. Comparative analysis of water stress regimes in avocado plants during the early development stage. Plants 2024, 13, 2660. https://doi.org/10.3390/plants13182660.

Ruano-Rosa, D.; Arjona-Girona, I.; López-Herrera, C.J. Integrated control of avocado white root rot combining low concentrations of fluazinam and *Trichoderma* spp. Crop Prot. 2018, 112, 363-370. https://doi.org/10.1016/j.cropro.2017.06.024.

Ruano-Rosa, D.; Cazorla, F.M.; Bonilla, N.; Martín-Pérez, R.; De Vicente, A.; López-Herrera, C.J. Biological control of avocado white root rot with combined applications of *Trichoderma* spp. and rhizobacteria. Eur. J. Plant Pathol. 2014, 138, 751-762. https://doi.org/10.1007/s10658-013-0347-8.

Ruano-Rosa, D.; del Moral-Navarrete, L.; López-Herrera, C.J. Selection of *Trichoderma* spp. isolates antagonistic to *Rosellinia necatrix*. Spanish Journal of Agricultural Research 2010, 8, 1084-1097. https://doi.org/10.5424/sjar/2010084-1403.

Ruano-Rosa, D.; Schena, L.; Ippolito, A.; López-Herrera, C.J. Comparison of conventional and molecular methods for the detection of Rosellinia necatrix in avocado orchards in southern Spain. Plant Pathol. 2007, 56, 2, 251-256. https://doi.org/10.1111/j.1365-3059.2006.01546.x.

Ruban, A.V. Nonphotochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage. Plant Physiol. 2016, 170, 1903-1916. https://doi.org/10.1104/pp.15.01935.

Salazar-García, S.; González-Durán, I.J.; Ibarra-Estrada, M.E. Identification of the appropriate leaf sampling period for nutrient analysis in 'Hass' avocado. HortScience 2015, 50, 130-136. https://doi.org/10.21273/HORTSCI.50.1.130.

Salazar-García, S.; Medina-Torres, R.; Ibarra-Estrada, M.E.; González-Valdivia, J. Influence of clonal rootstocks on leaf nutrient concentrations in 'Hass' avocado grown without irrigation. Rev. Chapingo Serie Hortic. 2016, 22, 161-175. https://doi.org/10.5154/r.rchsh.2015.06.013.

Salehi, M.; Moieni, A.; Safaie, N.; Farhadi, S. Whole fungal elicitors boost paclitaxel biosynthesis induction in *Corylus avellana* cell culture. PLoS ONE 2020, 15, e0236191. https://doi.org/10.1371/journal.pone.0236191.

Salleo, S.; lo Gullo, M.A.; de Paoli, D.; Zippo, M. Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: A possible mechanism. New Phytol. 1996, 132, 47-56. https://doi.org/10.1111/j.1469-8137.1996.tb04507.x.

Sánchez-Chávez, E.; Barrera-Tovar, R.; Muñoz-Márquez, E.; Ojeda-Barrios, D.L.; Anchondo-Nájera, A. Efecto del ácido salicílico sobre biomasa, actividad fotosintética, contenido nutricional y productividad del chile jalapeño. Rev. Chapingo Ser. Hortic. 2011, 17, 63-68.

Saniewski, M.; Miyamoto, K.; Ueda, J. Methyl jasmonate induces gums and stimulates anthocyanin accumulation in peach shoots. J. Plant Growth Regul. 1998, 17, 121-124. https://doi.org/10.1007/PL00007024.

Santos Casanova, B., Narváez Jurado, I., Martínez Ferri, E., Ribalta Cambos, L., Pliego Alfaro, F., Palomo Ríos, E., Pliego Prieto, C. Análisis transcriptómico de plantas de olivo tras la exposición al filtrado de *Rosellinia necatrix*. XXI Congreso de la Sociedad Española de Fitopatología, Córdoba, España, 2024

Sappl, P.G.; Carroll, A.J.; Clifton, R.; Lister, R.; Whelan, J.; Harvey Millar, A.; Singh, K.B. The Arabidopsis glutathione transferase gene family displays complex stress regulation and co-silencing multiple genes results in altered metabolic sensitivity to oxidative stress. Plant J. 2009, 58, 53-68. https://doi.org/10.1111/j.1365-313x.2008.03761.x.

Sappl, P.G.; Oñate-Sánchez, L.; Singh, K.B.; Millar, A.H. Proteomic analysis of glutathione S-transferases of *Arabidopsis thaliana* reveals differential salicylic acid-induced expression of the plant-specific phi and tau classes. Plant Mol. Biol. 2004, 54, 205-219. https://doi.org/10.1023/b:plan.0000028786.57439.b3.

Scholefield, P.B.; Sedgley, M.; Alexander, D.M. Carbohydrate cycling in relation to shoot growth, floral initiation and development and yield in the avocado. Sci. Hortic. 1985, 25:99-110. https://doi.org/10.1016/0304-4238(85)90081-0.

Schreiber, U.; Bilger, W.; Neubauer, C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. Pages 49-70 in: Ecophysiology of Photosynthesis. vol. 100. E.-D. Schulze and M. M. Caldwell, eds. Springer Study Edition, Berlin.

Schreiber, U.; Bilger, W.; Neubauer, C. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In Ecophysiology of Photosynthesis; Schulze, E.D., Caldwell, M.M., Eds.; Springer Study Edition; Springer: Berlin/Heidelberg, Germany, 1995; Volume 100, pp. 49-70.

Scora, W.R.; Wolstenholme, N.B.; Lavi, U. 2002. Taxonomy and botany. Pages: 15-37 in: The Avocado: Botany, Production and Uses. B. Schaffer, B.N. Wolstenholme, A.W. Whiley, eds. CAB International, Wallingford, Oxon, UK.

Selvaraj, K.; Fofana, B. 2012. An overview of plant photosynthesis modulation by pathogen attacks. Pages 466-484 in: Advances in Photosynthesis -Fundamental Aspects. CBS Publishers, Daryaganj, New Delhi.

Sharma, N.; Sharma, K.P.; Gaur, R.K.; Gupta, V.K. Role of chitinase in plant defense. Asian J. Biochem. 2011, 6, 29-37. https://doi.org/10.3923/ajb.2011.29.37.

Singh, R.; Sindhu, A.; Singal, H.R.; Singh, R. Biochemical basis of resistance in chickpea (*Cicer arietinum* L.) against Fusarium wilt. Acta Phytopathol. Entomol. Hung. 2003, 38, 13-19. https://doi.org/10.1556/aphyt.38.2003.1-2.3.

Sirhindi, G.; Mushtaq, R.; Gill, S.S.; Sharma, P.; Abd-Allah, E.F.; Ahmad, P. Jasmonic acid and methyl jasmonate modulate growth, photosynthetic activity and expression of photosystem II subunit genes in *Brassica oleracea* L. Sci. Rep. 2020, 10, 9322. https://doi.org/10.1038/s41598-020-65309-1.

Smith, C.E. Additional notes on pre-conquest avocados in Mexico. Econ. Bot. 1969, 23, 135-140. https://doi.org/10.1007/BF02860618.

Smith, C.E. Archeological evidence for selection in avocado. Econ. Bot. 1966, 20, 169-175. https://doi.org/10.1007/BF02904012.

Smith, L.A.; Dann, E.K.; Pegg, K.G.; Ehyley, A.W.; Giblin, F.R.; Doogan, V.; Kopittke, R. Field assessment of avocado rootstock selections for resistance to Phytophthora root rot. Australas. Plant Path. 2011, 40, 39-47. https://doi.org/10.1007/s13313-010-0011-0.

Smith, L.A.; Dann, E.K.; Pegg, K.G.; Whiley, A.W.; Giblin, F.R.; Doogan, V.; Kopittke, R. Field assessment of avocado rootstock selections for resistance to Phytophthora root rot. Australas. Plant Path. 2011, 40, 39–47. https://doi.org/10.1007/s13313-010-0011-0.

Spann, T. Advanced avocado rootstock selections move to commercial-scale field trials. From the Grove, Fall 2020, 16-17.

Srinivasan, T.; Kirti, P.B. Protease inhibitors and stress tolerance. Funct. Plant Sci. Biotechnol. 2012, 6, 59-66.

Srinivasan, T.; Kumar, K.R.R.; Kirti, P.B. Constitutive expression of a trypsin protease inhibitor confers multiple stress tolerance in transgenic tobacco. Plant Cell Physiol. 2009, 50, 541-553. https://doi.org/10.1093/pcp/pcp014.

Steduto, P.; Hsiao, T.C.; Fereres, E. On the conservative behavior of biomass water productivity. Irrig. Sci. 2007, 25, 189-207. https://doi.org/10.1007/s00271-007-0064-1.

Stout, A.B. A study in cross-pollination of avocado in southern California. California Avocado Association Annual Report 1923, 8, 29-45.

Svabova, L.; Lebeda, A. *In vitro* selection for improved plant resistance to toxin-producing pathogens. J. Phytopathol. 2005, 153, 52-64. https://doi.org/10.1111/j.1439-0434.2004.00928.x.

Szalai, G.; Kellos, T.; Galiba, G.; Kocsy, G. Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. J. Plant Growth Regul. 2009, 28, 66-80. https://doi.org/10.1007/s00344-008-9075-2.

Sztejnberg, A. 1994. *Rosellinia* (*Dematophora*) root rot. Pages: 80-81 in: Compendium of Tropical Fruit Diseases R.C. Ploetz, G.A. Zentmyer, W.T. Nishijima, H.D. Ohr, eds. APS Press, St Paul, Minnesota.

Sztejnberg, A.; Madar, Z. Host range of *Dematophora necatrix*, the cause of white root rot disease in fruit trees. Plant Dis. 1980, 64, 662-664. https://doi.org/10.1094/PD-64-662.

Tanaka, Y.; Sugano, S.S.; Shimada, T.; Hara-Nishimura, I. Enhancement of leaf photosynthetic capacity through increased stomatal density in Arabidopsis. New Phytol. 2013, 198, 757-764. http://doi.org/10.1111/nph.12186.

Teixeira de Sousa, A.J. Lutte contre *Rosellinia necatrix* (Hartig) Berlese, agent du «pourridié laineux»: Sensibilité de quelques espèces végétales et lutte chimique. Eur. J. For. Pathol. 1985, 15, 323-332. https://doi.org/10.1111/j.1439-0329.1985.tb01107.x.

ten Hoopen, G.M.; Krauss, U. Biology and control of *Rosellinia bunodes*, *Rosellinia necatrix* and *Rosellinia pepo*: A review. Crop Prot. 2006, 25, 89-107. https://doi.org/10.1016/j.cropro.2005.03.009.

Terry, L.A.; Joyce, D.C. Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. Postharvest Biol. Technol. 2004, 32, 1-13. https://doi.org/10.1016/j.postharvbio.2003.09.016.

Tesfay, S.Z.; Bertling, I.; Bower, J.P. Effects of postharvest potassium silicate application on phenolics and other anti-oxidant systems aligned to avocado fruit quality. Postharvest Biol. Technol. 2011, 60, 92-99. https://doi.org/10.1016/j.postharvbio.2010.12.011.

Thakur, M.; Sohal, B.S. Role of elicitors in inducing resistance in plants against pathogen infection: A review. ISRN Biochem. 2013, 762412. https://doi.org/10.1155/2013/762412.

Thomma, B.P.H.J.; Eggermont, K.; Broekaert, W.F.; Cammue, B.P.A. Disease development of several fungi on Arabidopsis can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem. 2000, 38, 421-427. https://doi.org/10.1016/S0981-9428(00)00756-7.

Tiwari, M.; Kidwai, M.; Dutta, P.; Narayan, S.; Gautam, N.; Chawda, K.; Shirke, P.A.; Mishra, A.K.; Chakrabarty, D. A tau class glutathione-S-transferase (OsGSTU5) confers tolerance against arsenic toxicity in rice by accumulating more arsenic in root. J. Hazard. Mater. 2022, 426, 128100. https://doi.org/10.1016/j.jhazmat.2021.128100.

Tobar, S.; Gil, P.M.; Schaffer, B.; Schwember, A.; Cautín, R.; Mártiz, J. Physiological and growth responses of W. Murcott Tangor grafted on four rootstocks under water restriction. Horticulturae 2024, 10, 352. https://doi.org/10.3390/horticulturae10040352.

Tucker, C.M. Report of the Plant Pathologist. Report of the Puerto Rico Agricultural Experiment Station 1929, 29-35.

Turc, B.; Sahay, S.; Haupt, J.; de Oliveira Santos, T.; Bai, G.; Glowacka, K. Up-regulation of non-photochemical quenching improves water use efficiency and reduces whole-plant water consumption under drought. J. Exp. Bot. 2024, 75, 3959-3972. https://doi.org/10.1093/jxb/erae113.

Uddling, J.; Gelang-Alfredsson, J.; Piikki, K.; Pleijel, H. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 2007, 91, 37-46. https://doi.org/10.1007/s11120-006-9077-5.

Untergasser, A.; Cutcutache, I.; Koressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. Primer3-new capabilities and interfaces. Nucleic Acids Res. 2012, 40, 115. https://doi.org/10.1093/nar/gks596.

Urdiales-Flores, D.; Zittis, G.; Hadjinicolaou, P.; Osipov, S.; Klingmüller, K.; Mihalopoulos, N.; Kanakidou, M.; Economou, T.; Lelieveld, J. Drivers of accelerated warming in

Mediterranean climate-type regions. npj Clim. Atmos. Sci. 2023, 6, 97. https://doi.org/10.1038/s41612-023-00423-1.

USDA (U.S. Department of Agriculture) Avocado, almond, pistachio and walnut Composition. Nutrient Data Laboratory. USDA National Nutrient Database for standard reference, release 24. Washington, DC: U.S. Department of Agriculture 2011. https://fdc.nal.usda.gov/fdc-app.html#/food-details/1102652/nutrients (accessed on 04 august 2024)

USDA (U.S. Department of Agriculture). https://www.mapa.gob.es/es/ministerio/servicios/informacion/aguacate_tcm30-103002.pdf.

Vadez, V.; Rao, S.; Sharma, K.K.; Bhatnagar-Mathur, P.; Devi, M.J. DREB1A allows for more water uptake in groundnut by a large modification in the root/shoot ratio under water deficit. J. SAT Agric. Res. 2007, 5, 1-5.

Vaghela, B.; Vashi, R.; Rajput, K.; Joshi, R. Plant chitinases and their role in plant defense: A comprehensive review. Enzyme Microb. Technol. 2022, 159, 110055. https://doi.org/10.1016/j.enzmictec.2022.110055.

van den Berg, N.; Christie, J.B.; Aveling, T.A.S.; Engelbrecht, J. Callose and β-1, 3-glucanase inhibit *Phytophthora cinnamomi* in a resistant avocado rootstock. Plant Pathol. 2018, 67, 1150-1160. https://doi.org/10.1111/ppa.12819.

van den Berg, N.; Mahomed, W.; Olivier, N.A.; Swart, V.; Crampton, B.G. Transcriptome analysis of an incompatible *Persea americana-Phytophthora cinnamomi* interaction reveals the involvement of SA- and JA-pathways in a successful defense response. PLoS ONE 2018, 13, e0205705. https://doi.org/10.1371/journal.pone.0205705.

van den Berg, N.; Swart, V.; Backer, R.; Fick, A.; Wienk, R.; Engelbrecht, J.; Prabhu, S.A. Advances in understanding defense mechanisms in *Persea americana* against *Phytophthora cinnamomi*. Front. Plant Sci. 2021, 12, 636339. https://doi.org/10.3389/fpls.2021.636339.

Van Rooyen, Z. 2011 New developments in horticultural research at Westfalia, South Africa. Proceedings of the VII World Avocado Congress, Cairns, Australia. https://www.avocadosource.com/wac7/section_09/vanrooyenz2011.pdf (accessed 07 October 2024).

Vasconsuelo, A.; Boland, R. Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Sci. 2007, 172, 861-875. https://doi.org/10.1016/j.plantsci.2007.01.006.

Verma, V.; Ravindran, P.; Kumar, P.P. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 2016, 16, 86. https://doi.org/10.1186/s12870-016-0771-y.

Vlot, A.C.; Dempsey, D.A.; Klessig, D.F. Salicylic acid, a multifaceted hormone to combat disease. Annu. Rev. Phytopathol. 2009, 47, 177-206. https://doi.org/10.1146/annurev.phyto.050908.135202.

von Caemmerer, S.; Farquhar, G.D. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 1981, 153, 376-387. https://doi.org/10.1007/BF00384257.

Vos, I.A.; Moritz, L.; Pieterse, C.M.J.; Van Wees, S.C.M. Impact of hormonal crosstalk on plant resistance and fitness under multi-attacker conditions. Front. Plant Sci. 2015, 6, 639. https://doi.org/10.3389/fpls.2015.00639.

Wally, O.; Jayaraj, J.; Punja, Z.K. Broad-spectrum disease resistance to necrotrophic and biotrophic pathogens in transgenic carrots (*Daucus carota* L.) expressing an *Arabidopsis* NPR1 gene. Planta 2009, 231, 131-141. https://doi.org/10.1007/s00425-009-1031-2.

Wang, C.; He, J.; Zhao, T.H.; Cao, Y.; Wang, G.; Sun, B.; Yan, X.; Guo, W.; Li, M.H. The smaller the leaf is, the faster the leaf water loses in a temperate forest. Front. Plant Sci. 2019, 10, 58. https://doi.org/10.3389/fpls.2019.00058.

Wang, Y.; Mostafa, S.; Zeng, W.; Jin, B. Function and mechanism of jasmonic acid in plant responses to abiotic and biotic stresses. Int. J. Mol. Sci. 2021, 22, 8568. https://doi.org/10.3390/ijms22168568.

Wardlaw, I.F. Transley Review No. 27 The control of carbon partitioning in plants. New Phytol. 1990, 116, 341-381. https://doi.org/10.1111/j.1469-8137.1990.tb00524.x.

Whalley, A.J.S. The xylariaceous way of life. Mycol. Res. 1996, 100, 897-922. https://doi.org/10.1016/S0953-7562(96)80042-6.

Whiley, A.W.; Schaffer, B. 1994. Avocado. Pages: 3-35 in: Handbook of environmental physiology of fruit crops. Vol 2. Sub-tropical and tropical crops. B. Schaffer, P.C. Anderson, eds. CRC Press, Boca Raton, Florida.

Whiley, A.W.; Whiley, D.G. 2011 Australian rootstock programme update. Proceedings of the VII World Avocado Congress, Cairns, Australia (abstract). https://www.avocadosource.com/wac7/Section_14/WhileyAnthony2011.pdf.

Wientjes, E.; Seijger, C. Less water in agriculture? Potential and challenges in optimizing water use efficiency. J. Exp. Bot. 2024, 75, 3754. https://doi.org/10.1093/jxb/erae227.

Willingham, S.L.; Pegg, K.G.; Anderson, J.M.; Cooke, A.W.; Dean, J.R.; Giblin, F.R.; Coates, L.M. Effect of rootstocks and nitrogen fertiliser on postharvest anthracnose development in Hass avocado. Australas. Plant Path. 2006, 35, 619-629. https://doi.org/10.1071/AP06062.

Willingham, S.L.; Pegg, K.G.; Cooke, A.W.; Coates, L.M.; Langdon, P.W.B.; Dean, J.R. Rootstock influences postharvest anthracnose development in 'Hass' avocado. Aust. J. Agr. Res. 2001, 52, 1017-1022. https://doi.org/10.1071/AR01015.

Witjaksono, Y.; Nugraheni, U.K.; Hoesen, D.H.; Litz, R.E. Regeneration from irradiated avocado (*Persea americana* Mill.) embryogenic cultures in: Induced Mutation in Tropical Fruit Trees, IAEA-TECDOC-1615. International Atomic Energy Agency, Vienna 2009, 83-90.

Wong, H.L.; Sakamoto, T.; Kawasaki, T.; Umemura, K.; Shimamoto, K. Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiol. 2004, 135, 1447-1456. https://doi.org/10.1104/pp.103.036384.

Xoca-Orozco, L.Á.; Aguilera-Aguirre, S.; Vega-Arreguín, J.; Acevedo-Hernández, G.; Tovar-Pérez, E.; Stoll, A.; Herrera-Estrella, L.; Chacón-López, A. Activation of the phenylpropanoid biosynthesis pathway reveals a novel action mechanism of the elicitor effct of chitosan on avocado fruit epicarp. Food Res. Int. 2019, 121, 586-592. https://doi.org/10.1016/j.foodres.2018.12.023.

Xoca-Orozco, L.Á.; Cuellar-Torres, E.A.; González-Morales, S.; Gutiérrez-Martínez, P.; López-García, U.; Herrera-Estrella, L.; Vega-Arreguín, J.; Chacón-López, A. Transcriptomic analysis of avocado Hass (*Persea americana* Mill) in the interaction system fruit-chitosan-*Colletotrichum*. Front. Plant Sci. 2017, 8, 956. https://doi.org/10.3389/fpls.2017.00956.

Xu, Z.; Zhou, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J. Exp. Bot. 2008, 59, 3317-3325. https://doi.org/10.1093/jxb/ern185.

Yang, Q.; Yang, L.; Wang, Y.; Chen, Y.; Hu, K.; Yang, W.; Zuo, S.; Xu, J.; Kang, Z.; Xiao, X.; et al. A High-Quality genome of *Rhizoctonia solani*, a devastating fungal pathogen with a wide host range. Mol. Plant-Microbe Interact. 2022, 35, 954-958. https://doi.org/10.1094/MPMI-06-22-0126-A.

Yordanov, I.; VelikoVa, V.; Tsonev, T. Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 2000, 38, 171-186. https://doi.org/10.1023/A:1007201411474.

Yoshida, C.H.P.; Pacheco, A.C.; Lapaz, A.d.M.; Gorni, P.H.; Vítolo, H.F.; Bertoli, S.C. Methyl jasmonate modulation reduces photosynthesis and induces synthesis of phenolic compounds in sweet potatoes subjected to drought. Bragantia 2020, 79, 319-334. https://doi.org/10.1590/1678-4499.20200203.

Yoshida, T.; Christmann, A.; Yamaguchi-Shinozaki, K.; Grill, E.; Fernie, A.R. Revisiting the basal role of ABA-roles outside of stress. Trends Plant Sci. 2019a, 24, 625-635. https://doi.org/10.1016/j.tplants.2019.04.008.

Yoshida, T.; Obata, T.; Feil, R.; Lunn, J.E.; Fujita, Y.; Yamaguchi-Shinozaki, K.; Fernie, A.R. The role of abscisic acid signaling in maintaining the metabolic balance required for Arabidopsis growth under nonstress conditions. Plant Cell 2019b, 31, 84-105. https://doi.org/10.1105/tpc.18.00766.

Young, D.H.; Michelotti, E.L.; Swindell, C.S.; Krauss, N.E. Antifungal properties of Taxol and various analogues. Experientia 1992, 48, 882-885. https://doi.org/10.1007/BF02118425.

Yu, X.; Zhang, W.; Zhang, Y.; Zhang, X.; Lang, D.; Zhang, X. The roles of methyl jasmonate to stress in plants. Funct. Plant Biol. 2018, 46, 197-212. https://doi.org/10.1071/fp18106.

Yuan, X.; Wang, H.; Cai, J.; Li, D.; Song, F. NAC transcription factors in plant immunity. Phytopathol. Res. 2019, 1, 3. https://doi.org/10.1186/s42483-018-0008-0.

Zafar, Z.; Rasheed, F.; Mushtaq, N.; Khan, M.U.; Mohsin, M.; Irshad, M.A.; Summer, M.; Raza, Z.; Gailing, O. Exogenous application of salicylic acid improves physiological and biochemical attributes of *Morus alba* saplings under soil water deficit. Forests 2023, 14, 236. https://doi.org/10.3390/f14020236.

Zentmyer, G.A. *Phytophthora cinnamomi* and the diseases it causes. Mongraph American Phytopathological Society 1980, 10.

Zentmyer, G.A. The duke avocado. Calif. Avocado Soc. Year. 1963, 47, 28-36.

Zentmyer, G.A. The origin of root rot resistant rootstocks. California Avocado Society Yearbook 1978, 62, 87-89.

Zhang, J.; Wang, F.; Liang, F.; Zhang, Y.; Ma, L.; Wang, H.; Liu, D. Functional analysis of a pathogenesis-related thaumatin-like protein gene TaLr35PR5 from wheat induced by leaf rust fungus. BMC Plant Biol. 2018, 18, 76. https://doi.org/10.1186/s12870-018-1297-2.

Zhang, L.; Marguerit, E.; Rossdeutsch, L.; Ollat, N.; Gambetta, G.A. The influence of grapevine rootstocks on scion growth and drought resistance. Theor. Exp. Plant Phy. 2016, 28, 143-157. https://doi.org/10.1007/s40626-016-0070-x.

Zhang, S.; Li, Q.; Ma, K.; Chen, L. Temperature-dependent gas exchange and stomatal/non-stomatal limitation to CO_2 assimilation of *Quercus liaotungensis* under midday high irradiance. Photosynthetica 2001, 39, 383-388. https://doi.org/10.1023/A:1015130326683.

Zheng, F.; Chen, L.; Zhang, P.; Zhou, J.; Lu, X.; Tian, W. Carbohydrate polymers exhibit great potential as effective elicitors in organic agriculture: A review. Carbohyd. Polym. 2020, 230, 115637. https://doi.org/10.1016/j.carbpol.2019.115637.

Zumaquero, A.; Kanematsu, S.; Nakayashiki, H.; Matas, A.; Martínez-Ferri, E.; Barceló-Muñoz, A.; Pliego-Alfaro, F.; López-Herrera, C.; Cazorla, F.M.; Pliego, C. Transcriptome analysis of the fungal pathogen *Rosellinia necatrix* during infection of a susceptible avocado rootstock identifies potential mechanisms of pathogenesis. BMC Genom. 2019a, 20, 1016. https://doi.org/10.1186/s12864-019-6387-5.

Zumaquero, A.; Martínez-Ferri, E.; Matas, A.J.; Reeksting, B.; Olivier, N.A.; Pliego-Alfaro, F.; Barceló, A.; van den Berg, N.; Pliego, C. *Rosellinia necatrix* infection induces differential gene expression between tolerant and susceptible avocado rootstocks. PLoS ONE 2019b, 14, e0212359. https://doi.org/10.1371/journal.pone.0212359.

Curriculum vitae

Esta Tesis Doctoral ha sido realizada en el Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA) centro IFAPA de Málaga, y dirigida por las Dras. Clara Pliego Prieto y Elsa Martínez Ferri pertenecientes a las Áreas de Mejora Vegetal y Biotecnología y de Recursos Naturales y Forestales, respectivamente.

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El trabajo realizado durante el periodo de desarrollo de esta tesis doctoral ha resultado en:

Publicaciones en revistas:

1. **Moreno-Pérez, A.**; Martínez-Ferri, E.; Pliego-Alfaro, F.; Pliego, C. Elicitors and Plant Defence. JOJ Hortic Arboric. 2020, 2,5, 555600. http://doi.org/10.19080/JOJHA.2020.19.555600.
2. **Moreno-Pérez, A.**; Zumaquero, A.; Martínez-Ferri, E.; López-Herrera, C.; Pliego-Alfaro, F.; Palomo-Ríos, E.; Pliego, C. A Comparative Transcriptome Analysis of Avocado Embryogenic Lines Susceptible or Resistant to *Rosellinia necatrix* Exudate. Agronomy 2023; 13, 1354. https://doi.org/10.3390/agronomy13051354.
3. **Moreno-Pérez, A.**; Martínez-Ferri, E.; van den Berg, N.; Pliego, C. Effects of Exogenous Application of Methyl Jasmonate and Salicylic Acid on the Physiological and Molecular Response of 'Dusa' Avocado to *Rosellinia necatrix*. Plant Dis. 2024. https://doi.org/10.1094/PDIS-11-23-2316-RE.
4. **Moreno-Pérez, A.**; Barceló, A.; Pliego, C.; Martínez-Ferri, E. Water Relations and Physiological Response to Water Deficit of 'Hass' Avocado Grafted on Two Rootstocks Tolerant to *R. necatrix*. Agronomy 2024, 14(9), 1959. https://doi.org/10.3390/agronomy14091959.

Otras publicaciones:

1. Martínez-Ferri, E.; Moreno-Ortega, G.; **Moreno-Pérez, A.**; Sarmiento, D.; Pliego, C. Manejo del área de mojado para mejorar la eficiencia de riego en plantaciones de aguacate con dotaciones de agua limitadas: avance de resultados. ECAFRUITS. 2021; 15: 44-47.
2. Martínez-Ferri, E.; Moreno-Ortega, G.; **Moreno-Pérez, A.**; Sarmiento, D.; Pliego, C. Consideraciones en el diseño de un proyecto de aguacate sostenible. Publicación del estudio sobre el cultivo del aguacate llevado a cabo por Agbar Agriculture junto con el IFAPA-Málaga. 2022.

Participación y comunicaciones a jornadas y congresos:

1. **Moreno-Pérez, A.**; Zumaquero, A.; Martínez-Ferri, E.; Barceló-Muñoz, A.; López-Herrera, C.; Pliego-Alfaro, F.; Palomo-Ríos, E.; Pliego, C. Póster: Transcriptome analysis of avocado embriogenic cultures selected for resistance to *Rosellinia necatrix* exudates. XV Meeting of Plant Molecular Biology. 26-27 noviembre 2020.

2. **Moreno-Pérez, A.**; Moreno-Ortega, G.; Pliego, C.; Martínez-Ferri, E. Póster: Physiological effects of exogenous application of methyl jasmonate and salicylic acid in avocado. 2nd PhD Meeting in Plant Science. 6 julio 2021.
3. **Moreno-Pérez, A.**; Moreno-Ortega, G.; Pliego, C.; Martínez-Ferri, E. Póster: Physiological effects of exogenous application of methyl jasmonate and salicylic acid in avocado. XXIV Reunión de la Sociedad Española de Biología de Plantas y el XVII Congreso Hispano-Luso de Biología de Plantas. 7-9 julio 2021
4. **Moreno-Pérez, A.**; Moreno-Ortega, G.; Martínez-Ferri, E.; Pliego, C. Póster: "Effects of exogenous application of methyl jasmonate and salicylic acid in avocado physiology and control of white root rot disease". III Jornada de seguimiento del Programa de Doctorado de Biotecnología Avanzada. 29 abril 2022.
5. **Moreno-Pérez, A.**; Moreno-Ortega, G.; Cazorla, F.; Martínez-Ferri, E.; Pliego, C. Póster: "Physiological and molecular responses of avocado following methyl jasmonate and salicylic acid applications: effects in the control of white root rot disease". XX Congreso de la Sociedad Española de Fitopatología (SEF), 24-26 octubre 2022.
6. **Moreno-Pérez, A.**; Guirado-Manzano, L.; Fernández-Pozo, N.; Cazorla, F.; Fernández-Ortuño, D.; Arrebola, E.; Pliego, C. Póster: "Análisis transcriptómico de la interacción *Neofusicoccum luteum* con rama y fruto de aguacate" XX Congreso de la Sociedad Española de Fitopatología (SEF), 24-26 octubre 2022.
7. Guirado-Manzano, L.; **Moreno-Pérez, A.**; Fernández-Pozo, N.; Cazorla, F.; Fernández-Ortuño, D.; Arrebola, E.; Pliego, C Póster "Transcriptome análisis of *Neofusicoccum luteum* during avocado branch and fruit infection". 16th European Conference of fungal genetics, 5- 8 marzo de 2023.
8. **Moreno-Pérez, A.**; Martínez-Ferri, E.; Pliego, C. Comunicación oral: "Estudios moleculares y fisiológicos de portainjertos de aguacate sometidos a estrés biótico y abiótico". IV Jornada de seguimiento del programa de doctorado de Biotecnología Avanzada. 5 mayo 2023
9. Pliego, C.; Narváez, I.; **Moreno-Pérez, A.**; Mercado, J.A.; Pliego-Alfaro, F.; Palomo-Ríos, E. Póster "Caracterización de variantes somaclonales de olivo obtenidos tras la exposición al filtrado crudo del hongo *Rosellinia necatrix*". XV Reunión de la Sociedad Española de cultivo *in vitro* de tejidos vegetales (SECIVTV), 6-8 septiembre 2023.

Estancia internacional:

Estancia en el Forestry and Agriculture Biotechnology Institute en Pretoria (Sudáfrica) bajo la supervisión de la Dra. Noëlani van den Berg. Del 15 de septiembre a 15 de diciembre de 2022 con una duración de 3 meses.

Docencia:

Participación de la actividad docente de la asignatura Fisiología Vegetal I, Grado de Biología, Universidad de Málaga (UMA) en el curso académico 2021/2022 (24h).

Actividades de Divulgación:

1. Café con Ciencia. Actividad “Café con Ciencia” del Centro IFAPA-MÁLAGA desarrollando el tema: Agua y Agricultura (adaptaciones de las plantas para ahorrar agua), celebrado el día 22 de noviembre del 2019 con una duración de 5 horas.
2. La Noche Europea de los Investigadores. Actividad de divulgación organizada por la Fundación DESQBRE el día 27 de noviembre de 2020 en la que se participó con la elaboración de un video titulado ‘Técnica de PCR para la identificación de enfermedades en plantas y humanos’.
3. Organización e impartición de actividad de divulgación científica en el CDP Los Rosales los años 2022 y 2023.
4. Actividad formativa “Gestión Integrada de plagas y enfermedades en aguacate y mango” organizada por el IFAPA de Málaga los días 22-26 mayo 2023.