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ALGUNOS ENFOQUES CONVENCIONALES Y MODERNOS ENCAMINADOS A LA MEJORA DE PORTAINJERTOS DE AGUACATE (*Persea americana* Mill.) PARA TOLERANCIA A DÉFICIT HÍDRICO DEL SUELO

TESIS

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Presenta:
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Bajo la supervisión de: Dr. Alejandro Facundo Barrientos Priego,

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ALGUNOS ENFOQUES CONVENCIONALES Y MODERNOS ENCAMINADOS A LA MEJORA DE PORTAINJERTOS DE AGUACATE (*Persea americana* Mill.) PARA TOLERANCIA A DÉFICIT HÍDRICO DEL SUELO.

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CONTENIDO

LISTA DE CUADROS

Capítulo 3

Capítulo 4

LISTA DE FIGURAS

Capítulo 3

Capítulo 4

DEDICATORIAS

Dedico mi tesis a Doña Mary y a mi querido amigo Don Chebo (†), quien siempre permanecerá en mi mente y mi corazón.

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RESUMEN GENERAL

ALGUNOS ENFOQUES CONVENCIONALES Y MODERNOS ENCAMINADOS A LA MEJORA DE PORTAINJERTOS DE AGUACATE (*Persea americana* Mill.) PARA TOLERANCIA A DÉFICIT HÍDRICO DEL SUELO

México, principal productor y exportador mundial de aguacate, enfrenta crecientes desafíos de sostenibilidad debido a la alta demanda hídrica del cultivo, especialmente en regiones como Michoacán y Jalisco. El cambio climático, con tendencias hacia mayor temperatura y menor precipitación, amenaza con reducir las áreas aptas para el cultivo, lo cual exige estrategias de adaptación como la selección de portainjertos tolerantes a la sequía. Esta investigación integró dos estudios enfocados en la evaluación de 18 accesiones del género *Persea* con potencial como portainjertos, mediante análisis fisiológicos, anatómicos y de eficiencia en el uso del agua bajo condiciones de déficit hídrico. En el primer estudio, las accesiones fueron sometidas a un estrés hídrico severo, por 70 horas, seguido de rehidratación. Se evaluaron variables como asimilación de CO_2, conductancia estomática, tasa de transpiración, defoliación y conductividad hidráulica. Se identificaron genotipos con baja defoliación y alta conductividad hidráulica (G8, G10, G13), propuestos como candidatos prometedores para climas áridos. El segundo estudio evaluó las mismas accesiones injertadas con 'Hass', en condiciones de riego, déficit hídrico y recuperación. Se identificaron tres grupos de respuesta, de los cuales destacó el Grupo 3 por mantener actividad fisiológica durante la sequía, alta recuperación de asimilación de CO_2 (+157 %), baja resistencia hidráulica y alta eficiencia en uso del agua (6.11 $\mu mol \cdot mmol^{-1}$). El genotipo G8 ('M-14') se posicionó como el más prometedor por sus mecanismos coordinados de regulación estomática y recuperación post-estrés. En conjunto, los resultados de este estudio demuestran una alta variabilidad genética en la tolerancia al estrés hídrico, y subrayan la relevancia de indicadores como la defoliación, la conductividad hidráulica y la eficiencia en el uso del agua para la selección de portainjertos adaptados a condiciones de déficit hídrico.

Palabras clave: *Persea americana* Mill., déficit hídrico, eficiencia en el uso del agua, conductividad hidráulica.

Tesis de Doctorado, Doctorado en Ciencias en Horticultura, Universidad Autónoma Chapingo

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GENERAL ABSTRACT

SOME CONVENTIONAL AND MODERN APPROACHES AIMED AT IMPROVING AVOCADO ROOTSTOCKS (*Persea americana* Mill.) FOR TOLERANCE TO SOIL WATER DEFICIT

Mexico, the world's foremost producer and exporter of avocados, is facing mounting sustainability challenges due to the crop's substantial water requirements, particularly in key production areas such as Michoacán and Jalisco. The ongoing climate change, characterized by rising temperatures and declining precipitation, poses a significant threat to the availability of suitable cultivation areas. This underscores the necessity for the development of adaptive strategies, including the selection of drought-tolerant rootstocks. This study employed a dual research approach, integrating physiological, anatomical, and water-use efficiency assessments under water-deficit conditions to evaluate 18 accessions of the genus *Persea* with potential as rootstocks. In the initial experiment, the accessions were subjected to severe water stress for a duration of 70 hours, subsequently followed by a rehydration process. The study measured several key parameters, including CO_2 assimilation, stomatal conductance, transpiration rate, defoliation, and hydraulic conductivity. Genotypes demonstrating low defoliation and high hydraulic conductivity (G8, G10, G13) were identified as promising candidates for arid environments. The second experiment assessed the same accessions grafted with 'Hass' under three conditions: irrigation, water deficit, and recovery. The analysis identified three distinct response groups, with Group 3 demonstrating superior performance by sustaining physiological activity during drought, high CO_2 assimilation recovery (+157 %), low hydraulic resistance, and enhanced water-use efficiency (6.11 µmol·mmol-1). It is noteworthy that the G8 (‘M-14’) genotype demonstrated the greatest resilience, a consequence of its coordinated stomatal regulation and rapid post-stress recovery. All together, these findings reveal significant genetic variability in water stress tolerance among *Persea* accessions and emphasize critical selection criteria such as defoliation resistance, hydraulic conductivity, and water-use efficiency for the selection of rootstocks adapted to water deficit conditions

Keywords: *Persea americana* Mill., water deficit, water-use efficiency, hydraulic conductivity

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1. INTRODUCCIÓN GENERAL

El cultivo de aguacate (*Persea americana* Mill.) es de gran relevancia económica, ecológica y social para México, país que lidera la producción mundial con una participación cercana a 24 % del volumen global (FAOSTAT, 2025; SIAP, 2025). Sin embargo, esta industria enfrenta serios desafíos debido a su alta demanda hídrica y sensibilidad al estrés por déficit de agua, condiciones que se agravan bajo los escenarios actuales y futuros de cambio climático (Yang et al., 2024). En regiones clave como Michoacán y Jalisco, donde se concentra más del 85 % de la producción nacional (SIAP, 2025), se proyecta una disminución significativa de la superficie apta para el cultivo debido al incremento en la temperatura, reducción de lluvias y mayor frecuencia de sequías (Charre-Medellín et al., 2021). Esto compromete no solo la productividad, sino también la sostenibilidad ambiental del sistema agrícola aguacatero, al incentivar la expansión hacia zonas forestales con impactos ecológicos negativos (Borrego & Allende, 2021; Denvir et al., 2022; Subercaseaux-Ugarte et al., 2025).

Frente a esta problemática, la selección y desarrollo de portainjertos de aguacate con tolerancia a sequía emerge como una estrategia prioritaria. Los portainjertos influyen de manera directa en la eficiencia en el uso del agua, la regulación estomática, la resistencia hidráulica y la capacidad de recuperación post-estrés del injerto (Crane et al., 2013; Warschefsky et al., 2016). En México, existe una amplia variabilidad genética aún subutilizada, proveniente de razas nativas y criollas locales, con potencial adaptativo a condiciones de escasez hídrica (Gutiérrez-Díez et al., 2015; Lahav & Lavi, 2013; Bedoya-Ramírez et al., 2023).

No obstante, actualmente no se dispone de portainjertos comerciales con tolerancia comprobada a la sequía, por lo que se requieren estudios experimentales que permitan identificar materiales promisorios para su inclusión

en programas de mejoramiento y sistemas de producción más resilientes (Herrera-González et al., 2013; Salazar-García et al., 2004).

La presente investigación se desarrolló con el objetivo general de evaluar la respuesta fisiológica, anatómica y de eficiencia en el uso del agua de diferentes accesiones de aguacate bajo condiciones de déficit hídrico, para identificar genotipos con características deseables para su uso como portainjertos tolerantes a la sequía. Se planteó la hipótesis de que existen diferencias significativas entre accesiones de aguacate en cuanto a su capacidad de adaptación al estrés hídrico, determinadas por variables como la conductividad hidráulica, la eficiencia en el uso del agua y la recuperación de asimilación de CO_2 tras el reabastecimiento de agua.

Para abordar el objetivo anterior se presenta en el Capítulo 2 una revisión de literatura relacionada con la selección de portainjertos tolerantes a sequía, que incluye los mecanismos de adaptación y metodologías de selección aplicadas en estudios previos. El Capítulo 3, se muestra la evaluación de dieciocho accesiones de aguacate bajo condiciones controladas de estrés hídrico, a partir de un enfoque en variables morfofisiológicas y anatómicas como la conductividad hidráulica, porcentaje de defoliación y características de xilema. Por su parte, en el Capítulo 3 se analizó la respuesta de las mismas accesiones injertadas con la variedad Hass durante las fases de riego, estrés y recuperación, a partir de variables como asimilación de CO_2, resistencia estomática y eficiencia en el uso del agua. Los resultados obtenidos evidenciaron una alta variabilidad en las respuestas de las accesiones, y permitieron identificar genotipos con mejor desempeño bajo déficit hídrico, entre los cuales destaca la accesión G8 (‘M-14’). Esta información constituye una base sólida para su evaluación a nivel de campo y eventual uso en programas de mejoramiento de portainjertos adaptados a escenarios de menor disponibilidad hídrica.

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2. REVISIÓN DE LITERATURA

2.1. Introducción

El aguacate (*Persea americana* Mill.) es un frutal de alta demanda comercial y creciente importancia en la agricultura mundial (Cheptoo et al., 2025). México es el principal productor global con 2.9 millones de tonelada anuales (SIAP, 2025) y una contribución del 24 % de la producción mundial (FAOSTAT, 2025). Sin embargo, su marcada sensibilidad el estrés hídrico constituye una limitante critica para su productividad, especialmente bajo los escenarios de cambio climático que pronostican un incremento en la frecuencia e intensidad de sequías (Yang et al., 2024). Estudios recientes demuestran que este estrés reduce los rendimientos, principalmente mediante la disminución del tamaño de los frutos (Kaneko et al., 2024), efecto que puede agravarse por la alta demanda hídrica del cultivo (800 a 1500 $m^3 \cdot t^{-1}$) (Fuerte-Velázquez & Gómez-Tagle, 2024), su baja eficiencia en el uso del agua y, transpiración elevada (Michelakis et al., 1993; Carr, 2013).

Michoacán y Jalisco concentran el 75.7 y 10.8 % de la producción nacional de aguacate, respectivamente (SIAP, 2025) y estudios recientes alertan que entre el 30 y 43 % de la superficie cultivada en las zonas aguacateras serán afectadas por los efectos del cambio climático (Charre-Medellín et al., 2021).

Esto provocaría un incremento en la demanda de evapotranspiración y una posible disminución del agua disponible en el suelo, que repercutirá en una mayor demanda de agua para mantener el rendimiento (Gómez-Tagle et al., 2022), y podría propiciar el cambio del cultivo a nuevas áreas boscosas con clima templado (Charre-Medellín et al., 2021)

Sin embargo, existe preocupación de los impactos ecológicos de estos posibles cambios de las regiones de cultivo (Denvir et al., 2022) pues se prevén efectos adversos en los procesos hidrológicos por la deforestación y la pérdida de biodiversidad, así como la erosión y degradación del suelo (Borrego & Allende, 2021; Subercaseaux-Ugarte et al., 2025).

En este contexto, la selección y uso de portainjertos tolerantes a sequía es una estrategia clave para mitigar los efectos del déficit hídrico y aumentar la resiliencia del cultivo en las regiones productoras de México. Los portainjertos no solo determinan el anclaje y la capacidad de exploración del suelo, sino que también influyen en la absorción y transporte de agua y nutrientes, en la fisiología del injerto y la regulación de respuestas adaptativas al estrés (Crane et al., 2013; Warschefsky et al., 2016).

Tradicionalmente en los sistemas de producción de aguacate se utilizan portainjertos de semillas, predominantemente de la raza mexicana, por lo que existe una gran variabilidad en su respuesta hídrica (Salazar-García et al., 2004), aunque algunas investigaciones han explorado las respuestas de genotipos nativos al déficit hídrico (Herrera-González et al., 2013), aun no se dispone de portainjertos con tolerancia a sequía que puedan aprovecharse de manera comercial en las áreas de producción. México, como centro de origen del cultivo del aguacate posee una amplia diversidad genética (Gutiérrez-Díez et al., 2015) presente en las razas botánicas y variantes criollas locales, muchas de las cuales han evolucionado bajo condiciones de escasez hídrica (Lahav & Lavi, 2013; Bedoya-Ramírez et al., 2023).

Esta revisión analiza los mecanismos, metodologías y avances recientes en la selección de portainjertos tolerantes a sequía, con los objetivos de sistematizar los mecanismos de tolerancia a nivel morfo-anatómico, bioquímico, molecular y analizar las metodologías de selección aplicadas en estudios previos.

2.2. Impacto del estrés hídrico en el cultivo de aguacate

En general, el aguacate es un cultivo con un sistema de raíces poco profundo, aunque puede extenderse hasta 1.5 m de profundidad, la mayoría de las raíces absorbentes se encuentran superficialmente (Carr, 2013). Por lo que el déficit hídrico es uno de los factores abióticos más limitantes para el rendimiento del aguacate (Beyá-Marshall et al., 2022), ya que presenta una limitada capacidad de exploración del suelo al mantener más del 70 % de sus raíces distribuidas en menos de 60 cm de profundidad (Lahav & Whiley, 2013; Erazo-Mesa et al., 2022). Esta afectación al rendimiento es provocada por la alteración de diversos procesos fisiológicos, bioquímicos y de desarrollo (Moreno-Ortega et al., 2021).

En condiciones de escasez de humedad en el suelo, las plantas de aguacate experimentan una disminución en la tasa de asimilación de CO_2, reducción en la conductancia estomática (Chartzoulakis et al., 2002), alteraciones en el balance hormonal y producción de especies reactivas de oxígeno (ROS), lo que conduce a un estrés oxidativo si no se activa una respuesta antioxidante eficiente (Cárceles et al., 2023).

A nivel morfológico se han documentado la reducción del área foliar y el cierre estomático como mecanismos de adaptación al déficit hídrico en aguacate, estos mecanismos buscan limitar la pérdida de agua por transpiración, no obstante, comprometen simultáneamente la fijación de dióxido de carbono y, por lo tanto, la acumulación de biomasa (Michelakis et al., 1993; Carr, 2013). En algunas investigaciones se ha demostrado que los cultivares de aguacate pueden presentar respuestas contrastantes ante la sequía al ser injertados en distintos portainjertos, lo que manifiesta la influencia del sistema radical en la capacidad de adaptación a la escasez de agua (Moreno-Ortega et al., 2021; Bedoya-Ramírez et al., 2023).

También, se ha demostrado que el estrés hídrico afecta directamente el crecimiento de las raíces y la absorción de nutrientes, lo que reduce la eficiencia en el uso del agua (Carr, 2013) y puede comprometer el crecimiento en distintas

etapas fenológicas como floración, cuajado y desarrollo del fruto (Rocha-Arroyo et al., 2011). Estas alteraciones, en última instancia se traducen en pérdidas económicas, especialmente en sistemas de producción de secano (Sommaruga & Eldridge, 2020).

La magnitud de las afectaciones en la producción de aguacate por déficit hídrico es variable y depende de la etapa fenológica en la que se presente, por ejemplo, la falta de agua en la floración provoca disminución en la viabilidad del polen y el amarre de frutos (Lovatt, 1990). La polinización inadecuada es un problema critico que incide en el rendimiento de los huertos (Alcaraz & Hormaza, 2024), en plantaciones con estrés hídrico el rendimiento se reduce entre 32 y 36 % en comparación de las plantas que disponen de humedad suficiente (Silber et al., 2019). Si este estrés ocurre durante el crecimiento de los frutos pueden presentarse disminuciones significativas en su tamaño (Moreno-Ortega et al., 2019), y los frutos además de ser más pequeños pueden presentar oscurecimiento enzimático del mesocarpio que acorta su calidad postcosecha (Bower et al., 1989). Por otra parte, se ha informado que las plantas bajo estrés son más susceptibles a patógenos y plagas, lo que agrava las pérdidas económicas (Ramirez-Guerrero et al., 2023). Por ejemplo, en los periodos de sequía las plantas suelen presentar un incremento en la incidencia de ácaros (Reyes-Bello et al., 2011), tisanópteros (“trips”) y escamas (Ramírez-Gil et al., 2023).

Incluso en etapa de vivero, el déficit hídrico afecta el crecimiento de las plantas y produce una mayor acumulación de carbono en tallo y raíces mientras que se reduce el área foliar (Rondon et al., 2024).

Desde un enfoque agronómico se han evaluado y propuesto estrategias como el uso de acolchado, cubiertas vegetales, el riego deficitario controlado y el uso de compuestos bioestimulantes para disminuir los efectos negativos del estrés hídrico (Aguirre-Arcos et al., 2022; Durán et al., 2021; Sleighter et al., 2023). No obstante, la selección de portainjertos con mayor tolerancia al déficit hídrico

representa una estrategia más efectiva y sostenible a largo plazo (Moreno-Pérez et al., 2024).

2.3. Papel de los portainjertos tolerantes a sequía

Los portainjertos desempeñan un lugar fundamental en la adaptación de las especies frutales a condiciones abióticas adversas (Nimbolkar et al., 2016), entre las cuales la sequía representa una de la más preocupantes y limitantes de la productividad (Iqbal et al., 2020). Los portainjertos, como componentes del sistema injerto-portainjerto no solo aportan soporte físico y acceso a los recursos edáficos, sino que modulan importantes funciones fisiológicas, bioquímicas y moleculares que influyen en el desarrollo, la eficiencia hídrica y el rendimiento del cultivo (Dhurve et al., 2023). La elección adecuada del portainjerto permite establecer sistemas agrícolas más resilientes frente a la variabilidad climática, especialmente en regiones con recursos limitados o regímenes de lluvias irregulares (Warschefsky et al., 2016).

Desde el punto de vista fisiológico los portainjertos tolerantes a sequía pueden contribuir al mantenimiento del balance hídrico de la planta al mejorar la eficiencia en la absorción de agua, regular la apertura estomática y mantener la turgencia celular (Nimbolkar et al., 2016). Estas respuestas permiten preservar el intercambio de gases y la actividad fotosintética bajo condiciones de estrés y en consecuencia reducir la pérdida de rendimiento asociada a la exposición a periodos de déficit hídrico (Ranjbar et al., 2021). Aunque las respuestas pueden variar entre especies y combinaciones injerto-portainjerto específicas, estudios realizados en frutales como manzano, cítricos y *Prunus* han demostrado que ciertos portainjertos inducen una mayor eficiencia en el uso del agua, que se traduce en una mejor adaptación frente al déficit hídrico (Liu et al., 2012; Jamshidi et al., 2021; Opazo et al., 2020).

Por otra parte, los portainjertos son responsables de la arquitectura del sistema de raíces, lo que incide en la capacidad de exploración del suelo y en la captación de agua en estratos profundos (Montesinos et al., 2021). Esta característica ha

sido ampliamente reconocida como uno de los principales mecanismos de tolerancia a sequía en especies leñosas (Martínez-Ballesta et al., 2010). Portainjertos con raíces más extensas, profundas o ramificadas favorecen el crecimiento y la producción en ambientes limitantes, como se ha documentado en especies como olivo y cerezo (Majikumna et al., 2024; Gonçalves et al., 2006). Aunque a nivel bioquímico y molecular, los portainjertos también pueden determinar la capacidad de adaptación de la planta en condiciones abióticas adversas (Gonçalves et al., 2019; Balfagón et al., 2022).

Desde una perspectiva productiva, el uso de portainjertos tolerantes a sequía contribuyen no solo al mantenimiento del rendimiento, sino también a la sostenibilidad del cultivo a largo plazo (Mozafarian & Kappel, 2020). Estos genotipos son materiales que permiten reducir la dependencia del riego, mejorar la eficiencia del uso del agua y minimizar las pérdidas por fallas en el establecimiento o estrés hídrico estacional (Tworkoski et al., 2021). En este sentido, el aprovechamiento del potencial de adaptación de los portainjertos representa una estrategia agronómica clave ante escenarios de cambio climático y escasez de agua (Flor et al, 2025).

2.4. Mecanismos de tolerancia a déficit hídrico en portainjertos

La tolerancia al déficit hídrico implica una interacción compleja entre respuestas fisiológicas, morfoanatómicas, bioquímicas y moleculares que les permiten a las plantas sobrevivir, adaptarse y mantener sus funciones vitales ante la falta de humedad aprovechable en el suelo (Farooq et al., 2009; Ilyas et al., 2020). Comprender estos mecanismos es fundamental para identificar genotipos con potencial de adaptación superior que puedan ser empleados para reducir la huella hídrica del cultivo de aguacate (Yang et al., 2022; Fuerte-Velázquez et al., 2024) así como afrontar los escenarios de escasez de agua que se prevén para los próximos años (Mozafarian & Kappel, 2020).

2.4.1. Mecanismos fisiológicos y morfo-anatómicos

Desde un punto de vista fisiológico, los portainjertos tolerantes a déficit hídrico muestran una mayor capacidad para mantener el potencial hídrico foliar (Olien & Lakso, 1986) e influyen directamente en la eficiencia en el uso del agua y la arquitectura hidráulica del sistema injerto-portainjerto (Gonçalves et al., 2006). Estas adaptaciones incluyen una mayor densidad y profundidad del sistema radical, la reducción controlada de la conductancia estomática y la regulación del potencial hídrico foliar, lo que permite que se mantenga la turgencia celular durante el periodo de estrés hídrico (Farooq et al., 2009; Bernardo et al., 2025).

A nivel anatómico, las características de xilema, como el diámetro y la densidad de elementos de vaso, determinan la vulnerabilidad de la planta a la cavitación durante periodos de déficit hídrico (Tyree & Sperry, 1989). Se ha identificado que portainjertos con estructuras de xilema más estrechas y numerosas tienden a mantener la conductividad hidráulica con mayor eficiencia bajo estrés, como se ha evidenciado en aguacate y otras especies leñosas (Reyes-Santamaría et al., 2002; Alemán-Sancheschúlz et al., 2019).

Además, la modificación en la arquitectura de la raíz con raíces profundas, representa un mecanismo fundamental para incrementar la captación de agua en estratos más profundos del suelo (Bernardo et al., 2025). Estos rasgos morfoanatómicos no solo aumentan la tolerancia al déficit hídrico, sino que también mejoran la productividad de los sistemas de producción a largo plazo (Mozafarian & Kappel, 2020).

2.4.2. Mecanismos bioquímicos y antioxidantes

En condiciones de déficit de agua, las plantas activan una red de respuestas bioquímicas destinadas a mantener la homeostasis celular y reducir el daño oxidativo provocado por especies reactivas de oxígeno (Anjum et al., 2011). Los portainjertos tolerantes suelen exhibir una mayor capacidad antioxidante, mediada por enzimas como el superóxido dismutasa (SOD), catalasa (CAT) y

ascorbato peroxidasa (APX), las cuales protegen los tejidos del estrés oxidativo (Ilyas et al., 2020; Farooq et al., 2012; Zia et al., 2021).

Asimismo, la acumulación de compuestos osmoprotectores como prolina, glicina-betaína y trehalosa permite mantener el potencial osmótico celular, estabilizar las proteínas y preservar la integridad de las membranas (Zivcak et al., 2016; Shao et al., 2022). Estos osmoprotectores mejoran la capacidad de retención hídrica y contribuyen a la tolerancia a mediano y largo plazo (Tworkoski et al., 2016).

Otro aspecto bioquímico importante es el balance hormonal que desempeña un papel fundamental en las respuestas de las plantas. Durante el estrés hídrico, se incrementa la concentración de ácido abscísico (ABA), que induce el cierre estomático y regula la expresión de genes relacionados con la respuesta a la sequía (Kim, 2014). En varios portainjertos con mayor tolerancia al estrés por déficit hídrico se ha corroborado una regulación más precisa del ABA y otras hormonas como etileno, auxinas y citocininas, lo que se traduce en una respuesta más equilibrada al estrés (Burgess & Huang, 2016; Labarga et al., 2023).

2.4.3. Mecanismos moleculares

La tolerancia a la sequía también está regulada por mecanismos moleculares que modulan la expresión génica en respuesta al déficit hídrico (Kaur & Asthir, 2017). Estos mecanismos incluyen tanto vías dependientes como independientes de ABA (Soma et al., 2021), e involucran factores de transcripción como DREB, NAC, WRKY, MYB y bZIP (Kaur et al., 2021) los cuales coordinan la activación de genes implicados en la protección celular, síntesis de osmoprotectores y detoxificación de ROS (Liu et al., 2018).

Estudios recientes han evidenciado que portainjertos con la capacidad de modular la expresión de genes reguladores de acuaporinas (PIP), proteínas relacionadas con el transporte de agua a través de las membranas celulares, presentan mayor capacidad para mantener la turgencia celular bajo sequía (Labarga et al., 2023). Este mecanismo permite optimizar el transporte de agua

en tejidos clave y ayuda a mantener la turgencia foliar, la conductancia estomática y tasa de crecimiento vegetal (Galaz et al., 2024).

Adicionalmente, en varias investigaciones ha tomado relevancia el estudio sobre los mecanismos epigenéticos de tolerancia a sequía, estas investigaciones sugieren que la metilación del ADN y la modificación de las histonas podrían participar en la memoria de estrés y adaptación a largo plazo de los portainjertos (Yang et al., 2022; Kapazoglou et al., 2021)

2.5. Diversidad genética de portainjertos de aguacate y su potencial para la tolerancia a sequia

En general, el aguacate se considera una especie altamente susceptible a la sequía (Carr, 2013), no obstante, la diversidad genética existente en las razas botánicas y poblaciones criollas de *P*. *americana* representa una fuente estratégica para la selección de portainjertos con mayor tolerancia al estrés hídrico (Ayala-Ledesma, 2014). El aguacate se clasifica en las razas mexicana (*P*. *americana* var. *drymifolia*), guatemalteca (*P*. *americana* var. *guatemalensis* L.) y antillana (*P*. *americana* var. *americana* Mill.), cada una con características morfofisiológicas adaptativas distintas, producto se su evolución en diferentes ambientes ecológicos (Galindo-Tovar et al., 2007; Rubinstein et al., 2019).

La raza mexicana, nativa de regiones de altitud media (sobre 2000 m) y clima semitropical a templado (Barrientos-Priego et al., 2015), ha demostrado poseer una mayor eficiencia en el uso del agua, arquitectura radical profunda y elevada capacidad de recuperación tras periodos de sequía. Estas características le confieren un valor agronómico elevado como fuente de genes de tolerancia a sequia (Reyes-Santamaría et al., 2002; Moreno-Ortega et al., 2021). Además, portainjertos de raza mexicana presentan plasticidad fenotípica en respuesta a condiciones de estrés hídrico, con mecanismos como cierre estomático temprano, la síntesis y acumulación de osmoprotectores y una expresión diferencial de genes relacionados con la respuesta al ácido abscísico (ABA), la

homeostasis redox y la organización de la pared celular (Moreno-Ortega et al., 2021).

Por otro lado, la raza guatemalteca, adaptada a climas más húmedos y altitudes entre 1000 y 2000 m (Barrientos-Priego et al., 2015), suele mostrar mayor susceptibilidad a la sequía, aunque ofrece vigor vegetativo y potencial de compatibilidad también puede presentar una buena adaptación a déficit hídrico (Reyes-Santamaría et al., 2002). En contraste, la raza antillana, típica de zonas bajas (<1000 m) y cálidas, exhibe crecimiento acelerado y cierta tolerancia a salinidad, pero es poco eficiente en condiciones de sequía severa (Ben-Ya'acov & Zilberstaine, 1999).

En estudios de evaluación de portainjertos se ha comenzado a investigar la respuesta de aguacate al déficit hídrico mediante enfoques genómicos y transcriptómicos (Moreno-Ortega et al., 2021; Moreno-Pérez et al., 2024), con el objetivo de identificar los mecanismos de tolerancia al estrés. Por ejemplo, en estudios recientes se han identificado respuestas vinculadas a la regulación de acuaporinas, transporte de ABA y expresión de factores de transcripción del tipo DREB (dehydration-responsive element binding protein) y NAC (NAM: no apical meristem, ATAF: Arabidopsis transcription activation factor, CUC: cup-shaped cotyledon), fundamentales en la respuesta a la sequía (Wang & Dane, 2013; Singh & Laxmi, 2015) y algunos investigadores proponen comprender claramente el proceso de tolerancia a la sequía antes de adoptar prácticas de manejo como la aplicación de hormonas exógenas, el mejoramiento genético y técnicas de ingeniería genética para combatir los problemas del déficit hídrico (Kaur et al., 2021).

2.6. Estrategias metodológicas para la evaluación de tolerancia a sequía

La evaluación de la tolerancia a sequía en portainjertos de aguacate requiere de un enfoque metodológico integral que combine herramientas experimentales en condiciones controladas y de campo, así como el uso de indicadores moleculares

y analíticos que permitan identificar genotipos sobresalientes por su respuesta al estrés hídrico (Guadarrama-Escobar et al., 2024).

2.6.1. Ensayos controlados y ensayos en campo

Los ensayos en condiciones controladas permiten establecer un control preciso de las variables ambientales (humedad, temperatura, fotoperiodo), lo que facilita el estudio detallado de respuestas fisiológicas y bioquímicas bajo niveles definidos de déficit hídrico (Carbonneau, 1985; Ollat et al., 2016). En estos ensayos es común aplicar metodologías de riego deficitario controlado, suspensión del riego o aplicación de soluciones osmóticas para simular condiciones de sequía (Farooq et al., 2012; Ilyas et al., 2020).

Por otro lado, los ensayos en campo aportan validez agronómica y ambiental a los resultados al hacer posible la evaluación de la plasticidad fenotípica de los portainjertos bajo condiciones reales de variabilidad climática y edáfica (Narandžić & Ljubojević, 2022). Su implementación es clave para la validación de genotipos en distintas regiones productoras (Salazar-García et al., 2004; Reyes-Santamaría et al., 2025).

2.6.2. Indicadores morfo-fisiológicos

La evaluación de la tolerancia a sequía en portainjertos de aguacate y otros frutales leñosos requiere la medición de diversos indicadores morfofisiológicos que reflejan el estado hídrico, el desempeño fisiológico y la integridad funcional de la planta bajo condiciones de estrés (Raoufi et al., 2019). Entre estos, el potencial hídrico foliar y el potencial de turgencia son parámetros esenciales que permiten cuantificar la capacidad de la planta para retener agua en sus tejidos (Solari et al., 2006). Valores más negativos en el potencial hídrico indican una mayor pérdida de agua, lo cual puede comprometer el funcionamiento celular, mientras que la persistencia de la turgencia es señal de una buena capacidad osmótica y adaptación (Farooq et al., 2009). La mortalidad del follaje en el dosel es un efecto visible del estrés por sequía, sin embargo, se ha identificado que la

resistencia de xilema a la embolia puede actuar como un amortiguador contra la muerte completa del dosel durante una sequía prolongada (Cardoso et al., 2020).

Otro conjunto de indicadores clave lo constituyen la conductancia estomática y la tasa de transpiración, que reflejan el grado de apertura estomática y, por ende, la capacidad de la planta para regular el intercambio gaseoso y la perdida de agua (Bartlett et al., 2021; Ranjbar et al., 2021). Una conductancia estomática moderada durante el estrés indica una estrategia de cierre parcial de estomas, que permite conservar agua sin afectar severamente la fotosíntesis (Burgess & Huang, 2016). En paralelo, la medición de la tasa de asimilación CO_2 y el índice de clorofila (SPAD) proporciona información sobre la eficiencia del aparato fotosintético y su estabilidad bajo condiciones limitantes, ya que el estrés hídrico suele inducir clorosis y reducción de la actividad fotosintética (Kaur et al., 2021).

Desde un enfoque estructural, el índice de área foliar (IAF) y la biomasa aérea y radical son indicadores morfológicos que permiten evaluar la capacidad de crecimiento de la planta y su arquitectura funcional (Sun et al., 2013). Un IAF adecuado durante el estrés hídrico indica un balance eficiente entre superficie fotosintética y transpiración, mientras la biomasa radical robusta puede asociarse con una mayor exploración del perfil edáfico y, por ende, con una mejor captación de agua (Reyes-Santamaría et al., 2002; Alemán-Sancheschúl et al., 2019).

Otro parámetro con relevancia es la medición de la eficiencia en el uso del agua, entendida como la relación entre la asimilación de CO_2 o tasa fotosintética y la cantidad de agua transpirada (Wang et al., 2018). Este es un indicador importante porque integra aspectos fisiológicos y eco fisiológicos, y es útil para seleccionar genotipos que puedan mantener productividad relativa con un uso más conservador de recurso hídrico (Farooq et al., 2012; Zia et al., 2021).

Finalmente, la longitud, densidad y volumen del sistema radical son determinantes para caracterizar la capacidad del portainjerto de explorar el suelo en busca de agua. Genotipos con raíces más profundas o con mayor ramificación lateral presentan generalmente mayor resiliencia al estrés hídrico, dado que

pueden acceder a zonas más profundas o retener agua con mayor eficacia (Bernardo et al., 2025; Labarga et al., 2023).

La evaluación combinada de estos indicadores permite establecer relaciones funcionales entre las características fisiológicas del portainjerto y su capacidad para mantener su funcionamiento vital bajo condiciones de escasez de agua. Esta información es fundamental para la identificación de genotipos superiores y para el diseño de estrategias de selección más eficientes en programas de mejoramiento genético del aguacate (Carr, 2013; Kaur et al., 2021).

2.6.3. Enfoques moleculares y genómicos

La aplicación de enfoques moleculares y genómicos en la evaluación de la tolerancia a sequía ha revolucionado la selección de portainjertos en especies frutales como el aguacate. Estos métodos permiten identificar y caracterizar genes, rutas metabólicas implicadas en las respuestas adaptativas al estrés hídrico, información que resulta fundamental para acelerar los programas de mejoramiento genético (Yang et al., 2022). Entre las estrategias más utilizadas se encuentran la transcriptómica, la genómica comparativa, el análisis de expresión génica mediante RT-qPCR, el RNA-seq, la identificación de QTLs (loci de caracteres cuantitativos), GWAS y marcadores moleculares asociados a rasgos de tolerancia (Li et al., 2024; Ksouri et al., 2016; Jones, 2012).

En portainjertos tolerantes a sequía se ha identificado la sobreexpresión de genes relacionados con la biosíntesis de osmoprotectores como poliaminas, prolina, glicina, betaina y trehalosa (Singh et al., 2015; Carraro & Di Iorio, 2022), enzimas antioxidantes (superóxido dismutasa, catalasa, ascorbato peroxidasa, glutatión peroxidasa) (Hussain et al., 2018), así como fatores de transcripción que modulan respuestas al estrés, como DREB, NAC, MYB, WRKY Y bZIP (Kaur et al., 2021). La activación de estos genes contribuye al mantenimiento de la homeostasis celular, la integridad de las membranas y el equilibrio osmótico bajo condiciones de déficit hídrico (Zhang et al., 2016).

Un aspecto que se considera clave en este enfoque de estudio es la distinción entre las vías de señalización dependientes e independientes del ABA (Soma et al., 2021; Yang et al., 2022). En condiciones de sequía, el ABA actúa como regulador central de las respuestas adaptativas al promover el cierre estomático, la acumulación de osmolitos y la activación de genes relacionados con el estrés (Mukherjee et al., 2023). Sin embargo, también se han documentado otras rutas, como las dependientes de calcio y MAP quinasas que también contribuyen a la regulación de respuestas especificas al déficit hídrico (Liu et al., 2022).

Además, se ha demostrado que la tolerancia a la sequía también puede estar mediada por la regulación de la expresión de acuaporinas, cuya modulación en raíces bajo estrés hídrico esta correlacionada con una mayor eficiencia en la absorción y transporte de agua, la cual es variable entre portainjertos (Labarga et al., Bernardo et al., 2025).

Los avances en secuenciación genómica han permitido desarrollar bases de datos de transcriptomas que contribuyen al estudio de la diversidad genética y la identificación de variantes genéticas asociadas a la tolerancia a estrés hídrico (Trenti et al., 2021). La integración de herramientas bioinformáticas, como el análisis de redes génicas y análisis basados en inteligencia artificial (Costa et al., 2020), permitirá la identificación de genotipos candidatos para futuros programas de edición génica (Jiang et al., 2022) o mejoramiento asistido por marcadores moleculares (Kumar et al., 2021; Ling et al., 2025).

2.7. Avances recientes en selección y mejoramiento de portainjertos tolerantes a sequía

En los últimos años, se han logrado avances importantes en la investigación sobre portainjertos de aguacate con el propósito de identificar genotipos que confieran mayor tolerancia a condiciones de déficit hídrico (Reyes-Santamaría et al., 2025). Este interés se ha impulsado por la necesidad de mantener la productividad del cultivo frente a escenarios de cambio climático, caracterizados por sequías intensas y frecuentes (Charre-Medellín et al., 2021).

Uno de los principales enfoques de estudio ha sido la caracterización fisiológica y morfológica de accesiones de aguacate criollo. Por ejemplo, se han evaluado variables como el índice de área foliar, la densidad estomática, la biomasa radical y la eficiencia en el uso del agua para seleccionar portainjertos elite con alto potencial de adaptación (Bedoya-Ramírez et al., 2023). Estos parámetros son clave para seleccionar portainjertos que no solo sobrevivan, sino que mantengan la funcionalidad fisiológica y rendimiento en condiciones de estrés (Cárceles et al., 2023).

Así mismo, se han analizado las respuestas fisiológicas y moleculares de portainjertos comerciales como 'Dusa' frente a déficits hídricos controlados, lo que ha permitido identificar mecanismos como la modulación de la expresión génica relacionada con acuaporinas, antioxidantes y señalización hormonal, particularmente el ABA (Moreno-Ortega et al., 2021; Moreno-Pérez et al., 2024). Estos hallazgos permiten el avance hacia una selección más precisa mediante la confirmación de respuestas fisiológicas y marcadores moleculares.

Desde una perspectiva hidráulica, se ha documentado que el comportamiento estomático isohídrico (Kaneko et al., 2024) y las propiedades hidráulicas del sistema radical pueden influir en la tolerancia a sequía, la conservación del agua en tejidos y el mantenimiento del flujo de savia hacia los frutos (Beyer et al., 2022). Este enfoque permite identificar portainjertos que minimizan los impactos sobre el rendimiento y la calidad del fruto.

En otras investigaciones, se han comenzado a integrar los enfoques ómicos para analizar las interacciones entre portainjerto e injerto a nivel transcriptómico y metabolómico, para identificar como estos factores influyen en el crecimiento, la acumulación de carbohidratos y el desarrollo del fruto bajo estrés hídrico (Núñez-Lillo et al., 2024).

Además, en estudios a nivel de campo se han registrado importantes diferencias en el comportamiento de los portainjertos. Por ejemplo, se ha mostrado como interacciones entre portainjertos e injertos modifican la eficiencia en el uso del

agua y la anatomía de xilema en condiciones de déficit hídrico (Reyes-Santamaría et al., 2025). Otros trabajos han proporcionado evidencia de la influencia del portainjerto sobre parámetros agronómicos como el amarre de frutos, la tasa de crecimiento y el rendimiento bajo riego limitado (Salazar-García et al., 2024; Mickelbart et al., 2012).

La selección de genotipos criollos adaptados a condiciones locales también ha sido una estrategia efectiva. Estudios en Colombia y México han resaltado el valor de estos recursos genéticos como base para programas de mejoramiento, al destacar la importancia de conservar y caracterizar la diversidad genética nativa (Cano-Gallego et al., 2024; Salazar-García et al., 2004).

Finalmente, a partir de los avances en las herramientas biotecnológicas como la transformación y edición genética se abren nuevas perspectivas que podrían utilizarse en un futuro cercano para introducir rasgos de tolerancia a sequía en portainjertos seleccionados, aunque este enfoque aun presenta limitaciones técnicas y regulatorias (Tamayo-Ramos et al., 2022). En conjunto, estos avances demuestran la necesidad de implementar enfoques integrativos para desarrollar portainjertos de aguacate más resilientes frente a la escasez hídrica.

2.8. Conclusiones

La selección y mejoramiento genético de portainjertos tolerantes a seguía representa una estrategia esencial para enfrentar los desafíos del cambio climático en los sistemas de producción de aguacate y otros frutales, sin embargo, la tolerancia a sequía es un rasgo complejo, controlado distintos por mecanismos que implican características morfológicas, anatómicas, fisiológicas, bioquímicas y moleculares que varían ampliamente entre genotipos y especies. En este contexto, la exploración de la diversidad y variabilidad genética disponible en el desarrollo de metodologías de evaluación del estrés hídrico han permitido algunos avances en la identificación de materiales promisorios con mejor capacidad de tolerancia al déficit hídrico.

A futuro, será fundamental fortalecer la investigación mediante un enfoque interdisciplinario y promover la colaboración entre centros y programas de investigación y productores, para acelerar la selección de portainjertos resilientes frente a condiciones hídricas restrictivas.

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3. HYDRAULIC CONDUCTIVITY AND DEFOLIATION: KEY INDICATORS OF WATER STRESS IN AVOCADO PLANTS

Type of the Paper (Original Research Paper)

Hydraulic conductivity and defoliation: key indicators of water stress in avocado plants

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Abstract: Mexico is positioned as the world's leading producer of avocados but faces sustainability concerns due to the high-water footprint of this crop, especially in the main producing regions of Michoacán and Jalisco. This study evaluated eighteen avocado accessions under conditions of water deficit and high temperatures, with the goal of identifying physiological and anatomical variables that can serve as indicators in the selection of rootstocks adapted to water scarcity. The plants were subjected to severe water deficit treatment for 70 hours, followed by water availability restoration to regain turgor. Before treatment, CO_2 assimilation (*A*), stomatal conductance (*g*), and transpiration rate (*E*) were measured. Twenty-one days after plant rehydration, defoliation percentage was recorded, along with the number of dead plants and those with vascular damage. The hydraulic conductivity (*k*) of the rootstocks was determined 60 days after the experiment's completion, and stem samples were collected to analyze vascular system characteristics, including vessel density (D_{eves}), size (A_{ves}), and the proportion of vessel elements in the xylem (P_{vesxil}). The non-parametric Kruskal-Wallis statistical test ($\alpha = 0.05$) was applied to determine significant differences among the accessions. Results showed high variability in characteristics and behavior, with significant differences in growth and tolerance to water deficit. Accessions with lower defoliation and higher hydraulic conductivity were identified, and it was proposed to continue evaluating their potential as rootstocks under drought conditions. The evaluation highlighted the importance of hydraulic conductivity and defoliation percentage as key indicators of water stress tolerance, allowing the grouping of plants according to their adaptive capacity.

Keywords: vascular system; CO_2 assimilation; high temperatures; rootstocks; drought stress.

3.1. Introduction

Mexico is the avocado producing country with the largest water footprint (Sommaruga and Eldridge, 2020). It has been estimated that a mature tree requires between 1000 and 1300 mm of annual rainfall for adequate production (Sommaruga and Eldridge, 2020), although there are estimates that have indicated that rainfed avocado plantations in Michoacán require 839 m^3 of water to produce one ton of fruit while those with irrigation can demand up to 2355 m^3 (Fuerte-Velázquez and Gómez-Tagle, 2024). The growing global demand for avocado has led to a significant increase in the cultivated area, which raises concerns about the sustainability of water use in these plantations and increasing temperatures coupled with climate change makes it imperative to seek alternatives. In particular, excessive irrigation not only increases the water footprint, but can also lead to salinity problems and soil degradation, which compromise long-term productivity (Sommaruga and Eldridge, 2020). In addition, recent studies have highlighted that domestic avocado production is competitive in the international market, contributing more than 45 % of world exports during recent seasons (Cruz-López et al., 2022). This situation underscores the urgent need to promote research and development of avocado rootstocks that require less water and are more tolerant to adverse climatic conditions, which could be key to maintaining the competitiveness and sustainability of the sector. On the other hand, it should be considered that 57.14 % of the established avocado area is under rainfed conditions (SIAP, 2024), which is also important for the development of rootstocks tolerant to lack of irrigation.

Rootstocks can exert significant physiological effects on the canopy, including the rate of nutrient uptake and translocation (Ibacache et al., 2020). If water availability is a problem, leaf stomata will begin to close and CO_2 assimilation will be reduced (Martinez-Ballesta et al., 2010). Therefore, rootstock can significantly influence cultivar productivity by affecting the water balance of the tree (Gijón et al., 2010).

The aim of this research was to evaluate eighteen avocado accessions with the potential to be used as rootstocks tolerant to soil water deficit in terms of their ability to optimize water use in the presence of water stress and high temperatures, in addition to identifying the

physiological and anatomical variables that allow the selection of plants for the development of new rootstocks that can adapt to adverse climatic conditions.

3.2. Materials and Methods

The study was conducted in a glass greenhouse of the Universidad Autónoma Chapingo located at 2264 m altitude (19.4901679, -98.8736711), during the growth period of the plants, from April 2021 to July 2022.

3.2.1. Plant material, growth conditions and experimental design

Eighteen avocado accessions (180 plants) from the Germplasm Bank of the Fundación Salvador Sánchez Colín-CICTAMEX, S.C. were evaluated (Table 1). The plants were established in 8 L PVC pots containing a substrate consisting of a 1:1 (v/v) mixture of agricultural soil and river sand. The agronomic management of the plants consisted of a periodic supply of nutrient solution to promote optimal conditions of water and nutrient availability. Nutrition was provided with a balanced solution (Table 2) two to three times per week. In addition, periodic applications of fungicide (copper oxychloride 3.5 g L-1) and insecticide (imidacloprid 1 mL L-1) were made during the growth stage of the plants until the time of evaluation to ensure the maintenance of plant health.

Table 1. Avocado accessions used in the selection of rootstocks with potential for tolerance to water deficit.

Code	Accession	Code	Accession
G1	'Colín V-33'	G10	Árbol 93 M-01 'Samuel'
G2	'Encinos'	G11	Árbol 139-141 'Boyce'
G3	'Derrumbe 92'	G12	Árbol 203 'Híbrido 23F'
G4	Árbol 247 Raza mexicana 12	G13	Árbol 110 'C9' (Islas Canarias)
G5	Árbol 55 'Fredy 7'	G14	Árbol 100 M-08 'Mariano'
G6	Árbol 144 'Frazer'	G15	Árbol 81 'Polo'
G7	Árbol 12 *Persea nubigena* 1/7 (CH-I-4)	G16	Árbol 164 'Juanita 39'
G8	Árbol 120 'M-14'	G17	Árbol 89 M-06 'Bladimiro'
G9	Árbol 66 *Persea nubigena* 'Rodeo II' (CH-G-76)	G18	Árbol 86 'Lamb Hass'

When the plants reached an average height of 130 cm, the number of leaves and stem diameter (mm) were recorded and subsequently subjected to water deficit under conditions

of high daytime temperatures (> 40 °C) and low relative humidity (< 50 %). A completely randomized design with ten replications was used for their distribution. The experimental unit was defined as a plant, which was subjected to substrate saturation prior to the water deficit treatment, to ensure that the initial conditions were uniform for all plants.

Table 2. Nutrient solution used for growing avocado plants during the experimental cycle.

CE (dS m^{-1})	pH	Macronutrients (mg L^{-1})					
		NO_3^-	$H_2PO_4^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}
		84	16	56	136	90	24
1.2	6.0	Micronutrients (mg L^{-1})					
		Fe	Mn	Cu	Zn	B	Mo
		3.0	0.5	0.03	0.05	0.5	0.05

3.2.2. Physiological evaluation and reaction to water deficit

The variables CO_2 assimilation (*A*, µmol m^{-2} s^{-1}), stomatal conductance (*g*, mmol m^{-2} s^{-1}), transpiration (*E*, mmol m^{-2} s^{-1}) and vapor pressure deficit (VPD, kPa) before the water deficit treatment were quantified using a portable infrared CO_2 analyzer (Cl-340 CID). Using the values of *A* and *E*, water use efficiency (WUE, µmol m^{-2} s^{-1}/ mmol m^{-2} s^{-1}) was calculated by dividing *A* by *E*. In addition, ambient temperature (T, °C), and relative humidity values (RH, %) were measured throughout the experiment. The duration of the treatment was 70 hours, after which the plants were irrigated to recover turgor and 21 days later the variables percent of defoliation (PD, %), plant death and internal stem damage (vascular damage) were recorded.

3.2.3. Measurements of hydraulic conductivity

The plants were subjected to an evaluation of hydraulic conductivity 60 days after the water deficit treatment. A 30 cm stem section was collected from each plant. The sections were placed in a container with distilled water and immediately prior to the determination of hydraulic conductivity, the segments were trimmed to 20 cm. The liquid used and forced through the stem segments for 15 minutes was a 0.01 M oxalic acid solution (Pire et al., 2007). The flow rate through the stem was determined gravimetrically with a precision electronic balance (Ohaus Discovery DV215CD, Ohaus Corporation, USA). Hydraulic

conductivity (k, kg m s^{-1} MPa^{-1}) was calculated as the flow rate passing through a given length of sample per unit applied pressure gradient (ΔP) according to Sperry et al. (1988).

$$k = \frac{F \times SL}{\Delta P}$$

where F is the flow rate (kg s^{-1}, assuming a water density of 1 g cm^{-3} at an ambient temperature of 25 °C), SL is the stem length and ΔP (expressed in MPa) is the hydrostatic gradient. The hydrostatic gradient was calculated by multiplying the effective height of the acidified water reservoir, with the density of the liquid (1000 kg m^{-3}) and the acceleration due to gravity (9.81 m s^{-2}).

Subsequently, hydraulic conductivity (k) was used to calculate the stem specific conductivity (k_{stem}) by dividing k by the stem cross-sectional area (A_{tt}, m^2) (k_{stem}, kg m s^{-1} MPa^{-1}). Vessel hydraulic conductivity (k_{ves}) was determined by dividing k by the total area of the vessel elements (A_{tves}, m^2) of each segment section (k_{ves} kg m s^{-1} MPa^{-1}).

3.2.4. Anatomical measurements

A 2 cm long portion was cut from each segment. The portions were fixed in a 2:1 (v:v) ethanol-acetic acid solution (J.T. Baker), dehydrated in ethanol series and then embedded in kerosene (Paraplast®). Nine 25 µm thick subsections were obtained from each portion using a microtome (MICROM HM 325, Thermo Scientific, Basingstoke, UK). The subsections were stained with safranin and fast green (Vazquez-Cooz and Meyer, 2002) and photographed with a Moticam® 2000 digital camera (Motic China Group, Ltd., Causeway Bay, Hong Kong) adapted to one of the eyepieces of a fluorescence microscope (BX53, Olympus Corporation, Tokyo, Japan). The study on the digital photographs was performed with ImageJ 1.54k image analysis software (Abràmoff et al., 2004). The variables evaluated were density of vessel elements (D_{eves}, vessels/mm^2), area of vessel elements (A_{ves}, μ^2). The proportion of vessel elements in the xylem ($P_{evesxyl}$, %) was calculated from these measurements.

3.2.5. Statistical analysis

The variables evaluated were analyzed with the GraphPad Prism Version 10.3.1 statistical software. Numerical variables were analyzed using the Kruskal-Wallis test after

the Shapiro-Wilk normality test. The results obtained are presented in graphs illustrating the significant differences found using Dunn's *post hoc* test. In addition, a hierarchical clustering analysis was performed by Ward's method, which allowed regrouping the individuals of each accession into groups sharing similar characteristics. The groups were analyzed with the Kruskal-Wallis nonparametric statistical test and compared with each other to determine if there were significant differences. Finally, a principal component analysis was performed to identify the variables that contributed most to the variability of the data and to corroborate the presence of relevant patterns in the data set analyzed.

3.3. Results and Discussion

Avocado plants that were subjected to water stress conditions presented a differential size (Figure 1), attributed to the genetic diversity of the accessions studied and their ability to adapt to environmental conditions during their development. Avocado is a species with high genetic variability, which allows contrasting characteristics to be observed even in plants from the same tree (Ayala and Ledesma, 2014).

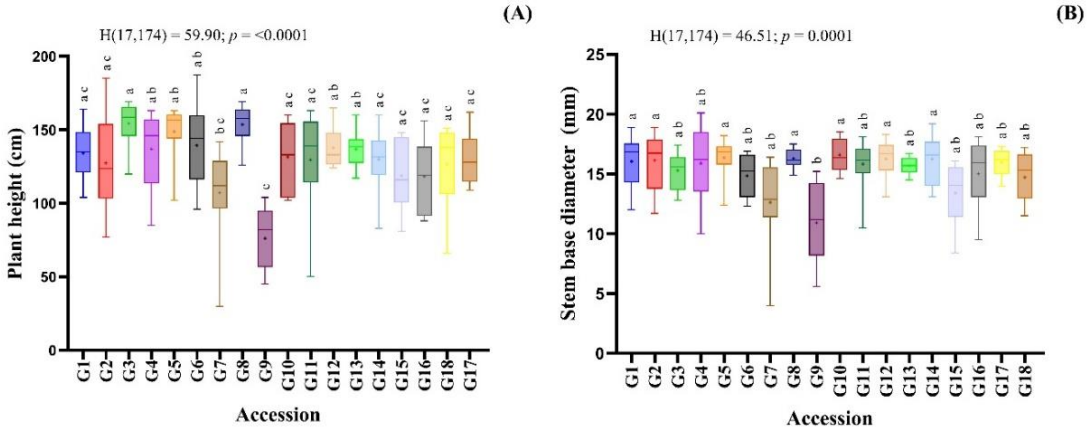

Figure 1. Variability in the growth of 18 avocado accessions evaluated under water deficit conditions. (A) Plant height (cm), (B) Stem base diameter (mm). Accessions were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). Boxes represent the interquartile range, while mean values are denoted by the symbol "+", and median values are represented by a horizontal line. The whiskers indicate the minimum and maximum values.

As can be seen in the analysis of height and diameter of the plants in this study. The plants with the smallest growth belonged to accessions G7 and G9 [*Persea americana* var. *nubigena* (L.O.Williams) L. E. Kopp] with an average height of 91.6 cm and a stem thickness

at its base of 11.7 mm, while those with the largest size corresponded to accession G5 (Árbol 55 ‘Fredy 7’) and G8 (Árbol 120 ‘M-14’) that presented an average height of 153.9 cm and a stem thickness of 15.8 mm (Figure 1).

When exposed to water deficit conditions, plants were placed in an unfavorable environment with temperatures above 40 °C during multiple hours of the day and relative humidity levels maintained below 50 %; the average vapor pressure deficit was 5.61 kPa at midday (Figure 2).

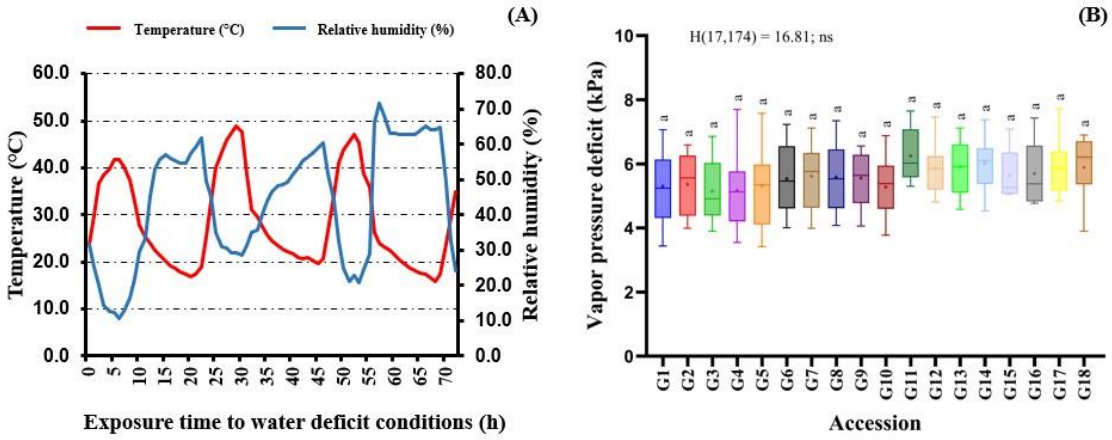

Figure 2. Environmental conditions during the application of water deficit stress to 18 avocado accessions. (A) Temperature and relative humidity values. (B) Vapor pressure deficit values. Accessions were categorized according to Dunn's *post hoc* statistical analysis ($\alpha = 0.05$). Boxes represent the interquartile range, while median values are denoted by the symbol “+”, and median values are represented by a horizontal line. The whiskers indicate the minimum and maximum values.

Although statistical differences in plant growth were determined, at the time of evaluation, the number of leaves had no significant differences ($P = 0.05$), indicating that the number of leaves was uniform among the 18 accessions. However, defoliation occurred variably among and within accessions (Figure 3). This caused some accessions to maintain greater uniformity in their response to water deficit stress. Water deficit can lead to significant reductions in leaf number and biomass (Moreira et al., 2021), and as in multiple cases in defoliation as a strategy to conserve resources and aid plant survival (Kaur et al., 2021).

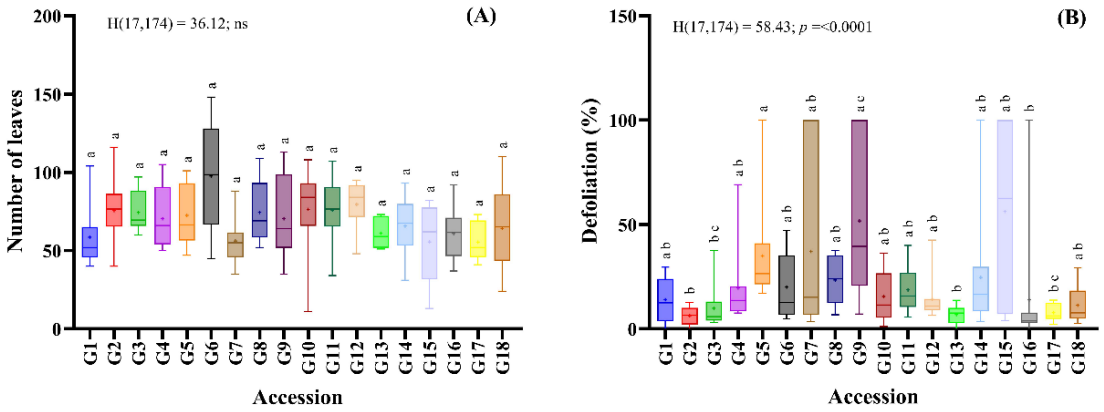

Figure 3. Variability in the number of leaves of 18 avocado accessions evaluated under water deficit conditions. (A) Number of leaves at the beginning of the evaluation. (B) Percentage of defoliation. Accessions were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). Boxes represent the interquartile range, while median values are denoted by the symbol "+", and median values are represented by a horizontal line. The whiskers indicate the minimum and maximum values.

In this context, it was observed that the accessions that exhibited greater variability in their defoliation percentages also presented a greater proportion of plants that did not survive the water stress, which would indicate that the accessions that exhibited greater resilience tended to show decreased defoliation percentages (Figure 3b). Plants of accessions G2 ('Encinos'), G13 (Árbol 110 'C9') and G17 (Árbol 89 M-06 'Bladimiro') showed less than 8 % defoliation and all plants survived the stress conditions (Figure 4a), and were not identified as showing damage to their vascular system (Figure 4b), while G7 and G9 which are *Persea americana* var. *nubigena* showed a higher percentage of defoliation (37 and 51 %, respectively) and number of dead plants (30 and 40 %), this may be due to the fact that these are wild specimens from mountain mesophyll forest where humidity conditions are high both in soil and environment. This suggests that the morphological and physiological characteristics of avocado accessions are determinant in their ability to tolerate adverse conditions, which could be key for the selection of more resistant rootstocks in breeding programs.

Although the mechanisms that induce plant defoliation under water deficit conditions are complex and multifactorial, identifying avocado accessions that can maintain their foliage even after exposure to high temperatures and low soil moisture is essential to help in the

identification of other variables related to water stress tolerance that, in subsequent analyses, would allow clarification of the mechanisms involved.

Plants employ different strategies to mitigate the effects of water deficit stress, which help them to reduce or avoid defoliation. Among the most studied are stomatal regulation (Bhattacharya, 2021), accumulation of low molecular weight osmolytes such as proline and glycine-betaine (Bhattacharya, 2021), antioxidant enzyme activity (Irfan et al., 2023) and compensatory mechanisms, such as increased stem water potential and higher concentrations of soluble sugars (Wang et al., 2021). In many cases, plant adaptation to water deficit stress does not depend on a single strategy, but is the result of altered morphological and physiological traits that help enhance water uptake and storage and regulate water loss through stomatal closure, protein and osmolyte synthesis to maintain osmotic balance at the cellular level (Kaur et al., 2021).

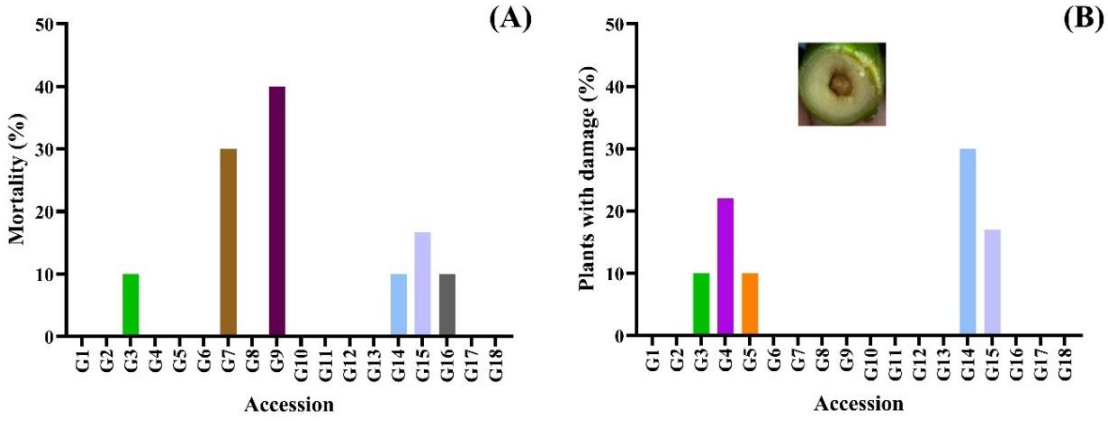

Figure 4. Response of 18 avocado accessions under water deficit conditions. (A) Percentage of mortality. (B) Percentage of plants with damage to the vascular system identified as the presence of darkened tissue areas.

Soil water deficit significantly impacts *A*, *g* and *E* in plants, leading to reduced growth and yield (Kudoyarova et al., 2013). Stomatic conductance impacts *A* and controls intracellular CO_2 concentration, such that a decrease in *g* can decrease the photosynthetic capacity of plants (Parkash et al., 2021; Zhao et al., 2020). In our research, it was found that *A* maintained a moderate positive correlation with *g* (r = 0.703; *P* = <0.0001) and *E* (r = 0.627; *P* = <0.0001). In addition, the correlation between *g* and *E* was observed to be r =

0.940 (P = <0.0001), indicating that an increase in stomatal conductance can significantly enhance transpiration rate and thus photosynthetic efficiency under optimal conditions.

In this research, the evaluation of physiological variables *A*, *E*, *g* and water use efficiency (WUE) was also carried out prior to the water deficit treatment and although no significant differences were found in *A* and WUE, *E* and g did show differences between accessions (Figure 5), where G11 (Árbol 139-141 'Boyce') stood out for having the lowest values of the 18 accessions evaluated.

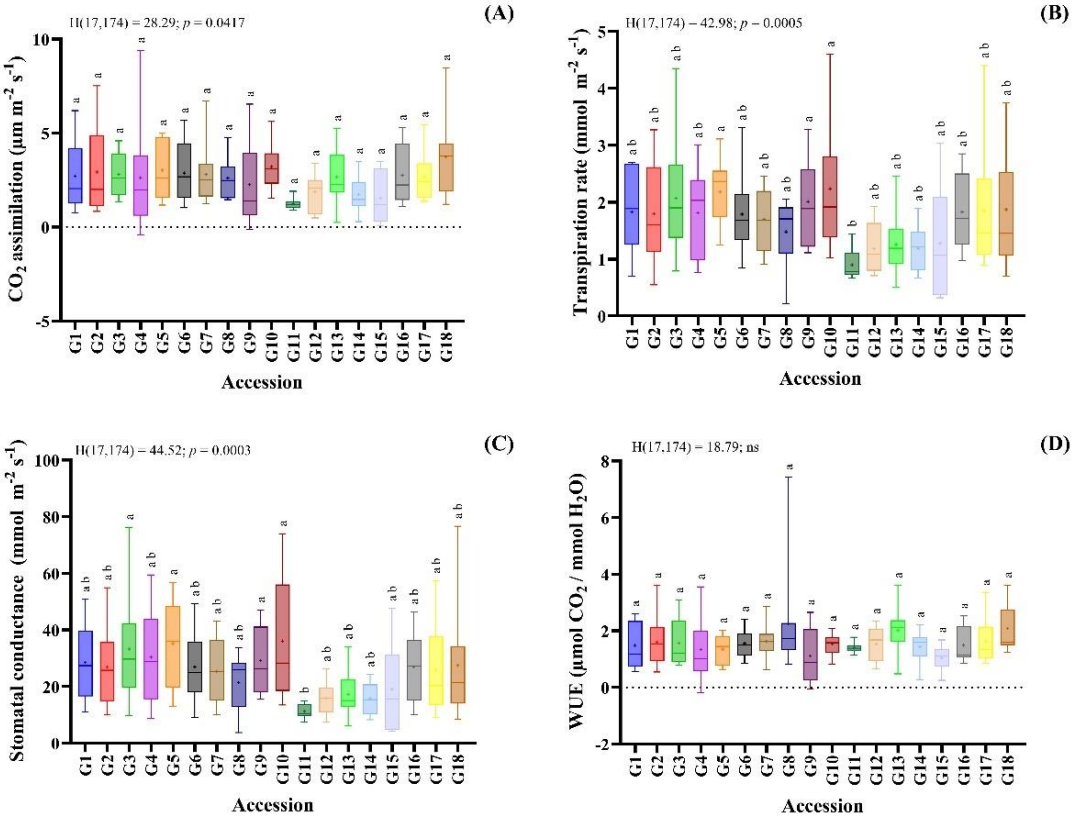

Figure 5. Values of physiological variables of 18 avocado accessions evaluated under water deficit conditions. (A) Stomatal conductance. (B) Transpiration rate. (C) Stomatal conductance. (D) Water use efficiency. Accessions were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). Boxes represent the interquartile range, while mean values are denoted by the symbol "+", and median values are represented by a horizontal line. The whiskers indicate the minimum and maximum values.

Physiological variables (*A*, *g*, *E* and WUE) had a low negative correlation (P = <0.0001; r = -0.490, -0.598, -0.351 and -0.315, respectively) with vapor pressure deficit (VPD), which is directly related to ambient temperature and relative humidity (Grossiord et al., 2020).

Vapor pressure deficit is the driving force of transpiration in plants (Amitrano et al., 2019), so it is fundamental to regulate stomata opening and closing, and thus affects water use efficiency (Yang et al., 2005). The accessions evaluated presented varied responses to water deficit, however; the values of physiological variables were not related to defoliation rate, plant survival, nor to the presence of internal stem damage, suggesting that other factors may be influencing tolerance to water stress and that the evaluation of physiological variables before water deficit treatment was not sufficient to predict plant response to stress conditions.

In our study, defoliation was correlated with plant height (r = -0.293; P = <0.001) and diameter (r = -0.495; P = <0.001), which indicated that taller plants with larger diameters tend to experience less defoliation, although it should be noted that the correlation coefficient was low. Thus, the anatomical characteristics of the vascular system of plants may play a more important role than their size in the capacity to tolerate water stress (Reyes-Santamaría et al., 2002).

The plant stem is formed by the tissues of the vascular system that is integrated by the xylem and phloem, which are tissues specialized in the transport of water and nutrients (Lacointe and Minchin, 2008). In this research it was observed that the accessions contrasted significantly in the variables related to xylem anatomy. Differences in vessel element density (D_{eves}) and vessel element area (A_{ves}) were identified (Figure 6). D_{eves} ranged from 243 to 382 vessels/mm^2 of xylem surface area, while A_{ves} showed a range from 316 to 585 µm^2, with considerable variability in water carrying capacity among the accessions studied (Figure 7b). Vessel elements are the conduits that transport water and nutrients from the roots to the leaves and their structure, size and density are crucial in the efficiency of the vascular system of plants (Shabala, 2007). In this context, accessions G8 (Árbol 120 ‘M-14’), G13 (Árbol 110 ‘C9') and G16 (Árbol 164 ‘Juanita 39’) with a higher proportion of vessel elements in the xylem ($P_{evesxyl}$) exhibited a higher specific hydraulic conductivity (k_{stem}), which indicated that a greater volume of water can be transported in their vascular system in contrast to accessions with a lower $P_{evesxyl}$ (Figure 7).

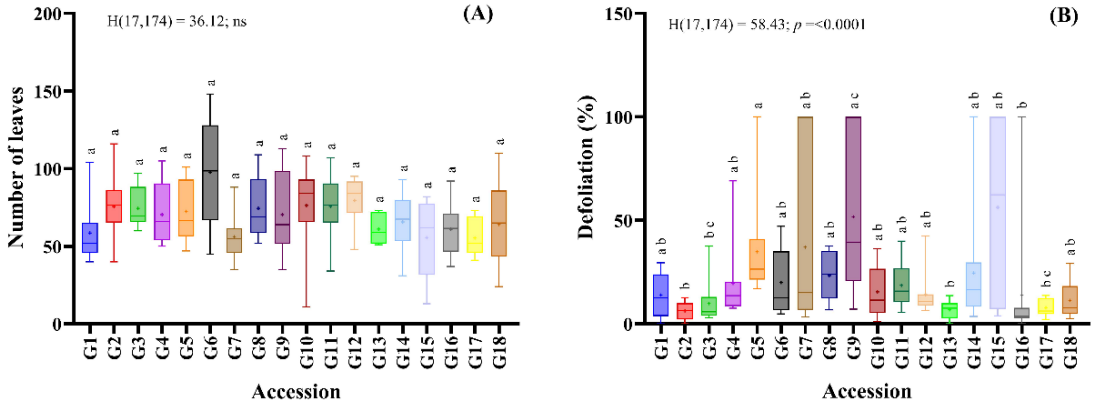

Figure 6. Variation in density (A) and size of xylem vessel elements (B) in 18 avocado accessions evaluated under water deficit conditions. Accessions were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). Boxes represent the interquartile range, while mean values are denoted by the symbol "+", and median values are represented by a horizontal line. The whiskers indicate the minimum and maximum values.

Although some accessions showed limited variation in the behavior of the plants that were evaluated, it was observed that the majority showed pronounced responses and this variability made it difficult to identify clear patterns in tolerance to water deficit. However, by means of hierarchical clustering and principal component analysis, it was possible to discern certain groups of plants that shared similar characteristics in their response to exposure to water deficit and high temperatures, suggesting the possibility of selecting individuals with potential for use as rootstocks tolerant to drought conditions.

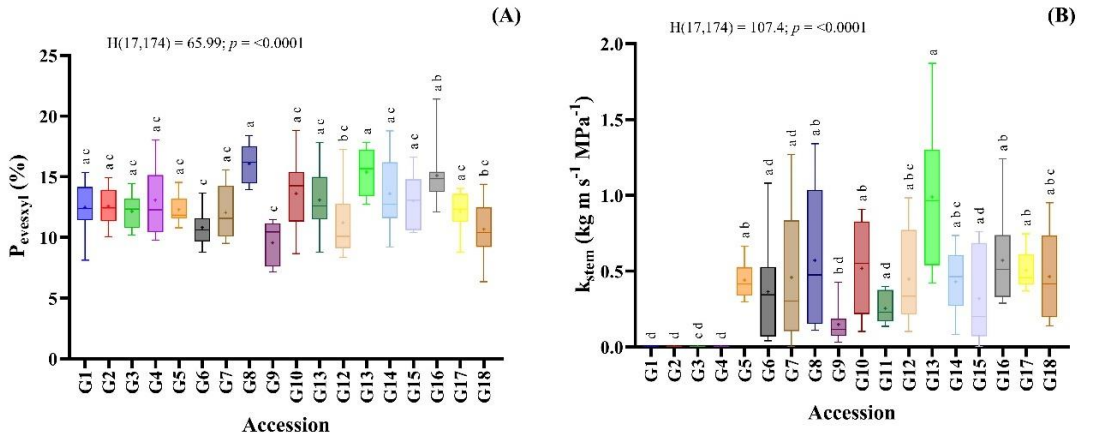

Figure 7. Variation in the proportion of vessel elements in xylem (A) and stem specific hydraulic conductivity (B) in 18 avocado accessions evaluated under water deficit conditions. Accessions were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05).

With regard to the physiological variables, it was possible to identify three groups that differed mainly in terms of their *A* and WUE. Groups 1 and 2 presented higher *A* and WUE than group 3 (Figure 8). In the stomatal conductance and transpiration rate, the groups had values that did not differ significantly from each other. As could be seen in the analysis of the behavior of the accessions, the physiological variables did not present outstanding information to establish a clear differentiation between the groups. However, the study of anatomical characteristics and their relationship with hydraulic conductivity was fundamental to better understand the response of avocado plants exposed to water deficit.

Group 1, although not so affected in leaf loss (17.46 %) did present a low specific hydraulic conductivity (0.21 kg m s^{-1} MPa^{-1}) as a consequence of a low density of vessel elements (275.42 vessels/mm^2) (Figure 9).

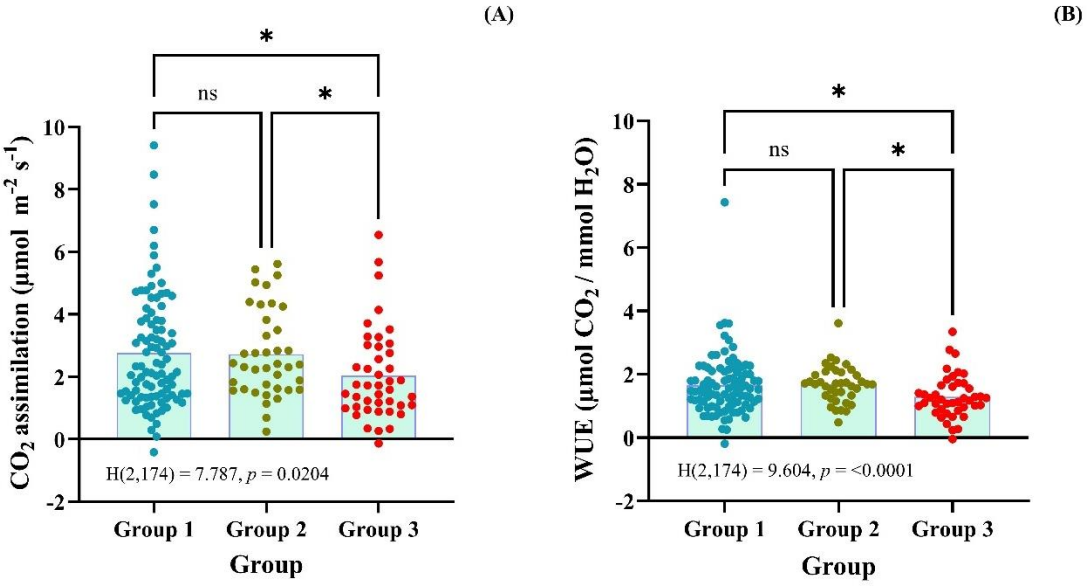

Figure 8. Variation in CO_2 assimilation (A) and water use efficiency (WUE) (B) of grouping 174 avocado plants under water deficit stress conditions. Groups were categorized according to Dunn's *post hoc* statistical analysis ($\alpha = 0.05$). ns: not significant; *: significant at $P \leq 0.05$.

Group 2 plants showed characteristics that allowed them to adapt more efficiently to water deficit stress conditions, had a higher proportion of vessel elements in the xylem (13.9 %), which resulted in a higher specific hydraulic conductivity (0.8936 kg m s^{-1} MPa^{-1}) and a low percentage of defoliation (11.2 %). While in group 3 a lower proportion of vessel elements (11.8 %) was observed in plants, resulting in a significantly lower k_{stem} (0.2021 kg m s^{-1} MPa^{-1}) and the highest defoliation percentage of the three groups (36.1 %) (Figure 9).

In addition, the most susceptible plants (Group 3) had a higher D_{ves} (364 vessels/mm^2) of small size (330.9 µm^2), which possibly had an impact on a lower water supply and flow capacity and consequently the highest vulnerability to water stress conditions, this result was in contrast with other studies, in which it has been hypothesized that smaller vessel elements present lower vulnerability to cavitation and better hydraulic conductivity (Reyes-Santamaría et al., 2002; Keret et al., 2024).

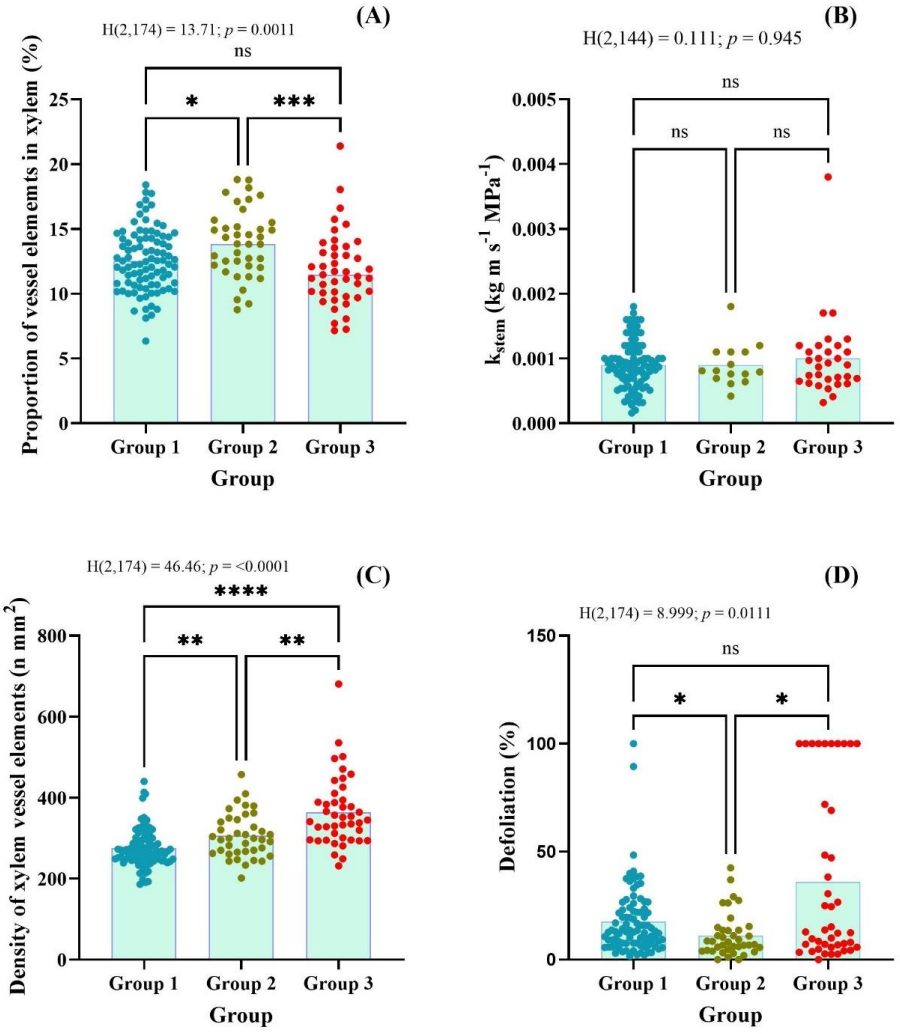

Figure 9. Variation in the proportion of vessel elements in xylem (A), specific hydraulic conductivity (k_{stem}) (B), vessel element density (C) and defoliation rate (D) of grouping 174 avocado plants under water deficit stress conditions. Groups were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). ns: not significant; *,**,****: significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

Several investigations have pointed out that xylem structure, especially the size and density of the vessel elements, are critical factors that influence plant resilience to

environmental changes (Hacke and Sperry, 2001). In forest species such as eucalyptus, the increase in the density of smaller vessel elements has been related to an improvement in xylem hydraulic conductivity (Keret et al., 2024), on the other hand, it is also noted that plants with larger vessel elements are more susceptible to cavitation under drought conditions (Atkinson and Taylor, 1996).

Maintenance of plant water potential despite the presence of severe water stress can prevent extensive xylem embolism and drought-induced mortality (Martorell et al., 2013). Plant mortality under conditions of extreme or prolonged water deficit has been related to the hydraulic failure hypothesis (Barigah et al., 2013), which postulates that reduced soil moisture availability results in significant cavitation within xylem vessels, as there is increased evaporative demand, the formation of vapor bubbles within the xylem would obstruct water transport and culminate in tissue desiccation and subsequent plant death (Brodribb and Cochard, 2008; Klein et al., 2018). Cavitation occurs whenever water pressure in the xylem drops below a critical threshold (Shen et al., 2002). In some species such as grapevine and poplar it has been found that broader vessel elements presented a higher susceptibility to cavitation (Gerin et al., 2024; Jacobsen et al., 2019), however; this vulnerability cannot be related solely to the size of the vessel elements, as in species such as eucalyptus it has been observed that the smallest vessels were the most susceptible to cavitation (Barigah et al., 2021).

In our study, the plants most affected by water deficit (group 3) had the greatest D_{eves} (Figure 9C) and their smaller vessel elements had a low hydraulic conductivity (k_{ves}) (Figure 10); this response was possibly due to internal damage to the vascular system by cavitation and embolism. In cases of severe damage, the presence of darkened areas on the stem was visible to the naked eye. A review of the integrity of the xylem in plants with internal damage showed the collapse of the parenchyma cells around the vessel elements (Figure 11A), which were deformed and consequently would have lost their functionality to transport water after rehydration of the substrate, at the end of the water deficit treatment. This response was contrasted by checking the xylem integrity of plants without visible internal damage in groups 1 and 2 (Figure 11B). Several of the accessions with internal damage presented a higher mortality rate (group 3), while others showed the capacity to maintain the functionality

of the vascular system and the affected plants remained alive until the end of their recovery period (Figure 4).

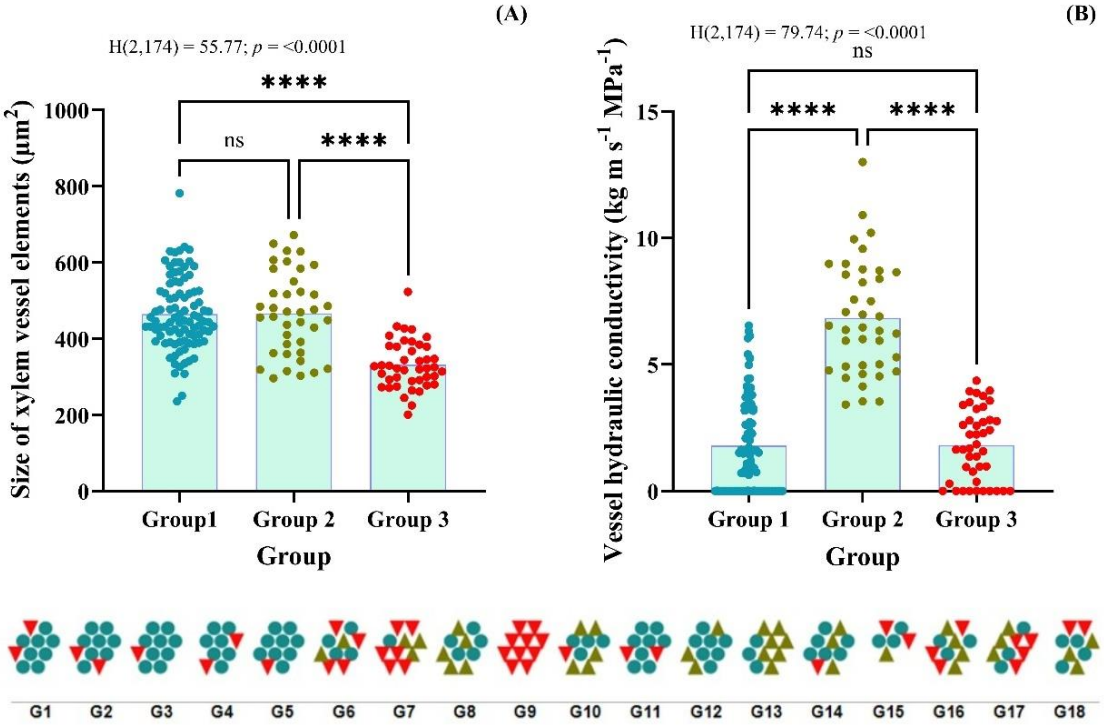

Figure 10. Variation in vessel element size (A), vessel element density (B) of grouping of 174 avocado plants under water deficit stress conditions. Groups were categorized according to Dunn's *post hoc* statistical analysis (α = 0.05). ns: not significant; **,****: significant at $P \leq 0.01$ and 0.001, respectively. The grouping of individuals of each accession is shown at the bottom of the figure: group 1 (blue circle), group 2 (green triangle) and group 3 (red triangle).

In woody plants, it has been identified that embolized ducts in the xylem can be restored through spontaneous bubble dissolution, which occurs as xylem pressure approaches atmospheric levels or in the presence of positive root or stem pressure (Nardini et al., 2017).

Additionally, vessel damage can be compensated by regeneration of new xylem tissue (Nardini et al., 2014). However, the occurrence of these adaptive mechanisms is only possible in plants that did not present excessive damage, in which case, the plant will most likely fail to recover and eventually die (Klein et al., 2018). This response of plants to water deficit highlights the importance of identifying avocado accessions or individuals that are tolerant to damage caused by the presence of conditions of lack of moisture in the soil and high temperatures, that maintain the functionality of their vascular system and ensure their

survival until the damage to the xylem is restored without significantly altering their growth and productivity. Among the outstanding accessions, individuals with anatomical and physiological characteristics that showed greater tolerance to water stress (group 2) will be key in the selection of rootstocks that have the potential to adapt to limited conditions of water availability in the soil. It should be noted that, after identifying the outstanding plants, their evaluation should be continued with the respective graft, since there are several relationships that affect gas exchange and even xylem anatomy in graft/rootstock combinations (Ayala-Arreola et al., 2010).

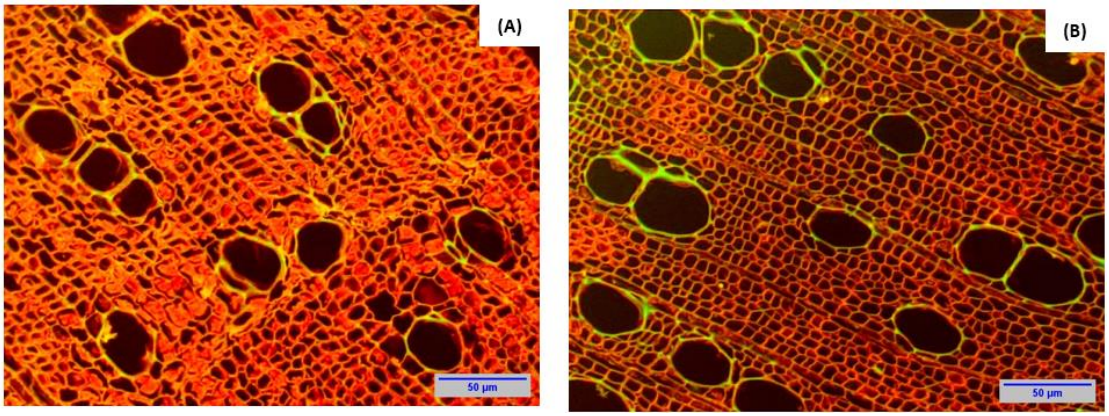

Figure 11. Comparison of the structure of the vascular system of an avocado plant susceptible to water deficit (collapsed cells) with visible stem damage (A) and a tolerant plant (non-collapsed cells) with no visible stem damage (B). Scale bars = 50 µm.

Finally, principal component analysis identified the relevance of percentage of defoliation and hydraulic conductivity as significant indicators of avocado plant response to water stress (Figure 12), suggesting that these variables can be used to optimize rootstock selection in breeding programs and be key indicators to distinguish germplasm accessions with greater potential for adaptation to drought conditions.

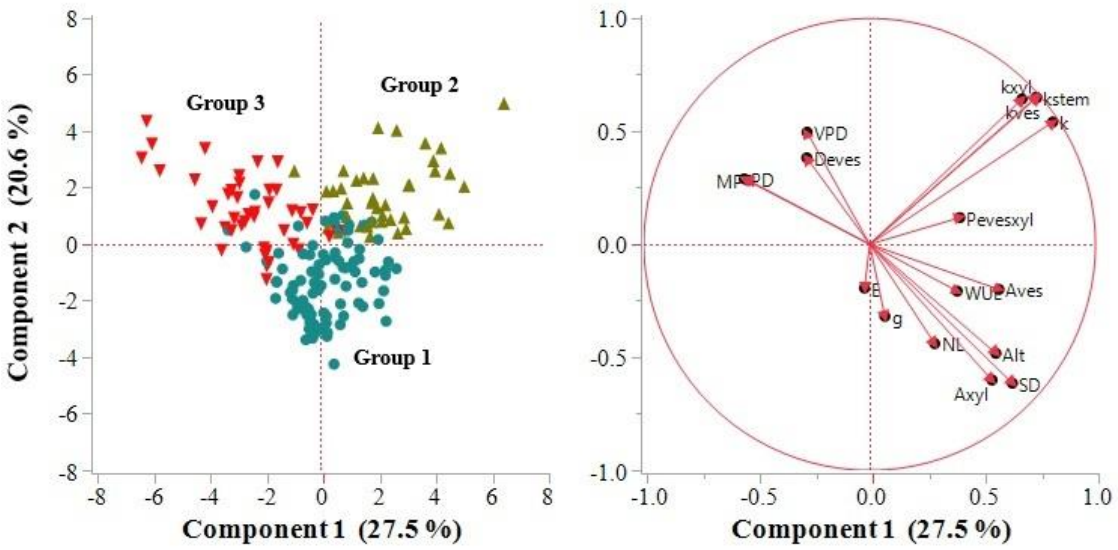

Figure 12. Principal component analysis on the correlations of the variables evaluated during the exposure of 174 avocado plants to water deficit and high temperatures. PD: percent defoliation, MP: plant death, D_{eves}: density of vessel elements, VPD: vapor pressure deficit, k_{stem}: stem specific hydraulic conductivity, k_{xyl}: xylem hydraulic conductivity, k_{ves}: vessel hydraulic conductivity, k: hydraulic conductivity, $P_{evesxyl}$: proportion of vessel elements in the xylem, A_{ves}: area of vessel elements, WUE: water use efficiency, Alt: plant height, SD: stem diameter, A_{xyl}: xylem area, NL: number of leaves, E: transpiration, g: stomatal conductance.

Of all the avocado accessions with plants that were placed in group 2, accessions G8 (Árbol 120 ‘M-14’), G10 (Árbol 93 M-01 ‘Samuel’) and G13 (Árbol 110 ‘C9’) had the highest number of outstanding plants, so it is recommended that they be included in future research to evaluate the adaptation of grafted cultivars in water-stressed environments.

3.4. Conclusions

Research on the adaptability of avocado plants to water stress has revealed a remarkable variability in characteristics and tolerance among different accessions of this species. Through the analysis of morphological, physiological and anatomical responses, it has been shown that the genetic characteristics of the accessions significantly influenced their ability to adapt to adverse conditions, such as high temperatures and low atmospheric and soil moisture. This study has identified accessions that not only maintain their foliage, but also exhibit lower defoliation and higher hydraulic conductivity (G8 (Árbol 120 ‘M-14’), G10 (Árbol 93 M-01 ‘Samuel’) and G13 (Árbol 110 ‘C9’), suggesting a promising potential in

breeding programs to carry out the selection of rootstocks that are able to adapt in regions with prolonged periods of drought. In addition, the importance of hydraulic conductivity and percent defoliation has been highlighted as key indicators in the evaluation of tolerance to water stress. The findings of this research not only contribute to the understanding of the adaptation mechanisms of avocado plants to water deficit, but also provide a solid basis for future research that seeks to optimize avocado agricultural production in contexts of water scarcity derived from climate change and the need to reduce the water footprint of this crop.

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Conflicts of Interest: "The authors declare no conflict of interest."

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4. SELECTION OF DROUGHT-TOLERANT AVOCADO ROOTSTOCKS: PHYSIOLOGICAL RESPONSES AND WATER-USE EFFICIENCY OF GRAFTED ‘HASS’

SELECTION OF DROUGHT-TOLERANT AVOCADO ROOTSTOCKS: PHYSIOLOGICAL RESPONSES AND WATER-USE EFFICIENCY OF GRAFTED 'HASS'

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4.1. Abstract

Avocado in Mexico faces critical challenges due to water scarcity under climate change, which threatens to reduce suitable cultivation areas. This study evaluated the influence of 18 *Persea* accessions grafted with 'Hass' under water deficit conditions, aiming to identify genotypes with greater tolerance to water scarcity and post-stress recovery capacity. An experimental design including irrigation, water deficit, and recovery phases, physiological and, morphological parameters were analyzed to group genotypes according to their response patterns. Group 3 (13 plants) maintained physiological activity for 16 days of water deficit, exhibited high recovery in CO_2 assimilation post-rehydration (*A*: +157 %), consistent with low rootstock hydraulic resistance (13,000 MPa·s·m·kg^{-1}), and high water-use efficiency (*WUE*: 6.11 µmol·mmol^{-1}) during recovery. In contrast, Group 1 (27 plants) showed high physiological performance under optimal conditions (*A*: 11.61 µmol ·m^{-2}·s^{-1} of CO_2) but marked stress vulnerability, evidenced by reductions in *A* (−56 %) and transpiration (*E*: −62 %), alongside drastic increases in stomatal resistance (R_s: +259 %), resulting in limited recovery—though without foliar damage (0 % defoliation). Group 2 (104 plants) displayed the poorest adaptation, characterized by defoliation (21.55 %), early leaf collapse (7 days), and drastic stomatal closure (R_s: +253 %), which failed to mitigate significant declines in *E* (−65 %). Key findings highlight that coordinated stomatal response and high water-use efficiency (*WUE*) are critical indicators of drought tolerance. Group 3 genotypes are valuable for breeding programs due to their enhanced physiological mechanisms to counteract water scarcity as rootstocks.

Keywords: *Persea americana* Mill., water stress, drought-tolerant rootstocks, leaf collapse, drought.

SELECCIÓN DE PORTAINJERTOS DE AGUACATE TOLERANTES AI DÉFICIT HÍDRICO: RESPUESTAS FISIOLÓGICAS Y EFICIENCIA EN EL USO DEL AGUA de 'HASS' INJERTADO

4.2. Resumen

El aguacate en México enfrenta desafíos críticos por la disponibilidad de agua ante el cambio climático, que amenaza con reducir las áreas aptas para su cultivo. Se evaluó la influencia de 18 accesiones de *Persea como* portainjertos de 'Hass' en condiciones de déficit hídrico, con el objetivo de identificar genotipos con mayor tolerancia a la falta de disponibilidad de agua y capacidad de recuperación post-estrés. Con un diseño experimental, que incluyó fases de riego, déficit hídrico y recuperación, se analizaron variables fisiológicas y morfológicas que permitieron agrupar a los genotipos acorde a sus patrones de respuesta. El Grupo 3 (13 plantas) mantuvo actividad fisiológica durante 16 días de déficit hídrico, mostró una alta recuperación en la asimilación de CO_2 post-rehidratación (*A*: +157 %), consistente con baja resistencia hidráulica de portainjertos (13,000 MPa·s·m^{-1}·kg^{-1}), y alta eficiencia en el uso del agua (*WUE*: 6.11 µmol·$mmol^{-1}$) en recuperación. En contraste, el Grupo 1 (27 plantas) mostró un alto rendimiento fisiológico en condiciones óptimas (*A*: 11.61 µmol m^{-2} s^{-1} de CO_2, pero marcada vulnerabilidad al estrés, evidenciada por reducciones de *A* (-56 %) y transpiración (*E*: -62 %) e incrementos drásticos en resistencia estomática (R_s: +259 %) que produjeron una recuperación limitada, aunque sin daño foliar (0 % defoliación). El Grupo 2 (104 plantas) presentó la menor adaptación, caracterizada por defoliación (21.55 %), colapso foliar temprano (7 días) y cierre drástico de estomas R_s (+253) % que no limitó las reducciones significativas en *E* (-65 %). Los resultados destacan que la coordinación entre respuesta estomática y alta eficiencia hídrica (*WUE*) son indicadores clave de tolerancia a déficit hídrico. Los genotipos del Grupo 3 representan material valioso para integrarse en programas de mejoramiento debido a que poseen mecanismos fisiológicos mejor adaptados para mitigar el estrés por déficit hídrico como portainjertos.

Palabras clave: *Persea americana* Mill., estrés hídrico, portainjertos tolerantes, colapso foliar, sequía.

4.3. Introduction

The avocado (*Persea americana* Mill.) is an economically and nutritionally significant crop in Mexico. This country holds a leading position as a global producer and exporter (Hurtado-Fernández et al., 2018). However, this crop faces growing challenges related to water availability, particularly in regions where agricultural water concessions are limited or where cultivation occurs under rainfed conditions (Fuerte-Velázquez & Gómez-Tagle, 2024).

Between 2016 and 2023, the average avocado yield in Mexico stabilized at 10.92 $t \cdot ha^{-1}$. During this period, irrigated orchards achieved an average yield of 11.72 $t \cdot ha^{-1}$, in contrast to rainfed orchards, which produced 10.11 $t \cdot ha^{-1}$ (SIACON, 2023). Nevertheless, this advantage contrasts with a notable difference in the water footprint of these production systems. Recent studies reveal that irrigated orchards require 40 to 180 % more water per ton of fruit produced (Fuerte-Velázquez & Gómez-Tagle, 2024). This disparity becomes highly relevant when considering that Mexico has the largest global water footprint for avocado cultivation (Sommaruga & Eldridge, 2020), with 48 % of the area irrigated (SIACON, 2023).

Climate change is expected to alter temperature and precipitation patterns in Michoacán, Mexico's primary avocado-producing region (Álvarez-Bravo et al., 2017), with a general trend toward decreased rainfall and increased temperatures (Ortiz-Paniagua & Gómez, 2018). These changes could drastically reduce the suitable land for cultivation, with recent estimates projecting a potential loss of 31.1 % to 43.0 % by 2050, depending on the severity of the climate scenario (Charre-Medellín et al., 2021).

Drought severely alters the water status of avocado plants and fruit growth. Physiological studies have shown that water deficit stress reduces stomatal conductance (by 40–60 %), limits photosynthesis, and decreases water flow to the fruits, resulting in up to 70 % slower growth compared to plants without water deficit (Kaneko et al., 2024). Even when 75 % of the water demand is supplied, avocado yields can decline by up to 15 % (Durán et al., 2021).

Although improvements in irrigation efficiency and agronomic management have been proposed to mitigate the water footprint of avocado cultivation (Fuerte-Velázquez & Gómez-Tagle, 2024), few studies address the potential of rootstocks as a genetic tool to optimize water use under current and projected climate change pressures. In this context, selecting genotypes that exhibit superior water-use efficiency and greater drought tolerance could not only narrow the productivity gap between rainfed and irrigated systems but also mitigate the negative effects of recurrent water deficits.

This study aimed to evaluate the influence of 18 avocado accessions used as rootstocks on the performance of the 'Hass' cultivar under water deficit conditions to identify individuals capable of minimizing damage during stress periods and promoting efficient recovery upon rehydration. We hypothesize that rootstocks will differentially influence the morphological and physiological responses of the grafted 'Hass' scion, with some exhibiting lower sensitivity to water stress and maintaining better performance in the evaluated physiological variables.

4.4. Materials and Methods

The study was conducted in a greenhouse at the Universidad Autónoma Chapingo, located at an elevation of 2,264 m (19.4904322, -98.8734917), during the plant growth and evaluation period from April 2021 to June 2023.

4.4.1. Plant material, growth conditions, and experimental design

Eighteen avocado accessions (Table 1) from the Germplasm Bank of the Fundación Salvador Sánchez Colín CICTAMEX S.C. were evaluated, selected for their genetic diversity and agronomic relevance. Plants were germinated in 250 mL individual containers on 29 April 2021 and transplanted on 9 July of the same year into polyvinyl chloride (PVC) pots (15.5 cm diameter × 38.5 cm height) filled with 8 L of substrate. The substrate consisted of a 1:1 (v/v) mixture of agricultural loam soil and river sand.

The substrate's water-holding capacity was quantified at 19.2 % (w/w) using the gravimetric method after saturation and free drainage for 24 h. The substrate's pH and electrical conductivity were 7.3 and 1.0 $dS \cdot m^{-1}$, respectively.

Table 3. *Persea americana* accessions employed in the selection of rootstocks with potential drought tolerance.

ID Accession	Accession	Origin	Plant number
G1	'Colín V-33'	Seedling of 'Fuerte', Ixtapan de la Sal, Mexico	10
G2	'Encinos'	Seedling of 'Hass', Coatepec Harinas, Mexico	10
G3	'Derrumbe 92'	Accession of Mexican race, Coatepec Harinas, Mexico	9
G4	Árbol 247 Raza mexicana 12	Seedling of Mexican race, Coatepec Harinas, Mexico	8
G5	Árbol 55 'Fredy 7'	Accession from Miahuatlán de Porfirio Díaz, Oaxaca, Mexico	10
G6	Árbol 144 'Frazer'	Variety from New Zealand	9
G7	Árbol 12 *Persea nubigena* 1/7 (CH-I-4)	Accession from Volcani Center, Bet-Dagan, Israel	5
G8	Árbol 120 'M-14'	Accession from Jungapeo, Michoacán, Mexico	10
G9	Árbol 66 *Persea nubigena* 'Rodeo II' (CH-G-76)	Accession from El Rodeo, Chiapas, Mexico	3
G10	Árbol 93 M-01 'Samuel'	Accession from Zitácuaro, Mexico	7
G11	Árbol 139-141 'Boyce'	Variety form California, USA	8
G12	Árbol 202 'Híbrido 23F'	Seeding of 'Colín V-33' from Coatepec Harinas, Mexico	9
G13	Árbol 110 'C9' (Islas Canarias)	Accession from Canary Islands, Spain	10
G14	Árbol 100 M-08 'Mariano'	Accession from Tacámbaro, Michoacán, Mexico	8
G15	Árbol 81 'Polo'	Variety from Coatepec Harinas, Mexico	3
G16	Árbol 164 M-39 'Juanita 39'	Accession from Zapopo, Michoacán, Mexico	8
G17	Árbol 89 M-06 'Bladimiro'	Accession from Tingambato, Michoacán, Mexico	8
G18	Árbol 86 'Lamb Hass'	Variety form California, USA	9

Plants were maintained under optimal substrate moisture conditions and fertilized three times per week with a balanced nutrient solution (Table 4; pH 5.8-6.0, EC 1.0-1.2 $dS \cdot m^{-1}$). Phytosanitary control included foliar applications of copper oxychloride (3.5

g·L⁻¹) and imidacloprid (1 mL·L⁻¹, applied as a substrate drench). On July 23, 2022, the seedling rootstocks were grafted with ‘Hass’ scions using the side-veneer grafting technique, and the growth of a single main stem was promoted. Morphological and physiological evaluations were conducted exclusively on the ‘Hass’ graft, aiming to characterize the influence of the rootstock on the cultivar performance.

Table 4. Nutrient solution composition for avocado cultivation during the experiment.

EC (dS m^{-1})	pH	Macronutrients (mg L^{-1})					
		NO_3^-	$H_2PO_4^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}
		80	16	56	136	90	24
1.0 - 1.2	5.8 - 6.0	Micronutrients (mg L^{-1})					
		Fe	Mn	Cu	Zn	B	Mo
		3.1	0.50	0.03	0.05	0.51	0.05

The trial was established under a completely randomized design, with a variable number of replicates per accession (3 to 10 plants) determined by graft-take success and post-stress survival during a preliminary evaluation phase under water deficit and high-temperature conditions (>40 °C). In total, 144 plants were evaluated, distributed across two experimental plots of 72 plants each, to ensure efficient logistical management during the experiment. At the onset of the water deficit stress evaluations, the rootstocks were 20.87 months old (1.74 years), with an average height of 164 cm, and 8.9 months had elapsed since grafting with ‘Hass’.

The evaluation of plants under water deficit stress conditions was conducted in consecutive periods: Plot 1 from April 4 to 22, 2023 (27 days); Plot 2 from May 4 to 20, 2023 (25 days). During these periods, mean temperature ranged between 16.0 and 20.52 °C, and relative humidity between 45.36 and 76.40 %. Specifically, the mean temperature and average relative humidity during Plot 1 evaluation were 18.41 °C and 64.0 %, respectively, while Plot 2 showed mean values of 18.67 °C and 62 % (Figure 13).

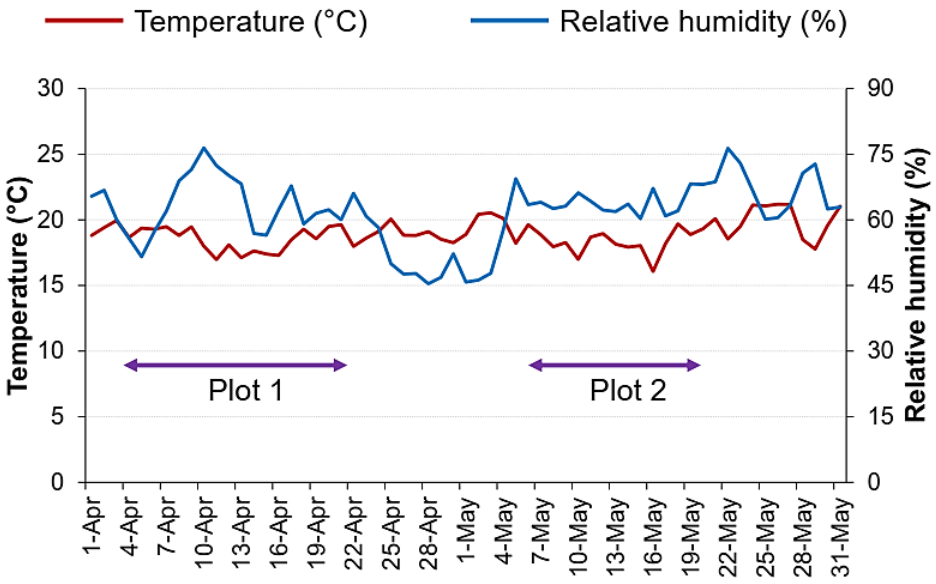

Figure 13. Monitoring of environmental conditions during water-deficit stress experiments in 18 avocado accessions using a HOBO® Pro v2 thermohygrometer (U23-001) with 5-minute logging intervals.

4.4.2. Evaluation of morphological and physiological parameters.

Each experimental unit consisted of an individual plant grafted with 'Hass', subjected to a standardized protocol comprising three phases: irrigation, water deficit, and recovery. During the irrigation phase (days 1-3), the substrate was maintained at maximum water-holding capacity. On the third day, leaf relative water content (RWC) was determined using the gravimetric method of Barrs & Weatherley (1962).

$$RWC\ (\%) = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

Prior to stress induction, plant height (m) was measured using a tape measure (±0.1 cm), and basal stem diameter (mm) was determined at 5 cm above the substrate level using a digital caliper (Mitutoyo®, ±0.01 mm). Leaf count was also recorded.

Throughout the three evaluation phases, physiological variables were recorded daily, including: CO_2 assimilation (A, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}). Measurements were taken at midday (12:00 h) using a portable infrared gas analyzer (CI-340, CID Bio-Science®). The mean

photosynthetically active radiation (PAR) during measurements was 577 ± 116 $\mu mol \cdot m^{-2} \cdot s^{-1}$ throughout the evaluation period.

From these measurements, the following physiological indices were calculated: water-use efficiency ($WUE = A/E$; $\mu mol\ CO_2 \cdot mmol^{-1}$), intrinsic water-use efficiency ($WUEi = A/g_s$; $\mu mol\ CO_2 \cdot mol^{-1}$), stomatal resistance ($R_s = 1/g_s$; $s \cdot m^2 \cdot mmol^{-1}$), and transpiration efficiency (E/g_s; dimensionless).

The water stress phase began with irrigation withholding and continued until each plant reached the permanent wilting point, defined as irreversible turgor loss in >80 % of the foliage. This point was determined through daily visual inspections at 08:00 h.

Upon reaching the permanent wilting point, leaf *RWC* was remeasured to assess post-stress water status. Plants were then gradually rehydrated through three applications of 500 mL water per plant at 2-hour intervals. Physiological variables were recorded during the five days following rehydration (recovery phase). Leaf collapse was documented during both water deficit and recovery stages.

At 25 days post-stress, defoliation percentage (PD, %) was quantified based on the ratio between remaining and initial leaf counts. At 65 days, graft ('Hass') hydraulic conductivity (*k*) was determined by collecting 30-cm stem segments, which were trimmed to 20 cm under distilled water to prevent embolism. These segments were perfused with 0.01 M oxalic acid (Pire et al., 2007), and flow rate (F, $kg \cdot s^{-1}$) was measured using a precision balance (Ohaus Discovery DV215CD). Hydraulic conductivity was calculated as $k = (F \times L)/\Delta P$ ($kg \cdot m \cdot s^{-1} \cdot MPa^{-1}$), where L is segment length (m) and ΔP is the pressure gradient (MPa), derived from $\Delta P = h \times \rho \times g$ (h: water column height, ρ: 1000 $kg \cdot m^{-3}$, g: 9.81 $m \cdot s^{-2}$) according to Sperry et al. (1988). Hydraulic resistance (R) was determined as the inverse of hydraulic conductivity.

4.4.3. Statistical analysis

The statistical analysis was conducted in three stages. First, redundant variables were identified through Pearson correlation analysis ($|r| > 0.8$) using JMP Pro v14.0 statistical software (SAS Institute Inc.), and those showing high collinearity were discarded. With

the selected variables, a principal component analysis (PCA) was performed, applying the Kaiser criterion (eigenvalues ≥ 1) for dimensionality reduction.

Subsequently, hierarchical cluster analysis was conducted using Ward's method (Euclidean distance) on the principal components, which enabled the classification of individuals into 3 groups with contrasting responses to water stress. Given the unequal number of replicates per accession and the lack of data homoscedasticity, non-parametric methods were applied for comparisons between groups and evaluation phases. Specifically, the Kruskal-Wallis test was used, followed by Dunn's post hoc analysis using GraphPad Prism v10.4.1 (GraphPad Software, LLC).

4.5. Results and Discussion

4.5.1. Grouping of accessions according to stress response

The Principal Component Analysis (PCA) reduced the 7 variables selected through Pearson correlation analysis to 3 principal components that together explained 75.55 % of the total variance (PC1: 39.97 %, PC2: 20.63 %, PC3: 14.93 %). PC1, associated with CO_2 assimilation under irrigation (*A*-Irrig) and transpiration under optimal irrigation (*E*-Irrig), represented the greatest source of variation. PC2 and PC3 were linked to stress response, highlighting days to leaf collapse (Days-LC) and water-use efficiency under water deficit (*WUE*-WD), respectively (Figure 14a).

Hierarchical clustering analysis defined three groups with contrasting average responses to water deficit stress (Figure 14b). Group 1 (n = 27) exhibited low tolerance (5.81 ± 2.2 days to wilting), with slight defoliation (9.29 ± 20.3 %) and low water-use efficiency during recovery (*WUE*-WD: 3.16 ± 1.02 μmol·mmol^{-1}). Group 2 (*n* = 104) was the most affected, displaying low tolerance (5.76 ± 2.1 days to wilting), moderate defoliation (34.7 ± 7.8 %), and the lowest recovery *WUE* (*WUE*-Rec: 2.83 ± 1.53 μmol·mmol^{-1}). In contrast, Group 3 (*n* = 13) exhibited high tolerance (15.0 ± 2.2 days to wilting), minimal defoliation (13.71 ± 12.71 %), and outstanding recovery efficiency (*WUE*-Rec: 6.11 ± 1.19 μmol·mmol^{-1}).

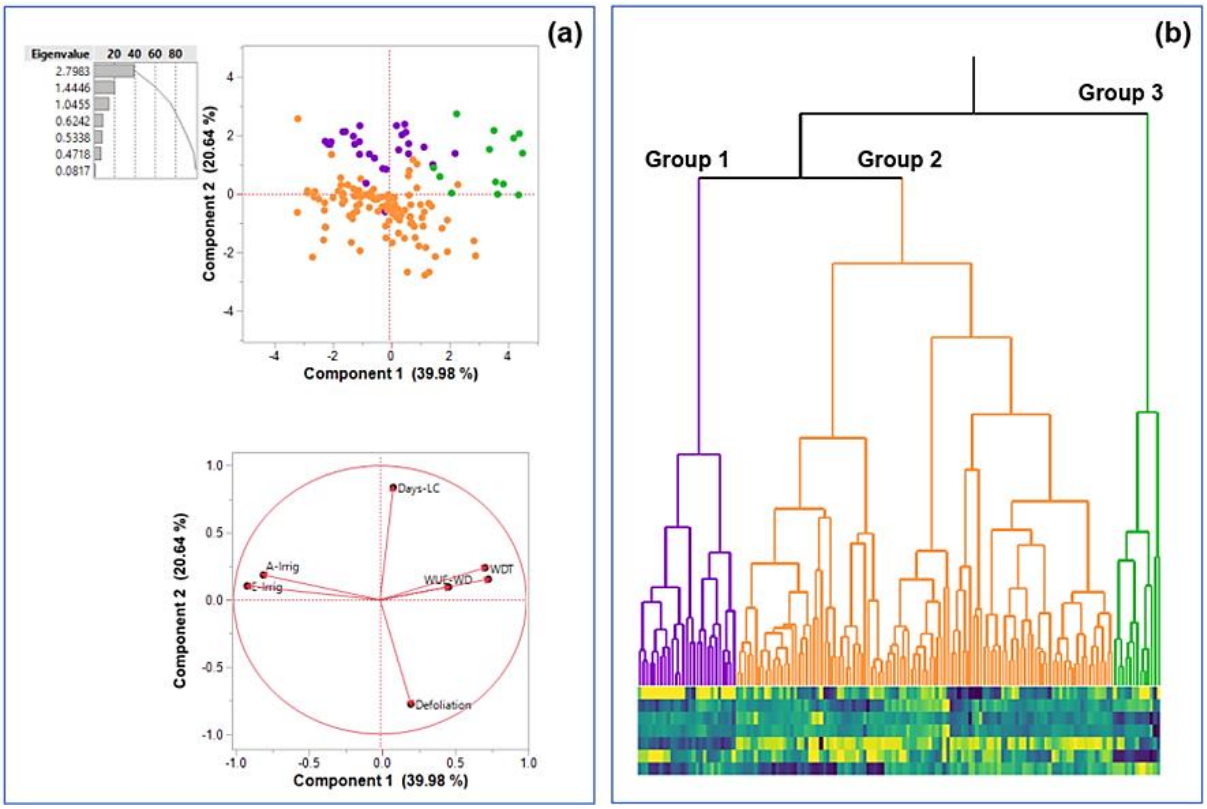

Figure 14. Multivariate analysis of drought response in avocado rootstocks grafted with 'Hass': a) PCA components (eigenvalues, scores and loadings); b) Ward-method dendrogram (*n* = 144).

These results indicate that Group 3 exhibited superior physiological traits for water stress tolerance, combining high leaf retention with efficient metabolic recovery. This suggests significant potential for these genotypes as drought-tolerant rootstocks. These differences were also phenotypically evident, as shown in Figure 15, which illustrates plant appearance under: (1) optimal irrigation, (2) severe stress (permanent wilting point), and (3) recovery (foliar appearance at 40 days post-stress).

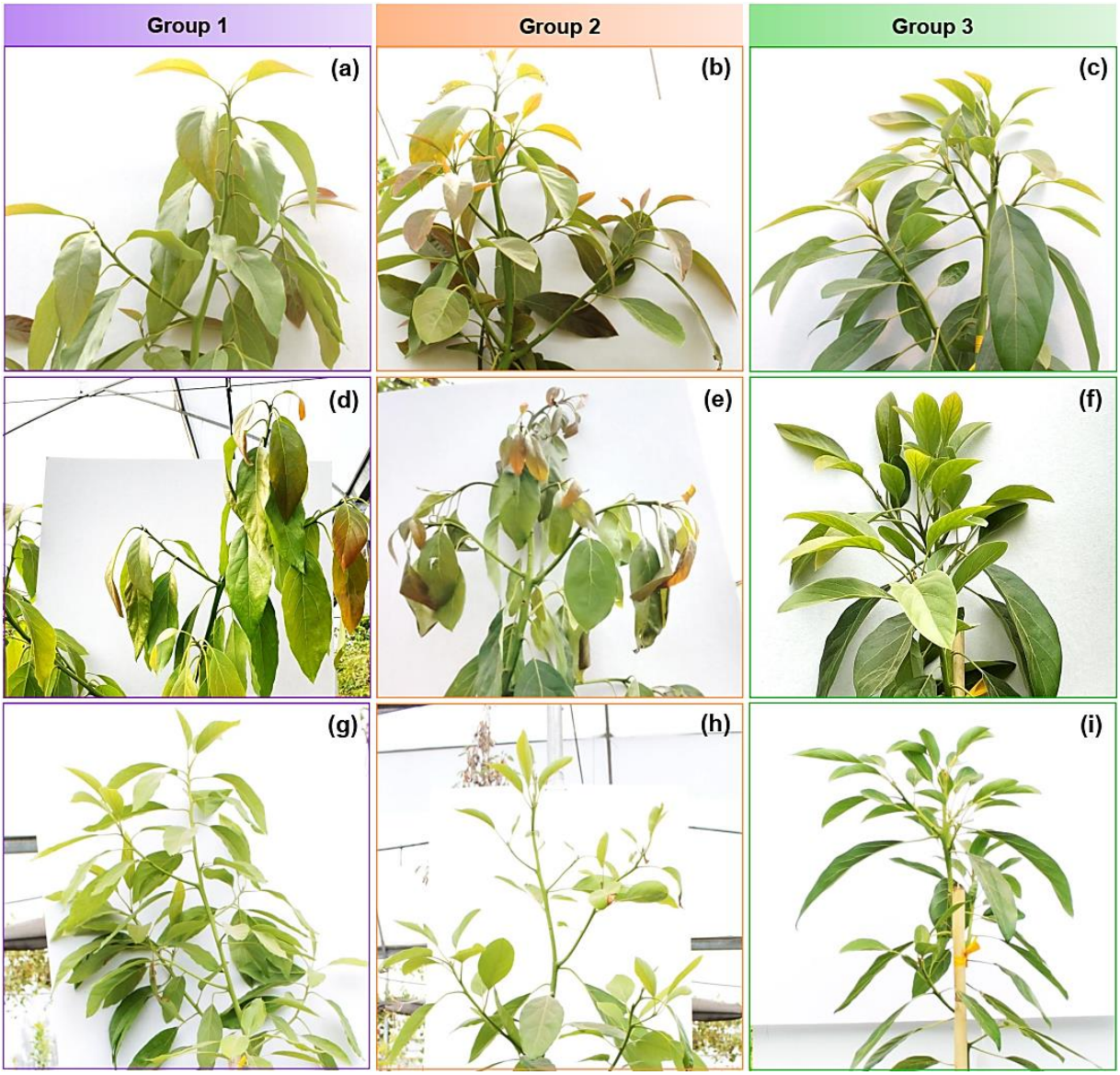

Figure 15. Phenotypic responses to drought stress of 'Hass' grafted on different rootstock tolerance groups. Optimal irrigation (a, b, c). Severe stress (d, e, f). Post-stress recovery (g, h, i).

4.5.2. Comparison of responses between groups

The evaluated groups showed uniform morphological characteristics (height, diameter, number of leaves; p >0.05), but exhibited significant differences in key variables associated with water tolerance (Table 5). In contrast, parameters such as relative water content (RWC) and graft hydraulic resistance (SHR) showed no variation between groups (p >0.05).

Table 5. Comparison of morphological and physiological variables of ‘Hass’ avocado grafted on rootstock groups under water deficit (*n* = 144): medians, Kruskal-Wallis *H* statistic, and significance (Dunn's *post hoc* test).

Variable	Group 1 (*n* = 27)	Group 2 (*n* = 104)	Group 3 (*n* = 13)	*H*	*p*-value
Height (cm)	166.00 a	167.00 a	157.00 a	3.835	0.147^{ns}
Diameter (mm)	21.60 a	21.30 a	22.30 a	1.767	0.413^{ns}
Number of leaves	80.00 a	86.00 a	74.00 a	2.295	0.317^{ns}
RWC-BWD (%)	93.39 a	93.29 a	92.05 a	2.672	0.263^{ns}
RWC-AWD (%)	77.71 a	78.47 a	76.11 a	0.458	0.795^{ns}
RHR (MPa s m^{-1} kg^{-1})	23000 ab	29500 a	13000 b	7.432	0.024*
SHR (MPa s m^{-1} kg^{-1})	5764700 a	5114350 a	6662900 a	4.998	0.082^{ns}
Defoliation (%)	0.00 b	21.55 a	13.56 ab	33.64	<0.001***
Leaf collapse (days)	0 b	7 a	13 a	43.51	<0.001***
WDT (days)	6 b	5 b	16 a	34.78	<0.001***

RWC_BWD: Relative water content (before water deficit), RWC-AWD: Relative water content (after water deficit); RHR: Rootstock hydraulic resistance; SHR: Scion hydraulic resistance; WDT: Water deficit tolerance.

Rootstock hydraulic resistance (RHR) varied significantly among groups (H = 7.43; *p* = 0.024), with Group 3 exhibiting the lowest values (13,000 MPa·s·m^{-1}·kg^{-1}) compared to Group 2 (29,500 MPa·s·m^{-1}·kg^{-1}). This difference suggests greater water transport efficiency in Group 3, potentially explaining its superior water deficit tolerance (WDT: 16 days *vs*. 5–6 days in other groups; *p* < 0.001; Figure 16b). Reduced hydraulic resistance facilitates xylem water flow, promoting hydraulic continuity and potentially enhancing drought tolerance (Garrido & Vergara, 2022).

In contrast, scion hydraulic resistance (SHR) showed no significant differences among groups (*p* = 0.082), indicating that the water stress response was primarily determined by the rootstock rather than the ‘Hass’ graft (Table 5). Avocado has been identified as a fruit crop with high vulnerability to water stress (Garrido & Vergara, 2022). This behavior was consistent with our findings, where 91 % of evaluated plants exhibited rapid irreversible leaf turgor loss and 78 % showed water deficit-induced defoliation.

Concerning defoliation percentage, Group 2 exhibited the highest levels (21.55 %), which were significantly greater than Group 1 (0.00 %; *p* < 0.001) but not statistically different from Group 3 (13.56 %; *p* = 0.013) (Figure 16a). This elevated defoliation in Group 2 was correlated with early leaf collapse (7 days; Figure 17) and may be associated with its higher rootstock hydraulic resistance (RHR) (Table 5). These results

are consistent with the physical principle that high hydraulic resistance increases the difficulty of water movement through the xylem (Tyree & Ewers, 1991), potentially explaining both the observed higher defoliation (21.55 %) and early leaf collapse (7 days). Under water deficit conditions, xylem conduction capacity becomes a critical limiting factor that triggers increased hydraulic resistance as cavitation progresses (Sperry et al., 2002). Extensive cavitation dramatically reduces plant hydraulic conductance and leads to progressive functional deterioration. According to Johnson et al. (2022), this process can reach a point of no return where the plant loses its ability to maintain water homeostasis, ultimately resulting in structural failures such as leaf collapse and massive defoliation.

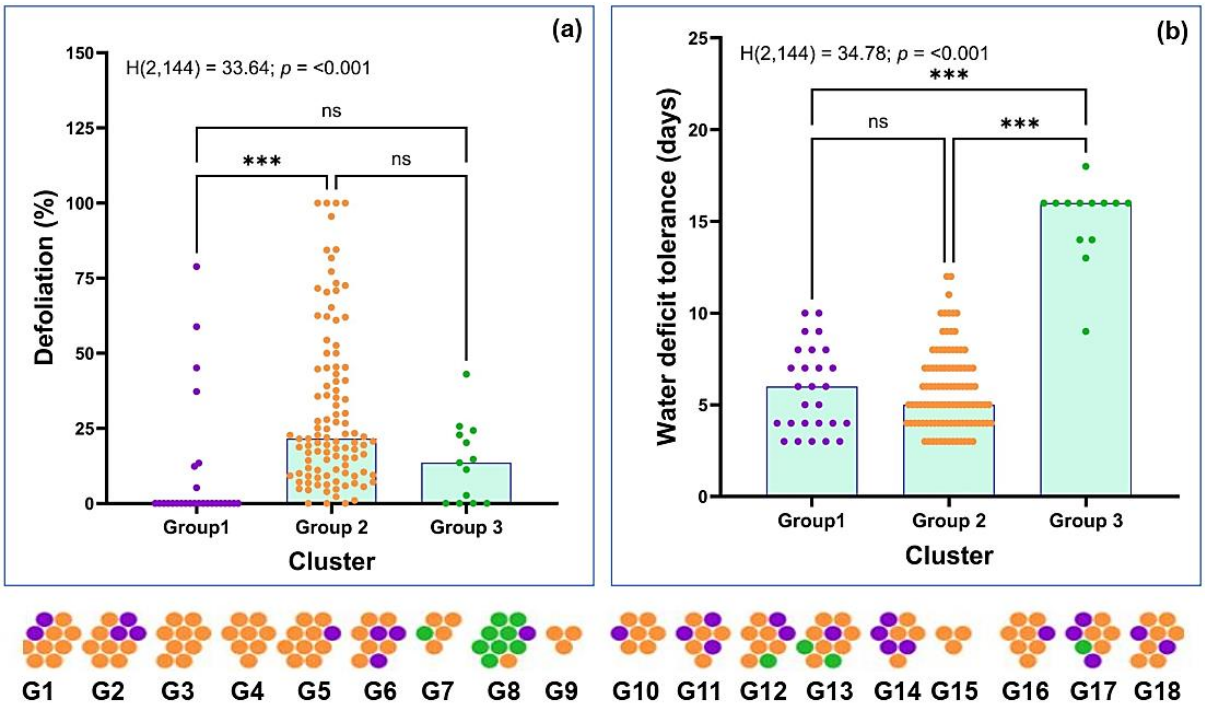

Figure 16. Comparison of response of 'Hass' avocado grafted on different rootstock groups and accessions distribution. a) Tolerance to water deficit stress. b) Defoliation. Individuals from each accession (G) are represented by colored circles at the bottom of the figure: purple = Group 1, orange = Group 2, and green = Group 3.

For its part, water deficit tolerance (WDT) was notably higher in Group 3 (16 days), which exceeded the tolerance period of Group 1 (6 days; p <0.001) and was three times greater than that of Group 2 (5 days; p <0.001) (Figure 16b). Additionally, leaf collapse occurred later in Group 3 (9 days) and Group 2 (5 days), while Group 1 showed no leaf

collapse (0 days; H = 43.51; p <0.001) (Figure 17), despite its overall low stress tolerance. These results highlight that, while the groups showed no differences in morphological traits, Group 3 combined low rootstock hydraulic resistance (efficient water flow) with high stress tolerance (Table 5).

The 13 individuals classified in Group 3 originated from genetically diverse accessions, with predominant representation from Tree 120 ‘M-14’ (G8), which constituted 69.2 % of the group (9 plants). This distribution underscores that this particular accession possesses physiological mechanisms better adapted to water stress management compared to the other evaluated accessions. Notably, while 72.2 % of accessions showed a homogeneous stress response by being classified exclusively in Groups 1 or 2, accessions such as Tree 110 ‘C9’ (Canary Islands, G13), Hybrid 23F (G12), and M-06 ‘Bladimiro’ (G17) exhibited remarkable intra-accession diversity, with individuals distributed across all three tolerance groups (Figure 16).

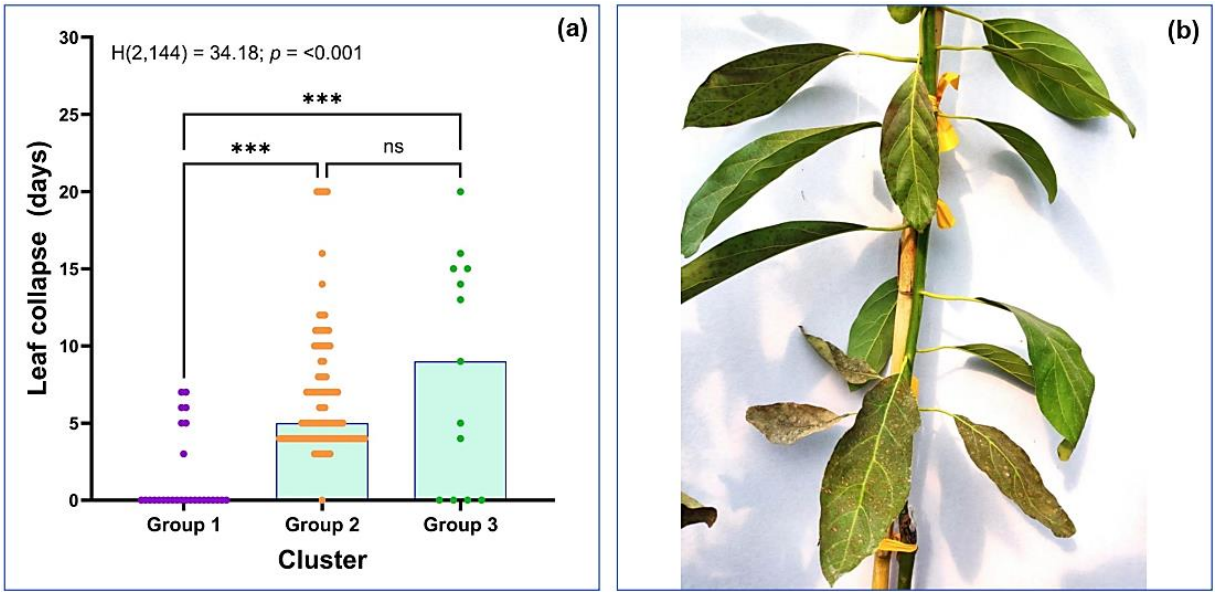

Figure 17. Comparison of response among avocado rootstock groups. a) Leaf collapse. b) Leaf collapse visual appearance.

The marked predominance of ‘M-14’ in Group 3 indicates that its genetic basis confers unique physiological advantages as a rootstock under water deficit conditions. Recent studies suggest this superiority may be linked to more efficient regulation of abscisic acid (ABA) response genes (Moreno-Ortega et al., 2021), which would enhance the

plant's ability to maintain water homeostasis. These findings emphasize the importance of selecting rootstocks based on their physiological characteristics rather than geographic origin, particularly considering the heterogeneity observed in accessions such as ‘C9’ (G12) and ‘Hybrid 23F’ (G13) (Figure 16), where genetically related individuals showed contrasting stress responses. Stress tolerance is a quantitative trait that typically decreases during domestication processes (Fita et al., 2015; Smýkal et al., 2018).

4.5.3. Comparison of physiological responses by group and water condition

The evaluated avocado rootstock groups exhibited three distinct physiological strategies in response to water deficit (Table 6), revealing important implications for material selection in breeding programs.

Table 6. ‘Hass’ avocado grafted in different rootstock group median values of leaf CO_2 assimilation (*A*), stomatal conductance (g_s), and transpiration (*E*) in three avocado rootstock groups under irrigation (Irrig), water deficit (WD), and recovery (Rec) conditions, with Kruskal-Wallis *H* statistics and *p*-values.

Variable	Group 1 (*n* = 27)	Group 2 (*n* = 104)	Group 3 (*n* =13)	*H*	*p*-value
A-Irrig (µmol m⁻² s⁻¹)	11.61 a	10.18 a	7.19 b	15.02	<0.001***
A-WD (µmol m⁻² s⁻¹)	5.13 a	3.45 b	2.08 c	17.73	<0.001***
A-Rec (µmol m⁻² s⁻¹)	4.63 a	3.95 a	5.36 a	6.32	0.042*
g_s-Irrig (mmol m⁻² s⁻¹)	121.70 a	93.07 a	41.60 b	27.12	<0.001***
g_s-WD (mmol m⁻² s⁻¹)	33.88 a	26.32 a	12.71 b	28.14	<0.001***
g_s-Rec (mmol m⁻² s⁻¹)	42.63 a	35.66 a	30.41 a	3.04	0.218ns
E-Irrig (mmol m⁻² s⁻¹)	3.99 a	3.62 a	1.62 b	28.41	<0.001***
E-WD (mmol m⁻² s⁻¹)	1.52 a	1.25 a	0.48 b	34.63	<0.001***
E-Rec (mmol m⁻² s⁻¹)	1.44 a	1.23 a	0.83 b	10.97	0.004**

Group 1 showed high performance under optimal conditions but was vulnerable to stress. This group achieved the highest values for CO_2 assimilation (*A*: 11.61 µmol·m⁻²·s⁻¹), stomatal conductance (g_s: 121.70 mmol·m⁻²·s⁻¹), and transpiration (*E*: 3.99 mmol·m⁻²·s⁻¹), consistent with a high-yield phenotype in favorable environments. However, this strategy proved limiting under water deficit, with drastic reductions in gas exchange parameters: *A* (−56 %), g_s (−72 %), and *E* (−62 %). These reductions were

associated with low stress tolerance (Figure 18), evidenced by early permanent wilting (6 days).

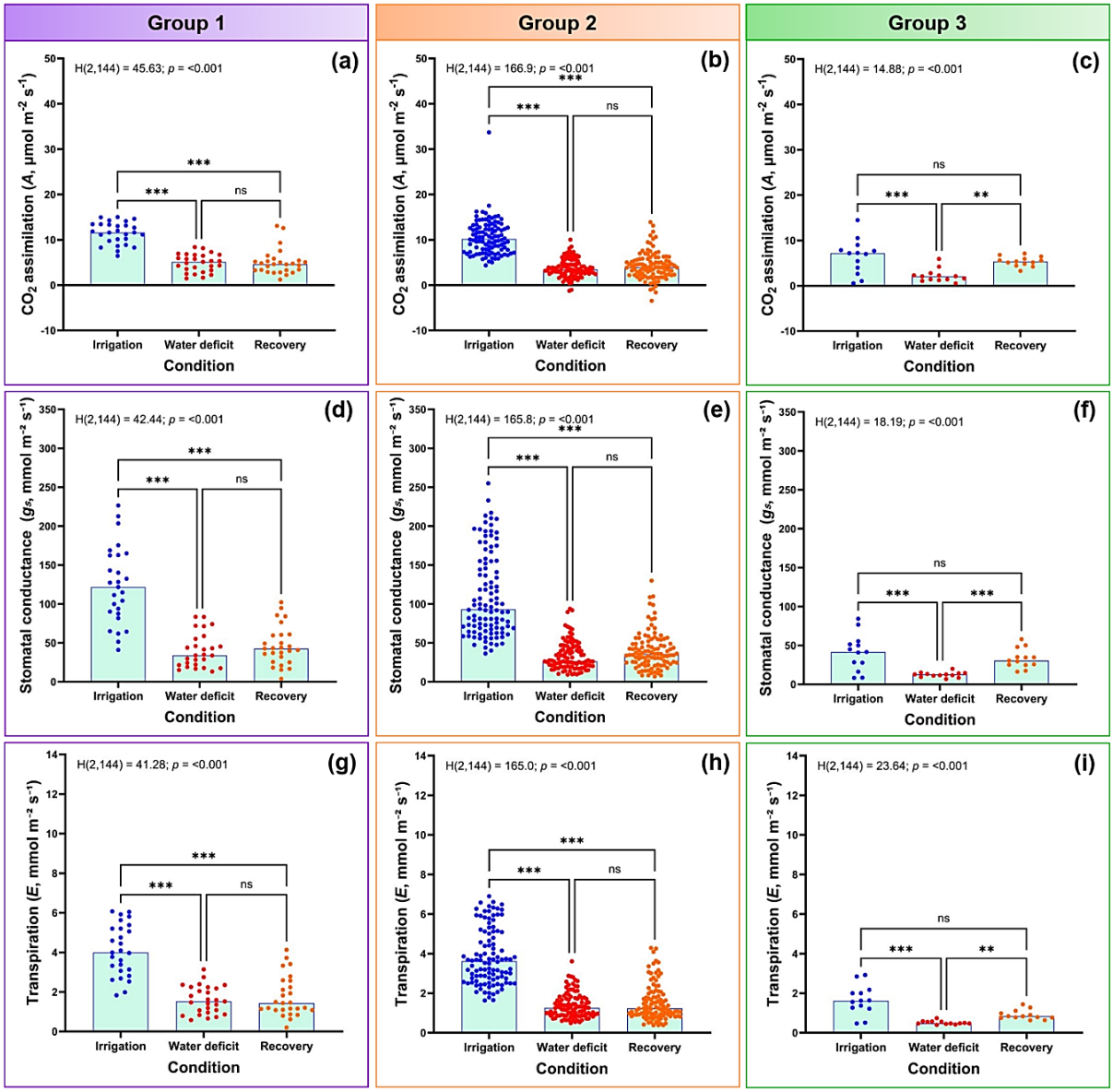

Figure 18. 'Hass' avocado grafted in different rootstock group comparison of values of leaf CO_2 assimilation (*A*; a, b, c), stomatal conductance (g_s; d, e, f), and transpiration (*E*; g, h, i), under optimal irrigation, water deficit, and recovery conditions.

During recovery, although leaves regained turgor, *A*, g_s, and *E* values remained at levels equivalent to those recorded during water deficit (Figure 18). Despite maintaining foliar integrity (0 % defoliation), the inability to restore stomatal function post-stress suggests damage to the photosynthetic apparatus. This dysfunction may be attributed

to oxidative stress, which could impair stomatal regulation mechanisms, compromising stomatal reopening and, consequently, the plant's capacity to restore CO_2 fluxes and regain baseline assimilation rates (Flexas et al., 2006a).

Group 2 showed insufficient protective mechanisms against stress. Its physiological performance was similar to Group 1 under optimal irrigation conditions, but under water deficit it exhibited marked vulnerability in its CO_2 assimilation capacity (Figure 18b). First, it displayed an apparently delayed and insufficient stomatal response, evidenced by drastic reductions in stomatal conductance (-72 %) and transpiration (-65 %) of similar magnitude to the decrease in CO_2 assimilation (-66 %), suggesting primarily stomatal rather than metabolic limitation (Flexas et al., 2006). This inefficient response was associated with severe tissue damage reflected in the greatest foliage loss (21.55 %) among evaluated groups, early permanent wilting (5 days), and notable post-rehydration leaf collapse (Figure 18a), which are indicative of failures in cellular protection mechanisms (Hussain et al., 2018).

The post-rehydration leaf collapse indicated a disruption of cellular protection systems, which may manifest as irreversible loss of cellular integrity due to membrane lipid peroxidation (Lei, 2008) and critical accumulation of reactive oxygen species (ROS) (Sun et al., 2020). This chain reaction was phenotypically expressed through a characteristic sequence consisting of initial punctate necrotic lesions that progressed to unified brown spots, followed by complete leaf blade desiccation (Figure 17b). The persistence of damaged leaves attached to the stem further suggests a disruption in programmed senescence mechanisms (Lim et al., 2007).

As a survival strategy, programmed leaf senescence serves a physiological function by reducing water loss through transpiration, maintaining plant water balance, and facilitating nutrient mobilization from senescent leaves to young organs to optimize resources during stress periods (Munné-Bosch & Alegre, 2004). However, in the plants evaluated in this study, the drastic foliar response would compromise vegetative development (Zhang et al., 2024). This phenomenon is particularly critical in traditional cultivation systems using rootstocks with high genetic diversity, where variability in senescence patterns may exacerbate production losses and is consistent with drastic

yield reductions under water limitation for ‘Hass’ growth and productivity (Duran et al., 2021).

Group 3, by contrast, adopted a strategy that enabled coordinated physiological processes. Initially, it showed the lowest values for CO_2 assimilation (7.19 $\mu mol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (41.60 $mmol \cdot m^{-2} \cdot s^{-1}$), and transpiration (1.62 $mmol \cdot m^{-2} \cdot s^{-1}$) under optimal irrigation conditions (Table 6). During water deficit, a drastic reduction in g_s (-69 %) was observed, suggesting high sensitivity to substrate water status, likely mediated by the root system which detects changes in water availability and initiates signaling pathways leading to whole-plant physiological and morphological responses (Gorgues et al., 2022).

This precise response allowed maintenance of foliar turgor for 16 days of water deficit, an effect also attributed to avocado rootstock influence on the scion (Reyes-Santamaría et al., 2025). The second key feature was metabolic resilience, evidenced by rapid recovery of CO_2 assimilation (+157 %) during rehydration (5 days post-rehydration) (Table 6; Figure 18).

This recovery suggested effective photosynthetic protection, likely via proline and glycine betaine accumulation. These compounds help maintain cellular osmotic balance and protect cellular structures under water deficit conditions (Bhattacharya, 2021). In avocado, these metabolites have been shown to accumulate during drought (Barrientos Priego & Rodríguez Ontiveros, 1994). and their synthesis may further contribute to preserving Rubisco activity (Ghosh et al., 2021). Furthermore, recent studies identified that other metabolites like trehalose and sinapoyl aldehyde play a key protective role during early stress stages in avocado, with their concentrations potentially decreasing as stress persists, possibly due to plant maladaptation to prolonged stress conditions (Zheng et al., 2024).

The maintenance of photosynthetic systems in Group 3 contrasted sharply with the irreversible damage observed in Groups 1 and 2, which exhibited similarly reduced physiological parameters during both water deficit and recovery phases (Figure 6).

The water-use efficiency and stomatal regulation parameters under the different evaluated water conditions confirmed the contrasting responses in the influence of avocado rootstock groups on the 'Hass' scion (Table 7). Group 1 showed stability in its water-use efficiency (*WUE*; μmol·mmol⁻¹) across all conditions, with no significant differences between optimal irrigation (2.91), water deficit (2.97), and recovery (2.94) (Figure 20). However, its intrinsic water-use efficiency (*WUEi*) increased under stress (+30 %) (Table 7), suggesting moderate stomatal adjustment to conserve water. Nevertheless, the increased E/g_s ratio during water deficit (+22 %) could indicate either that stomata might be poorly regulated by facilitating excessive water losses despite partial closure (Figure 20d) or that it may be part of a leaf thermoregulation mechanism (Chada et al., 2023) to cope with the stress produced by photorespiration stimulated by stomatal closure (Flexas & Medrano, 2002b).

Table 7. Median values of water-use efficiency (WUE), intrinsic water-use efficiency (WUEi), transpiration-stomatal conductance ratio (E/gs), and stomatal resistance (R_s) in 'Hass' avocado grafted on three rootstock groups under irrigation (Irrig), water deficit (WD), and recovery (Rec) conditions, with Kruskal-Wallis H statistics and p-values.

Variable	Group 1 (*n* = 27)	Group 2 (*n* = 104)	Group 3 (*n* =13)	*H*	*p*-value
WUE-Irrig (μmol·mmol⁻¹)	2.91 a	2.69 a	3.68 a	6.33	0.042^{ns}
WUE-WD (μmol·mmol⁻¹)	2.97 a	2.64 a	3.03 a	8.33	0.015^{ns}
WUE-Rec (μmol·mmol⁻¹)	2.94 b	2.885 b	6.11 a	30.92	<0.001***
WUEi-Irrig (μmol·mol⁻¹)	0.1047 b	0.1019 b	0.1621 a	20.06	<0.001***
WUEi-WD (μmol·mol⁻¹)	0.1357 ab	0.1099 b	0.1636 a	8.74	0.013*
WUEi-Rec (μmol·mol⁻¹)	0.1242 b	0.1110 b	0.1711 a	21.87	<0.001***
E/g_s-Irrig (mmol·mmol⁻¹)	0.0356 b	0.0354 b	0.0393 a	8.62	0.013*
E/g_s-WD (mmol·mmol⁻¹)	0.0433 a	0.0425 a	0.0408 a	0.07	0.086^{ns}
E/g_s-Rec (mmol·mmol⁻¹)	0.0392 a	0.0371 a	0.0266 b	15.74	<0.001***
R_s-Irrig (s·m²·mmol⁻¹)	0.00821 b	0.01075 b	0.02404 a	27.12	<0.001***
R_s-WD (s·m²·mmol⁻¹)	0.02951 b	0.03800 b	0.07867 a	28.14	<0.001***
R_s-Rec (s·m²·mmol⁻¹)	0.02334 a	0.02805 a	0.03288 a	3.05	0.217^{ns}

Group 2, for its part, showed limited physiological performance under water deficit conditions (Table 7). Although its intrinsic water-use efficiency (*WUEi*) increased slightly (+8 %; p <0.05), and the transpiration-stomatal conductance ratio (E/g_s) also showed a significant increase (+20.0 %; p <0.001), this moderate stomatal adjustment would indicate a decoupling between transpiration and CO_2 assimilation, revealed by

the loss of synchronization between these processes. Furthermore, the severe increase in R_s during water deficit (+253 %) did not limit water losses through transpiration, a behavior that persisted even during the recovery phase (Figure 19).

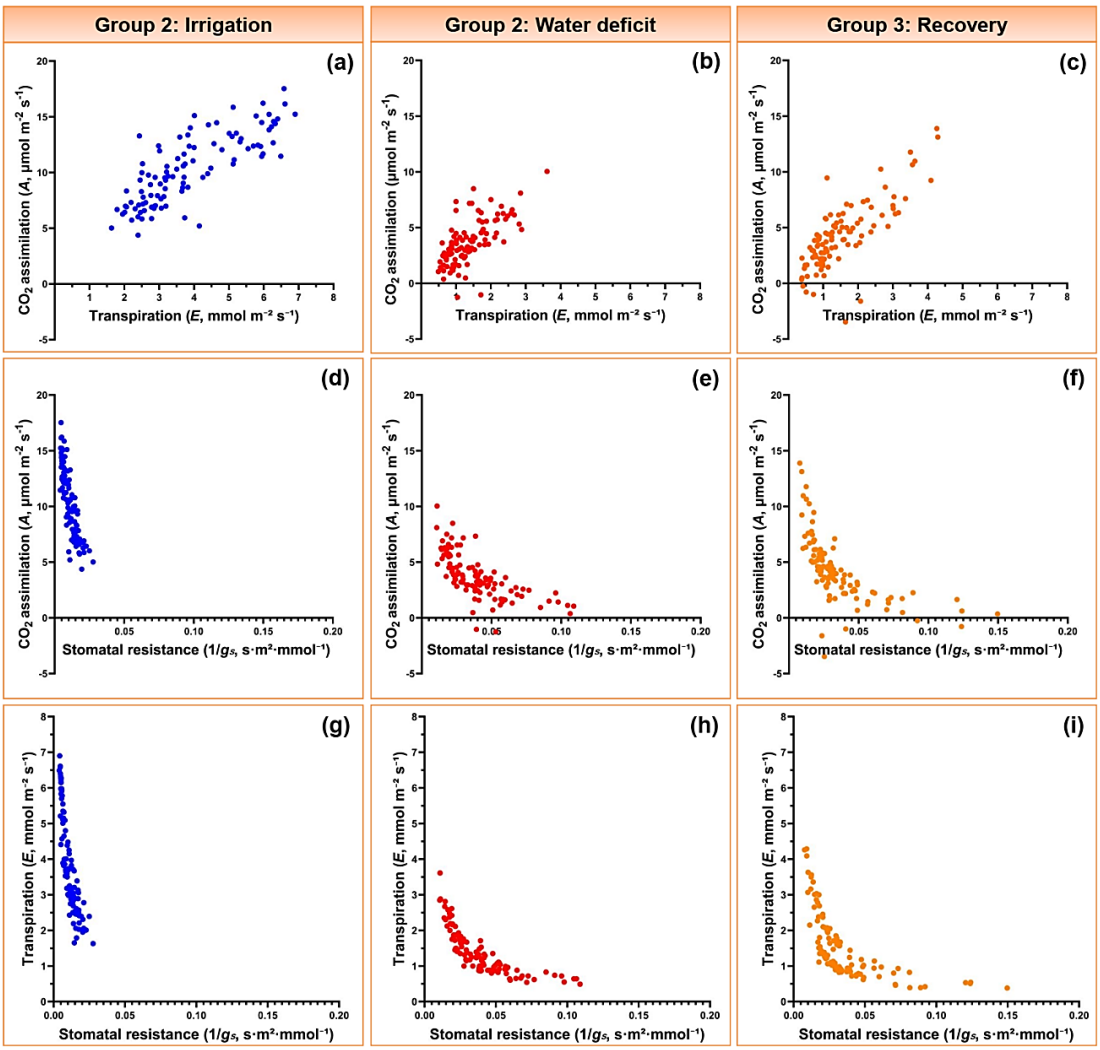

Figure 19. 'Hass' leaf functional relationships of Group 2 rootstock individuals under irrigation, water deficit, and recovery conditions. Transpiration-CO_2 assimilation coupling (a-c). Stomatal resistance-CO_2 assimilation coupling (d-f). Stomatal resistance-transpiration coupling (g-h).

This moderate stomatal adjustment, similar to that observed in Group 1 (Figure 20), indicates that under water deficit conditions, the increase in stomatal resistance (R_s) does not necessarily minimize transpiration efficiently, as reported by Martinez-Maldonado & Marin (2023). Despite this, the decoupling did not significantly affect water-use efficiency (*WUE*) across different water regimes (Figure 20b), suggesting

that the carbon-water relationship would remain stable at the physiological level through processes such as enhanced Rubisco activity (Flexas et al., 2006).

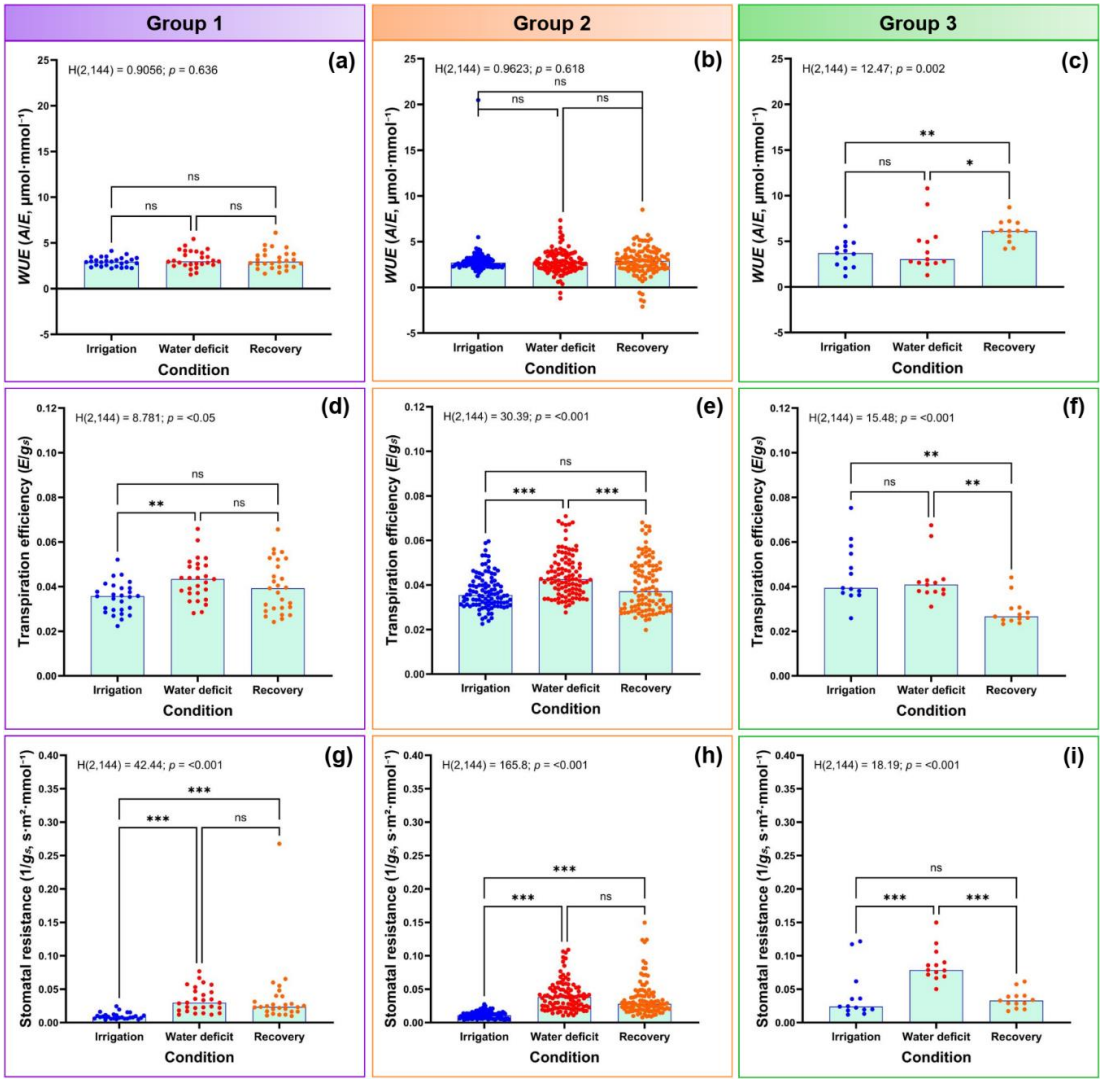

Figure 20. Comparison of values of water-use efficiency (*WUE*; a-c), transpiration efficiency (E/g_s; d-f), and stomatal resistance ($1/g_s$; g-i) in 'Hass' avocado grafted on three rootstock groups under optimal irrigation, water deficit, and recovery conditions.

In contrast, Group 3 stood out for its high-water-use efficiency (*WUE*) during recovery (6.11 µmol·mmol^{-1}), showing a 102 % increase compared to water deficit conditions (p <0.01). This group exhibited the highest intrinsic water-use efficiency (*WUEi*) across all evaluated conditions (Table 7), along with a biphasic stomatal response characterized by a dramatic increase in stomatal resistance (R_s) during stress (+227 %; p <0.001), followed by a significant reduction in the E/g_s ratio during recovery (-34 %; p <0.001;

Figure 20f), demonstrating effective readjustment between transpiration and stomatal aperture.

Despite intermediate defoliation (13.56 %; Table 5), these plants maintained controlled relative water content loss (15.94 %) and withstood stress for 16 days. The coordinated physiological response enabled preservation of photosynthetic function through stress, and rapid metabolic recovery post-rehydration, outperforming other groups in both water conservation and carbon assimilation efficiency.

The remarkable recovery capacity of Group 3 can be explained by the coordinated implementation of multiple physiological strategies. Recent studies have demonstrated that precise regulation of stomatal conductance, increased water-use efficiency, and more efficient abscisic acid (ABA) signaling systems enable certain genotypes to maintain significant CO_2 assimilation rates under water stress conditions (Jiménez-Arias et al., 2023). This physiological response may be enhanced by the rootstock's ability to facilitate water flow, as it exhibited the lowest recorded hydraulic resistance (Table 5). Recent evidence suggests that rootstocks actively participate in regulating key molecular mechanisms such as modulation of aquaporin gene expression, hormonal balance, and mRNA transfer through the graft union (Labarga et al., 2023; Yang et al., 2022; He et al., 2022). These combined mechanisms could confer greater tolerance to drought conditions.

These attributes, combined with its post-stress water efficiency (6.11 $\mu mol \cdot mmol^{-1}$, positioned this group as promising material for rootstock selection under drought conditions. However, future studies should evaluate its balance between tolerance and yield in optimal environments, as well as its stability under recurrent stress and the molecular mechanisms conferring water deficit tolerance. Among the thirteen Group 3 rootstocks, nine (69.2 %) showed tolerance to prolonged water deficit (16 days) and were classified into three subgroups according to their leaf collapse pattern (Table 8). Of these nine genotypes, most belonged to accession G8 (Árbol 120 ‘M-14’), highlighting the potential of this genetic line for drought conditions. Additionally, two promising genotypes from other accessions were identified: one from G7 (Árbol 12 *Persea nubigena* 1/7 CH-I-4) and another from G13 (Árbol 110 ‘C9’).

Table 8. Comparison of physiological parameters in ‘Hass’ avocado grafted on rootstocks with prolonged tolerance to water deficit (16 days), classified by their leaf collapse response: resistant (CRG), pre-rehydration collapse (PRG), and post-rehydration collapse (PoRG).

Variable	CRG (n = 4)	PRG (n = 3)	PoRG (n = 2)
A-Irrig (µmol m^{-2} s^{-1})	2.416	6.927	5.022
A-WD (µmol m^{-2} s^{-1})	1.524	3.150	1.335
A-Rec (µmol m^{-2} s^{-1})	4.719	5.906	4.223
g_s-Irrig (mmol m^{-2} s^{-1})	15.487	42.318	29.432
g_s-WD (mmol m^{-2} s^{-1})	11.111	13.593	9.627
g_s-Rec (mmol m^{-2} s^{-1})	28.571	35.538	22.038
E-Irrig (mmol m^{-2} s^{-1})	0.830	1.650	1.482
E-WD (mmol m^{-2} s^{-1})	0.475	0.525	0.474
E-Rec (mmol m^{-2} s^{-1})	0.791	0.906	0.694
WUE-Irrig (µmol·$mmol^{-1}$)	2.570	4.120	3.254
WUE-WD (µmol·$mmol^{-1}$)	3.313	5.060	2.460
WUE-Rec (µmol·$mmol^{-1}$)	6.176	6.374	5.163
R_s-Irrig (s·m^2·$mmol^{-1}$)	0.091	0.029	0.044
R_s-WD (s·m^2·$mmol^{-1}$)	0.109	0.116	0.129
R_s-Rec (s·m^2·$mmol^{-1}$)	0.047	0.047	0.097

A: CO_2 assimilation, g_s: Stomatal conductance; *E*: Transpiration; *WUE*: Water-use efficiency; E/g_s: Transpiration-stomatal conductance ratio; R_s: Stomatal resistance.

The three rootstock subgroups developed distinct physiological strategies in response to prolonged water stress, as revealed by the quantitative parameters in Table 8. The collapse-resistant genotypes (CRG; n = 4) demonstrated highly balanced stomatal regulation, characterized by a moderate increase in stomatal resistance (R_s: +20 %), controlled reductions in CO_2 assimilation (*A*: -37 %) and stomatal conductance (g_s: -28 %), and post-stress recovery that exceeded initial values (*A*: +95 %; g_s: +84 %). This coordinated response, associated with complete leaf preservation (0 % defoliation) and a recovery *WUE* of 6.176 µmol·$mmol^{-1}$ (+140 % compared to irrigation - the highest among subgroups), suggested precise ABA signaling modulation and particularly efficient cellular protection mechanisms (Hsu et al., 2020; Madritsch et al., 2019).

In strong contrast, the pre-rehydration collapse genotypes (PRG; n = 3) exhibited a dysregulated physiological response resulting in stomatal hypersensitivity (R_s: +300 %) and drastic reductions in *A* (-55 %), g_s (-68 %), and *E* (-68 %) during water deficit, along with incomplete recovery (*A*: -15 %; g_s: -25 %; *E*: -53 %) relative to optimal irrigation

conditions (6.927 µmol·m^{-2}·s^{-1}), despite increased *WUE* (+25 %) (Table 8). This dissociation between water efficiency and recovery capacity suggests structural damage to photosystems (Flexas & Medrano, 2002a), likely due to lipid peroxidation and accumulated oxidative stress (Meyer & de Kouchkovsky, 1993), where apparent water conservation success (elevated *WUE*) masks physiological damage by failing to restore post-stress chloroplast functionality (Flexas et al., 2012). The early leaf collapse (day 7) and recorded defoliation (20.95 %) not only confirm water efficiency problems but also indicate hydraulic stress manifested through xylem embolisms (Brodribb et al., 2016), while revealing an adaptive physiological pruning response. According to Peters & Choat (2024), this selective process would minimize productivity losses by preserving tissues with higher photosynthetic value or strategic positions in foliar architecture.

The post-rehydration leaf collapse genotypes (PoRG, n = 2) displayed a distinctive adaptive strategy characterized by two differentiated phases. During water stress, they showed the greatest initial impact, with a 73.4 % reduction in CO_2 assimilation (from 5.022 to 1.335 µmol·m^{-2}·s^{-1}) and the lowest water-use efficiency among subgroups (*WUE*: 2.460 µmol·mmol^{-1}). However, in the recovery phase, they demonstrated remarkable delayed tolerance by achieving CO_2 assimilation of 4.223 µmol·m^{-2}·s^{-1} (+216 % compared to the stress period) and a *WUE* of 5.163 µmol·mmol^{-1} (+110 %). This pattern, along with a 120 % increase in post-rehydration stomatal resistance, suggests the late activation of cellular repair mechanisms (Ruehr et al., 2019) that might involve mobilization of reserves accumulated in non-foliar organs (Scholz et al., 2016), progressive resynthesis of photosynthetic apparatus components (Correia et al., 2016), reorganization of cellular membranes, and restoration of xylem hydraulic capacity (Zwieniecki & Secchi, 2017). The daily monitoring of physiological parameters during the three experimental phases (irrigation: days 1-3, water deficit: days 4-19, recovery: days 20-25) revealed distinctive patterns validating the adaptive strategies of each subgroup (Figure 21 and 10). The CRG genotypes (G8: P2, P5 and P7) demonstrated notable physiological stability, evidenced by minimal fluctuations in CO_2 assimilation rate (*A*) during water stress (particularly G8 P7) (Figure 21a).

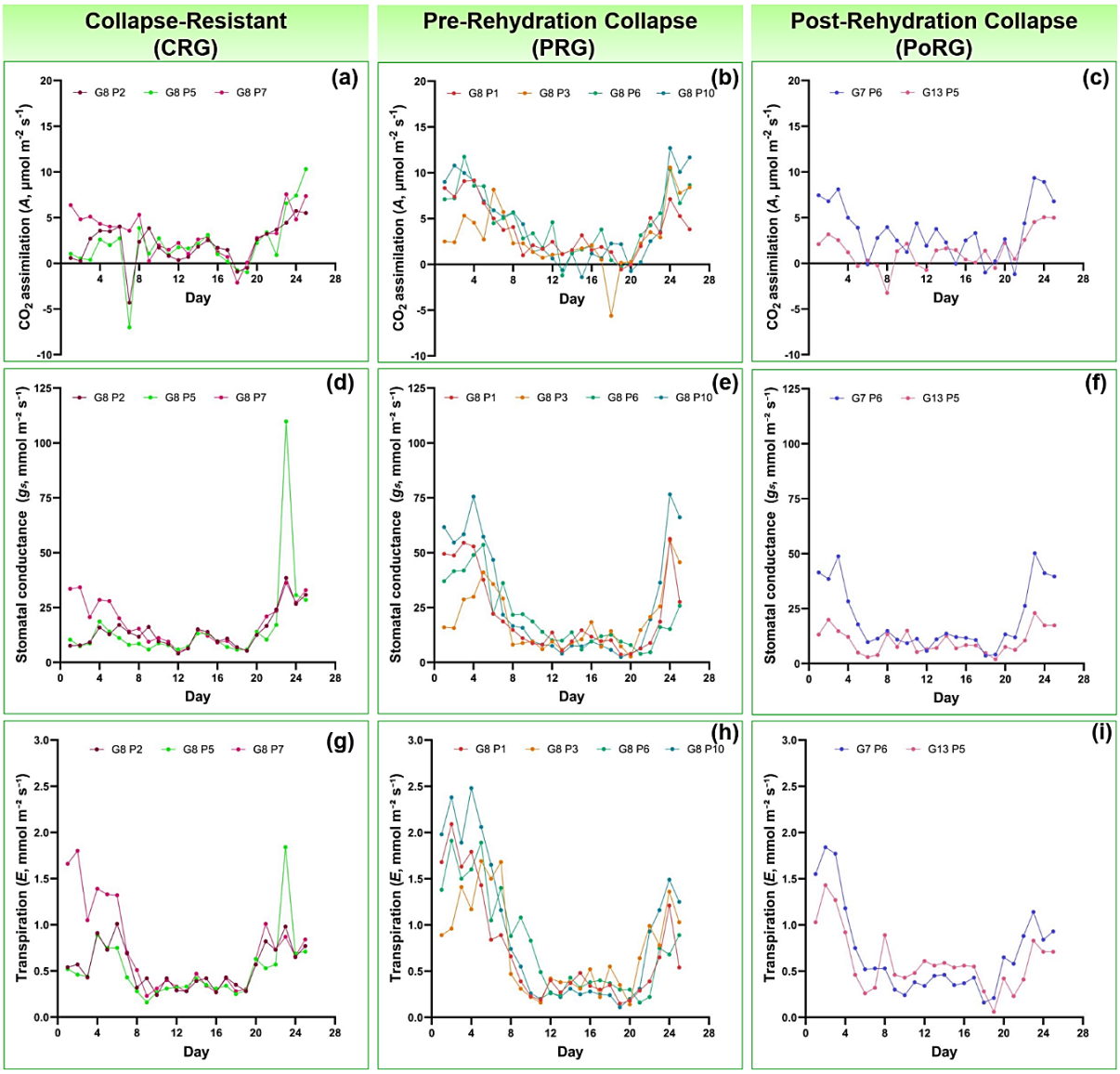

Figure 21. Daily physiological response patterns in three drought-tolerant avocado rootstock groups grafted with 'Hass', across optimal irrigation (days 1-3), water deficit (days 4-19), and recovery (days 20-25) periods: (a-c) CO_2 assimilation, (d-f) stomatal conductance, and transpiration.

This stability was accompanied by coordinated regulation of stomatal conductance (g_s: reduction of 0.5-1.2 mmol·m^{-2}·s^{-1}·day^{-1}; reaching 5.31 mmol·m^{-2}·s^{-1} by day 19) and transpiration (E), which progressively decreased from 1.66 to 0.23 mmol·m^{-2}·s^{-1} during the same period (Figure 21). Stomatal resistance (R_s) remained within an optimal range (0.03-0.20 s·m^2·mmol^{-1}), enabling stable water-use efficiency (*WUE*: 3.84-8.75 μmol

mmol^{-1}) and complete recovery of transpiration (0.84 mmol·m^{-2}·s^{-1} by day 25) (Figure 22).

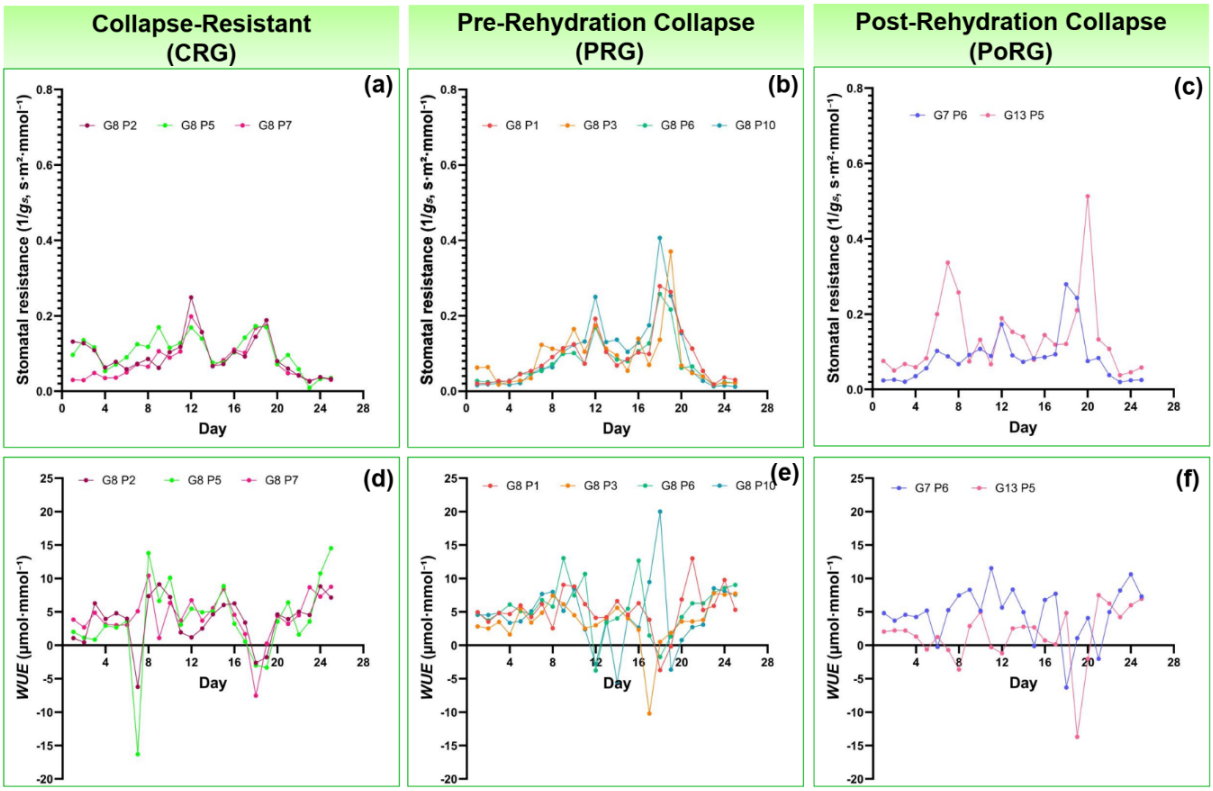

Figure 22. Daily physiological response patterns in three drought-tolerant avocado rootstock groups grafted with 'Hass', across optimal irrigation (days 1-3), water deficit (days 4-19), and recovery (days 20-25) periods: (a-c) stomatal resistance, water-use efficiency (*WUE*).

These results highlighted that stomatal resistance behavior was determinant for water tolerance (Figure 22). The collapse-resistant genotypes (CRG) particularly stood out for their ability to maintain physiological balance through fine stomatal regulation, simultaneously optimizing CO_2 assimilation (Figure 21) and water conservation (Figure 22). This capacity manifested in a gradual but sustained reduction in transpiration (-78 %; days 4-19) without severely compromising photosynthetic activity (*A* >0 µmol·m^{-2}·s^{-1}). Through this physiological regulation, these rootstocks demonstrated a remarkable ability to maintain water balance, with positive *WUE* during 87.5 % of stress days (group average). Among them, G8 P7 excelled with 93.8 % positive days (*WUE*: 1.09-10.41 µmol·mmol^{-1}), followed by G8 P5 (87.5 %, *WUE*: -16.30 to 13.79 µmol·mmol^{-1}) and G8 P2 (81.3 % positive days: *WUE* -6.23 to 9.12 µmol·mmol^{-1}). This

variability reflected differentiated water conservation strategies, where all genotypes showed exceptional negative values (day 18) associated with extreme stress conditions (Figure 22d).

The pre-rehydration leaf collapse genotypes (G8 P1, P3, P6, P10) showed contrasting responses, with extreme oscillations in R_s (>300 % between consecutive days; G8 P6: 0.053 to 0.370 $s \cdot m^2 \cdot mmol^{-1}$, days 7-12) (Figure 22b) that triggered comprehensive physiological collapse (Figure 22e). Transpiration (*E*) in these genotypes underwent abrupt reductions (G8 P1: from 1.68 to 0.18 $mmol \cdot m^{-2} \cdot s^{-1}$, days 1-19), accompanied by critical *WUE* values (-10.22 $\mu mol \cdot mmol^{-1}$ in G8 P3) and complete loss of CO_2 assimilation function (*A* ≤0 $\mu mol \cdot m^{-2} \cdot s^{-1}$). This stomatal control dysregulation resulted in an inability to recover baseline transpiration levels post-stress (0.72 $mmol \cdot m^{-2} \cdot s^{-1}$ on day 25 *vs*. initial 1.68 in G8 P1) (Figure 21h).

The PoRG group displayed heterogeneous responses. G7 P6 showed a biphasic pattern with a tolerance phase (days 4-12) featuring stable transpiration (1.18 to 0.53 $mmol \cdot m^{-2} \cdot s^{-1}$) until reaching a critical point (day 15: *E* = 0.35 $mmol \cdot m^{-2} \cdot s^{-1}$; *WUE* = -0.11 $\mu mol \cdot mmol^{-1}$), which after rehydration achieved gradual recovery up to 0.93 $mmol \cdot m^{-2} \cdot s^{-1}$ (day 25; *WUE* = 7.29 $\mu mol \cdot mmol^{-1}$). In contrast, G13 P5 exhibited erratic behavior, with extreme transpiration fluctuations (0.92 to 0.06 $mmol \cdot m^{-2} \cdot s^{-1}$) reflecting its stomatal dysregulation, showing 43.8 % days with negative *WUE* (-13.72 to 6.95 $\mu mol \cdot mmol^{-1}$) and extreme transpiration variations (0.92 to 0.06 $mmol \cdot m^{-2} \cdot s^{-1}$). This genotype reached its minimum *WUE* value on day 19 (-13.72 $\mu mol \cdot mmol^{-1}$), which would reflect severe metabolic damage (Figure 22).

This analysis demonstrated that the coordination between stomatal response and maintenance of positive water-use efficiency (*WUE*) was a consistent indicator for differentiating water stress tolerance among avocado rootstocks (Figure 22). The CRG genotypes stood out for their ability to preserve optimal metabolic balance by maintaining transpiration (*E*) values above 0.3 $mmol \cdot m^{-2} \cdot s^{-1}$, preventing abrupt drops in *WUE* (positive during 87.5 % of stress days). This critical transpiration threshold was identified as a determining factor for preventing physiological damage, supported by efficient coupling between CO_2 assimilation (*A*) and water loss (r > 0.78). In contrast,

the PRG genotypes showed metabolic decoupling, with negative *WUE* during 43.8 % of days and uncoordinated stomatal regulation (erratic R_s). Among the PoRG group, G7 P6 excelled due to its gradual recovery, while G13 P5 exhibited extreme fluctuations and critical *WUE* values (-13.72 μmol·mmol^{-1}). These findings not only highlight the importance of stomatal resistance (R_s) modulation and maintenance of stable *WUE*, but also establish quantitative criteria for rootstock selection in breeding programs. The CRG genotypes and G7 P6 emerged as the most promising for drought conditions, due to their capacity to sustain integrated physiological functionality even under prolonged stress.

4.6. Conclusions

This study classified 18 avocado genotype accessions used as rootstocks into three groups with contrasting morphological and physiological responses influencing 'Hass' scion performance under water deficit conditions. Group 3 rootstocks, particularly accession G8 ('M-14'), exhibited high drought tolerance through three key mechanisms: (1) significantly lower hydraulic resistance (13,000 MPa·s·m^{-1}·kg^{-1}) enabling efficient water transport, (2) rapid metabolic recovery (+157 % in post-rehydration CO_2 assimilation), and (3) superior water-use efficiency (WUE: 6.11 μmol·mmol^{-1}) during recovery. These traits were associated with precise stomatal regulation and likely photosynthetic apparatus protection, contrasting sharply with Groups 1 and 2, which showed vulnerability through drastic stomatal closure (R_s: +259 %), transpiration collapse (-65 %), and either limited recovery (Group 1) or moderate defoliation (21.55 %, Group 2). These results highlight stomatal response coordination with high WUE as key drought tolerance indicators.

The identification of G8 ('M-14') as a superior rootstock for 'Hass' with high physiological performance offers a genetic solution to bridge yield gaps between irrigated and rainfed systems, particularly critical for avocado-growing regions facing projected precipitation reductions due to climate change. Future research should validate these genotypes in commercial orchards, decipher their adaptive mechanisms, and evaluate performance under recurrent drought scenarios.

4.7. References

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