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Aspectos fisiológicos del déficit hídrico en el jinjolero (*Ziziphus jujuba* Mill). Efectos en la calidad comercial y compuestos bioactivos del fruto

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Zulma Natali Cruz Pérez

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Memoria presentada para optar al grado de Doctor
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Informan

Que la Tesis Doctoral titulada "Aspectos fisiológicos del déficit hídrico en el jinjolero (*Ziziphus jujuba* Mill). Efectos en la calidad comercial y compuestos bioactivos del fruto", de la que es autora la Ing. Zulma Natali Cruz Pérez, ha sido realizada bajo nuestra dirección y supervisión en el Centro de Edafología y Biología Aplicada al Segura (CEBAS-CSIC).

En Murcia, a 5 de Mayo de 2014

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Certifica

Que da su conformidad a la lectura de la Tesis doctoral presentada por Zulma Natali Cruz Pérez con el título de *"Aspectos fisiológicos del déficit hídrico en el jinjolero (Ziziphus jujuba* Mill*). Efectos en la calidad comercial y compuestos bioactivos del fruto"*, realizada dentro del programa de doctorado de Recursos y Tecnologías Agroalimentarias, y de la que han actuado como directores los Drs. Arturo Torrecillas Melendreras, Ángel Gil Izquierdo y Pedro Rodríguez Hernández. Los cuales han manifestado su conformidad con la forma y contenido de la mencionada tesis para que pueda procederse a su exposición pública.

Y para que conste a los efectos oportunos, expido el presente en Orihuela a 26 de Mayo de 2014

Fdo: José Ramón Díaz

La ingeniera agrónoma Zulma Natali Cruz Pérez ha disfrutado, para la realización de su Tesis Doctoral, de una beca de la Agencia Española de Cooperación Internacional parta el Desarrollo (AECID).

Este trabajo se encuadra dentro de las actividades del Departamento de Riego del CEBAS (CSIC), en los siguientes proyectos de investigación: *Acciones para el fortalecimiento y consolidación de un grupo de investigación de excelencia en el INCA-UNAH (Cuba) sobre optimización del uso del agua en agricultura* (AECID, D/016779/08, D/023231/09, D/030431/10, A1/035430/11) y *Estrategias de manejo del riego deficitario para optimizar la calidad y saludabilidad del melocotón extratemprano y la granada* (CICYT, AGL2010-19201-C04-01AGR).

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Internacional del Instituto Nacional de Ciencias Agrícolas. San José de las Lajas, La Habana (Cuba). Poster.

Estructura de la Tesis Doctoral

La presente memoria ha sido elaborada de acuerdo con la Normativa Interna de la Universidad Miguel Hernández de Elche para la **presentación de Tesis Doctorales con un conjunto de publicaciones.** La estructura de la misma corresponde a los siguientes apartados:

- **Resumen**, donde se exponen de manera abreviada los resultados y conclusiones más relevantes.
- **Introducción**, dónde se plantea la oportunidad de la investigación realizada y los antecedentes correspondientes.
- **Objetivos**, donde se detallan cada uno de los objetivos parciales de la investigación.
- **Materiales y métodos**, donde se indican las características agronómicas de la parcela experimental y las condiciones culturales, así como todas las metodologías empleadas tanto para el estudio de las relaciones agua-suelo-planta como para determinar las características físicas y químicas de los frutos.
- **Publicaciones**, donde se incluyen tres artículos, dos de ellos ya publicados y otro aceptado
    - En la primera de las publicaciones (***Plant Science*** 197: 77–83 (2012)) se estudió por primera vez los mecanismos desarrollados a nivel foliar por el jinjolero para afrontar situaciones de déficit hídrico.
    - En la segunda de las publicaciones, aceptada para publicar en el ***Macedonian Journal of Chemistry and Chemical Engineering***, se analizó el efecto del déficit hídrico sobre las características físicas y la calidad de los jínjoles, con especial referencia al contenido de aminoácidos.

- En la última, publicada en el ***Journal of Agricultural and Food Chemistry*** (61: 6187-6197 (2013)) se abordó como afectaba tanto el déficit hídrico como el almacenamiento doméstico de los frutos al contenido en procianidinas y a los procesos de agregación de las mismas.

- **Resultados y Discusión**, donde se analizan y discuten los resultados más importantes y las posibles causas.
- **Conclusiones**, donde se enumeran, las conclusiones definitivas obtenidas.
- **Bibliografía**, donde se detallan las referencias bibliográficas utilizadas en otros apartados complementarios a los artículos aportados
- **Abreviaturas y símbolos**, donde se indican las abreviaturas y símbolos más importantes de los utilizados en la memoria.

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Índice

1. Resumen y Abstract

Resumen

El objetivo global del trabajo se centró en el estudio de algunos aspectos fisiológicos de la respuesta del jinjolero (*Zizyphus jujuba* Mill) al déficit hídrico durante la fase de maduración de los frutos, abordando facetas hasta ahora no estudiadas, tales como los mecanismos desarrollados a nivel foliar para afrontar este tipo de estrés y su influencia en algunos importantes atributos físicos y químicos de la calidad de los jínjoles, prestando especial atención al contenido de aminoácidos y al perfil y autoagregación de proantocianidinas en los frutos, así como la evolución de los niveles de estos compuestos tras tres meses de almacenamiento doméstico en frío.

Los resultados obtenidos mostraron cómo el jinjolero desarrolla de forma simultánea y complementaria mecanismos de evitación y tolerancia al déficit hídrico, lo que le permite mantener la turgencia celular y considerables niveles de intercambio gaseoso, incluso a niveles muy severos de estrés. Los mecanismos de evitación del estrés se basan en una progresiva regulación estomática y un acortamiento de la duración diaria de la máxima apertura estomática para disminuir las pérdidas de agua vía transpiración. La tolerancia al estrés se basa en el desarrollo de una acumulación activa de solutos (ajuste osmótico) y altos contenidos de agua apoplástica.

Por primera vez se identificó un aminoácido esencial (cistina (Cys-cys)) y siete no esenciales (4-hidroxiprolina (p-Hyp), ácido α-aminoadipico (AADA), ornitina (orn), ácido β-aminoisobutírico (BAIB), ácido α-amino-n-butírico (AABA), cistationina (Cysta), y homocistina (Hcys-cys)). El déficit hídrico moderado aceleró la maduración de los frutos mejorando su calidad comestible. El déficit hídrico severo indujo no sólo un mayor grado de madurez, mejorando la mayoría de las características químicas que determinan el sabor y valor nutricional sino las características físicas. En estos frutos, cabe destacar que la disminución de los contenidos de

asparagina (Asn) es un aspecto positivo ya que disminuye el riesgo de formación de acrilamida durante el procesado de los jínjoles en caliente.

Igualmente, se detectó la presencia de procianidinas no detectadas previamente en los jínjoles. Hasta eses momento, sólo se habían detectado dos procianidinas (la (epi)catequina y su dímero). Sin embargo en este trabajo se han detectado y cuantificado dos trímeros, dos tetrámeros y seis pentámeros de la procianidina. Todas las procianidinas detectadas en los jínjoles fueron tipo B y sus niveles totales aumentan por efecto del déficit hídrico durante la maduración de los frutos. Estos aumentos se debieron fundamentalmente al incremento de compuestos de bajo peso molecular, por lo que el déficit hídrico aumenta la biodisponibilidad de las procianidinas, aumentando los efectos fisiológicos potenciales sobre la salud humana. La tendencia de estas moléculas a autoagregarse no se vio afectada por el déficit hídrico, resultando similar a la observada en otros frutos.

Adicionalmente, el almacenamiento doméstico en frío de los frutos bien regados indujo un significativo aumento del contenido en procianidinas, mientras que los frutos sometidos a un estrés hídrico severo disminuyeron el contenido de estos compuestos durante el almacenamiento en frío.

Abstract

The purpose of the present study was to analyse some physiological aspects of the response of jujube (*Zizyphus jujuba* Mill) plants to deficit irrigation during fruit maturation. In this sense, the resistance mechanisms (avoidance and tolerance) developed in response to a water stress and during recovery, the effect of deficit irrigation during fruit maturation on jujube yield and fruit characteristics, including amino acids and procyanidins content were studied.

Jujube trees exposed to water stress depend strongly on stress avoidance and stress tolerance mechanisms. From the beginning of water stress to the time of maximum water stress, leaf turgor was maintained allowing substantial gas exchange levels and, as a consequence, good leaf productivity. This leaf turgor maintenance was mainly due to two simultaneous and complementary mechanisms: decreased leaf conductance and a shorter period of maximum stomatal opening in order to control water loss via transpiration (stress avoidance mechanisms). The gradual recovery of leaf conductance (g_l) after rewatering can also be considered as a mechanism for promoting leaf rehydration. In addition, from the beginning of the stress period, active osmotic adjustment operated, which could have contributed to the maintenance of leaf turgor (stress tolerance mechanism). The high leaf apoplastic relative water content (RWC_a) levels and the possibility of increasing the accumulation of water in the apoplasm in response to water stress, supporting a steeper gradient in water potential between the leaf and the soil, which can be considered another drought tolerance characteristic in pear-jujube leaves.

Jujube yield and fruit characteristics can be clearly modified by water deficit imposed during fruit maturation. One essential (cystine (Cys-cys)) and seven non-essential (4-hydroxyproline (p-Hyp), α-aminoadipic acid (AADA), ornithine (orn), β-aminoisobutyric acid (BAIB), α-amino-n-butyric acid (AABA), cystathionine (Cysta), and homocystine (Hcys-cys)) amino acids were identified for the first time. Fruits from plants exposed to

moderate water deficit during the maturation stage initiated the ripening phase earlier than control fruits and had an improved eating quality. Fruits subjected to severe water deficit showed changes in their physical characteristics and reached a more advanced degree of ripening than T0 and T1 fruits, with not only most of the fruit chemical characteristics that determine taste being improved but also the nutritional value. The decrease in the asparagine (Asn) content of the fruit as a result of severe water deficit is a positive aspect, which prevents acrylamide formation during heat-processing of the fruit.

The current work demonstrates the occurrence of novel procyanidins in pear-jujube. To date, only two procyanidins [(epi)catechin and its dimer] have been described. In the present study, two trimers, two tetramers, and six procyanidin pentamers have been tentatively identified and quantified for the first time in pear-jujube. The results confirm that proanthocyanidins in pear-jujube fruits consist exclusively of B type procyanidins, whose levels are increased by water deficit during the fruit maturation stage. The fact that the total procyanidin content of the edible portion of fruits under water deficit is based mainly on an increase in the low molecular mass compounds leads us to conclude that pear-jujube fruits from trees exposed to water deficit increase procyanidin bioavailability and enhance the potential physiological effects on human health. The tendency of these molecules to selfaggregate does not change with the portion of the fruit or the irrigation treatment and is similar to that observed in other fruits. Additionally, fruits from well watered trees may increase their procyanidin content during fruit cold storage, whereas fruits from trees that were exposed to severe water stress decrease their procyanidins content during cold storage.

2. Introducción

La escasez de recursos hídricos constituye un problema clave para la producción agraria en zonas áridas y semiáridas, las cuales suponen más de la tercera parte de la superficie terrestre. En estas zonas los frutales se encuentran sometidos a frecuentes situaciones de estrés hídrico, por lo que el uso eficiente del riego resulta indispensable para mantener su productividad. Además, la disponibilidad de agua de riego para la agricultura se encuentra en clara recesión, incluso en zonas donde llueve abundantemente (Cai y Rosegrant, 2003).

Esta situación hace insostenible la continua expansión de las dotaciones de agua para riego, por lo que los agrosistemas de zonas áridas y semiáridas deben afrontar la imperiosa necesidad de convivir con la escasez de agua (Pereira et al., 2002). Por ello, la fruticultura en dichas zonas debe enfocarse hacia el uso de materiales vegetales menos demandantes de agua y resistentes al déficit hídrico, lo cual, junto con la búsqueda de vías para reducir el consumo de agua, podría permitir no sólo el ahorro de importantes cantidades de agua de riego, sino la producción de alimentos de alta calidad (Greenwood et al., 2010; Jiménez et al., 2010).

El jinjolero (*Zizyphus jujuba* Mill) (Fotografías 2.1 y 2.2) pertenece a la familia Rhamnaceae y es nativo de zonas templadas de Asia, particularmente China y las zonas vecinas de Mongolia y repúblicas de Asia Central. Su cultivo se extendió hacia el oeste, llegando al Mediterráneo a través de oriente próximo y el SO de Asia. Igualmente, su expansión hacia el este alcanzó Corea y Japón (Azam-Ali et al., 2006).

En muchos países del mundo, los jinjoleros se consideran un cultivo *menor*, por lo que los diversos gobiernos no han potenciado ni el desarrollo de plantaciones, ni la investigación sobre su cultivo. Sin embargo, cabe subrayar que estos frutos forman parte de la cultura y forma de vida de millones de personas en Asia e incluso de África después de la introducción de algunas especies del género *Zizyphus* (Williams et al., 2006). Esta aceptación deriva de los efectos beneficiosos para la salud humana de los jínjoles, por lo que son considerados

alimentos funcionales por sus propiedades nutricionales y medicinales (Heo et al., 2003; Huang et al., 2007; Li et al., 2007; Zhao et al., 2008; Mahajan y Chopda, 2009; Xue et al., 2009; Choi et al., 2011; Sun et al., 2011; Choi et al., 2012).

Fotografía 2.1. Vista parcial de una plantación de jinjoleros

Los mecanismos desarrollados por los cultivos leñosos para afrontar situaciones de estrés hídrico se basan, principalmente, en estrategias de evitación o de retraso del estrés, o simplemente de tolerancia (Savé et al., 1995; Torrecillas et al., 1996). La valoración de en qué medida, tras la recuperación de una situación de estrés hídrico, se reanuda el intercambio gaseoso y el crecimiento, manteniéndose la productividad, resulta particularmente relevante en cultivos perennes de zonas áridas y semiáridas. En este sentido, el jinjolero es una especie frutal muy interesante debido a su capacidad para afrontar sequías severas durante el ciclo de cultivo y tolerar tanto muy bajas temperaturas

durante el reposo invernal como el riego con aguas salinas (Dahiya et al., 1981; Ming et al., 1986; Jain y Dass, 1988).

Fotografía 2.2. Jínjoles (cv. Grandes de Albatera) en distintos grados de maduración

Concretamente, situaciones de déficit hídrico severo desde la rotura de yemas al inicio de la aparición de las hojas y situaciones de estrés hídrico moderado durante el periodo de maduración de los frutos permite el ahorro de agua y la mejora de la productividad del agua (Cui et al., 2009a,b). Sin embargo, la bibliografía relativa al efecto del riego deficitario sobre la calidad y el contenido en compuestos funcionales de los jínjoles es muy escasa. Una excepción es el trabajo de Cui et al. (2008), quienes mostraron que el déficit hídrico suave durante la maduración de los frutos no afectan el peso y tamaño de los jínjoles, pero sí induce una ligera disminución de los niveles de humedad, permitiendo reducir la incidencia de podredumbres durante el almacenamiento postrecolección (Cui et al., 2008).

En relación a los compuestos funcionales, cabe destacar la importancia de los aminoácidos no sólo por su valor nutricional sino por su contribución al sabor de los frutos y su papel en los mecanismos de resistencia frente al déficit hídrico. Las procianidinas son compuestos de elevado interés nutricional y medicinal dada su potente capacidad antioxidante y posibles efectos protectores de la salud humana (Santos Buelga y Scalbert, 2000). En este sentido, Carnésecchi et al. (2002) descubrieron que las procianidinas poseen actividad anti-proliferativa de las células cancerosas en humanos. Adicionalmente, Saito et al. (1998) y Mao et al. (1999) demostraron que el carácter anti-proliferativo y anti-canceroso de los flavonoles y procianidinas están relacionados con el grado de polimerización de estos compuestos.

En África, el almacenamiento postrecolección de frutas indígenas, como las del género *Ziziphus*, es una de las estrategias más importantes de las comunidades rurales para reducir el hambre, mejorar la nutrición y generar retornos económicos (Mithofer et al., 2006). Si bien, tras la recolección, la senescencia de los jínjoles a temperatura ambiente es muy rápida (Wang et al., 2009), Tembo et al. (2008) demostraron que la proporción de frutos de *Z. mauritiana* deshidratados aumentaba con la temperatura de almacenamiento entre 5 y 20 ºC, y que la proporción de frutos deshidratados al cabo de 12 semanas de almacenamiento a 5 ºC era muy baja.

A pesar de la importancia del jinjolero, la información relativa al comportamiento de esta especie en zonas áridas y semiáridas es muy escasa. No existe ninguna información sobre los mecanismos foliares desarrollados a nivel de las relaciones hídricas para afrontar una situación de sequía. Igualmente, no existen datos relativos a cómo afecta el riego deficitario a los niveles de algunos compuestos funcionales, tales como los aminoácidos y procianidinas en los frutos, y la información referente al efecto del déficit hídrico sobre otros aspectos de la calidad de los jínjoles, tanto químicos (azúcares, vitamina C, ácidos orgánicos, etc.) como físicos (tamaño, firmeza, color, etc.) han sido mínimamente estudiados. Además,

el posible efecto del riego deficitario sobre algunos compuestos funcionales durante el almacenamiento doméstico en frío de estos frutos puede constituir un aspecto clave para la alimentación de algunas comunidades rurales en riesgo de desnutrición.

3. Objetivos

El objetivo global de esta tesis fue **profundizar en el conocimiento de la respuesta del jinjolero (*Zizyphus jujuba* Mill, cv. Grande de Albatera) al déficit hídrico**. Para su consecución se abordaron los siguientes objetivos parciales:

- *Evaluación de las relaciones hídricas a nivel foliar para establecer los mecanismos de resistencia (evitación y tolerancia) desarrollados tanto durante una situación de déficit hídrico como durante la recuperación*.

- *Determinar el efecto de distintos niveles de déficit hídrico sobre la respiración y emisión de etileno y las características físicas y químicas de los jínjoles.*

- *Profundizar en el conocimiento del contenido de aminoácidos libres, tanto esenciales como no esenciales, en los jínjoles procedentes de distintos tratamientos de riego*.

- *Determinar el perfil de procianidinas en los jínjoles, así como su grado de agregación y la respuesta al déficit hídrico.*

- *Establecer el efecto del riego deficitario en el contenido de procianidinas tras un almacenamiento doméstico en frío de tres meses.*

4. Materiales y métodos

En este apartado se incluye un resumen de las características más importantes de las condiciones experimentales, tratamientos de riego, metodologías empleadas para la medida de las variables consideradas, el diseño estadístico y el análisis de los datos obtenidos en cada trabajo de los que conforman la tesis doctoral. Para mayor detalle, se pueden consultar las tres publicaciones aportadas.

Condiciones experimentales, material vegetal y tratamientos

La parcela experimental se encuentra ubicada en una finca comercial localizada a 3 km de la ciudad de Albatera (Alicante) (38º 12' N, 0º 51' W), con un suelo Torrifluvent de textura franco-arenosa, de muy baja salinidad, alto contenido en caliza, muy bajos niveles de materia orgánica, baja capacidad de intercambio catiónico, y bajos niveles de potasio y fósforo asimilable (Fotografía 4.1). El agua de riego presentó una conductividad eléctrica de entre 1.7 y 2.2 dS/m y unos contenidos en Cl^- entre 36 y 48 mg l^{-1}.

Fotografía 4.1. Vista de la parcela experimental de jinjoleros

Se utilizaron jinjoleros (*Zizyphus jujuba* Mill), cv. Grande de Albatera) de unos 7 años de edad a un marco de 2 m x 6 m. La fertilización y los tratamientos fitosanitarios fueron los habitualmente

utilizados por los agricultores de la zona. Se controló la proliferación de malas hierbas con herbicidas. El clima de la zona es típicamente mediterráneo, con inviernos suaves, baja pluviometría y veranos secos y calurosos.

El riego se realizó por la noche, usando un sistema localizado con una tubería por hilera de árboles. Se llevaron a cabo tres tratamientos de riego consistentes en un tratamiento control (T0) en el que las plantas se regaron a fin de asegurar condiciones no limitantes de agua en el suelo, las plantas del tratamiento T1 se regaron según los criterios del agricultor y las plantas del tratamiento T2 se regaron como las del T0 pero suprimiéndoles el riego durante 36 días. La recuperación de las plantas del tratamiento T2 se realizó reanudando el riego a nivel de las plantas del T0 durante 14 días.

Medidas

Estado hídrico de las plantas

El intercambio gaseoso, fotosíntesis (P_n) y conductancia foliar (g_l), se midió en el envés de hojas de la orientación sur y del tercio medio de los árboles, con un medidor de fotosíntesis (LICOR 6400, LICOR Inc., Lincoln, USA) (Fotografía 4.2).

El potencial hídrico foliar (Ψ_l) se determinó con una cámara de presión (modelo 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA), siguiendo las recomendaciones de Turner (1988). El potencial de tallo al mediodía (12 h solares) se determinó sobre hojas envueltas con lámina de aluminio y enfundadas en una bolsa de plástico al menos 2 h antes de la medida con cámara de presión (Fulton et al., 2001; Shackel, 2011) (Fotografía 4.3).

Fotografía 4.2. Medida de conductancia foliar (g_l) y fotosíntesis neta (P_n) con un medidor LI-6400 (LICOR Inc., Lincon, USA)

Fotografía 4.3. Hoja cubierta con lámina de aluminio y cámara de presión (Soilmoisture Equipment Corp., modelo 3005) utilizada para medir el potencial hídrico de tallo (Ψ_{stem}).

El potencial osmótico foliar antes del alba y mediodía se determinó en las mismas hojas utilizadas para la medida del potencial hídrico foliar antes del alba (Ψ_{pd}) y mediodía (Ψ_{md}). Las hojas se introdujeron en nitrógeno líquido y se conservaron a – 80 ºC hasta la medida. Tras dejarlas descongelar, se extrajo la savia y se midió en un osmómetro de presión de vapor (Wescor 5600, Logan, USA) (Fotografía 4.4). El potencial de turgencia antes del alba (Ψ_{ppd}) y mediodía (Ψ_{pmd}) se estimó como la diferencia entre los valores de potencial hídrico y osmótico. Para estimar el potencial osmótico a plena turgencia antes del alba (Ψ_{os}) se procedió a la rehidratación de las hojas inmediatamente después de muestreadas introduciendo los peciolos en agua destilada durante 24 h a 4 ºC. Posteriormente, se introdujeron en nitrógeno líquido y se procedió como se ha indicado anteriormente (Sánchez-Blanco et al., 2002; Torrecillas et al., 2003)

Fotografía 4.4. Osmómetro de presión de vapor (Wescor 5600, Logan, USA) para medida de potencial osmótico.

Al final del periodo de estrés, se muestrearon hojas antes del alba, se rehidrataron como se ha indicado en el caso de Ψ_{os} y se procedió a la

elaboración de curvas presión–volumen (PV) a fin de conocer los valores de Ψ_{os}, el potencial hídrico en el punto de pérdida de turgencia (Ψ_{tlp}), el módulo de elasticidad (Є), el contenido relativo de agua en el punto de pérdida de turgencia (RWC_{tlp}) y el contenido relativo de agua apoplástica (RWC_a) (Tyree and Hammel, 1972; Tyree and Richter, 1981, 1982; Savé et al., 1993). Las hojas rehidratadas se pesaron utilizando una balanza analítica (± 0.1 mg de precisión) y se introdujeron en una cámara de presión aumentado la presión lentamente (0.025 MPa s^{-1}) hasta la aparición de savia en la superficie del peciolo foliar. Tras despresurizar la cámara, cada hoja se volvió a dejar transpirar libremente en el laboratorio a temperatura ambiente (22 ± 2 °C). El proceso se repitió hasta que las medidas con la cámara de presión alcanzaron el límite del manómetro (Kikuta y Richter, 1986). Con los valores de peso inicial (hojas rehidratadas), tras cada medida con la cámara de presión (correspondientes a valores de Ψ_l) y peso final seco (a 80 °C durante 48 h) se calculó el contenido relativo de agua (RWC) (Barrs y Weatherley, 1962).

Las curvas PV se elaboraron utilizando una transformación tipo II (Tyree y Richter, 1982). La representación del inverso del potencial hídrico foliar (Ψ_l) frente al RWC mostró un tramo lineal y otro curvilíneo. La extrapolación del tramo lineal a valores de RWC iguales a la unidad proporciona el valor inverso de Ψ_{os}, y la extrapolación sobre el eje de abscisas proporciona el valor del RWC_a. El punto de intersección del tramo lineal y el curvilíneo suministra los valores de Ψ_{tlp} y RWC_{tlp}. El módulo de elasticidad (Є) de los tejidos foliares al 100 % de RWC (RWC_o) se estimó siguiendo el procedimiento de Patakas y Noitsakis (1999) como $Є\ (MPa) = (\Psi_{os} - \Psi_{stlp})\ (100 - RWC_a)/(100 - RWC_{tlp})$, donde Ψ_{stlp} es el potencial osmótico en el punto de pérdida de turgencia y los valores de Ψ_{os} corresponden a los obtenidos con el análisis de las curvas PV.

Características físicas del fruto

Los frutos se recolectaron al alcanzar la madurez comercial (estado S7, según Choi et al. (2012)) (Fotografía 4.5) y se transportaron al laboratorio para su análisis. La parte comestible (piel + pulpa) se separó de la no comestible (endocarpo + semilla).

Fotografía 4.5. Jínjoles en estado de maduración S7 (Choi et al., 2012).

El peso medio de los frutos se determinó pesando 25 frutos de cada repetición. El contenido de humedad se determine mediante secado a 105 ºC hasta peso constant (AOAC, 2000). La firmeza de los frutos se evaluó como la carga necesaria para romper la pulpa de los frutos en la zona ecuatorial desprovista de piel. Para ello, se utilizó un penetrómetro tipo Penefel con punta de 8 mm (Copa-Technology, Tarascon, Francia) (Egea et al., 2007)

El color interno y externo se midió por reflectancia utilizando un colorímetro Minolta CR 2000 (Osaka, Japón). Los resultados se expresaron como el sistema CIELAB *L**, *a**, *b**, calculando los valores medios de luminosidad (*L**), rojo-verdoso (*a**), y azul-amarillento (*b**). El color objetivo se calculó como cromaticidad o croma ($C^* = (a^{*2} + b^{*2})^{1/2}$) y ángulo Hue ($H^o$ = arctan (*b** / *a**))

Características químicas del fruto

Los sólidos solubles totales (TSS) se estimaron midiendo el índice de refracción del jugo del fruto con un refractómetro digital. La acidez valorable (TA) se determinó mediante potenciometría con NaOH 0.1N a pH 8.1 (Egea et al., 2010). El índice de madurez se estimó como la relación entre TSS y TA. El contenido de vitamina C se evaluó siguiendo la metodología de Egea et al. (2010) y los contenidos de azúcares y ácidos orgánicos según Sánchez-Bel et al. (2008).

Para el análisis de bioelementos se utilizó el material seco procedente de las medidas de humedad. El contenido en N se determinó utilizando un analizador elemental Thermo-Finnigan 1112 (Thermo-Finnigan, Milan, Italy). Los contenidos de P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B se realizaron sobre la digestión nítrico-perclórica (5:3, *v/v*) de este material seco en microondas (CEM Mars Xpress, NC, USA), utilizando espectrometría de emisión óptica de plasma acoplado inductivamente (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

Los aminotioles y aminoácidos libres se extrajeron e inmediatamente se derivatizaron siguiendo la metodología de Salazar et al. (2012). Se analizaron por cromatografía líquida de ultra alta presión (UHPLC) trabajando en fase reversa y acoplado a un espectrómetro de masas triple cuadrupolo (Agilent Technologies, Waldbronn, Alemania). (Nagumo et al., 2009; Salazar et al., 2012).

Las proantocianidinas se extrajeron siguiendo el procedimiento indicado por Buendía et al. (2010). Estos compuestos se analizaron por cromatografía líquida de alta presión (HPLC) trabajando en fase normal y acoplado a un espectrómetro de masas trampa de iones (ultra HCT Bruker, Bremen, Alemania) (Buendía et al., 2010). La autoagregación de las procianidinas se evaluó utilizando la técnica de dispersión de luz dinámica (DLS) (Zetasizer Nano-ZS, Malvern Instruments, Malvern, RU)

Tasa respiratoria y emisión de etileno

Se colocaron 16 frutos en un contenedor hermético de volumen conocido y provisto de un septum de silicona. Transcurridas 2 h se muestreó la atmósfera interna para la cuantificación de etileno utilizando un Hewlett–Packard HP5890 (Bristol, United Kingdom), equipado con un detector de ionización de llama y una columna de acero inoxidable (3 m x 3.2 m) rellena de alúmina activa de 80/100 mesh (Martínez-Madrid et al., 1996). El CO_2 se cuantificó directamente en el espacio de cabeza del contenedor, utilizando un analizador de gases PBI Dansensor, CheckMate 9900.

Diseño experimental y análisis estadístico

El diseño del experimento fue completamente aleatorio con cuatro repeticiones. Cada repetición consistió en tres filas adyacentes de once árboles, realizándose las medidas en el árbol central de la fila intermedia de cada repetición y utilizando el resto de árboles como bordes. Los árboles de cada repetición fueron de aspecto (área foliar, sección del tronco, altura, superficie de suelo sombreada, etc.) muy similar. Los datos se analizaron con el software SPSS para Windows (SPSS Inc., Chicago). Los valores de cada repetición se promediaron antes de calcular el valor medio y el error estándar correspondiente a cada tratamiento. Tras la realización del análisis de la varianza, se compararon los valores medios de cada tratamiento utilizando un test de rango múltiple.

5. Publicaciones

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Leaf mechanisms for drought resistance in *Zizyphus jujuba* trees

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ABSTRACT

No information exists on the mechanisms developed at the level of leaf water relations by pear-jujube trees (*Zizyphus jujuba* Mill.) to confront drought. For this reason, the purpose of the present study was to analyse its leaf water relations in order to clarify the resistance mechanisms (avoidance and tolerance) developed in response to a water stress and during recovery. Field-grown 7-year-old pear-jujube trees (cv. Grande de Albatera) were subjected to three irrigation treatments. Control (T0) plants were drip irrigated (112% ETo) in order to guarantee non-limiting soil water conditions, T1 plants (deficit irrigation, 64% ETo) were drip irrigated according to the criteria used by the grower and T2 plants irrigated as T0 but subjected to water withholding for 36 days and a subsequent re-irrigation at the levels used in T0 for 14 days, during the summer of 2011. The results indicated that pear-jujube plants confront water stress by developing stress avoidance and stress tolerance mechanisms. From the beginning of deficit irrigation (T1) and water withholding (T2) to when maximum water stress levels were achieved, leaf turgor was maintained allowing substantial gas exchange levels and, consequently, good leaf productivity. This leaf turgor maintenance was mainly due to two simultaneous and complementary mechanisms. Leaf conductance and the duration of maximum stomatal opening in water stressed plants decreased in order to control water loss via transpiration, contributing to maintain leaf turgor (stress avoidance mechanisms). Also, the gradual recovery of g_l observed after rewatering the plants can be considered as a mechanism for promoting leaf rehydration. In addition, from the beginning of the stress period, active osmotic adjustment operated, also contributing to the maintenance of leaf turgor (stress tolerance mechanism). The high RWC_a levels and the possibility of increasing the accumulation of water in the apoplasm in response to water stress, supporting a steeper gradient in water potential between the leaf and the soil, which can be considered another drought tolerance characteristic in pear-jujube.

1. Introduction

The shortage of water resources is a problem of paramount importance for fruit production in arid and semi-arid lands, which occupy more than one third of the total land area on the earth. In such areas, most fruit crops are submitted to frequent water stress situations and the effective use of irrigation water during the growing season is a key component for preventing crop stress and for maintaining productivity. Moreover, the portion of fresh water currently available for agriculture is decreasing not only in arid and drought prone areas but also in regions where rainfall is abundant [1].

Given that the continuous expansion of the water supply is unsustainable, arid and semi-arid agrosystems must face up to coping with water scarcity [2]. Fruit tree culture in arid and semiarid areas must be directed towards the use of less water-demanding and more stress-resistant plant materials, which, together with the search for ways to reduce irrigation water consumption, should permit considerable water savings and the profitable production of high quality fruits [3,4].

Pear-jujube tree (*Zizyphus jujuba* Mill.), belonging to the plant family Rhamnaceae, is native to temperate Asia, particularly China and neighbouring areas of Mongolia and the Central Asian Republics. Its cultivation has spread westwards to the Mediterranean, throughout the Near East and SW Asia and east-

Abbreviations: €, leaf bulk modulus of elasticity; Ψ_l, leaf water potential; Ψ_{md}, midday leaf water potential; Ψ_{pd}, predawn leaf water potential; Ψ_{pmd}, midday leaf turgor potential; Ψ_{ppd}, predawn leaf turgor potential; Ψ_{os}, predawn leaf osmotic potential at full turgor; Ψ_{stem}, midday stem water potential; Ψ_{tlp}, leaf water potential at the turgor loss point; g_l, leaf conductance; g_{lmd}, midday leaf conductance; P_n, leaf net photosynthesis; P_{nmd}, midday leaf net photosynthesis; RWC, relative leaf water content; RWC_a, relative leaf apoplastic water content; RWC_o, relative leaf water content at full turgor; RWC_{tlp}, relative leaf water content at the turgor loss point.

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wards to Korea and Japan [5]. Pear-jujubes are considered a fruit of minor importance and, from a research and development point of view, they have received little attention from most governments. However, pear-jujube fruits are an integral part of the culture and way of life of millions of people and have also become important for many regions of the world following their introduction [5]. *Z. jujuba* fruits are rich in carbohydrates, fibre, protein, fat and several other essential vitamins and minerals [6]. Moreover, pear-jujubes are used in many traditional medicines and have been shown to exhibit numerous health-promoting effects, including antimicrobial and antiviral properties [7], the alleviation of brain nerve disorder [8], the antiproliferation of cancer cell [9] and regulation of the immune function [10]. Consequently, pear-jujubes can be considered as a so-called functional food, since they have nutritional as well as medicinal uses [11].

Physiological mechanisms to confront drought in woody crops are mainly based on avoiding or delaying desiccation, or simply on tolerating desiccation [12,13]. The problem of assessing to what extent gas exchange and growth can resume and productivity be maintained once water stress is relieved, is particularly relevant for perennial crops cultivated in arid and semiarid regions. In this sense, pear-jujube is a very interesting fruit tree species due to its ability to withstand severe drought during the growing season and to tolerate both very low winter temperatures during its dormancy and saline irrigation water [14–16]. In this sense, severe water deficit at the bud-burst to leafing stage and moderate and severe water deficit at the fruit maturation stage reduce water consumption and improve the ratio of fruit yield to total water use of pear-jujube trees [17,18]. Moreover, deficit irrigation shortens the fruit maturation period by 10–15 d, raising the market price of the fruits [19]. Low water deficit during the pear-jujube fruit growth stage and low, moderate and severe water deficits during the fruit maturation stage have no significant effect on the fruit weight and fruit volume but reduce fruit water content slightly, which led to much reduced rotten fruit percentage during the post-harvest storage period [19].

Despite the importance of pear-jujube, to the best of our knowledge, no information exists on the mechanisms developed at the level of leaf water relations by this crop to confront drought. For this reason, the purpose of the present study was to analyse its leaf water relations in order to clarify the resistance mechanisms (avoidance and tolerance) developed in response to a water stress and during recovery. The extent of elastic and osmotic adjustments, turgor maintenance, gas exchange, midday stem water potential, predawn and midday leaf water potential, leaf water potential at the turgor loss point, relative water content at the turgor loss point and relative apoplastic water content were measured.

2. Materials and methods

2.1. Experimental conditions, plant material and treatments

The experiment was carried out during the summer of 2011, from the day of the year (DOY) 202 to 252, at a farm located 3 km from the city of Albatera (Alicante, Spain) (38°12′N, 0°51′W). The soil of the orchard is a Torrifluvent with sandy loam texture, very low electrical conductivity (109 μS/cm, 1:10, w:v), high lime content (57%), very low organic matter content (0.3%), low exchangeable potassium (40 mg/kg) and available phosphorus (20 mg/kg) levels. The irrigation water had an electrical conductivity of between 1.7 and 2.2 dS/m and a Cl^- concentration ranging from 36 to 48 mg l^{-1}.

The plant material consisted of 7-year-old pear-jujube trees (*Z. jujuba* Mill., cv. Grande de Albatera), planted at 2 m × 6 m. Fertilization and pest control practices were those usually used by the growers, and no weeds were allowed to develop within the orchard.

The climate of the area is typically Mediterranean, with mild winters, low annual rainfall, and hot dry summers. During the experimental period, average daily maximum and minimum air temperatures were 32 and 22 °C, respectively, while mean daily air vapour pressure deficit (VPD_m) [20] ranged from 1.25 to 3.25 kPa, and reference crop evapotranspiration (ETo) [20] was 248 mm. Total rainfall achieved only 20 mm, which took place mainly on DOY 245 and 246 (16 mm).

Three irrigation treatments were considered, in which irrigation was carried out daily and during the night using a drip irrigation system with one lateral pipe per tree row. Control plants (treatment T0) were irrigated in order to ensure non-limiting soil water conditions (112% ETo), and T1 plants were irrigated according to the criteria used by the farmer 64% ETo). T2 plants were irrigated as T0 but irrigation was withheld for 36 days (from day of the year (DOY) 202 to 238). The recovery of T2 plants was ensured by re-irrigation at the levels used in T0 for 14 days (DOY 239–252).

2.2. Measurements

Leaf water potential (Ψ_l) was measured in mature leaves located on the south facing side, from the middle third of the tree (two leaves per tree and four trees per treatment), using a pressure chamber (model 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA), as recommended by Turner [21]. Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in a similar number and type of leaves as used for Ψ_l, enclosing leaves in a small black plastic bag covered with aluminium foil for at least 2 h before measurements in the pressure chamber [22,23].

Gas exchange in attached leaves, leaf conductance (g_l) and net photosynthesis (P_n), was measured with a steady-state porometer (LI-1600, LICOR Inc., Lincon, USA) on the abaxial surface of the leaves in a similar type of leaves as used for the Ψ_l measurements. Two measurements were taken on four trees per treatment.

Predawn and midday leaf osmotic potentials were determined in the same leaves used for predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials. Leaves were frozen in liquid nitrogen and the osmotic potential was measured after thawing the samples and expressing sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Predawn (Ψ_{ppd}) and midday (Ψ_{pmd}) leaf turgor potentials were derived as the difference between osmotic and water potentials. To estimate predawn leaf osmotic potential at full turgor (Ψ_{os}), excised leaves were sealed in plastic bags immediately after excision and subjected to a rehydration treatment by dipping their petioles in distilled water for 24 h at 4 °C. Rehydrated leaves were frozen in liquid nitrogen before measuring the osmotic potential in an osmometer as indicated above [24,25].

The diurnal patterns of Ψ_l, g_l and P_n in response to the three irrigation treatments were performed on DOY 209, 216, 223, 229, 234, 238 and 252. Measurements were performed from sunrise to sunset on two leaves per tree and four trees per treatment, using the above described procedures.

At the end of the stress period (DOY 238), leaves were excised at predawn, resaturated as indicated for Ψ_{os}, and pressure–volume (PV) curves were performed in order to determine values of Ψ_{os}, leaf water potential at the turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ), relative water content at the turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) [26–29]. Four PV curves per each irrigation treatment (a PV curve per replicate) were performed. The resaturated leaves were weighed using an analytical balance (±0.1 mg precision), placed in the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s^{-1}) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the

chamber). Once depressurized, the leaf was allowed to transpire outside the pressure chamber on the laboratory bench at room temperature (22 ± 2 °C). Leaves were repeatedly weighed and their balance pressures determined over the full range of the pressure gauge [30]. Data for initial saturated weight, intermediate fresh weight (corresponding to values for Ψ_l), and final dry weight (at 80 °C for 48 h) were used to calculate the relative water content (RWC) [31].

The curves were drawn using a type II transformation [28]. When the reciprocal of water potential (Ψ_l) was plotted against RWC, the resultant relationships showed both linear and non-linear regions. Extrapolation on the straight portion of the curve obtained for a value of RWC = 1 gave the reciprocal of the Ψ_{os} and extrapolation to the abscissa gave RWC_a. Ψ_{tlp} and RWC_{tlp} were estimated as the intersection between the linear and curvilinear portions of the PV curve. The leaf bulk modulus of elasticity (ϵ) of leaf tissue at 100% RWC (RWC_o) was estimated according to Patakas and Noitsakis [32] as ϵ (MPa) = $(\Psi_{os} - \Psi_{stlp})(100 - RWC_a)/(100 - RWC_{tlp})$, where Ψ_{stlp} is the osmotic potential at the turgor loss point and Ψ_{os} values correspond to those obtained from the analysis of the PV curves.

2.3. Statistical design and analysis

The design of the experiment was completely randomized with four replications, each replication consisting of three adjacent tree rows, each with eleven trees. Measurements were taken on the inner tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), while the other trees served as border trees. Data were analysed using SPSS [33] software. Analysis of variance was performed and means values were compared by Duncan's multiple range test. All means were compared at the 0.05 level of significance. Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

The Ψ_l values in T0 and T1 plants on DOY 209, 216, 223, 229, 234, 238 and 252 showed differences between treatments but a similar diurnal time course, decreasing progressively early in the morning, stabilizing at minimum values at around midday and recovering in the afternoon (Fig. 1). In contrast, from DOY 209 to 238 Ψ_l values in T2 plants showed a different circadian rhythm, characterized by a slight variation in Ψ_l values every day. Also, Ψ_l values in T2 plants were lower than those in T0 and T1 plants, a difference that tended to increase during the water stress period. Ψ_l values at the end of the recovery period (DOY 252) pointed to differences between treatments, reflecting that total recovery of Ψ_l values in T2 plants was not accomplished (Fig. 1).

The diurnal time course of g_l and P_n values in T0 plants was characterized by an increase from sunrise to reach high and maximum values between 10:00 and 17:00 h, after which it decreased (Fig. 1). In contrast to T0 and T1 plants, T2 plants showed a gradual decrease in the duration of maximum stomatal opening in response to the withholding of irrigation water, and low g_l and P_n values were registered during most of the day at the end of the water stress period (DOY 238). From DOY 209 to 238, the daily course of g_l and P_n values in T1 plants was below those of T0 plants and higher than in T2 plants. As consequence of rewatering, the circadian pattern of g_l and P_n of T2 plants substantially increased on DOY 252, but they were still generally below those of the T0 treatment (Fig. 1).

Midday leaf conductance (g_{lmd}) and net photosynthesis (P_{nmd}) values in T0 were high and nearly constant during the measurement period (Fig. 2). The g_{lmd} values of T1 plants were also nearly constant and intermediate between T0 and T2 values (Fig. 2A). However, g_{lmd} and P_{nmd} values in T2 plants gradually decreased during the stress period, reaching minimum values of 111.00 mmol m^{-2} s^{-1} and 2.57 μmol m^{-2} s^{-1}, respectively (on DOY 238), before increasing when irrigation was restarted.

Significant differences between treatments from DOY 209 to 238 were noted in Ψ_{pd} and Ψ_{stem} values, with the characteristic that T1 plants showed lower values than T0 plants and higher than T2 plants (Fig. 3A and F). Ψ_{pd} values in T0 and T1 plants and Ψ_{stem} values in T0 plants remained constant during the experimental period, whereas Ψ_{pd} and Ψ_{stem} values in T2 plants and Ψ_{stem} values in T1 plants showed a tendency to gradually decrease, reaching minimum values of −3.20, −3.14 and −2.28 MPa, respectively, on DOY 238. Ψ_{stem} and Ψ_{pd} in T2 plants showed values intermediate to those in T0 and T1 plants when irrigation was recommenced (DOY 252) (Fig. 3A and F).

Midday leaf water potential (Ψ_{md}) values in the three irrigation treatments were lower than the Ψ_{stem} values, and in T0 and T1 plants decreased slightly from DOY 209 to 238 (Fig. 3B). Ψ_{md} values in T2 plants gradually decreased during the stress period, reaching a minimum value of −4.22 MPa. Also, on DOY 216, 223, 238 and 252, Ψ_{md} values in T1 plants were higher than those in T2 plants and lower than those of T0 plants. After rewatering the plants, Ψ_{md} values in T2 plants partially recovered, reaching similar values to those of T1 plants but lower than those of T0 plants (Fig. 3B).

The Ψ_{os} values of the three treatments were almost constant throughout the experimental period (Fig. 3E). However, there were differences between the treatments from DOY 216 to the end of the experimental period. Ψ_{os} values in T1 plants were frequently lower than those in T0 plants and higher than those in T2 plants (Fig. 3E).

Ψ_{ppd} and Ψ_{pmd} values in the three treatments were always above zero, indicating that turgor was maintained during the experimental period (Fig. 1C and D). However, significant differences between treatments were noted in Ψ_{ppd} values during the measurements period. They were nearly constant in T0 plants and also higher than in the other treatments. The Ψ_{ppd} values in T2 plants gradually decreased during the stress period, by the end of which they had reached minimum values but rapidly recovered when irrigation was resumed. Ψ_{ppd} values in T1 plants were higher than in T2 plants, remaining nearly constant from the beginning of the experiment to DOY 229 when they began to fall until DOY 252 (Fig. 1C and D). No differences between T0 and T1 were found in Ψ_{pmd} values, except on DOY 234 and 252. On the other hand, Ψ_{pmd} values in T2 plants were lower than in the other two treatments from the beginning of the experiment and rapidly recovered after rewatering the plants (Fig. 1C and D).

At the end of the period during which irrigation was withheld (DOY 238), Ψ_{tlp}, ϵ and RWC_{tlp} values were high and were not affected by the irrigation treatment (Table 1). However, values of RWC_a were significantly higher in T2 plants.

4. Discussion

Taking into consideration that Ψ_{pd} values mainly depend on soil moisture levels [34–36], the high and constant Ψ_{pd} and Ψ_{ppd} values in T0 plants during the measurement period (Fig. 2A and C) suggested that the amount of water supplied was sufficient. The fact that Ψ_{pd} and Ψ_{ppd} values in T1 plants were significantly lower than those in T0 plants (Fig. 2A and C) suggested that the irrigation criteria used by the grower did not adequately satisfy pear-jujube water requirements.

At the end of the stress period, T2 plants showed Ψ_{pd}, Ψ_{stem} and Ψ_{md} values of −3.20, −3.14 and −4.22 MPa, respectively, indicating severe water stress in the plants of this treatment (Fig. 3A, B and F). In this sense, the delay in recovery of Ψ_{pd}, Ψ_{md} and Ψ_{stem}

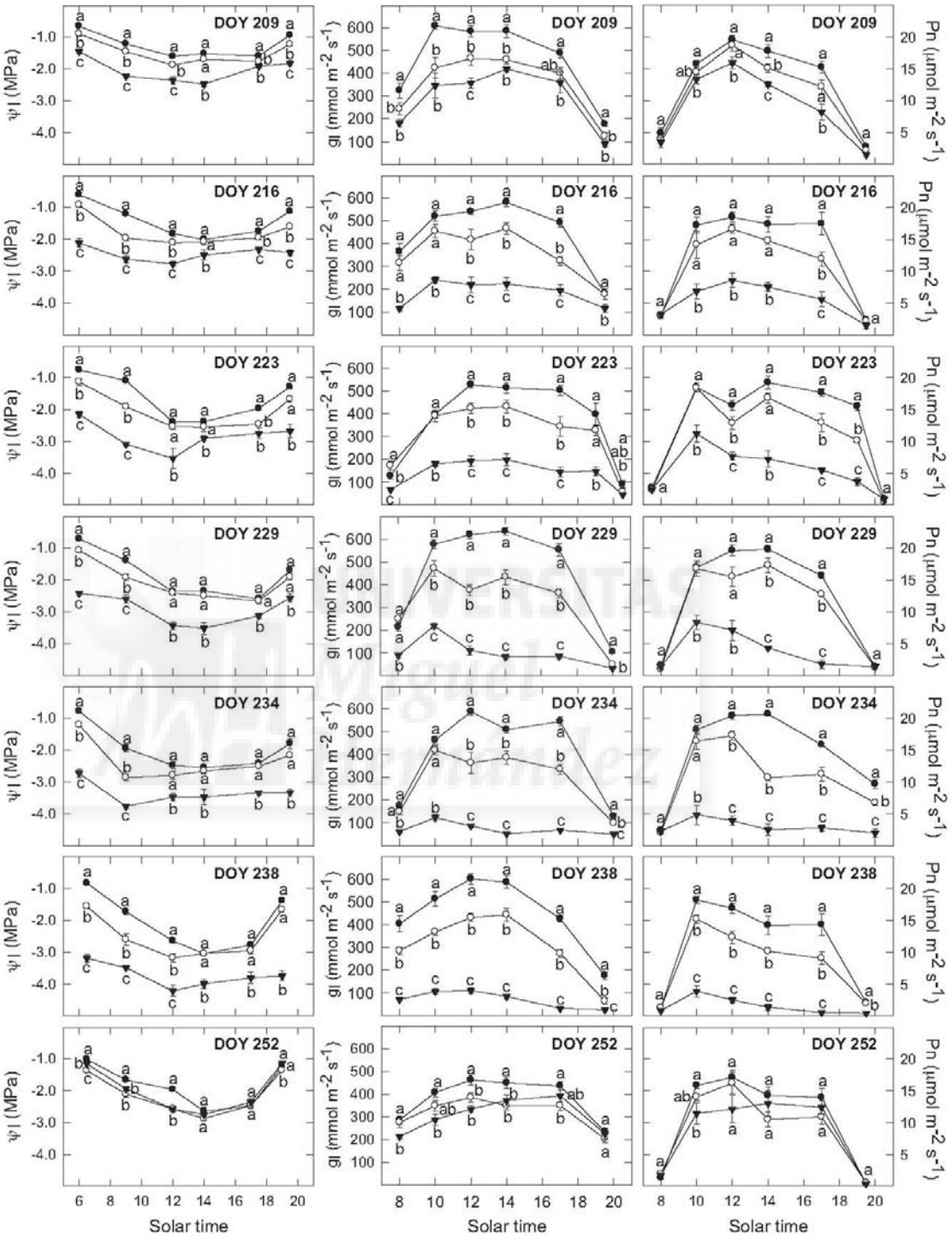

Fig. 1. Diurnal course of leaf water potential (Ψ_l), leaf conductance (g_l) and net photosynthesis (P_n) values (mean ± SE, not shown when smaller than symbols, $n = 4$) for pear-jujube plants in T0 (closed circles), T1 (open circles) and T2 (closed triangles) treatments at six different times during the stress period (DOY 209, 216, 223, 229, 234 and 238) and at the end of the T2 recovery period (DOY 252). Different letters on data points at each time indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

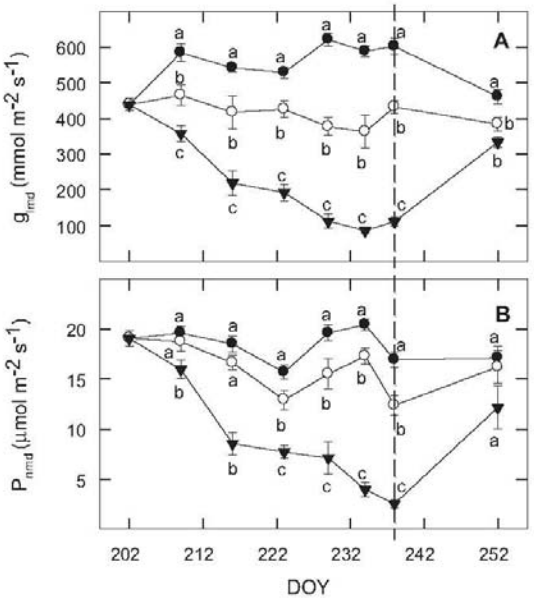

Fig. 2. Midday leaf conductance (g_{lmd}) and net photosynthesis (P_{nmd}) values (mean ± SE, not shown when smaller than symbols, $n = 4$) for pear-jujube plants in T0, T1 and T2 treatments during the experimental period. Vertical dashed line indicates the end of the T2 water stress period. Symbols as in Fig. 1.

values (Fig. 3A, B and F) in T2 plants when irrigation was resumed may be related with reduced hydraulic conductivity, perhaps as a result of xylem cavitation events [37]. However, the existence of differences between Ψ_{pd} and Ψ_{md} values in T2 plants during the

Table 1
Effect of water stress on leaf osmotic potential at turgor loss point (Ψ_{tlp}), leaf bulk modulus of elasticity (ϵ) relative water content at turgor loss point (RWC_{tlp}) and relative apoplastic water content (RWC_a) of pear-jujube plants in T0, T1 and T2 treatments at the end of the stress period. Means with the same letter across each row do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$).

Parameters	T0	T1	T2
Ψ_{tlp} (MPa)	−4.44a	−4.70a	−5.46a
ϵ (MPa)	4.60a	5.42a	5.20a
RWC_{tlp} (%)	63.30a	60.90a	66.20a
RWC_a (%)	29.50b	26.87b	41.57a

water withholding period showed that the capacity of the conduct system to transport water was not completely annulled, allowing some rehydration of leaves from midday onwards (Fig. 3A and B).

The observed decrease in Ψ_{os} values in T1 and T2 plants (0.35 and 0.72 MPa, respectively) can be attributed to the active accumulation of solutes and, therefore, active osmotic adjustment in mature leaves of the pear-jujube plants [38]. The greater osmotic adjustment in T2 plants could be attributed to the accumulative effect of water stress in this treatment. The present results agree with those observed in other fruit trees such as apricot [39], apple [40], pomegranate [41] and peach [42,43]. It has been shown that osmoregulation is a function of species and cultivar and only takes place when water stress develops gradually over a prolonged period of time [42,44].

The fact that leaf turgor was maintained in T1 and T2 plants, even at maximum water stress levels (Fig. 1C, D and F), indicated that active osmoregulation contributed to maintaining turgor (Ψ_{ppd} and Ψ_{pmd} above zero) (Fig. 1C and D). Despite the fact that several authors have shown that Ψ_{os} affects Ψ_{tlp} in some woody crops [13,45], the fact that Ψ_{tlp} values in pear-jujube leaves did not change as a result of water stress (Table 1) could indicate that the osmotic adjustment reached was not able to modify the Ψ_{tlp} values in T1 and T2 leaves. In addition to the contribution of active

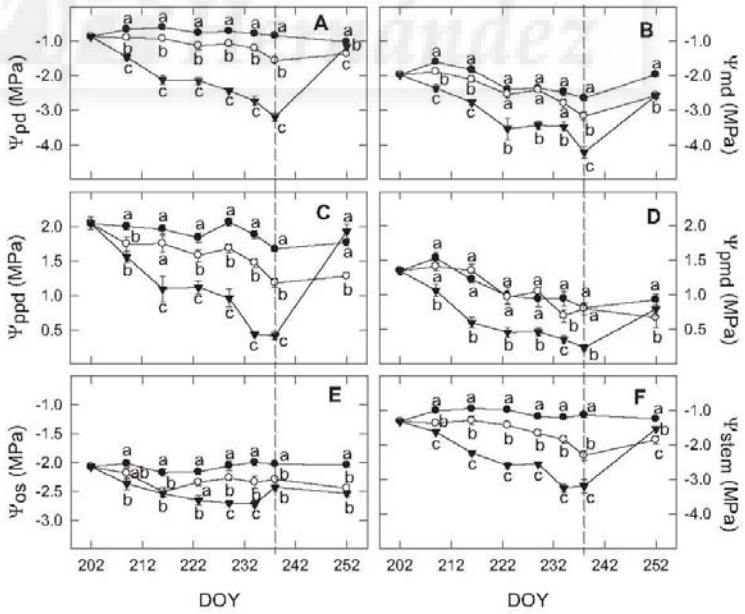

Fig. 3. Predawn (Ψ_{pd}, A) and midday (Ψ_{md}, B) leaf water potential, predawn (Ψ_{ppd}, C) and midday (Ψ_{pmd}, D) leaf turgor potential, predawn leaf osmotic potential at full turgor (Ψ_{os}, E) and midday stem water potential (Ψ_{stem}, F) values (mean ± SE, not shown when smaller than symbols, $n = 4$) for pear-jujube plants in T0, T1 and T2 treatments during the experimental period. Other symbols as in Figs. 1 and 2.

osmoregulation to the maintenance of leaf turgor, the stomata regulation observed in T1 and T2 plants (Figs. 1 and 2) could be considered a complementary mechanism for maintaining turgor. The decrease in stomatal conductance in woody crop leaves under water stress has been described as a primary response to water deficit, when it plays an active task in controlling leaf water status [41,43,46].

The partial recovery of g_{lmd} values in T2 plants after rewatering (DOY 252) (Fig. 2A) indicated that stomatal closure was not a simple passive response to water deficit; therefore, the pattern of g_{lmd} under water stress may be related to hormonal changes within the leaf, such as an increase in abscisic acid and/or a decrease in the cytokinin content [47,48].

In agreement with the behaviour observed in peach and pomegranate plants [41,43], but in contrast to the behaviour detected in other woody crops [12,49], pear-jujube leaves were not able to develop elastic adjustment (ϵ decrease) in response to water stress. The fact that RWC_{tlp} values did not change under water stress (Table 1) confirmed that the ϵ controlled the RWC_{tlp} values [12]. In species which show osmoregulation, the maintenance of cell wall rigidity may be a prerequisite for maintaining cell tissue integrity upon rehydration following a period of stress [50,51].

RWC_a values in pear-jujube plants (30–42%) (Table 1) were similar to those found in other fruit crops such as apricot (27–42%) [39] and peach (29–44%) [43] and to the lower limit of the range found for pomegranate (42–58%) [41] and almond (42–59%) [13]. Nevertheless, pear-jujube RWCa values were high compared with other tree species, such as *Eucalyptus globulus* (14–27%) [52] and *Quercus alba* (26–31%) [53], but lower than those found for grapes (51–63%) [54]. High RWC_a values are common in xeromorphic plants [55], and the increase in the apoplastic water fraction as a result of water stress might be interpreted as an adaptive mechanism [56], because structural changes in cell wall of pear-jujube leaves in response to water stress could allow a greater accumulation of water in the apoplasm and lead to lower leaf water potential values, resulting in a steeper gradient in water potential between the leaf and the soil, thus favouring water absorption [57].

The above results indicate that pear-jujube trees exposed to water stress depend strongly on stress avoidance and stress tolerance mechanisms. From the beginning of deficit irrigation (T1) and water withholding (T2) to the time of maximum water stress, leaf turgor was maintained allowing substantial gas exchange levels and, as a consequence, good leaf productivity. This leaf turgor maintenance was mainly due to two simultaneous and complementary mechanisms: decreased leaf conductance and a shorter period of maximum stomatal opening in order to control water loss via transpiration (stress avoidance mechanisms). The gradual recovery of g_l after rewatering can also be considered as a mechanism for promoting leaf rehydration. In addition, from the beginning of the stress period, active osmotic adjustment operated, which could have contributed to the maintenance of leaf turgor (stress tolerance mechanism). The high RWC_a levels and the possibility of increasing the accumulation of water in the apoplasm in response to water stress, supporting a steeper gradient in water potential between the leaf and the soil, which can be considered another drought tolerance characteristic in pear-jujube leaves.

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EFFECTS OF WATER DEFICIT DURING MATURATION ON AMINO ACIDS AND JUJUBE FRUIT EATING QUALITY

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Jujube yield and fruit characteristics can be clearly modified by water deficit imposed during fruit maturation. One essential (cystine (Cys-cys)) and seven non-essential (4-hydroxyproline (p-Hyp), α-aminoadipic acid (AADA), ornithine (Orn), β-aminoisobutyric acid (BAIB), α-amino-n-butyric acid (AABA), cystathionine (Cysta), and homocystine (Hcys-cys)) amino acids were identified for the first time. Fruits from plants exposed to moderate water deficit during the maturation stage (T1) initiated the ripening phase earlier than control (T0) fruits and had an improved eating quality. Fruits subjected to severe water deficit (T2) showed changes in their physical characteristics and reached a more advanced degree of ripening than T0 and T1 fruits, with not only most of the fruit chemical characteristics that determine taste being improved but also the nutritional value. The decrease in the asparagine (Asn) content of the fruit as a result of severe water deficit is a positive aspect, which prevents acrylamide formation during heat-processing of the fruit.

Keywords: ethylene emission; fruit quality; plant water relations; fruit respiration; amino acids

ВЛИЈАНИЕ НА НЕДОСТИГОТ НА ВОДА ЗА ВРЕМЕ НА СОЗРЕВАЊЕ ВРЗ АМИНОКИСЕЛИНИТЕ И ХРАНЛИВИОТ КВАЛИТЕТ НА *ZIZYPHUS JUJUBA*

Приносот и карактеристиките на *Zizyphus jujuba* очигледно можат да се модифицираат со наметнат недостиг на вода при созревањето. За прв пат се идентификувани една есенцијална (Cys–cys) и седум неесенцијални аминокиселини: 4-хидроксипролин (p-Hyp), α-аминоадипинска киселина (AADA), орнитин (orn), ß-аминоизобутерна киселина (BAIB), α-амино-n-бутерна киселина (AABA), цистатионин (Cysta) и хомоцистин (Hcys-cys). Овошките изложени на умерен недостиг на вода за време на созревањето (T1) побрзо започнувале со фазата на созревање во однос на овошките од контролната група (T0) и имале подобар прехранбен квалитет. Овошките изложени на голем недостиг на вода (T2) покажале промени во физичките карактеристики и достигнувале понапреден степен на созревање од овошките од T0 и T1, и тоа не само со подобрени хемиски карактеристики кои го подобруваат вкусот, но и со подобрена нутрициска вредност. Намалувањето на содржината на аспарагин (Asp) во овошките како резултат на голем недостиг на вода има позитивна страна затоа што го спречува образувањето на акриламид за време на топлинската обработка на овошјето.

Клучнизборови: емисија на етилен; квалитет на овошје; однос на вода и растенија; респирација кај растенија; аминокиселини

1. INTRODUCTION

Jujube or Chinese jujube tree (*Zizyphus jujuba* Mill.) is a native fruit tree of China, where it has been cultivated for more than 5000 years, and yields about 450,000 tons annually in that country alone [1]. Although species of the *Zizyphus* genus are considered as minor crops, they are an integral part of the culture and way of life of millions of Asian and African people and are considered as crops with substantial growth potential by the International Centre for Underutilized Crops in Southampton, U.K. [2].

Z. jujuba is admired for its wide adaptation, easy management, early bearing, and the nutritional value of its fruit, which is eaten fresh, dried, or processed into confectionary. It is a nutritionally valuable fruit for the variety of essential nutrients it contains such as carbohydrates, crude fibre, crude protein, flavonoids, and several essential minerals and vitamins [3–7]. Moreover, jujube fruits have been shown to possess biological activities associated with the antiproliferation of cancer cells [8], the alleviation of brain nerve disorder [9], regulation of immune function [10], antimicrobial and antiviral properties [11], and the reduction of blood triglyceride levels[12]. It has analeptic, palliative, and antibechic uses [7, 13]. Nevertheless, Choi *et al.* [4, 6] recently identifiedhigh levels of free asparagine (Asn), which is the major precursor of acrylamide, a potentially toxic compound formed during the heat-processing of plant foods [14, 15].

From the agronomic point of view, *Z. jujuba* is a very interesting crop because it is able to withstand severe drought during the growing season. Cruz *et al.*[16] showed that the jujube tree is able to maintain leaf turgor and productivity under severe water deprivation thanks to two simultaneous and complementary mechanisms: stomatal regulation and active osmotic adjustment. Despite the increasing water shortage in the areas considered most suitable for jujube tree cultivation, hardly any reports exist on the effect of irrigation and its relationship with the jujube fruit yield and quality. An exception is the work of Cui *et al.*[17], who showed that regulated deficit irrigation improves fruit quality and water use efficiency. It is well known that the free amino acids present in some fruits contribute to their taste and to defense mechanisms against water deficit. Clarifying the changes in amino acid contents is therefore an important step in elucidating the response of jujube fruits to water deficit.

For these reasons, the aim of the present paper was to ascertain whether water deficit during the fruit maturation period is a useful tool for the management of some important quality attributes in jujube fruits. For this, fully irrigated trees were compared with deficit-irrigated trees and trees submitted to a period of non-irrigation during fruit maturation.

2. EXPERIMENTAL

The experiment was carried out in 2011 at a farm near the town of Albatera (Alicante, Spain) (38º 12' N, 0º 51' W). The plant material consisted of 7-year-old jujube trees (*Zizyphus jujuba* Mill. cv. Grande de Albatera), planted at 2 m×6 m. The soil of the orchard is a Torrifluvent with a sandy loam texture, very low electrical conductivity (109 µS/cm, 1:10 *w:v*), high lime content (57 %), very low organic matter content (0.3 %), and low exchangeable potassium (40 mg/kg) and available phosphorus (20 mg/kg). The irrigation water had an electrical conductivity of between 1.7 and 2.2 dS/m and a Cl^- concentration ranging from 36 to 48 mg/ l.

The climate of the area is strictly Mediterranean, with mild winters, low annual rainfall, and hot, dry summers. During the experimental period, the average daily maximum and minimum air temperatures were 32 and 22 ºC, respectively, while the mean daily air vapour pressure deficit (VPD_m) [18] ranged from 1.25 to 3.25 kPa and the reference crop evapotranspiration (ETo) [18] was 189 mm. The total rainfall was negligible (1.8 mm on day of the year, DOY, 221).

Three irrigation treatments were considered, in which irrigation was carried out daily and during the night using a drip irrigation system with one lateral pipe per tree row. Control plants (treatment T0) were irrigated to ensure non-limiting soil water conditions (112 % ETo), T1 plants were irrigated according to the criteria used by the farmer (64 % ETo), and T2 plants were irrigated as in T0 but irrigation was withheld for 36 days (from DOY 202 to 238). The total water amounts applied during the measurement period were 213 and 122 mm for treatments T0 and T1, respectively.

2.1. *Chemicals*

The AQC reagent was purchased from Chemos GmbH (Regenstauf, Germany). The Bis-

Tris reagent and all amino acid standards (histidine (His), 1-methyl-histidine (Met-His), 4-hydroxyproline (p-Hyp), asparagine (Asn), phosphoethanolamine (PEA), arginine (Arg), glutamine (Gln), serine (Ser), glycine (Gly), ethanolamine (EA), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), threonine (Thr), alanine (Ala), γ-Amino-n-butyric acid (GABA), α-Aminoadipic acid (AADA), proline (Pro), Ornithine (Orn), β-Aminoisobutyric acid (BAIB), α-Amino-n-butyric acid (AABA), lysine (Lys), Cystine (Cys-cys), cystathionine (Cysta), tyrosine (Tyr), valine (Val), Methionine (Met), homocystine (Hcys-cys), leucine (Leu), isoleucine (Ile), tryptophan (Trp), and phenylalanine (Phe)) were obtained from Sigma Aldrich (Madrid, Spain). Acetonitrile and methanol (both of LC-MS grade), sulfuric acid, nitric acid, perchloric acid, formic acid, ammonium acetate, ascorbic acid, and sodium hydroxide were obtained from Panreac Química S.A. (Barcelona, Spain), and metaphosphoric acid was from Merck (Darmstadt, Germany). Boric acid was bought from Probus (Badalona, Spain), while arabinose, glucose, sucrose, malic acid, citric acid, oxalic acid, and calcium disodium EDTA were purchased from Sigma (Steinheim, Germany). The Milli-Q water used was produced by an Elix® 3 Millipore water purification system (Molsheim, France).

2.2. *Plant water status*

The predawn leaf water potential (Ψ_{pd}) was measured in mature leaves located on the south facing side, from the middle third of the tree (two leaves per tree and four trees per treatment), using a pressure chamber (model 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA). The midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in a similar number and type of leaves as Ψ_{pd}: leaves were enclosed in a small, black plastic bag covered with aluminum foil for at least 2 h before the measurements in the pressure chamber [19].

Midday gas exchange in attached leaves, leaf conductance (g_{lmd}), and net photosynthesis (P_{nmd}), were measured with a field-portable, closed gas-exchange photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA), in a similar type of leaf as used for the Ψ_l measurements. Two measurements were taken on four trees per treatment.

2.3. *Fruit weight, firmness, and color*

Jujube fruits were harvested in their commercial ripening state (the S7 stage of growth, according to Choi *et al.* [4]) and immediately transported to the laboratory, on 27 August (DOY 239). The fruits were divided into the edible portion (peel and pulp) and pit (shell and seed), and the average weight, moisture content, firmness, peel and flesh color, total soluble solids, ethylene emission, and respiratory rate were immediately measured in each fresh edible portion sample. Aliquots of all samples were stored at –20 °C and freeze-dried before other analyses.

Average fruit weight was estimated by weighing 25 fruits per replicate. For the moisture content, samples were oven-dried at 105 °C to constant weight (AOAC, 2000). The flesh firmness was determined as the load needed to break the flesh after removal of the peel in the equatorial zone, according to Egea *et al.* [20], using a Penefel-type penetrometer (Copa-Technology, Tarascon, France) equipped with a rod 8 mm in diameter.

The external and internal color were measured by reflectance with a Minolta CR 2000 colorimeter (Osaka, Japan) and the results were expressed in the CIELAB L^*, a^*, b^* system. The mean values of the lightness (L^*), red-greenness (a^*), and blue-yellowness (b^*), parameters for each fruit were calculated. The objective color was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($H^o$ = arctan (b^* / a^*)) [21].

2.4. *Fruit ethylene emission and respiratory rate*

Ethylene and CO_2 production were determined by placing 16 fruits in a hermetically sealed container, of known volume and equipped with a silicone septum. After 2 h, 1 mlof the internal atmosphere of the container was extracted with a syringe for the determination of ethylene. The ethylene was quantified in a Hewlett–Packard HP5890 (Bristol, United Kingdom), equipped with a flame ionization detector and a stainless-steel column (3 m × 3.2 mm diameter) packed with 80/100 mesh activated alumina. The quantification was carried out following calibration, point-by-point, with an external standard[22]. The CO_2 quantification was performed directly in the head space of the container, 2 h after closure, using a bench-top headspace gas analyzer (PBI Dansensor, CheckMate 9900).

2.5. *Fruit total soluble solids, vitamin C, sugar, organic acids, and bioelements*

Total soluble solids (TSS) were determined by measuring the refractive index of the juice extracted from the fruit, with a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) was determined by potentiometric titration with 0.1N NaOH at pH 8.1 [23]. The ripening index was estimated as the ratio between TSS and TA.

Vitamin C was determined according to Egea *et al*. [23]. The lyophilized powder of the edible portion (0.5 g) was diluted with 10 ml of cold 50 ml/lmetaphosphoric acid, making up the homogenized mixture to 50 ml with the same solvent. The final solution was kept on ice in darkness for 30 min and then centrifuged at 20 000 g for 25 min. Then, the supernatant was passed through a C18 Plus Sep-Pack cartridge and a 0.2-µm filter. Quantification was carried out with a high-performance liquid chromatograph (Shimadzu LC-10Atvp, Kyoto, Japan), using a thermostatted ion exchange column (ION-300) at 30 °C with isocratic elution. The absorbance at 254 nm was recorded with a UV-visible detector. A standard curve in the concentration range 10–100 mg/kg ascorbic acid was used.

To determine the sugar and organic acid contents, lyophilized powder of the fruit edible portion (0.5 g) was diluted with Milli-Q water (50 ml) and then centrifuged (1200 g) at 4 °C for 30 minutes. After centrifugation, the supernatant was filteredthrough a Durapore 0.45 µm HV filter (Millipore Corporation, USA) and then passed through a C18 Plus Sep-Pack cartridge (Waters Corporation, Massachusetts, USA). Quantifications were carried out by a Shimadzu LC-10Atvp HPLC (Kyoto, Japan), using a thermostated ion-exchange column (ION-300, Teknochroma) at 30 °C, with isocratic elution of the mobile phase (2.5 mM H_2SO_4). Two detectors were connected in tandem: a Shimadzu Refractive Index Detector, to detect the sugars, and a Shimadzu UV-Vis Detector for the spectrophotometric detection of the organic acids. The detection wavelength was 210 nm for oxalic acid and 230 nm for citric and malic acids. The sugar and organic acid quantifications were performed by means of calibration curves for each compound prepared with solutions made from standards of each organic acid and sugar [24].

Dried peel and flesh samples from the fruit moisture content determination were finely crushed in an agate mortar prior to determination of the mineral concentrations. The peel and flesh N concentrations were measured using a Thermo-Finnigan 1112 EA elemental analyzer (Thermo-Finnigan, Milan, Italy). The peel and flesh powders were subjected to nitro-perchloric acid attack (5:3, *v/v*) in a microwave oven (CEM Mars Xpress, NC, USA), reaching 200 °C in 20 min and holding this temperature for 2 h. The P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B concentrations of the arils were quantified by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

2.6. *Amino acids*

Individual amino acids (authentic standards) were prepared by dissolving each amine in Bis-Tris (pH 6.5). Calibration standards were generated by diluting the stock solutions to 1, 0.5, 0.25, 0.12, 0.06, and 0.03 mM. In the case of amino thiols, calibration standards were produced by diluting the stock solutions to 2, 1, 0.5, 0.25, 0.12, and 0.06 µM in Milli-Q water.

Free amino acids were determined by following a method described previously [25]. Briefly, 20 mg of the fine powdered edible portion or pit of the jujube fruits were homogenized with 500 µl of extraction buffer (MeOH / water, 1:1, *v/v*) using an ultra turrax (IKA, T10, Germany), for 30 seconds on ice. The samples were then incubated on dry ice for 5 minutes. The homogenates were sonicated in an ultrasound bath for 1 minute followed by centrifugation (Eppendorf centrifuge 5804 R, Hamburg, Germany) for 10 minutes at 17900 g, at 4 °C. The supernatants were transferred to limited volume vials and the precipitates were re-extracted with 500 µl of extraction buffer, homogenized, incubated on ice, and centrifuged again. All supernatants were combined. The extracts were immediately derivatized.

The derivatization of amino acids and amino thiols was accomplished by following the Waters AccQ Tag™ Ultra UHPLC amino analysis procedures, as described by Nagumo *et al*. [26] and Salazar *et al*. [25]. Briefly, 350 µl of borate derivatization buffer (0.2 M sodium borate, pH 8.8, with 5 mM calcium disodium EDTA), 50 µl of amino acid standard or jujube extract, and 100 µl of reconstituted AQC (10 mM AQC dry powder in acetonitrile) were placed in a 2-ml propylene vial [27, 28]. This solution was vortexed for several seconds, allowed to stand for 1 minute at room temperature, and then heated in a heating block for 10 minutes at 55 °C. After removing the vial from the heating block, the sample was injected into a UHPLC-MS/MS.

Amino acids and thiols were analyzed by reverse phase UHPLC as reported by Nagumo *et al*. [26] and Salazar *et al*. [25], with slight modifications. Briefly, chromatographic separation was carried out on an AccQ Tag Ultra BEH column (2.1×100 mm, 1.7 µm) (Waters Corp., Ireland). Two types of eluent were used for gradient separation. Mobile phase A consisted of 50 ml of an aqueous solution (acetonitrile, formic acid, and 5 mM ammonium acetate in water) (10: 6: 84, *v/v/v*) diluted with 950 ml of Milli-Q water. Mobile phase B was a mixture of acetonitrile and formic

acid (99.9: 0.1, *v/v*). The injection volume was 20 µl and the elution was performed at a flow rate of 0.5 ml/min^{-1}. The gradient profile was: 99.9 % A at 0–0.5 min, 90.9 % A at 5.7 min, 78.8 % A at 7.7 min, 40.4 % A at 8–10 min, 10 % A at 10.01–12.00 min, and 99.9 % A at 12.01–14.00 min. These compounds were identified using a UHPLC system coupled to a 6460 tandem mass spectrometer (Agilent Technologies, Waldbronn, Germany). Data acquisition and processing were performed using MassHunter software version B.04.00, from Agilent Technologies. The MS analysis was applied in the multiple reaction monitoring (MRM) mode, which was performed using the positive ionization mode. The MS parameters fragmentor (ion optics; capillary exit voltage) and collision energy were optimized for each analyte (Table 6). The allocation of these parameters, along with preferential MRM transition of the corresponding analytes, generated the most abundant product ions. The MRM transition used for each derivatized amino acid/thiol corresponded, in most cases, to the AMQ moiety (171^+) – which results from the collision-induced cleavage at the ureide bond of the AMQ adduct of each amino acid/thiol [25, 29]. The working conditions for the MS parameters of the electrospray source were as follows: gas flow, 9 l/ min; nebulizer, 40 psi; capillary voltage, 4000 V; nozzle voltage, 1000 V; gas temperature, 325 °C; sheath gas temperature, 390 °C; and jetstream gas flow, 11 l/min. The acquisition time was 12 min for each sample.

2.7. *Statistical design and analysis*

The experiment had a completely randomized design with four replications, each consisting of three adjacent tree rows; each row had 11 trees. Physiological measurements were taken on the inner tree of the central row of each replicate, these trees being healthy, uniform, and very similar in appearance, while the other trees served as border trees. The data were processed using SPSS software version 19 for Windows [30]. One-way analysis of variance was carried out and mean values were compared by Tukey´s multiple range test. All means were compared at the 0.05 level of significance. Values for each replicate were averaged before the mean and the standard deviation of each treatment were calculated.

3. RESULTS AND DISCUSSION

3.1. *Plant water status*

Significant differences between the irrigation treatments were noted in the Ψ_{pd} and Ψ_{stem} values from DOY 209 to 238 (Figures 1B and 1 D).

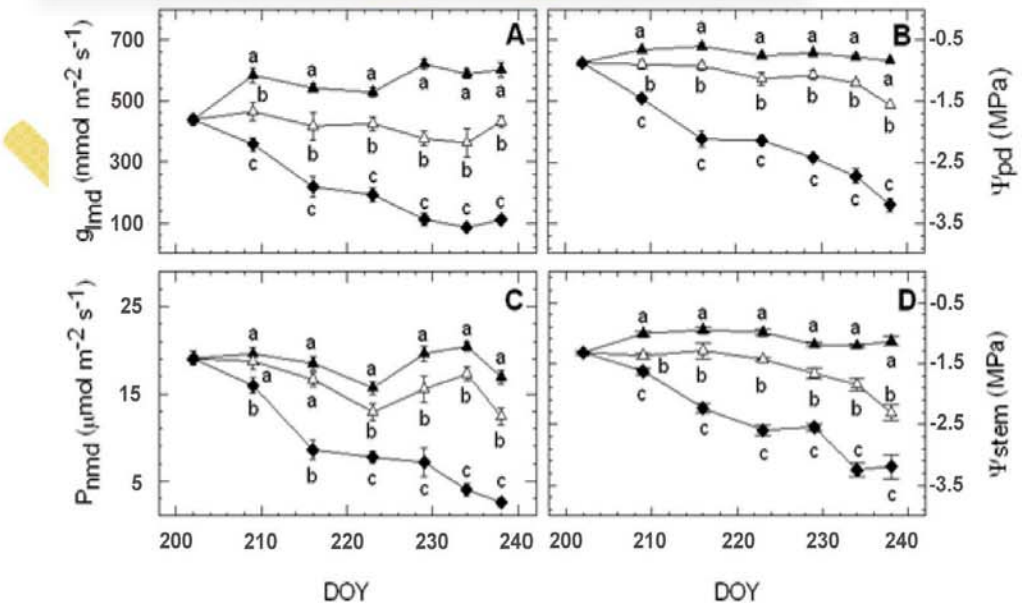

Fig. 1. Midday leaf conductance (g_{lmd}, A), predawn leaf water potential (Ψ_{pd}, B), midday net photosynthesis (P_{nmd}, C), and midday stem water potential (Ψ_{stem}, D) values (mean ± SE, not shown when smaller than symbols, $n = 4$), for jujube plants in treatments T0, T1, and T2 during the experimental period. Different letters on data points at each date indicate significant differences according to Tukey's test ($P \leq 0.05$).

The Ψ_{pd} and Ψ_{stem} values in T1 plants were lower than in T0 plants and higher than in T2 plants. In addition, the Ψ_{pd} values in T0 and T1 plants and Ψ_{stem} values in T0 plants remained constant during the experimental period, whereas the Ψ_{pd} and Ψ_{stem} values in T2 plants and Ψ_{stem} values in T1 plants showed a tendency to gradually decrease, reaching minimum values on DOY 238.

The midday leaf conductance (g_{lmd}) and net photosynthesis (P_{nmd}) values during the measurement period were high and nearly constant in T0 plants (Figures 1A and 1C). Moreover, the g_{lmd} values of T1 plants were also nearly constant, and intermediate between the T0 and T2 values (Figure 1A). However, the g_{lmd} and P_{nmd} values in T2 plants gradually decreased during the stress period, reaching minimum values on DOY 238.

Considering that Ψ_{pd} values depend on soil moisture levels [31, 32], the high and constant Ψ_{pd} values in T0 plants during the experiment indicate that these plants were not exposed to limiting soil water conditions (Figure 1B). Moreover, the existence of significant differences in the Ψ_{pd}, Ψ_{stem}, g_{lmd}, and P_{nmd} values among treatments point to a water deficit situation in T1 and T2 plants (Figure 1). The water deficit in the T1 plants can be considered as moderate because the Ψ_{pd} and Ψ_{stem} values in T1 plants reached minimum values of –1.56 and –2.28 MPa, respectively, while the g_{lmd} and P_{nmd} values were relatively high and constant, although lower than those in T0. At the end of the measurement period, the T2 plants showed very low Ψ_{pd} and Ψ_{stem} values (–3.20 and –3.14 MPa, respectively) (Figures 1B and D) and a considerable degree of stomatal regulation compared with the T1 plants (Figures 1A and C), indicating that treatment T2 induced a more severe water deficit.

3.2. *Fruit weight, firmness and color*

The total marketable jujube yield of the T1 plants was higher than that of the T2 plants and lower than for the T0 plants (Table 1). The fruit from T0 and T1 trees showed similar average weight, moisture content, and firmness. In contrast, T2 fruits showed the lowest average weight and moisture content, but the greatest firmness (Table 1).

Changes in fruit peel and flesh color were only observed in T2 fruits (Table 2). In this sense, the peel and flesh H^o values of T2 fruits were lower than those of T0 and T1 fruits, and the peel and flesh a^* and flesh b^* values of T2 fruits were higher than in the other treatments. The flesh L^* values of T2 fruits were decreased with respect to those of T0 and T1 fruits.

Fruit yield in the three irrigation treatments (833 – 2500 g m^{-2} or 10 –30 kg $tree^{-1}$) (Table 1) can be considered as adequate according to the yields mentioned by Gao *et al.* [33] for different cultivars (130 – 1800 g m^{-2}) and those obtained by Cui *et al.* [17], who considered the effect of different deficit irrigation levels during different growth stages (14 – 24 kg/tree). However, in contrast with our results, these authors indicated that low, moderate, or severe water deficit during the fruit maturation stage (stage III, early August – early September) had no significant effect on fruit yield and size but reduced the fruit water content. Unfortunately, Cui *et al.* [17] did not evaluate quantitatively the water status of the plants under severe water deficits and so our results cannot be compared with theirs.

Table 1

Effect of irrigation treatments on jujube yield (kg $tree^{-1}$), average fruit weight (FW, g), fruit moisture content (MC, %), and fruit firmness (FF, N)

Treatment	Yield	FW	MC	FF
T0	31.81a	30.23a	83.47a	64.00b
T1	21.36b	27.98a	79.66a	66.67b
T2	9.59c	14.40b	64.95b	82.33a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

The fruit size reduction observed in T2 fruits (Table 1) could be related to the results obtained recently (data not shown) in a very similar experiment by Cruz, who found that, in contrast with the axiom that expansive cell growth requires cell turgor, jujube fruit size in plants under severe water stress was reduced even though fruit turgor was maintained. This may have been due to enhancement of the cell elasticity mechanism (elastic adjustment), to maintain fruit turgor by reducing fruit cell size. The fact that jujube fruits under severe water deficit (T2) showed greater firmness could be related to the decreased moisture content in these fruits (Table 1), leading to a gummy and cork-like flesh structure. In this sense, Wu *et al.* [34] showed that *Z. Jujube* fruit firmness increased at the end of the ripening period (fruit surface 100% red) as a consequence of the lower fruit water content. This phenomenon has been observed in other fruits, such as plums, which became stiff, leading to an increase in the firmness values expressed as the load needed to break the flesh [35].

Changes in fruit peel and flesh color were evident in the T2 irrigation treatment (Table 2). The severe water stress induced changes in the fruit peel a^* and H^o values (from green towards a more intense red color). These changes were less pronounced in the fruit flesh, which remained greener than the peel and showed a lower degree of luminosity while its colour was yellower than that of the T0 and T1 fruit flesh (Table 2).

T a b l e 2

Effect of irrigation treatments on jujube fruit peel and flesh lightness (L), red-greenness (a*), blue-yellowness (b*), chroma (C*), and hue angle (H^0) values*

	L^*		a^*		b^*		C^*		H^0	
Treatment	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh
T0	78.02a	88.43a	–12.09b	–12.21b	45.04a	24.42b	46.64a	27.30a	105.04a	116.57a
T1	79.26a	88.17a	–11.18b	–12.13b	44.66a	25.17b	46.05a	27.94a	104.06a	115.73a
T2	67.55a	79.27b	3.04a	–9.91a	40.37a	27.16a	41.08a	28.91a	84.27b	110.02b

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

3.3. *Fruit ethylene emission, respiratory rate, and total soluble solids*

Fruit ethylene emission was increased in T1 and T2 fruits with respect to the T0 fruits (Table 3). However, the fruit respiratory rate was highest in T1 fruits and lowest in T2 fruits, while T0 fruits showed intermediate values (Table 3). Flesh TSS showed their minimum values in T0 fruits and were highest in T2 fruits, T1 fruits showing intermediate values. The TA values were unaffected by the irrigation treatment, but the TSS/TA ratio in T2 fruits was significantly higher than in T0 and T1 fruits (Table 3).

T a b l e 3

Effect of irrigation treatments on jujube fruit ethylene emission (C_2H_4, nL/ h g FW), respiratory rate (RR, CO_2 mg / h kg FW), total soluble solids (TSS, ºBrix), titratable acidity (TA, % malic acid), and ripening index (TSS/TA)

Treatment	C_2H_4	RR	TSS	TA	TSS/TA
T0	0.027b	59.35b	15.47c	0.25a	61.31b
T1	0.143a	66.37a	20.20b	0.32a	62.56b
T2	0.118a	56.17c	26.83a	0.36a	77.53a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

Kader *et al.* [36] concluded that *Z. jujuba* fruits followed a non-climateric pattern. In contrast, Abbas and Saggar [37] and Abbas and Fandi [38], in *Z. mauritiana* fruits, and Al-Niami *et al.* [39], in *Z. spina-christi* fruits, showed a climacteric behaviour. Moreover, coinciding with these observations in closely related species, Zhang *et al.* [40] and Wang *et al.* [41] recently indicated that *Z. jujuba* fruits are climacteric. In this sense, the increase in ethylene emission and respiratory rate in T1 fruits reflect the fact that under moderate water deficit the fruits entered the ripening phase earlier than control (T0) fruits [39]. In addition, the increase in ethylene emission in T2 fruits – compared with T0 fruits – and the decrease in fruit respiratory rate in T2 fruits – compared with both T0 and T1 fruits – reflect the finding that under severe water deficit (T2) the fruits reach a higher degree of ripening [38].

3.4. *Fruit vitamin C, sugar, organic acids and bioelements*

The T2 fruit showed higher concentrations of vitamin C, sucrose, glucose, arabinose, malic acid, and oxalic acid and a lower citric acid content than T0 fruits (Table 4). These organic compounds showed similar values in T0 and T1 fruits, except sucrose and arabinose, which increased significantly in T1 fruits (Table 4).

Table 4

Effect of irrigation treatments on jujube fruit vitamin C (mg/100 g FW), sucrose (mg/100 g FW), glucose (mg/100 g FW), arabinose (mg/100 g FW), malic acid (MA, mg/100 g FW), citric acid (CA, mg/100 g FW), and oxalic acid (OA, mg/100 g FW)

Treatment	Vitamin C	Sucrose	Glucose	Arabinose	MA	CA	OA
T0	375.33b	8954.03b	4029.59b	3567.76c	520.34b	272.71a	34.46b
T1	380.65b	23808.80a	3833.23b	4368.44b	530.88b	284.76a	26.66b
T2	590.04a	18564.92a	8815.84a	9217.86a	715.37a	191.92b	57.12a

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

The most abundant bioelements in the edible portion of jujube fruits were K, N, Ca, P, and Mg, followed, in descending order, by S, Na, B, Zn, Mn, Fe, and Cu (Table 5). Some changes in the nutrient content of the fruit edible portion were observed among the three irrigation treatments. In this respect, the mineral content of T1 fruits was very similar to that observed in T0 fruits, except that the K, P, and Cu contents were lower (Table 5). In contrast, the K, N, P, Mg, S, B, Mn, Fe, and Cu contents in the edible portion of T2 fruits were lower than in T0 fruits, whereas the Ca, Na and Zn levels were similar to those in T0 and T1 fruits (Table 5).

Under our experimental conditions, the jujube T0 fruits showed a vitamin C content (Table 4) similarto those found by Cui *et al.* [17] in fruits from cv. Lizao and by Gao *et al.* [42] in fruits from 10 promising jujube selections (Lingbaozao, Puaisanhao, Fengmiguan, Junzao, Zaowangzao, Jinchangyihao, Taigumizao, Qingjianmuzao, and Jiaxianmuzao) and higher than those found by Li *et al.* [7] in fruits from five different *Z. jujuba* cultivars (Jinsixiaozao, Yazao, Jianzao, Junzao, and Sanbianhong). These values confirm that the jujube fruit is a rich source of ascorbic acid compared with other fruit species: according to the Food and Nutrition Board of the National Research Council [43], one 20-g portion of jujube fruit (a medium size fruit) could provide almost the entire daily vitamin C requirement of an adult. The increase in vitamin C content in fruits under severe water deficit (T2) (Table 4) is in line with the results of Cui *et al.* [17] and could have resulted from a concentration effect due to the decrease in fruit moisture content (Table 1).

Table 5

Effect of irrigation treatments on some mineral nutrient contents (mg/100g DW) in the edible portion (peel and flesh) of jujube fruit

Treatment	K	N	Ca	P	Mg	S	Na	B	Zn	Mn	Fe	Cu
T0	1052a	839a	66a	54a	49a	24a	6a	1.61a	0.70a	0.68a	0.51a	0.23a
T1	864b	749a	52a	45b	41ab	21a	5a	1.69a	0.61a	0.63ab	0.50ab	0.16b
T2	630c	587b	57a	39b	35b	13b	4a	1.10b	0.57a	0.51b	0.32b	0.06c

Means within a column that do not have a common letter are significantly different according to Tukey's test ($P \leq 0.05$).

In contrast with the findings of other authors [7],the sugars detected in the Grande de Albatera cultivar fruits were mainly sucrose, glucose, and arabinose, along with traces of fructose. The presence of arabinose, one of the major neutral sugars in insoluble and soluble dietary fiber, suggests another favourable nutritional aspect of these fruits. High amounts of arabinose could be due to neutral arabinogalactan type polysaccharides or also the pectic type, which are linked to galacturonic acid residues [44]. The fact that the arabinose content of T2 fruits was higher than that of T0 and T1 fruits agrees with the effect of water deficit on the cell wall of the date palm fruit observed by Gribaa *et al.* [45]. These authors showed that the arabinose content was significantly higher in the pectin-enriched extracts from fruits under non-irrigated conditions and suggested that this could be involved in maintaining the hydration of the cell and cell wall flexibility under water deficit.

Moderate water deficit (T1) increased the sucrose and arabinose contents, as reflected in the higher TSS levels (Tables 3 and 4), indicating that moderate water deficit during fruit maturation im-

proves jujube fruit eating quality because the fruits taste sweeter. Severe water deficit (T2) improved not only the fruit taste but also the nutritional value, because these fruits showed increases in sucrose, glucose, arabinose, vitamin C, malic acid, and oxalic acid, but also a decrease in citric acid (Table 4).

The overall level of bioelements in the edible portion of jujube fruits (Table 5) differed slightly with respect to those found in five different cultivars by Li *et al.* [7]. For example, K was the predominant bioelement in both cases but we observed levels higher than those in cultivar Yazao, the richest source of K among the Chinese jujube cultivars studied by Li *et al.* [7]. Also, the Zn contents were above those reported by Favier *et al.* [46] and Li *et al.* [7] , although the Ca content was similar to that reported by Favier *et al.* [46] and that found in cultivar Jinsixiaozao by Li *et al.* [7]. The Mg and Na contents were within the range found by Favier *et al.* [46] and Li *et al.* [7], although P and the micronutrients Cu, Fe, and Mn showed values clearly lower than those reported by Li *et al.* [7]. These differences could be ascribed to the cultivar's efficiency for nutrient uptake, the edaphoclimatic conditions, or cultural practices [47]. Severe water deficit (T2) induced decreases in the K, N, P, Mg, S, B, Mn, Fe, and Cu contents in the edible portion of the fruits, compared with T0, while moderate water deficit (T1) induced no changes in the fruit content of most of the bioelements, except K, P, and Cu, which were lower than in T0 fruits (Table 5). These findings agree with the idea that drought restricts not only water acquisition but also nutrient uptake [20, 21, 48].

3.5. *Amino acids*

Thirty-two free amino acids were found in the pitand the edible portion of jujube fruits, 11 of them essential and 21 non-essential (Tables 6–8). In this sense, two essential (Cys-cys and Met) and two non-essential (Cysta and Hcys-cys) amino acids fall within the definition of amino thiols since they have a sulfhydryl group.

The edible portion of T2 fruits showed significantly lower contents of total free and total essential free amino acids than that of T0 fruits (Table 7). While the edible portion of T1 fruits had a total free amino acid content similar to that of T0 fruits, the total essential free amino acid content was similar to that of T2 fruits and lower than that of T0 fruits (Table 7). Differences were observed among the responses of the free amino acids to T1 and T2 deficit irrigation. For example, while the levels of free amino acids in the edible portion of T2 fruits had an overall tendency to decrease with respect to those in T0 fruits, Pro, Trp, and Cysta increased while His, Cys-cys, Met-his, BAIB, and Hcys-cys did not change. In the edible portion of T1 fruits, the levels of most of the free amino acids were similar to those in T0 fruits, except for 13 amino acids, 11 of which (Tyr, Leu + Ile, Phe, PEA, Gln, Asp, Cit, AADA, Orn, BAIB, and AABA) showed lower values and two (Cys-cys and Cysta) higher values (Table 7).

The effects of T1 and T2 on the concentrations of pit free amino acids were very different from those observed in the edible portion of the fruit (Tables 7 and 8). The total free amino acids and total essential free amino acids contents were similar for all three irrigation treatments. However, the responses of the amino acids to the T1 and T2 treatments differed greatly. For example, T2 decreased the levels of 12 amino acids (Thr, Lys, p-Hyp, Asn, Gln, Ser, Gly, Glu, Ala, GABA, Orn, and Hcys-cys), compared with T0, and increased the levels of 11 (Cys-cys, Met, Trp, Phe, PEA, Arg, EA, AADA, Pro, AABA, and Cysta), while eight amino acids (His, Tyr, Val, Leu + Ile, Met-his, Asp, Cit, and BAIB) showed similar levels in the T2 and T0 pits. As regards T1, 20 amino acids (His, Thr, Lys, Tyr, Leu + Ile, Trp, Phe, Met-his, p-Hyp, Asn, Arg, EA, Gly, Asp, Cit, Ala, AADA, BAIB, AABA, and Hcys-cys) showed levels similar to those of T0 pits, whereas five (Cys-cys, Met, PEA, Pro, and Cysta) showed higher concentrations and six (Val, Gln, Ser, Glu, GABA, and Orn) lower concentrations, compared with T0 pits (Table 8).

It is important to emphasize that the free amino acids identified in the edible portion (peel and flesh) and pit (shell and seed) of jujube fruits (Table 6) showed some differences with respect to those identified in three Korean cultivars (Boeumdeachu, Mechu, and Sanzoin) by Choi *et al.* [4, 6] These authors identified cysteine (Cys) and hydroxylysine (Hyl), which were not found in the fruits of the Grande de Albatera cultivar. However, to the best of our knowledge, this is the first time that the following have been identified in *Z. jujuba* fruits: one essential amino acid (Cys-cys) and seven non-essential amino acids (p-Hyp, AADA, Orn, BAIB, AABA, Cysta, and HCys-Cys) (Table 6). The presence of four amino thiols (Cys-cys, Met, Cysta and Hcys-cys) in jujube fruits can also be considered a valuable aspect, because biothiols are the most important antioxidants that protect cells from oxidative damage [49]. Also, Zagorchev *et al.* [50] indicated that the data available so far suggest that thiols play a central role in the abiotic stress tolerance of plants.

The levels of total amino acids in the edible portion of T0 fruit (Table 7) were similar to those found in the fruit pulp of the Boeum-deachu and Mechu cultivars [6]. The total essential amino acid content in the T0 fruit edible portion (Table 7) was also similar to that found in the fruit pulp of cultivar Sanzo in [6]. However, the sums of the total and total essential amino acids in T0 pits (Table 7) were clearly lower than those found in the seeds of three Korean cultivars by Choi *et al.* [6], probably due to a dilution effect of the shell portion.

T a b l e 6

MS parameters of the free amino acids identified in jujube fruits under UHPLC-QqQ-MS/MS conditions by the use of AQC derivatization [25, 26]

ID	Amino acid	Parent ion (*m/z*)	Daughter ion (*m/z*)	Fragmentor	Collision energy	t_R (min)
1	His	326.2	171	100	0	3.53
2	Met-his	340.2	171	100	10	3.61
3	p-Hyp	302.1	171	120	0	3.64
4	Asn	303.1	171	100	0	3.99
5	PEA	312.4	171	80	0	4.00
6	Arg	345.2	171	100	0	4.16
7	Gln	317.2	171	110	0	4.43
8	Ser	276.1	171	80	0	4.47
9	EA	232.1	171	100	0	4.48
10	Gly	246.1	171	90	0	4.72
11	Asp	304.1	171	140	0	5.13
12	Cit	340.2	171	100	10	5.20
13	Glu	318.1	171	110	0	5.39
14	Thr	290.1	171	120	0	5.66
15	Ala	260.2	171	100	0	6.12
16	GABA	274.1	171	90	0	6.18
17	AADA	332.3	171	110	0	6.64
18	Pro	286.2	171	90	0	6.69
19	Orn	473.2	171	110	5	7.09
20	BAIB	274.1	171	80	0	7.12
21	AABA	274.1	171	110	0	7.26
22	Lys	487.2	171	90	5	7.34
23	Cys-cys	581.6	171	90	0	7.38
24	Cysta	563.3	171	100	0	7.54
25	Tyr	352.2	171	90	0	7.88
26	Val	288.2	171	110	0	7.99
27	Met	320.2	171	90	0	8.00
28	Hcys-cys	609.4	171	130	0	8.32
29	Leu + Ileu	302.2	171	100	0	8.65
30	Trp	375.2	171	120	0	8.68
31	Phe	336.2	171	100	0	8.69

Abbreviations used: Histidine (His), 1-methyl-hystidine (Met-His), 4-hydroxyproline (p-Hyp), asparagine (Asn), phosphoethanolamine (PEA), arginine (Arg), glutamine (Gln), serine (Ser), ethanolamine (EA), glycine (Gly), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), threonine (Thr), alanine (Ala), γ-amino-*n*-butyric acid (GABA), α-aminoadipic acid (AADA), proline (Pro), ornithine (Orn), β-aminoisobutyric acid (BAIB), α-amino-n-butyric acid (AABA), lysine (Lys), cystine (Cys-Cys), cysthationine (Cysta), tyrosine (Tyr), valine (Val), methionine (Met), homocystine (HCys-Cys), leucine (Leu), alloisoleucine (Ileu), tryptophan (Trp), phenylalanine (Phe).

Table 7

Effect of irrigation treatments (T0, T1, and T2) on the concentrations of free amino acids (g/kg DW) in the edible portion (peel and flesh) of jujube fruits

Amino acids	Treatment					
	T0	T1			T2	
Essential						
His	0.6±8.2E-02	a	0.4 ±4.8E-02	a	0.9 ±0.2	a
Thr	0.6 ±1.4E-02	a	0.5 ±4.0E-02	a	0.2 ±3.4E-02	b
Lys	0.1 ± 2.1E-03	a	0.1± 3.4 E-03	a	6.0E-02 ± 1.4 E-03	b
Cys-cys	8E-05 ±1E-05	b	1.2E-04 ±1.0E-05	a	8.0E-05 ±1.0E-05	b
Tyr	1.4 ±0.2	a	0.7 ±5.3E-02	b	0.5 ±1.0E-02	b
Val	0.3 ± 5E-03	a	0.2 ±1.3E-02	a	0.1 ±9.5E-03	b
Met	1.1E-03 ±1.0E-04	a	8.7E-04 ±4 E-05	ab	4.5E-04 ±3 E-05	b
Leu + Ile	0.1 ±4.6E-03	a	9.7E-02 ±5.0E-03	b	4.7E-02 ±5.2E-03	c
Trp	0.2 ±3.2E-02	b	0.2 ±1.1E-02	b	0.4 ±5.0E-02	a
Phe	5.1E-02 ±7.6E-03	a	2.4E-02 ±7.7E-04	b	1.8E-02 ±2.1E-03	b
Non-essential						
Met-his	6.0E-02 ± 5.9E-03	a	7.0E-02 ±2.0E-02	a	5.0E-02 ±5.2E-03	a
p-Hyp	2.8 ±6.8E-02	a	2.3 ±0.1	a	1.5 ±0.2	b
Asn	37.9 ±1.4	a	33.6 ±3.3	a	14.0 ±3.0	b
PEA	1.2E-02 ±7.5E-04	a	7.7E-03 ±8.8E-04	b	6.2E-03 ±4.0E-04	b
Arg	4.0 ±0.3	a	3.4 ±0.3	a	1.4 ±0.2	b
Gln	1.1 ±1.8E-02	a	0.9 ±6.2E-02	b	0.3 ±2.9E-02	c
Ser	7.1 ±0.2	a	6.3 ±0.6	a	2.5 ±0.1	b
EA	1.4 ± 2.6E-02	a	1.3 ±9.9E-03	a	1.0 ±2.3E-02	b
Gly	0.4 ±2.3E-02	a	0.4 ±1.8E-02	ab	0.2 ±3.4E-02	b
Asp	13.5 ±0.1	a	9.7 ±0.7	b	2.1 ±6.8E-02	c
Cit	5.8E-02 ±1.5E-03	a	3.8E-02 ±1.2 E-04	b	2.0E-02 ±1.9E-03	c
Glu	6.3 ±0.4	a	6.4 ±0.4	a	1.3 ±0.3	b
Ala	0.9 ±4.3E-02	a	0.8 ±4.6E-02	a	0.2±8.1E-02	b
GABA	1.4 ±6.3E-02	a	1.3 ±6.3E-02	ab	1.0 ±6.3E-02	b
AADA	6.4E-02 ±3E-03	a	3.8E-02 ±2.7E-03	b	4.7E-02 ±3.5E-03	b
Pro	17.8 ±2.3	b	22.6 ±2.4	ab	26.9 ±2.0	a
Orn	0.3 ±1.0E-02	a	7.8E-02 ±8.0E-4	b	6.2E-02 ±5.3E-04	b
BAIB	8.3E-02 ±1.4E-03	a	5.5E-02 ±2.6E-03	b	6.4E-02 ±5.6E-03	ab
AABA	0.4 ±3.2E-03	a	0.3 ±7E-04	b	0.2 ±3.4 E-02	b
Cysta	4.0E-03 ±2 E-04	b	5.9E-03 ±4.1 E-04	a	5.4E-03 ±1.3E-03	a
Hcys-cys	1.9E-04 ±2E-05	a	2.4E-04 ±2.0E-05	a	1.6E-04 ±4.0E-05	a
Total	99.3 ± 5.3	a	92.0 ± 7.5	a	55.2 ± 6.5	b
Total essential	3.4 ± 0.3	a	2.2 ± 0.1	b	2.2 ± 0.4	b

Means within a row followed by a different letter are significantly different according to Tukey's test ($P \leq 0.05$). Abbreviations as in Table 6.

Although the same amino acids were found in the fruit edible portion and pit, differences in the contribution of each free amino acid to the total free amino acids were evident (Tables 6–8). In the edible portion of T0 fruit, His, Thr, and Tyr constituted 77 % of the total essential amino acid content. However, in pits, which had much lower amino acid content than the edible portion, His, Thr, and Tyr constituted only 42 % of the total essential amino acid content. Moreover, considering both essential and non-essential amino acids, Asn, Asp, Glu, and Pro constituted 76 % of total amino acids in the T0 fruit edible portion but only 59 % in pits (Tables 7 and 8).

Amino acids are the main transport forms of N in plants [51] and the irrigation treatments pro-

duced similar changes in the total N and total free amino acid contents in the edible part of the fruit (Tables 5 and 7). This suggests that the decrease in total free amino acids in T2 fruits could have been due to a decrease in the transport of amino acids into fruits under this severe water deficit [52]. Alternatively, or as a complementary possibility, the decrease in total free amino acids in T2 fruits could have been caused by the partial inactivation of amino acid biosynthesis pathways in the fruit [52].

In addition to the fact that our results indicate high amounts of free amino acids in jujube fruits, it is important to emphasize the relatively high Asn content found in the edible portion of the

T a b l e 8

Effect of irrigation treatments (T0, T1, and T2) on the concentrations of free amino acids (g/kg DW) in the pits (shell + seed) of jujube fruits

Amino acids	Treatment					
	T0		T1		T2	
Essential						
His	0.2 ± 7.2E-02	a	0.3 ± 3.0E-02	a	0.3 ± 2.7E-02	a
Thr	0.2 ± 6.5E-04	a	0.2 ± 9.1E-03	a	0.1 ± 5.6E-03	b
Lys	6.4E-02 ± 3.9E-03	a	6.5E-02 ± 3.1E-03	a	5.4E-02 ± 3.3E-04	b
Cys-cys	1.7E-04 ± 3.3E-06	b	2.0E-04 ± 2.0E-06	a	2.0E-04 ± 3.6E-06	a
Tyr	0.1 ± 7.2E-03	a	0.1 ± 1.2E-02	a	0.1 ± 1.4E-02	a
Val	6.3E-02 ± 2.1E-03	a	4.6E-02 ± 6.3E-04	b	6.6E-02 ± 3.3E-03	a
Met	3.4E-04 ± 1.1E-05	b	6.6E-04 ± 4.6E-05	a	6.6E-04 ± 8.4E-05	a
Leu + Ile	2.1E-02 ± 7.5E-04	a	2.1E-02 ± 1.8E-04	a	2.2E-02 ± 8.6E-04	a
Trp	0.5 ± 5.2E-02	b	0.4 ± 1.9E-02	b	0.6 ± 3.2E-02	a
Phe	6.7E-03 ± 6.6E-05	b	6.5E-03 ± 5.8E-05	b	8.2E-03 ± 1.1E-03	a
Non-essential						
Met-his	1.1E-02 ± 4.8E-04	ab	9.0E-03 ± 8.7E-04	b	1.2E-02 ± 7.5E-04	a
p-Hyp	1.1 ± 4.6E-02	a	1.1 ± 7.1E-02	a	0.7 ± 2.9E-02	b
Asn	6.5 ± 0.3	a	6.4 ± 0.9	a	3.9 ± 4.9E-02	b
PEA	8.8E-04 ± 2.9E-05	b	1.3E-03 ± 1.2E-04	a	1.8E-03 ± 9.4E-05	a
Arg	4.6 ± 4.1E-01	b	4.5± 2.6E-01	b	5.6 ± 0.3	a
Gln	0.3 ± 4.2E-03	a	0.2 ± 1.1E-02	b	0.2 ± 9.9E-04	b
Ser	1.0 ± 4.4E-02	a	0.7 ± 1.5E-02	b	0.5 ± 1.3E-02	c
EA	0.1 ± 6.0E-03	b	0.1 ± 1.9E-03	b	0.2 ± 1.9E-03	a
Gly	7.6E-02 ± 2.6E-03	a	8.1E-02 ± 3.7E-03	a	6.4E-02 ± 4.2E-03	b
Asp	1.7 ± 8.3E-02	a	1.5± 1.5E-01	a	1.6 ± 5.5E-02	a
Cit	1.3E-02 ± 1.3E-03	a	1.2E-02 ± 4.7E-04	a	1.3E-02 ± 1.4E-03	a
Glu	1.4 ± 4.2E-02	a	1.2 ± 6.8E-02	b	1.0 ± 0.2	b
Ala	0.4 ± 7.4E-03	a	0.4 ± 8.1E-03	a	0.3 ± 1.2E-02	b
GABA	0.3 ± 1.3E-02	a	0.2 ± 6.1E-03	b	0.2 ± 9.9E-03	b
AADA	1.2E-02 ± 1.9E-03	b	1.4E-02 ± 1.2E-03	ab	1.9E-02 ± 4.2E-04	a
Pro	3.1 ± 0.2	b	5.1 ± 0.8	a	6.7 ± 0.7	a
Orn	3.6E-02 ± 2.1E-03	a	2.7E-02 ± 1.4E-03	b	2.5E-02 ± 7.6E-04	b
BAIB	1.8E-03 ± 1.2E-04	ab	2.3E-03 ± 1.7E-04	a	1.5E-03 ± 2.2E-05	b
AABA	1.8E-03 ± 9.7E-05	b	2.0E-03 ± 2.2E-04	ab	1.0E-02 ± 6.4E-03	a
Cysta	5.4E-04 ± 3.5E-05	b	1.7E-03 ± 2.2E-04	a	2.0E-03 ± 2.1E-04	a
Hcys-cys	3.2E-04 ± 1.4E-05	a	3.1E-04 ± 7.8E-06	a	1.8E-04 ± 4.0E-06	b
Total	21.6 ± 1.3	a	22.7 ± 2.3	a	22.3 ± 1.5	a
Total essential	1.2± 7.4E-02	a	1.1 ± 7.5E-02	a	1.3 ± 8.4E-02	a

Means within a row followed by a different letter are significantly different according to Tukey's test ($P \leq 0.05$). Abbreviations as in Table 6.

fruit because Asn serves as a precursor of the potentially toxic acrylamide, which may be formed during the heat-processing of plant foods [15]. Recently, Choi *et al*. [4] showed that the Asn content of jujube fruit pulp decreased during the last stages of fruit growth, from 14.7 to 9.2 g/kg DW; representing 54.9 and 31.9 %, respectively, of the total free amino acid content. In this sense, at harvest we found slightly higher Asn levels in the edible portion of the Grande de Albatera T0 fruits (37.9 g/kg DW) (Table 7), which contributed 38.2 % to the total free amino acid content. Despite the fact that most of the amino acids in both the edible portion and pits of jujube fruits showed a tendency to decrease as a result of water deficit, the decrease in the Asn content from 37.9 (T0) to 14.0 g/kg DW (T2) in the fruit edible portion and from 6.5 (T0) to 3.9 g/kg DW (T2) in the pits must be considered advantageous. In other words, severe water deficit during fruit maturation can be considered as a helpful tool to decrease the fruit Asn content, hence reducing the risk of formation of the potentially toxic acrylamide.

A common response to water deficit is the accumulation in plant tissues of some amino acids, mainly Pro (Tables 7 and 8), which may be used to achieve osmotic adjustment [53]. In this study, Pro was the most abundant amino acid in both the edible portion and pit. Indeed, in the T0 fruit edible portion Pro contributed 18 % to the total free amino acid content, whereas in T1 and T2 fruits this contribution was 25 and 49 %, respectively. Likewise, in the T0 pits Pro contributed 14 % to the total free amino acid content, whereas in T1 and T2 fruits this contribution was 23 and 30 %, respectively (Tables 7 and 8). These increases suggest that Pro could contribute to osmotic adjustment in water stressed jujube fruits. The role of Pro may not be confined to osmotic adjustment, because it also helps the cell to overcome water stress-induced oxidative stress [52, 53]. Whatever the case, it is important to indicate that the response of Pro in jujube fruits to water deficit was not as sensitive as expected, because it is well known that Pro levels in leaves increase in response to drought in direct proportion to the magnitude of the water deficit [54, 55].

4. CONCLUSIONS

In contrast to previous findings, a major conclusion of our study is that, under our experimental conditions, jujube fruit maturation was sensitive to water deficit, because the yield and fruit size decreased and most of the chemical characteristics of the fruit were modified. To the best of our knowledge, this is the first time that one essential amino acid (Cys-cys) and seven non-essential amino acids (p-Hyp, AADA, Orn, BAIB, AABA, Cysta and HCys-Cys) have been identified in *Z. jujuba* fruits. The presence of four amino thiols (Cys-cys, Met, Cysta and Hcys-cys) underlines the functional character of these fruits.

Certain proportionality was observed in the response of jujube fruits to both deficit irrigation treatments. Fruits from plants exposed to moderate water deficit (T1) showed no change in fruit size, moisture content, firmness, or fruit peel and flesh color, but a decrease in fruit yield was accompanied by changes in ethylene emission, respiratory rate, and some fruit chemical characteristics, such as increases in the sucrose and arabinose contents, although no changes were found for most of the bioelements. This behavior indicates that T1 fruits entered the ripening phase earlier than control fruits and that moderate water deficit during fruit maturation improves jujube fruit eating quality because the fruits taste sweeter. Fruits from the more pronounced water deficit treatment (T2) showed decreased growth, resulting in smaller fruit size, lower moisture content and yield, and changes in firmness and peel and flesh color. Furthermore, changes in ethylene emission and the respiratory rate, likewise changes in chemical characteristics (increases of sugars and vitamin C and changes in organic acids), indicate that T2 fruits achieved a higher degree of ripening than T0 and T1 fruits, improving not only most of the fruit chemical characteristics that make up the taste but also the nutritional value.

The response of fruit amino acids to water deficit was not as sensitive as expected, since it did not show a direct relationship with the magnitude of the deficit. These results together with others, such as the decrease in fruit Asn content due to severe water deficit, may be the key to jujube fruit cultivation in arid and semiarid zones, due to a lower risk of acrylamide formation during fruit heat-processing.

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Effect of Water Deficit and Domestic Storage on the Procyanidin Profile, Size, and Aggregation Process in Pear-Jujube (*Z. jujuba*) Fruits

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ABSTRACT: No information exists on the proanthocyanidin content of pear-jujube (*Ziziphus jujuba* Mill) fruit, their polymeric types and sizes, and their self-aggregation, or on the effect of different water deficit levels during the fruit maturation period on these compounds. Two trimers, two tetramers, and six B type procyanidin pentamers were identified and quantified for the first time. Water deficit increased the content of procyanidins of low molecular mass, improving their potential bioavailability and possible physiological effects on human health. The tendency of procyanidins to self-aggregate was similar in the edible portion and pit, and was not affected by water deficit. The procyanidin content of fruit from well watered trees increased during domestic cold storage, whereas the fruits from trees suffering severe water stress lost some of their procyanidin content.

KEYWORDS: *deficit irrigation, plant water relations, water deficit, procyanidins, LC-MS/MS*

INTRODUCTION

Pear-jujube (*Ziziphus jujuba* Mill), which belongs to the plant family Rhamnaceae, is a very interesting crop because it is able to withstand severe water deficits, while maintaining leaf turgor, which allows good gas exchange levels and, as a consequence, good leaf productivity.[1] Such leaf turgor maintenance is mainly due to two simultaneous and complementary mechanisms: stomata regulation and active osmotic adjustment. Cui et al.[2] indicated that the fruit maturation stage is the optimal stage for implementing water deficit in pear-jujube, because low, moderate, and severe water deficits have no effect on the fruit weight and volume, the fruits taste sweeter, and eating quality is improved. In addition, the fruit maturation period is shortened, increasing the market value of the fruit, while fruit firmness is enhanced and the percentage of rotten fruit after storage is reduced.

Jujubes are considered to be minor fruits and, from a research and development point of view, have not received any major emphasis. However, the fruits are an integral part of the culture and way of life of millions of diverse Asian peoples and have also become so for large regions of Africa after the major cultivated species were introduced. [3] A key characteristic of pear-jujube fruit is its health-promoting effects, and it is considered a "functional food", since it has nutritional as well as health-beneficial uses reported by *in vitro*, *in vivo*, and nutritional trials with humans.[4−11]

The presence of flavonoids may help explain the antioxidant, antitumor, antiproliferative, anti-inflammatory, and proapoptotic activities of *Z. jujuba* fruits, as well as its protective effect against cardiovascular diseases and type II diabetes.[12−16] In addition, the presence of phenolic acids provides potent antioxidant and anti-inflammatory effects, as well as a protective effect against the oxidative damage associated with diseases such as coronary heart disease, stroke, and cancers.[17−21]

Proanthocyanidins are of great interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health.[22] In this sense, Carnésecchi et al.[23] were the first to describe the antiproliferative effect of procyanidins on human cancer cells. Mao et al.[24] and Saito et al.[25] indicated that the antiproliferative and antitumoral properties of flavanols and procyanidins are presumably related to their degree of polymerization. However, to the best of our knowledge, no information exists on the proanthocyanidin contents, their polymeric types and sizes, and their self-aggregation in *Z. jujuba* fruit.

Flavonoids and phenolic acids have been detected in cultivars of *Z. jujuba*. Hudina et al.[26] and Bekir San et al.[27] reported the following quantitative amounts (mg × 100 g^{-1} dry weight) of phenolics in *Z. jujuba*: rutin (0.89−5.87), (+)-catechin (0.74−1.37), (−)-epicatechin (0.48−5.13), chlorogenic acid (0.22−0.95), caffeic acid (0.09−0.37), ferulic acid (0.22), and *p*-hydroxybenzoic acid (0.08−0.18). Moreover, Choi et al.[10] indicated the presence (mg × 100 g^{-1} dry weight) of procyanidin B2 (14−30.7), epicatechin (258.8−352.), querce-

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tin-3-*O*-rutinoside (295.5−1147), quercetin-3-*O*-galactoside (1.4−15.7), kaempferol-glucosyl-rhamnoside (18.7−32.6) in pulp, and saponarin (133.0−170.4), spinosin (1303.8−2237.7), vitexin (44.7−134.0), swertish (10.9−14.8), 6″-hydroxybenzoylspinosin (54.0−84.6), 6″-feruloylspinosin (1237.3−1242.9) in seeds. Of note is the fact that phenolic compounds in the skin were 5−6 times higher than in the pulp.[9]

According to Mithofer et al.[28] the storage of indigenous fruits, such as those of *Ziziphus* genus, is one of the main strategies adopted by rural communities in Africa to reduce hunger, improve nutrition, and generate income. Although *Z. jujuba* fruit senesces rapidly at ambient temperature after harvest,[29] Tembo et al.[30] reported that the proportion of shriveled *Z. mauritiana* fruits increased as the storage temperature rose from 5 to 20 °C, and that fruits stored at low temperature (5 °C) had a very low proportion of shriveled fruits after 12 weeks of storage.

Information on the effect of plant water status on secondary metabolite contents is scarce, and many results could be considered contradictory, probably because of the fact that in most fruits it is not possible establish a linear correlation between these parameters.[31,32] Castellarin et al.[33] showed that water deficit has limited effects on proanthocyanidins in grape berries, and Tovar et al.[34] indicated that polyphenol contents of olive flesh increased as irrigation decreased. A decrease in phenolics during fruit development seems to be a general trend.[35,36] Conversely, there is some divergence with reference to changes in phenolics during fruit maturation and storage. In this sense, catechin and epicatechin are reduced during fruit storage at 25 °C, particularly at high relative humidity (75%).[37] Moreover, some authors indicated that the concentration of catechins has a tendency to decrease during fruit cold storage.[36,38] In contrast, Burda et al.[39] showed that procyanidins remained near constant from the maturation period to the end of cold storage.

The aim of this paper was to evaluate the profile of proanthocyanidin and their self-aggregation in pear-jujube fruits and to study the effect of different water deficit levels during fruit maturation on the proanthocyanidin content. In addition, we studied the changes in the proanthocyanidin content of the fruit after domestic cold storage for three months.

MATERIALS AND METHODS

Experimental Conditions, Plant Material, and Treatments. The experiment was carried out in 2011, at a farm near the city of Albatera (Alicante, Spain) (38° 12′ N, 0° 51′ W), planted with 7 year-old pear-jujube trees (*Zizyphus jujuba* Mill.) of the autochthonous cultivar "Grande de Albatera" at 2 m × 6 m. The soil of the orchard is a Torrifluvent with sandy loam texture, very low electrical conductivity (109 μS/cm, 1:10 w:v), high lime content (57%), very low organic matter content (0.3%), low exchangeable potassium (40 mg/kg), and low available phosphorus (20 mg/kg) levels. The irrigation water had an electrical conductivity of between 1.7 and 2.2 dS/m and a Cl^- concentration ranging from 36 to 48 mg L^{-1}.

The climate of the area is strictly Mediterranean, with mild winters, low annual rainfall, and hot dry summers. During the experimental period, average daily maximum and minimum air temperatures were 32 and 22 °C, respectively, while mean daily air vapor pressure deficit (VPD_m)[40] ranged from 1.25 to 3.25 kPa, and reference crop evapotranspiration (ETo)[40] was 189 mm. Total rainfall was negligible (1.8 mm on day of the year (DOY) 221).

Three irrigation treatments were considered, in which irrigation was carried out daily and during the night using a drip irrigation system with one lateral pipe per tree row. Control plants (treatment T0) were irrigated in order to ensure nonlimiting soil water conditions (112% ETo), and T1 plants were irrigated according to the normal criteria used by the grower (64% ETo). T2 plants were irrigated as T0, but irrigation was withheld for 36 days (from DOY 202 to 238). Total water amounts applied during the measurement period were 213 and 122 mm for T0 and T1 treatments, respectively.

Pear-jujube fruits were harvested when T0 fruits reached commercial ripening state (S7 stage of growth, according to Choi et al.[4]) and immediately transported to the laboratory on 27 August (DOY 239). Harvested fruits of each replicate were divided into two groups. Fruits from one of these groups were divided into edible portion (peel and pulp) and pit (shell and seed) and directly frozen at −20 °C until analysis. The fruits of the other group were stored at 5 °C and 65% relative humidity for 12 weeks. All samples were freeze-dried before analyzing the proanthocyanidins and their self-aggregation by LC-MS/MS and light scattering, respectively.

Plant Water Status. Predawn (Ψ_{pd}) leaf water potential was measured in mature leaves located on the south facing side, from the middle third of the tree (two leaves per tree and four trees per treatment). Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in a similar number and type of leaves as used for Ψ_{pd}, enclosing leaves in a small black plastic bag covered with aluminum foil for at least 2 h before measurements in the pressure chamber (model 3005, Soil Moisture Equipment Co., Santa Barbara, CA).[41−43]

Midday gas exchange in attached leaves, leaf conductance (g_{lmd}), and net photosynthesis (P_{nmd}) were measured with a steady-state porometer (LI-1600, LI-COR Inc., Lincoln, NE) on the abaxial surface of the leaves in a similar type of leaf as used for the Ψ_l measurements. Two measurements were taken on four trees per treatment.

Reagents and Standards. Epigallocatechin, which was used as standard, was purchased from Phytoplan (Heidelberg, Germany). Acetonitrile and methanol, both of LC-MS grade, and acetone of HPLC grade were obtained from Panreac Química S.A. (Barcelona, Spain), and acetic acid of LC-MS grade was from Scharlau (Sentmenant, Spain).

Extraction of Proanthocyanidins. Proanthocyanidins were extracted as described by Buendía et al.[44] with some modifications. Briefly, 1.6 g of each freeze-dried and powdered portion was weighed and homogenized with 0.025 L of extraction solution (acetone/water/acetic acid; 70/29.5/0.5) by using an ultraturrax (Ika, Staufen, Germany) for 1 min. The samples were kept on ice before and during homogenization. The homogenates were then sonicated in an ultrasound bath for 15 min followed by centrifugation (JP Selected Centronic Centrifuge, Barcelona, Spain) for 10 min at 1765 *g* (3200 rpm) at room temperature. Supernatants were concentrated in a rotary evaporator at 35 °C, and the aqueous residue was filtered through a C18 Sep-Pak cartridge (Waters Associates, Milford, MA), previously activated with 0.010 L of methanol, water, and air sequentially. Retained phenolic compounds were eluted with 0.008 L of methanol. The methanol was evaporated in a rotary evaporator at 35 °C, the residue was dissolved in 0.001 L of acetonitrile/acetic acid (2%), which was filtered through a 0.22 μm PDVF filter (Millex HV13, Millipore, Bedford, MA), and 3 μL of the solution was directly injected into LC-MS/MS for identification and quantification of flavan-3-ols compounds.

Normal Phase LC-MS/MS. The analysis of proanthocyanidins was performed by normal phase analysis, as previously reported.[44] Chromatographic separation was carried out on a Develosil 100 Å normal phase column (250 mm × 0.5 mm, 5 μm particle size) (Phenomenex, Seto, Japan). Two types of eluents were used to separate the gradients: a mixture of acetonitrile−acetic acid (98/2 v/v) as solvent A and a mixture of methanol−water−acetic acid (95/3/2 v/v/v) as solvent B. The injection volume was 3 μL, and elution was performed at a flow rate of 10 μL min^{-1}. The linear gradient started with 0% B, reaching 40% B at 40 min and 80% at 50 min, and keeping isocratic conditions for 2 min, reaching 0% B at 55 min and finally 0% B at 70 min. Identification of the compounds was made in a 1200 series micro-HPLC-DAD system (Agilent Technologies, Waldbronn, Germany) equipped with a degasser (model G1379B), a thermostatted autosampler (model G1377A), a capillary pump (model G1376A), and photodiode array detector (model G1315D). HPLC

was coupled to an ion trap mass spectrometer (ultra HCT Bruker, Bremen, Germany) equipped with electrospray ionization (ESI) and operated in negative ion mode. Data acquisition and processing were performed using software B.01.03-SR2 [204] for ChemStation for LC 3D system from Agilent Technologies. The capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and daughter (MS-MS) spectra were measured from m/z 100 to 1500. Collision-induced fragmentation experiments were realized in the ion trap using helium as the collision gas, with the collision energy set at 50%.

The different compounds were identified and quantified by their UV spectra, which were recorded at 280 nm, and their molecular mass and daughter ions acquired in the negative mode on the mass spectrometer.

The calibration curve was made using 4, 8, 15.6, 31.3, 62.5, 125, 250, and 500 μM of (−)-epigallocatechin standard solutions.

Dynamic Light Scattering. Self-aggregation of proanthocyanidins was analyzed using the dynamic light scattering technique (DLS).[45,46] After the proanthocyanidins had been extracted as described above, to ensure that no other insoluble components are present, and after dissolving the residue in acetonitrile/acetic acid (2% v/v), the samples were filtered through a 0.22 μm PDVF filter (Millex HV13, Millipore, Bedford, MA) to observe the degree of self-aggregation. After standing for a minimum of 24 h, the samples were filtered again through the same type of filter to remove macroaggregates. In some cases, these compounds were observed after the second filtration. These samples were allowed to stand for a new period of 24 h before a third filtration. In all cases, after the final filtration, DLS measurements were performed immediately (within 90 s).

DLS measurements were made in a Zetasizer Nano-ZS (Malvern Instruments, Malvern, U.K.) with a laser wavelength of 633 nm and a quartz cell of minimum volume ZEN2112. This system uses backscatter detections at 175°, and all measurements were performed at 293 K. The time-dependence of the scattered light was monitored, and the autocorrelation function of the particles was measured. The cumulant method was used to fit the autocorrelation curves. This method provides the z-averaged hydrodynamic radius, which is a measure of the average size of the aggregates. The results shown in this work are those of the first of 10 consecutive runs involving between 13 and 15 subruns each (a number chosen in the automatic mode of the apparatus). Final results are the average of 4 different experiments from 4 different samples.

Statistical Design and Analysis. The design of the experiment was randomized with four replications, each replication consisting of three adjacent tree rows, each with 11 trees. Physiological measurements were taken on the inner tree of the central row of each replicate, which were healthy, uniform, and very similar in appearance, while the other trees served as border trees. Data were processed using SPSS software version 19 for Windows (2010; SPSS Inc., Chicago). Two-way analysis of variance was carried out, and mean values were compared by Tukey's multiple range test. All means were compared at the 0.05 level of significance. Values for each replicate were averaged before the mean and the standard deviation of each treatment were calculated.

RESULTS

Plant Water Status. Midday leaf conductance (g_{lmd}) and net photosynthesis (P_{nmd}) values in T0 were high and nearly constant throughout the measurement period (Figure 1A,B). The g_{lmd} values of T1 plants were also almost constant and intermediate between T0 and T2 values. However, g_{lmd} and P_{nmd} values in T2 plants gradually decreased during the stress period, reaching minimum values of 111.00 mmol m^{-2} s^{-1} and 2.57 μmol m^{-2} s^{-1}, respectively, on DOY 238.

Ψ_{stem} values in T0 plants remained constant during the experimental period, whereas Ψ_{stem} values in T1 and T2 plants showed a tendency to gradually decrease, reaching minimum values of −2.28 and −3.14 MPa, respectively, on DOY 238

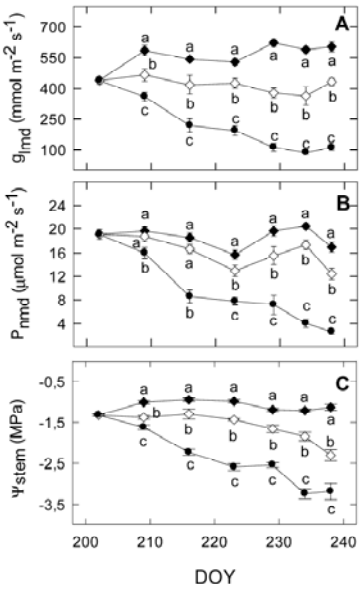

Figure 1. Midday leaf conductance (g_{lmd}, A), midday net photosynthesis (P_{nmd}, B), and midday stem water potential (Ψ_{stem}, C) values (mean ± SE, not shown when smaller than symbols, n = 4) for pear-jujube plants in T0 (◆), T1 (◇), and T2 (●) treatments during the experimental period (DOY, day of the year). Different letters on data points at each date indicate significant differences according to Tukey's test ($P \leq 0.05$).

(Figure 1C). Significant differences between treatments from DOY 209 to 238 were noted in Ψ_{stem} values, with the particular characteristic that T1 plants showed lower values than T0 plants and higher values than T2 plants (Figure 1C).

Effect of Water Deficit on the Proanthocyanidin Content, Their Self-Aggregation, and Size. The proanthocyanidin profile of pear-jujube fruits is shown in Figures 2 and 3 and Table 1, which illustrates the presence of one monomer of (epi)catechin and oligomers of this compound as a monomeric unit, which are known as procyanidins. Moreover, all of these procyanidins were type B because they contain only the single interflavan linkages (Figure 3). Procyanidins were tentatively identified on the basis of their mass spectra considering their mass (m/z 289, 577, 865, 1151, 1153, and 1441), their most characteristic fragmentations, and their elution order as previously described in Table 1.

Pear-jujube pits showed some essential differences in the proanthocyanidins content compared with observations made in the edible portion (Tables 2 and 3). These differences were due to the fact that the pentamers corresponding to peaks **9**–**12** were not detected in pits at harvest time.

The total procyanidins content in the edible portion of the fruits increased as a result of water deficit effect, although the differences between T1 and T2 contents were not significant (Table 2). However, the behavior observed in each procyanidin was not similar. In this sense, the (epi)catechin, the dimer (peak **2** in Figure 2), one trimer (peak **4** in Figure 2), and three pentamers (peaks **9** and **10** in Figure 2) showed a response to water deficit similar to that observed considering the total

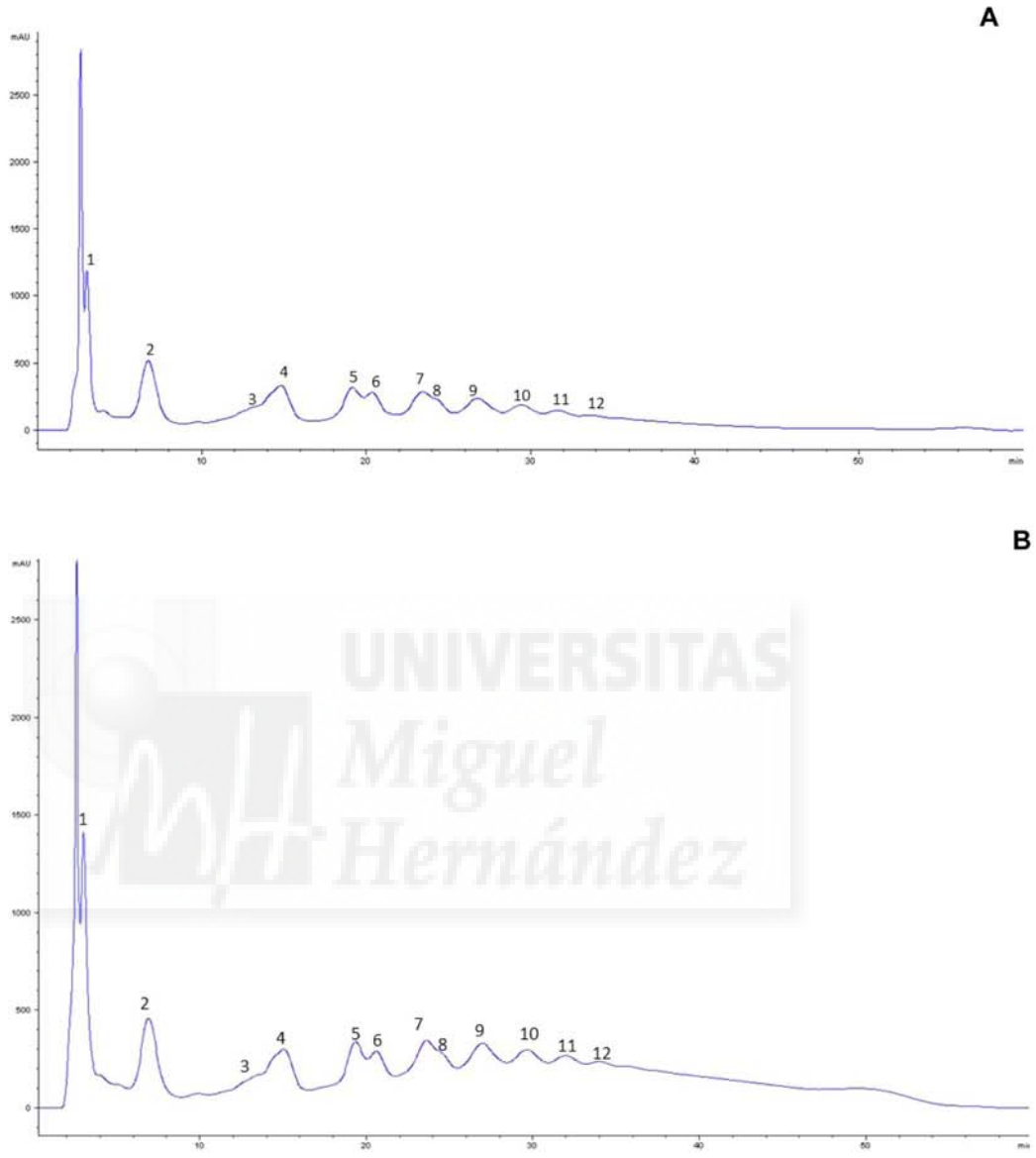

Figure 2. HPLC-DAD chromatogram (obtained at 280 nm) of edible portion (A) and pit (B) proanthocyanidins in pear-jujube fruits. Peak numbering as in Table 1.

proanthocyanidin contents, while the other proanthocyanidins did not change following water stress effect (Table 2).

The total procyanidin content in pits showed minimum values in T0 plants and maximum values in T2 plants. The behavior of each proanthocyanidins was not uniform (Table 3). In this respect, the dimer (peak **2** in Figure 2), the trimers (peaks **3** and **4** in Figure 2), and one tetramer (peak **6** in Figure 2) were more abundant under severe water stress (T2) conditions. The (epi)catechin and one pentamer (peak **8** in Figure 2) showed a progressive response to water deficit, the content in T2 pits being the highest and the contents in T1 intermediate between T0 and T2. An average behavior in response to water deficit was observed in one tetramer (peak **5** in Figure 2) and one pentamer (peak **7** in Figure 2), which increased in pits from T1 and T2 plants, although the differences between these treatments were not significant (Table 3).

Proanthocyanidin self-aggregates were considerably larger than oligomers, varying from 130 to 328 nm (Table 4). Self-aggregates in T1 and T2 fruit peel were larger than those from pit and flesh, respectively. Moreover, the effect of irrigation treatments on the self-agregate size from the different fruit portions was not significant (Table 4).

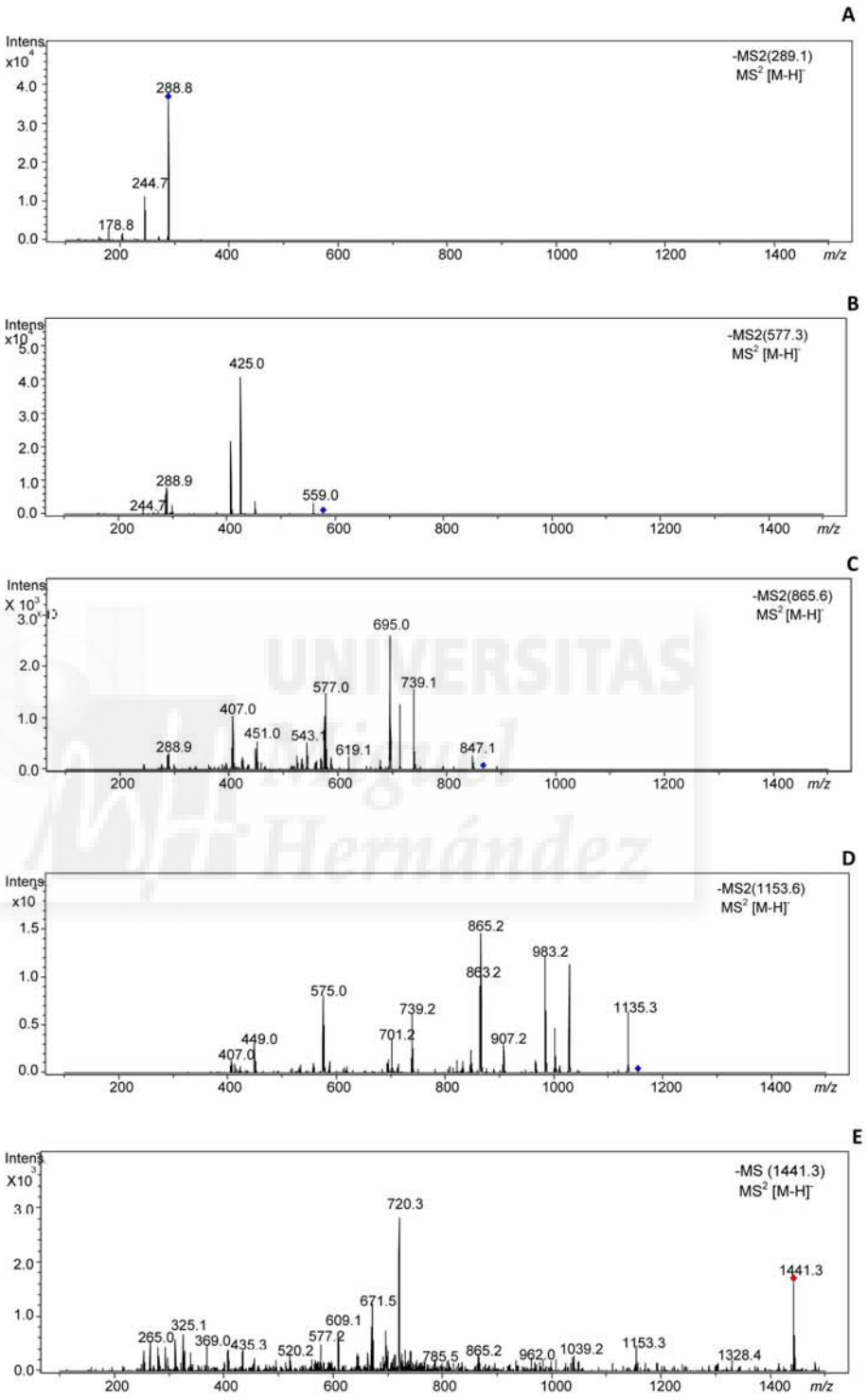

Figure 3. HPLC-MS-MS spectra in negative ionization mode of proanthocyanidins in edible portion of pear-jujube fruit: (epi)catechin (A), dimer B type (B), trimer B type (C), tetramer B type (D), and pentamer (E) (peaks 1, 2, 3, 5, and 9, respectively, in Figure 2).

Table 1. Tentative Proanthocyanidin Oligomer Compound Identification in Pear-Jujube Fruits Identified by LC-MS/MS[a]

ID	proanthocyanidin	$[M-H]^-$	product ions (m/z)	t_R (min)	refs
1	(epi)catechin	289	245, 179	2.9	Buendia et al., (2010); Vallejo et al., (2012); Gu et al., (2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
2	dimer B type [(E)C–B–(E)C]	577	425, 407, 289	6.7	Buendia et al.,(2010); Vallejo et al., (2012); Gu et al.,(2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
3	trimer B type [(E)C–B–(E)C–B–(E)C]	865	739, 695, 577, 575, 451, 407, 289	11.3	Buendia et al.,(2010); Vallejo et al., (2012); Gu et al.,(2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
4	trimer B type [(E)C–B–(E)C–B–(E)C]	865	739, 695, 577, 575, 451, 425, 407, 287	13.3	Buendia et al.,(2010); Vallejo et al., (2012); Gu et al., (2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
5	tetramer B type [(E)C–B–(E)C– B–(E)C–B–(E)C]	1153	1135, 1027, 865, 863, 739, 575, 449, 407	19.3	Gu et al., (2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
6	tetramer B type [(E)C–B–(E)C– B–(E)C–B–(E)C]	1153	1137, 865, 695, 577, 476, 407	20.6	Gu et al., (2003)(1); Gu et al., (2003)(2); Sarnoski et al., (2012)
7	pentamer B type [(E)C–B–(E)C– B–(E)C–B–(E)C–B–(E)C]	1441	1152, 983, 865, 739, 577, 407	23.4	Gu et al., (2003)(1); Gu et al., (2003)(2)
8	pentamer B type [(E)C–B–(E)C– B–(E)C–B–(E)C–B–(E)C]	1441	1152, 865, 577, 407	24.6	Gu et al., (2003)(1); Gu et al., (2003)(2)
9	pentamer B type [(E)C–B–(E)C– B–(E)C–B–(E)C–B–(E)C]	1441	1153, 865, 720, 695, 577	26.8	Gu et al., (2003)(1); Gu et al., (2003)(2)
10	pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	1441	1153, 1135, 863, 575, 407, 285	29.5	Gu et al., (2003)(1); Gu et al., (2003)(2)
11	pentamer B type [(E)C–B–(E)C– B–(E)C–B–(E)C–B–(E)C]	1441	1153, 1135, 863, 577	31.7	Gu et al, (2003)(1); Gu et al, (2003)(2)
12	pentamer B type [(E)C–B–(E)C– B–(E)C–B–(E)C–B–(E)C]	1441	1153, 1135, 863, 577	33.8	Gu et al., (2003)(1); Gu et al., (2003)(2)

[a]Abbreviations used: identification (ID), molecular ion ($[M-H]^-$), retention time (t_R), (epi)catechin ((E)C), B type linkage (B). Peaks 3–12 have been described for the first time in pear-jujube fruit. References indicate previous MS^n spectra of procyanidins described in other fruits or fruit products coincident with those found in pear-jujube fruit.

Effect of Domestic Cold Storage in Normal and Water Stressed Fruits. The total proanthocyanidin content of the edible portion of the fruits increased with cold storage in T0 fruits and decreased in T2 fruits, while the storage effect in T1 fruits was not significant (Table 2). The behavior observed for each proanthocyanidin in T0 fruits reflected a significant increase as a result of cold storage, except (epi)catechin, whose content remained constant during storage (Table 2). The content of each proanthocyanidin in T1 fruit tended to remain constant during storage except the (epi)catechin (peak **1** in Figure 2) and the dimer (peak **2** in Figure 2), which decreased, and one pentamer (peak **11** in Figure 2), which increased. Although the total proanthocyanidin content decreased significantly during storage, the observed behavior of each proanthocyanidin differed. The (epi)catechin (peak **1** in Figure 2), the dimer (peak **2** in Figure 2), one trimer (peak **4** in Figure 2), one tetramer (peak **5** in Figure 2), and one pentamer (peak **12** in Figure 2) contents decreased; the content of the others proanthocyanidins did not change during storage (Table 2).

The storage effect on the total proanthocyanidin content in fruit pits from the three irrigation treatments was similar to that observed in the edible portion (Tables 2 and 3). In T0 fruit pits the content of each proanthocyanidin increased with storage except two pentamers (peaks **7** and **8** in Figure 2), which decreased. In contrast, the behavior of each proanthocyanidin contents in fruit pits from T1 and T2 was not so uniform. In T1 pits, the (epi)catechin (peak **1** in Figure 2), the dimer (peaks **2** in Figure 2), the trimers (peaks **3** and **4** in Figure 2), and the tetramers (peaks **5** and **6** in Figure 2) did not change, whereas two pentamers (peaks **7** and **8** in Figure 2) decreased and four pentamers (peaks **9**–**12** in Figure.2) increased (Table 3). In T2 pits, the contents of the dimer (peak **2** in Figure 2), one trimer (peak **4** in Figure 2), and two pentamers (peaks **11** and **12** in Figure 2) were constant during storage. However, the (epi)catechin, (peak **1** in Figure 2), one trimer (peak **3** in Figure 2), one tetramer (peak **5** in Figure 2), and two pentamers (peaks **9** and **10** in Figure 2) increased. Also, one tetramer (peak **6** in Figure 2) and two pentamers (peaks **7** and **8** in Figure 2) did not change, but showed a similar behavior to that observed for the total proanthocyanidins content (Table 3).

DISCUSSION

The fact that Ψ_{stem}, g_{lmd}, and P_{nmd} values in T0 plants were very high and almost constant during the measurement period (Figure 1) suggested that the irrigation applied to this treatment was sufficient to avoid any water deficit during the measurement period. The differences in Ψ_{stem}, g_{lmd}, and P_{nmd} values between T0, T1, and T2 plants clearly indicated a water deficit situation in T1 and T2 plants. However, the fact that at maximum stress Ψ_{stem} values in T2 plants were very low (−3.14 MPa) and that a strong degree of stomatal regulation was observed in the plants of this treatment (Figure 1A and B) indicated that T2 represented a severe water deficit situation. The decrease in Ψ_{stem} values resulting from the T1 deficit irrigation treatment led to low Ψ_{stem} values (−2.28 MPa). However, g_{lmd} and P_{nmd} values, despite being lower than those in T0 (Figure 1A,B), were still very high and nearly constant, indicating that water deficit in T1 can be considered as moderate.

The fact that the proanthocyanidins in pear-jujube fruits consisted exclusively of B type procyanidins, is in agreement with Gu et al.[47] who also indicated that fruits are the major source of these compounds in the diet. Previously, the presence of (+)catechin, procyanidin B2, and (epi)catechin has been demonstrated in in pear-jujube fruits.[10,26,27] However, the

Table 2. Effect of Irrigation Treatments (T0, T1, and T2) at Different Times (0, at Harvest; 1, after 12 Weeks at Domestic Cold Storage) on the Proanthocyanidin Oligomeric Species Content (mg/kg DW) in the Edible Part (Peel and Flesh) of Pear-Jujube Fruits[a]

proanthocyanidin	time	treatment T0		treatment T1		treatment T2	
(epi)catechin	0	252.8 ± 43.2	bA	739.2 ± 51.5	aA	718.7 ± 41.2	aA
	1	435.7 ± 6.7	aA	374.7 ± 2.9	bB	247.0 ± 14.2	cB
dimer B type [(E)C−B−(E)C]	0	385.2 ± 81.8	bB	1074.3 ± 77.7	aA	1143.6 ± 141.5	aA
	1	758.7 ± 17.6	aA	672.1 ± 20.9	bB	246.5 ±9.85	cB
trimer B type [(E)C−B−(E)C−B−(E)C]	0	274.2 ± 50.9	aB	465.6 ± 43.5	aA	442.1 ± 40.8	aA
	1	539.9 ± 12.7	aA	497.6 ± 37.2	aA	260.8 ± 25.1	bA
trimer B type [(E)C−B−(E)C−B−(E)C]	0	182.5 ± 47.5	bB	669.3 ± 47.9	aA	658.3 ± 34.7	aA
	1	790.5 ± 27.2	aA	512.0 ± 29.2	bA	389.9 ±10.7	bB
tetramer B type [(E)C−B−(E)C−B−(E)C−B−(E)C]	0	411.3 ± 84.8	aB	682.5 ±46.9	aA	805.3 ± 87.2	aA
	1	885.6 ±10.5	aA	678.5 ± 22.6	bA	472.3 ± 13.5	cB
tetramer B type [(E)C−B−(E)C−B−(E)C−B−(E)C]	0	282.1 ± 57.3	aB	476.0 ± 40.2	aA	461.9 ± 18.9	aA
	1	673.3 ± 11.8	aA	592.3 ± 12.8	aA	287.6 ± 27.9	bA
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	541.0 ± 105.3	aB	751.5 ± 107.9	aA	874.2 ± 103.4	aA
	1	1339.5 ± 33.2	aA	1124.9 ± 36.4	aA	499.3 ± 75.9	bB
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	250.3 ± 80.6	aB	393.8 ±41.3	aA	376.3 ± 22.7	aA
	1	649.0 ± 37.2	aA	511.0 ± 16.0	abA	377.4 ± 36.8	bA
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	288.4 ± 71.0	bB	820.2 ± 118.9	abA	1018.2 ± 134.8	aA
	1	1333.3 ± 18.3	aA	1224.3 ± 15.4	aA	755.4 ± 63.9	bA
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	80.8 ± 23.1	bB	606.9 ± 99.4	aA	828.4 ± 126.2	aA
	1	1162.8 ± 46.0	aA	942.7 ± 102.7	abA	666.5 ± 65.7	bA
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	nd		404.6 ± 150.0	aB	693.4 ± 191.1	aA
	1	915.0 ± 29.9	a	899.9 ± 23.3	aA	486.1 ± 29.0	bA
pentamer B type [(E)C−B−(E)C−B−(E)C−B−(E)C−B−(E)C]	0	nd		378.6 ± 86.4	aA	551.4 ± 95.4	aA
	1	565.2 ± 13.5	a	472.2 ± 11.8	bA	419.0 ± 11.9	bB
total	0	2948.6 ± 644.6	bB	7547.6 ± 911.6	aA	8571.7 ± 961.6	aA
	1	10048.4 ± 104.1	aA	8502.2 ± 92.7	bA	5107.7 ± 160.3	cB

[a]Means within a row for each proanthocyanidin and storage time followed by different small letter, and within a column for each proanthocyanidin and treatment followed by different capital letter, are significantly different at $P = 0.05$ by Tukeys test. Abbreviations used: (epi)catechin ((E)C), not detected (nd).

occurrence of the other procyanidins (Figures 2 and 3, Table 1) has not been reported, and two trimers, two tetramers, and six pentamers are described here in pear-jujube fruit for first time to our knowledge.

Hudina et al.[26] studied the catechin and (epi)catechin contents in seven Chinese pear-jujube fruit varieties (Bianhesuanzao, Yuanlingzao, Fupingdazao (Syn. Pozao), Zanhuangdazao, Zizao, Huizao, and Jinsixiaozao) and a wild ancestor of pear-jujube (Acid jujube), showing that these compounds varied from 0.01 to 0.02 and from 0.01 to 0.05 g/kg DW, respectively. Also, Choi et al.[10] reported that the (epi)catechin and one dimer contents in the pulp of three Korean varieties (Boeun-deachu, Mechu, and Sanzoin) of pear-jujube fruits ranged between 2.6 and 3.5 and 0.1 and 0.3 g/kg DW, respectively. In our case, the (epi)catechin content in the fruits from the three irrigation treatments (0.3−0.7 g/kg DW, Table 2) was higher than those reported in the Chinese varieties and lower than those reported in Korean varieties. Also, the dimer contents in the Korean fruit varieties were lower than those found in the fruits from T0, T1, and T2 treatments (0.4−1.1 g/kg DW, Table 2).

Taking into consideration that in our experiment the pear-jujube fruit moisture levels varied between 65% (T2) and 84% (T0) (data not shown), the procyanidin content would correspond to a range of 0.5 to 3.0 g/kg FW. These values could be considered as intermediate according to the concentrations of proanthocyanidins in 21 different fruits (from 0.04 g/kg FW (kiwi) to 6.64 g/kg FW (choke berries)) reported by Gu et al.[48] However, our results point to a higher procyanidin content than those observed in other stone fruits like apricots, peaches, and plums.[48,49]

The increase in procyanidins in pear jujube fruits following water deficit effect (Tables 2 and 3) agrees with observations of Sun et al.[11] who found that pear-jujube fruits from semiarid regions had the highest antioxidant activity, and with Guo et al.[50] who also indicated that flavonoid levels increased under harsh growing conditions, even though flavonoid levels were more dependent on plant material than growing conditions. In addition, when the procyanidin content increased in the fruit edible portion in response to water deficit (Tables 2 and 3), no significant differences were observed in its content between T1 and T2 fruits. This fact could be related with the gas exchange levels observed in T1 and T2 plants despite the water stress (Figure 1). In this sense, it is important to take into account that plant growth begins to decline at a water deficit level lower than that at which stomatal closure takes place. Therefore, in plants under water deficit (T1 and T2 plants), when carbohydrates exceed the amount used for growth concentrations, the considerable CO_2 assimilation levels observed could increase the biosynthesis of carbon-based secondary metabolites.[32] Moreover, the increase in the procyanidin content through a water stress effect could also be related with the fact that water deficit can lead to an increase in the levels of free phenylalanine,[51] a precursor in the procyanidin

Table 3. Effect of Irrigation Treatments (T0, T1, and T2) at Different Times (0, at Harvest; 1, after 12 Weeks at Domestic Cold Storage) in Proanthocyanidin Oligomeric Species Content (mg/kg DW) in Pits (Shell + Seed) of Pear-Jujube Fruits[a]

proanthocyanidin	time	treatment T0		treatment T1		treatment T2	
(epi)catechin	0	350.7 ± 9.2	cB	436.4 ± 7.0	bA	541.5 ± 9.9	aA
	1	564.4 ± 69.7	aA	345.8 ± 11.4	abA	290.2 ± 10.0 b	bB
dimer B type [(E)C–B–(E)C]	0	219.5 ± 1.2	bB	219.8 ± 14.8	bA	280.5 ± 2.5	aA
	1	979.4 ± 131.2	aA	365.1 ± 6.6	bA	228.1 ± 7.5	bA
trimer B type [(E)C–B–(E)C–B–(E)C]	0	277.2 ± 6.2	bB	310.6 ± 10.2	bA	400.8 ± 11.0	aA
	1	665.5 ± 69.4	aA	363.4 ± 22.4	abA	216.3 ± 14. 8	bB
trimer B type [(E)C–B–(E)C–B–(E)C]	0	277.6 ± 6.7	bB	329.5 ± 9.1	bA	493.1 ± 14.1	aA
	1	1061.5 ± 196.3	aA	466.6 ± 23.6	bA	164.3 ± 10. 5	bA
tetramer B type [(E)C–B–(E)C–B–(E)C–B–(E)C]	0	573.4 ± 12.5	bB	801.9 ± 10.2	aA	894.9 ± 27.1	aA
	1	1020.6 ±167.7	aA	488.5 ± 7.5	bA	252.4 ± 11.1	bB
tetramer B type [(E)C–B–(E)C–B–(E)C–B–(E)C]	0	378.0 ± 14.3	bB	453.8 ± 22.6	bA	1004.6 ± 61.1	aA
	1	674.5 ± 37.5	aA	464.4 ± 8.1	bA	221.3 ± 20.7	cB
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	1281.7 ± 24.0	bA	1429.2 ± 10.7	abA	1586.1 ± 80.4	aA
	1	798.3 ± 52.7	aB	465.1 ± 19.5	bB	229.0 ± 14.3	cB
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	1121.5 ± 39.3	cA	1324.3 ± 18.2	aA	1508.2 ± 12.7	aA
	1	613.7 ± 26.1	aB	430.2 ± 16.2	bB	227.6 ± 43.9	cB
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	nd		nd		nd	
	1	1592.7 ± 108.1	a	1055.5 ± 31.7	b	444.8 ± 30.3	c
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	nd		nd		nd	
	1	1098.9 ± 109.9	a	826.9 ± 36.0	a	306.9 ± 57.9	b
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	nd		nd		nd	
	1	987.4 ± 18.9	a	650.9 ± 12.6	b	nd	
pentamer B type [(E)C–B–(E)C–B–(E)C–B–(E)C–B–(E)C]	0	nd		nd		nd	
	1	1082.0 ± 32.8	a	660.6 ± 56.8	b	nd	
total	0	4479.6 ± 74.0	cB	5305.5 ± 46.2	bA	6709.8 ± 165.9	aA
	1	10010.5 ± 535.6	aA	6229.9 ± 148.9	bA	2504.8 ± 222.5	cB

[a]Means within a row for each proanthocyanidin and storage time followed by different small letter, and within a column for each proanthocyanidin and treatment followed by different capital letter, are significantly different at $P = 0.05$ by Tukeys test. Abbreviations used: (epi)catechin ((E)C), not detected (nd).

Table 4. Effect of Irrigation Treatments (T0, T1, and T2) on the Average Molecular Radius (nm) of Procyanidin Self-Aggregates in Peel, Flesh, and Pit of Pear-Jujube Fruits[a]

treatment	peel	flesh	pit
T0	222 ± 85 aA	130 ± 41 aA	149 ± 56 aA
T1	262 ± 78 aA	193 ± 75 aAB	140 ± 42 aB
T2	328 ± 61 aA	171 ± 78 aB	190 ± 82 aAB

[a]Means within a column for each fruit portion or within a row for each treatment followed by different small letter or capital letter, respectively, are significantly different at $P = 0.05$ by Tukeys test.

synthesis, and an increase in L-phenylalanine ammonia lyase (PAL) activity[52] and, probably, PAL synthesis.[34,53]

It is important to emphasize that it has been suggested that the majority of proanthocyanidins transit into the small intestine intact and are degraded mainly by colonic microflora in the cecum and large intestine.[54,55] According to Santos-Buelga and Scalbert,[22] low molecular mass proanthocyanidins can be absorbed in the human gastrointestinal tract. Déprez et al.[56] showed that proanthocyanidins with a polymerization degree higher than three appear not to be absorbed directly from the gastrointestinal lumen. Furthermore, Holt et al.[57] detected dimers in blood after human subjects consumed a proanthocyanidin-rich diet, and trimers have been shown to be absorbed through the human intestinal cell line Caco-2.[56] In our study, the increase in total procyanidin content in the edible portion of the fruits was based mainly on an increase in the low molecular mass compounds (Table 2), which allows concluding that pear-jujube fruits from trees under water deficit produce procyanidins of higher potential bioavailability and with greater potential physiological effects for human health.

The self-aggregation is a new parameter that we underline at this point since it influences the bioaccesibility of the gut microbiota to the procyanidins to metabolize them. Therefore, a lower self-aggregation of the procyanidins could favor the absorption of them, and it could affect the total bioavailability of these compounds. Poncet-Legrand et al.,[58] using a hydroalcoholic solvent system, detected aggregation of procyanidins in apple and pear parenchyma and grape seeds. In this sense, self-aggregation is due to the preferred interaction of molecules with others of the same nature but not with those of the solvent. However, kinetics of the process and final size of aggregates can be affected by the nature of the solvent. For this, the fact that proanthocyanidin self-aggregates in pear-jujube fruits (Table 4) showed similar size to those found by Poncet-Legrand et al.[58] in other fruits could indicate that self-aggregate conformation and interaction with both hydroalcoholic and acetonitrile/acetic acid solvents were similar.

To explain why procyanidin content changes take place in the edible portion and pit of pear-jujube fruits during domestic cold storage (Tables 2 and 3), it is important to consider that water deficit accelerates the onset of ripening.[33,59] Despite the nonclimateric pattern of *Z. jujuba*,[60] other authors such as Abbas and Fandi[61] demonstrated that evergreen species of the genus *Ziziphus* (*Z. mauritiana*) showed changes in respiration

rates and ethylene production during fruit development that were typical of climacteric fruits, while Wang et al.[29] demonstrated that *Z. jujuba* fruits senesce rapidly at room temperature due to their climacteric character. In this respect, the fact that the proanthocyanidin content decreased in T2 fruits during cold storage, whereas the storage effect in T1 fruits was not significant and induced a proanthocyanidin content increase in T0 fruits (Tables 2 and 3), can be explained if we consider that at harvest time the ripening degree would be proportional to the water deficit achieved (Figure 1). In this sense, the changes observed in procyanidins in T2 fruits (Tables 2 and 3) would agree with the decrease in phenolics observed in grape berries during overripening by Nadal[62] and in maoluang fruits by Butkhup and Samappito.[63] Therefore, at harvest time, T0 fruits would be less ripe than T1 and T2 fruits. So, during domestic cold storage, these fruits would ripen more, increasing phenolics due to an increase in PAL activity, as indicated by Tovar et al.[34] and Nadal.[62] However, it is difficult to explain why the procyanidin content of T1 fruits did not change during cold storage, although an intermediate ripening degree at harvest time would have led to constant level of procyanidins.

The current work demonstrates the occurrence of novel procyanidins in pear-jujube. To date, only two procyanidins [(epi)catechin and its dimer] have been described. In the present study, two trimers, two tetramers, and six procyanidin pentamers have been tentatively identified and quantified for the first time in pear-jujube. The results confirm that proanthocyanidins in pear-jujube fruits consist exclusively of B type procyanidins, whose levels are increased by water deficit during the fruit maturation stage. The fact that the total procyanidin content of the edible portion of fruits under water deficit is based mainly on an increase in the low molecular mass compounds leads us to conclude that pear-jujube fruits from trees exposed to water deficit increase procyanidin bioavailability and enhance the potential physiological effects on human health. The tendency of these molecules to self-aggregate does not change with the portion of the fruit or the irrigation treatment and is similar to that observed in other fruits. Additionally, fruits from well watered trees may increase their procyanidin content during fruit cold storage, whereas fruits from trees that were exposed to severe water stress (T2) decrease their procyanidins content during cold storage.

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Notes

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6. Resultados y discusión

Mecanismos de resistencia a la sequía

Atendiendo al primer objetivo parcial, se realizó un ensayo cuyas condiciones experimentales se encuentran detalladas en la publicación recogida el apartado 5.1 de esta memoria.

Considerando que los valores del potencial hídrico antes del alba (Ψ_{pd}) dependen fundamentalmente del nivel de humedad del suelo (Elfving y Kaufman, 1972; Torrecillas et al., 1988; Sellin et al., 1996), el hecho de que durante todo el periodo experimental las plantas del tratamiento T0 presentasen valores altos y casi constantes de Ψ_{pd} y del potencial de presión antes del alba (Ψ_{ppd}) indicó que las dosis de riego aplicadas a este tratamiento no indujeron ninguna situación de déficit hídrico. El hecho de que los valores de Ψ_{pd} y Ψ_{ppd} en la plantas del tratamiento T1 fuesen significativamente menores que las del tratamiento T0 evidenció que los criterios desarrollados por los agricultores no satisfacen los requerimientos hídricos de los jinjoleros.

Al final del periodo de supresión del riego, las plantas del tratamiento T2 mostraron valores de Ψ_{pd}, potencial hídrico de tallo al mediodía (Ψ_{stem}) y potencial hídrico al mediodía (Ψ_{md}) de - 3.20, - 3.14 y - 4.22 MPa, respectivamente, lo que vino a indicar niveles muy severos de déficit hídrico en las plantas de este tratamiento. Además, el retraso en la recuperación de los niveles de Ψ_{pd}, Ψ_{md} y Ψ_{stem} tras la reanudación del riego podría estar relacionada con una reducida conductividad hidráulica, quizás como resultado de cavitaciones en el xilema (Massai et al., 2000). Sin embargo, la existencia de diferencias entre los valores de Ψ_{pd} y Ψ_{md} en las plantas del tratamiento T2 durante el periodo de supresión del riego mostró como la capacidad para transportar agua a través del sistema conductor no estuvo completamente anulada, permitiendo una cierta rehidratación de las hojas a partir del mediodía.

La significativa disminución de los niveles de potencial osmótico a plena turgencia (Ψ_{os}) en la plantas de los tratamientos T1 y T2 (0.35 y

0.72 MPa, respectivamente) podría atribuirse a una acumulación activa de solutos y, por tanto, al desarrollo de un proceso de ajuste osmótico activo (Wilson et al., 1989), que adquirió mayores dimensiones en las hojas de las plantas del tratamiento T2 debido al efecto acumulativo del déficit hídrico en las plantas de este tratamiento. Este comportamiento concuerda con los observados en otras especies frutales como albaricoquero Torrecillas et al., 1999), manzano (Wang et al., 1995), granado (Rodríguez et al., 2012) y melocotonero (Arndt et al., 2000; Mellisho et al., 2011). Es importante señalar que este proceso de osmoregulación depende no sólo de la especie y cultivar y sólo se desarrolla cuando el déficit hídrico se impone de forma gradual durante un prolongado periodo de tiempo (Lakso, 1990; Arndt et al., 2000).

El hecho de que las hojas de los tratamientos T1 y T2 mantuviesen la turgencia foliar, incluso a niveles máximos de estrés, mostró como la osmoregulación activa contribuye al mantenimiento de la turgencia celular (Ψ_{ppd} y Ψ_{pmd} mayores que cero).

A pesar de que varios autores han indicado que en cultivos leñosos los valores de Ψ_{os} pueden afectar a los del potencial hídrico en el punto de pérdida de turgencia (Ψ_{tlp}) (Torrecillas et al., 1996; Sánchez-Blanco et al., 1991), el hecho de que los niveles de Ψ_{tlp} en las hojas de jinjolero no cambiasen como consecuencia del déficit hídrico podría indicar que el ajuste osmótico no fue suficiente para modificar los valores de Ψ_{tlp} en las hojas de las plantas de los tratamientos T1 y T2.

La importante regulación estomática observada en las plantas de los tratamientos T1 y T2 pudo ser un mecanismo complementario del ajuste osmótico para mantener la turgencia celular. La disminución de la conductancia estomática en las hojas de plantas leñosas bajo estrés hídrico ya ha sido descrita como una respuesta primaria al déficit hídrico, jugando un papel activo en el control del estado hídrico foliar (Rieger y Duemmel, 1992; Mellisho et al., 2011; Rodríguez et al., 2012).

La recuperación parcial de la conductancia foliar al mediodía (g_{lmd}) tras la reanudación del riego (día del año (DA) 252) en las plantas del tratamiento T2 indicó que la regulación estomática acontecida durante el periodo de déficit hídrico no fue una simple respuesta pasiva al déficit hídrico, sino que la conducta de g_{lmd} bajo esta situación puede estar relacionada con cambios hormonales, tales como un incremento en ácido abcísico y/o una disminución en los niveles de citoquininas (Mansfield., 1987; Davies y Zhang, 1991).

De forma similar a lo detectado previamente en melocotonero y granado (Mellisho et al., 2011; Rodríguez et al., 2012), pero contrariamente a lo observado en otros cultivos leñosos (Savé et al., 1995; Sharon et al., 2001), las hojas de los jinjoleros no son capaces de desarrollar ajuste elástico (disminución de Є) en respuesta al estrés hídrico. Comportamiento que puede considerarse como una clara confirmación de cómo los valores de Є influyen en los de RWC_{tlp} (Savé et al., 1995). En especies que desarrollan ajuste osmótico se ha demostrado que el mantenimiento de la rigidez de la pared celular puede ser un prerrequisito para mantener la integridad de las células de los tejidos durante la rehidratación subsiguiente a un periodo de estrés hídrico (Clifford et al., 1998; Álvarez et al., 2009).

Los contenidos foliares de agua apoplástica (RWC_a) (30 - 42 %) resultaron similares a los encontrados en otros frutales como el albaricoquero (27 – 42 %) (Torrecillas et al., 1999) y el melocotonero (29 – 44 %) (Mellisho et al., 2011) y cercanos al límite inferior detectado en granado (42 – 58 %) (Rodríguez et al., 2012) y almendro (42 – 59 %) (Torrecillas et al., 1996). A su vez, los valores de RWC_a en jinjolero resultaron altos en relación a otras especies tales como *Eucalyptus globulus* (14–27%) (Correia et al., 1989) y *Quercus alba* (26–31%) (Parker y Pallardi, 1987), e inferiores a los encontrados en viña (51 – 63 %) (Rodrigues et al., 1993). Altos contenidos de RWCa es una característica muy común en plantas xeromórficas (Cutler et al., 1977), y su aumento en condiciones de déficit hídrico puede considerarse como un mecanismo

adaptativo (Serrano y Peñuelas, 2005), ya que estos cambios estructurales en la pared celular de las hojas de jinjolero podrían permitir una mayor acumulación de agua en el apoplasto, originando una disminución del potencial hídrico foliar y, en consecuencia, un mayor gradiente de potencial hídrico entre las hojas y el suelo, lo que favorecería la absorción de agua (Fereres et al., 1979).

Los resultados obtenidos en este ensayo mostraron como las plantas de jinjolero expuestas a déficit hídrico desarrollan mecanismos de tolerancia y evitación del estrés. Desde el inicio del riego deficitario (T1) y la supresión del riego (T2) hasta el momento de máximo estrés, se mantuvo la turgencia celular, permitiendo importantes niveles de intercambio gaseoso, y como consecuencia, una buena productividad. Los niveles de turgencia celular se mantuvieron gracias a dos mecanismos complementarios: disminución de la conductancia foliar y un muy corto periodo de máxima apertura estomática para controlar las pérdidas de agua vía transpiración (mecanismos de evitación del estrés). La progresiva recuperación de las tasas de conductancia foliar tras la reanudación del riego también puede considerarse como un mecanismo para favorecer la rehidratación. Además, desde el inicio del periodo de déficit hídrico se desarrolló un proceso de ajuste osmótico activo, el cual pudo intervenir en el mantenimiento de la turgencia foliar (mecanismo de tolerancia al estrés). Los altos contenidos de RWCa y la posibilidad de aumentar en respuesta al déficit hídrico, originando un mayor gradiente de potencial hídrico entre la hoja y el suelo, puede considerarse como otro mecanismo de tolerancia al estés.

Efecto del riego deficitario en la calidad del fruto y contenido de aminoácidos

Para abordar el segundo y tercer objetivo parcial, se utilizaron los frutos de los árboles del ensayo anterior, cuyas condiciones experimentales se encuentran detalladas en la publicación recogida en el

apartados 5.1 y 5.2 de esta memoria. Concretamente, la recolección se efectuó cuando los frutos del tratamiento T0 alcanzaron mayoritariamente un estado de madurez comercial equivalente al estado S7, según Choi et al. (2012).

Las plantas del tratamiento control (T0) no se vieron sometidas a déficit hídrico durante todo el ensayo. Las plantas del tratamiento T1 se vieron sometidas a un déficit hídrico moderado y las plantas del tratamiento T3 se vieron sometidas a un déficit hídrico más severo.

La producción de los árboles del tratamiento T0 fue similar a la indicada por Cui et al. (2008) y Gao et al. (2011) para distintas variedades de la especie objeto de estudio. Sin embargo, en contra de algunos trabajos de otros autores, el jinjolero resultó altamente sensible al déficit hídrico durante la maduración, ya que no sólo disminuyó la producción y el tamaño del fruto sino que la mayoría de las características químicas del fruto resultaron modificadas.

El aumento de la firmeza de los frutos procedentes de las plantas bajo condiciones de estrés severo (T2), pudo deberse a que la deshidratación de los frutos produce una estructura más viscosa y acorchada (Wu et al., 2012). Igualmente, estos frutos modificaron significativamente los valores de a^* y H^o en la piel, evolucionando desde verde a un rojo más intenso. La modificación del color de la pulpa fue menos importante, ya que permaneció más verdosa y menos luminosa que la piel, aunque alcanzó un tono amarillento más importante que el los frutos del tratamiento T1 y T2.

Partiendo del hecho de que el jínjol es un fruto climatérico (Wang et al., 2009; Zhang et al., 2012), el aumento de la emisión de etileno y la tasa respiratoria en los frutos del tratamiento T1 avala la idea de que bajo déficit hídrico moderado los jínjoles adelantan la maduración. Además, el hecho de que los frutos del tratamiento T2 presentasen un aumento de la emisión de etileno respecto de la observada en los frutos del tratamiento T0, y que la tasa respiratoria en los frutos del tratamiento T2 disminuyese,

respecto de los frutos de los tratamientos T0 y T1, reflejó como el estrés severo produce una mayor madurez de los frutos.

El contenido de bioelementos en la parte comestible de los frutos fue similar a la encontrada por Li et al. (2007) en distintos cultivares, siendo el K, N, Ca, P, y Mg los nutrientes más abundantes seguido, en orden descendente, de S, Na, B, Zn, Mn, Fe y Cu. Los frutos bajo déficit hídrico moderado (T1) sólo disminuyeron los contenidos de K, P y Cu, mientras que los frutos bajo déficit hídrico severo (T2) presentaron significativas disminuciones de los contenidos en K, N, P, Mg, S, B, Mn, Fe y Cu (Hussein y Camilia, 2011; Mellisho et al., 2012).

Otros aspectos nutritivos del jínjol, en comparación con otros frutos, son los altos contenidos de vitamina C (Li et al., 2007; Gao et al., 2012), ya que un fruto de tamaño medio (20 g) puede casi cubrir las necesidades diarias de un adulto. Además, otro aspecto de suma importancia es la presencia de arabinosa, uno de los principales azúcares neutros de la fibra dietética soluble e insoluble.

El déficit hídrico aumenta la calidad comestible de los jínjoles de forma proporcional al nivel alcanzado. Bajo déficit hídrico moderado parece que acrecienta el sabor dulce de los mismos debido a los aumentos en sacarosa y arabinosa. Bajo déficit hídrico severo, además de acrecentar el sabor dulce, debido a los aumentos de sacarosa, glucosa y arabinosa, se produce un aumento del valor nutricional al incrementar significativamente los niveles de vitamina C (Gribaa et al. 2013).

Cabe destacar que por primera vez se identificó en jínjoles un aminoácido esencial (Cys-cys) y siete no esenciales (p-Hyp, AADA, Orn, BAIB, AABA, Cysta, and HCys-Cys). Además, la presencia de cuatro aminotioles (Cys-cys, Met, Cista, and Hcys-cys) respalda el carácter funcional de estos frutos. Cabe señalar que los aminoácidos identificados, tanto en la parte comestible como en el hueso de los jínjoles, mostraron algunas diferencias respecto a los identificados en variedades coreanas (Boeum-deachu, Mechu y Sanzoin) por Choi et al. (2011, 2012), ya que

estos autores identificaron Cys e Hyl que no se encontraron en los frutos de Grande de Albatera.

La respuesta de los jínjoles al déficit hídrico no fue tan sensible como cabría esperar, ya que no se encontró una relación directa con la magnitud del déficit. En este sentido, los niveles de aminoácidos en la parte comestible de los frutos del tratamiento T2 tendieron a disminuir, mientras que los contenidos en los frutos del tratamiento T1 tendieron a ser similares a los del tratamiento control. Por otra parte, el contenido de aminoácidos totales en el hueso no resultó afectado por el déficit hídrico, y a nivel individual se produjeron aumentos y disminuciones muy poco coincidentes en los frutos de los tratamientos T1 y T2.

Por otra parte, cabe destacar que los jínjoles presentaron un relativamente alto contenido de Asn, el cual es precursor de la acrilamida, compuesto potencialmente tóxico. Sin embargo, los niveles de Asn disminuyeron de forma muy importante, tanto en la parte comestible como el hueso, por efecto del déficit hídrico. Por tanto, el déficit hídrico durante la maduración del fruto debe considerarse como una herramienta de utilidad a fin de reducir los riesgos de formación de acrilamida durante el procesado de los frutos en caliente.

Efecto del riego deficitario en el contenido de procianidinas en el fruto y el almacenamiento doméstico en frío

Los resultados relativos al cuarto y quinto objetivo parcial se detallan en la publicación recogida en el apartado 5.3.

El hecho de que todas las proantocianidinas en los jínjoles fuesen exclusivamente procianidinas tipo B, coincide con los resultados de Gu et al. (2003a) quienes habían indicado que los frutos son la principal fuente de estos compuestos en la dieta humana. Anteriormente, se había demostrado la presencia de (+)catequina, procianidina B2 y (epi)catequina en jínjoles (Hudina et al., 2008; San et al., 2010; Choi et al., 2011). Sin

embargo, no se conocía la existencia de otras procianidinas ni dos trímeros, dos tetrámeros y seis pentámeros.

Los contenidos de catequina y (epi)catequina en siete variedades chinas de jínjol (Bianhesuanzao, Yuanlingzao, Fupingdazao (Syn. Pozao), Zanhuangdazao, Zizao, Huizao y Jinsixiaozao) y un ancestro silvestre (jínjol ácido) se encuentran entre 0.01 y 0.02 y entre 0.01 y 0.05 g/kg DW, respectivamente (Hudina et al., 2008). Por otra parte, en tres variedades coreanas de jínjol (Boeun-deachu, Mechu y Sanzoin) se han encontrado niveles de (epi)catequina y un dímero de entre 2.6 y 3.5 y entre 0.1 y 0.3 g/kg DW, respectivamente. En nuestro caso, los niveles de (epi)catequina (0.3 - 0.7 g/kg DW) fueron superiores a los encontrados en las variedades chinas, pero inferiores a los de las coreanas. Sin embargo, los contenidos del dímero en olas variedades coreanas fueron inferiores a los detectados en los frutos de los tres tratamientos de riego (0.4 – 1.1 g/kg DW). Considerando que los niveles de humedad en el fruto oscilaron entre el 65 % (T2) y el 84 % (T0) (datos no mostrados), el nivel de procianidinas en relación al peso fresco oscilaría entre 0.5 to 3.0 g/kg, lo que puede considerarse como intermedio en relación a los veintiún tipos de frutos estudiados por Gu et al. (2003b), pero muy superior al nivel de procianidinas encontrados en otros frutos de hueso como albaricoques, melocotones y ciruelas (Gu et al., 2003b; Buendía et al., 2008).

Los aumentos de los niveles de procianidinas observados por efecto del déficit hídrico resultaron coincidentes con las observaciones de Sun et al. (2011), quienes indicaron que los jínjoles procedentes de regiones semiáridas poseen una actividad antioxidante mayor. Igualmente, Guo et al. (2011) mostraron como los niveles de flavonoides son mayores en condiciones medioambientales adversas.

El hecho de que los niveles de procianidinas fuesen similares en los jínjoles de los tratamientos T1 y T2 podría estar relacionado con los considerables niveles de intercambio gaseoso observado en las plantas de estos tratamientos a pesar del déficit hídrico alcanzado. En este sentido, es importante considerar que el crecimiento vegetal comienza a

disminuir a un nivel de déficit hídrico bastante más suave que al que se produce el cierre estomático. Por tanto, en plantas bajo déficit hídrico (plantas T1 y T2), cuando los carbohidratos exceden las cantidades necesaria para el crecimiento, la asimilación de CO_2 podría incrementar la biosíntesis de metabolitos secundarios basados en el carbono (Horner et al., 1990). Además, el aumento en el contenido de procianidinas por efecto del déficit hídrico podría también estar relacionado con el hecho de que el déficit hídrico podría conducir a un incremento en los niveles de fenilalanina (Saunier et al., 1968), precursor en la síntesis de procianidina, y a un incremento en la actividad L-fenilalanina amonioliasa (PAL) (Roby et al., 2004) y, probablemente, la síntesis de PAL (Chalker-Scott et al., 1989; Tovar et al., 2002)

Conviene considerar que se ha sugerido que la mayoría de la proantocianidinas transitan intactas a lo largo del intestino delgado y se degradan fundamentalmente por acción de la microflora del colon en el ciego e intestino grueso (Rios et al., 2002; Gu et al., 2004). Según Santos-Buelga y Scalbert (2000), las proantocianidinas de bajo peso molecular pueden ser absorbidas en el tracto gastrointestinal humano. Deprez et al. (2001) demostró que las proantocianidinas con un grado de polimerización mayor de tres parece que no pueden ser absorbidas directamente en el lumen gastrointestinal. Además, Holt et al. (2002) detectaron dímeros en la sangre de humanos que habían consumido una dieta rica en proantocianidinas, y se ha demostrado que los trímeros se absorben a través las células Caco-2 del intestino humano. (Deprez et al., 2001). Por tanto, el hecho de que el aumento del contenido en procianidinas en la parte comestible de los jínjoles sea debido esencialmente a un aumento de los compuestos de bajo peso molecular, permite concluir que los frutos de jinjoleros bajo déficit hídrico poseen procianidinas de mayor biodisponibilidad y con mayor potencialidad para realizar efectos fisiológicos en la salud humana.

La autoagregación de las procianidinas es un aspecto altamente interesante, ya que condiciona la bioaccesibilidad de los microorganismos

del intestino para metabolizar las procianidinas. Una menor autoagregación de las procianidinas podría favorecer su absorción y, por tanto, su biodisponibilidad. En este sentido, Poncet-Legrand et al. (2003), utilizando un disolvente hidroalcohólico, detectaron agregación de procianidinas en el parénquima de manzanas y peras y en las semillas de uvas. En este caso, la autoagregación se debió a una interacción preferencial de las moléculas con otras de la misma naturaleza, pero no con las del disolvente. No obstante, el disolvente parece afectar tanto a la cinética del proceso como a tamaño final de los agregados. Por tanto, el hecho de que los autoagregados de proantocianidinas en los jínjoles mostrasen un tamaño similar al encontrado por Poncet-Legrand et al. (2003) en otros frutos podría indicar que la conformación de los autoagregados y la interacción con ambos disolventes (hidroalcoholico a acetonitrilo/ácido acético) son similares.

Para explicar los cambios en los niveles de procianidinas tanto en la parte comestible como en el hueso de los jínjoles es importante considerar que el déficit hídrico acelera la maduración (Castellarin et al., 2007; Mellisho et al. 2012). A pesar de que inicialmente se adscribió un carácter no climatérico al *Z. jujuba* (Kader et al., 1982), otros autores como Abbas y Fandi (2002) demostraron que las especies de hoja perenne del género *Ziziphus* (*Z. mauritiana*) presentan cambios en la respiración y emisión de etileno durante el desarrollo del fruto característicos de los frutos climatéricos, y Wang et al. (2009) demostraron que los frutos de *Z. jujuba* a temperatura ambiente realizan un rápido proceso de senescencia debido a su carácter climatérico. A este respecto, el hecho de que durante el almacenamiento en frío el contenido de procianidinas disminuyese en los frutos del tratamiento bajo estrés severo (T2), mientras que frutos del tratamiento con estrés moderado (T1) no modificasen su contenido de procianidinas, y se produjese un aumento de las mismas en los frutos del tratamiento control (T0), podría explicarse partiendo del hecho de que en el momento de la cosecha el nivel de madurez de los frutos podría haber sido proporcional al nivel del déficit

hídrico alcanzado. De esta manera, los cambios en las procianidinas de los jínjoles de las plantas del tratamiento T2 concuerdan con los observados por Nadal (2010) en uva y por Butkhup y Samappito (2011) en frutos de mao luang durante la sobremaduración. La inferior madurez de los jínjoles del tratamiento T0 en el momento de la cosecha haría que durante el almacenamiento doméstico en frío madurasen en mayor medida, aumentando los niveles de compuestos fenólicos debido a un aumento de la actividad PAL (Tovar et al., 2002; Nadal, 2010). Sin embargo, es difícil explicar por qué el contenido de procianidinas de los jínjoles del tratamiento T1 no se modificó durante el almacenamiento, aunque un nivel de madurez intermedio en la recolección puede que no originase variaciones significativas en el contenido de procianidinas.

Posiblemente, uno de los aspectos más importantes de este ensayo fuese la identificación de nuevas procianidinas en los jínjoles, ya que hasta ese momento sólo dos procianidinas ((epi)Catequina y un dímero) habían sido descritas y los resultados obtenidos posibilitaron la identificación tentativa y la cuantificación de dos trímeros, dos tetrámeros, y seis pentámeros. Las proantocianidinas existentes en los jínjoles son exclusivamente procianidinas tipo B, cuyos niveles aumentan por efecto del déficit hídrico durante el periodo de maduración de los frutos.

El hecho de que el contenido total de procianidinas en la parte comestible de los frutos de plantas bajo déficit hídrico se basa esencialmente en un incremento en compuestos de bajo peso molecular permite concluir que los jínjoles bajo déficit hídrico aumentan la bioasimilabilidad de las procianidinas y los posibles efectos fisiológicos asociados a estos compuestos sobre la salud humana. La tendencia de estas moléculas a autoagregarse fue similar en las distintas partes del fruto y no se vio afectada por los tratamientos de riego, resultando del mismo orden que la observada en otros frutos. Finalmente, cabe señalar que los frutos de árboles bien regados pueden incrementar el contenido de procianidinas durante el almacenamiento doméstico en frío, mientras que los frutos procedentes de árboles con estrés hídrico severo (T2)

disminuyen el contenido de procianidinas durante su almacenamiento en dichas condiciones.

7. Conclusiones

1. Las plantas de jinjolero son capaces de afrontar niveles muy severos de déficit hídrico mediante el desarrollo simultáneo y complementario de mecanismos de evitación y tolerancia al estrés.

2. Los mecanismos de evitación del estrés se basaron tanto en un progresivo acortamiento de la duración diaria de la máxima apertura estomática, como de una progresiva regulación de los niveles globales de apertura estomática.

3. La tolerancia al estrés se basó en el desarrollo tanto de una acumulación activa de solutos (ajuste osmótico) como de altos contenidos de agua apoplástica.

4. De forma contraria a lo indicado en la bibliografía precedente, el periodo de maduración del fruto resultó altamente sensible al déficit hídrico, ya que resultan afectadas la producción, el tamaño del fruto y la mayoría de las características químicas del fruto.

5. Los frutos bajo condiciones de déficit hídrico modifican en mayor medida el color de la piel que el de la pulpa, evolucionando hacia una piel de color rojo más intenso y una pulpa más amarillenta y menos luminosa que la piel.

6. Los jínjoles mostraron ser una fuente muy rica de vitamina C y nutrientes, especialmente de K, N, Ca, P, y Mg. Sin embargo, el déficit hídrico severo produce significativos aumentos de la vitamina y disminuciones de los bioelementos.

7. Cabe destacar que por primera vez se identificó en jínjoles un aminoácido esencial (Cys-cys) y siete no esenciales (p-Hyp, AADA, Orn, BAIB, AABA, Cysta, y HCys-Cys). Además, la presencia de cuatro aminotioles (Cys-cys, Met, Cista, y Hcys-cys) respalda el carácter funcional de estos frutos.

8. Resulta muy importante resaltar que los niveles de Asn disminuyen de forma muy importante por efecto del déficit hídrico. Por tanto, el déficit hídrico durante la maduración del fruto debe considerarse como una herramienta de utilidad a fin de reducir los riesgos de formación de acrilamida durante el procesado de los frutos en caliente.

9. Se han identificado y cuantificado por primera vez en jínjoles dos trímeros, dos tetrámeros y seis pentámeros de procianidinas tipo B.

10. El hecho de que el contenido de procianidinas de bajo peso molecular aumente bajo déficit hídrico, convierte a este tipo de estrés en una herramienta de utilidad para mejorar la biodisponibilidad de las procianidinas, potenciando sus efectos fisiológicos sobre la salud humana.

11. El almacenamiento doméstico en frío de los jínjoles procedentes de árboles bien regados constituye una adecuada opción durante periodos prolongados de tiempo, ya que produce un incremento del contenido de procianidinas en los frutos.

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9. Abreviaturas y símbolos

Alfabetizables

AABA	Ácido α-amino-n-butírico
AADA	Ácido α-aminoadípico
Ala	Alanina
AMQ	6-aminoquinolina
Arg	Arginina
Asn	Asparagina
Asp	Ácido aspártico
B	Enlace tipo B
BAIB	Ácido β-aminoisobutírico
Cit	Citrulina
cv	Cultivar
Cys-cys	Cistina
Cysta	Cistationina
DOY	Día del año
EA	Etanolamina
ETo	Evapotranspiración del cultivo de referencia
GABA	Ácido γ-amino-n-butírico
Gln	Glutamina
Glu	Ácido glutámico

Gly	Glicina
Hcys-cys	Homocistina
His	Histidina
HPLC	Cromatografía líquida de alta eficacia
HPLC-DAD	Cromatografía líquida de alta eficacia acoplada a un detector de diodos
ID	Identificación
LC-MS/MS	Cromatografía líquida acoplada a un tándem de masas
Leu + Ileu	Leucina + aloisoleucina
Lys	Lisina
Met	Metionina
Met-his	1-metil-histidina
MRM	Monitorización de reacciones múltiples
MS/MS	Tándem de masas
nd	No detectado
Orn	Ornitina
PEA	Fosfoetanolamina
Phe	Fenilalanina
p-Hyp	4-hidroxiprolina
Pro	Prolina
PV	Presión-Volumen

Rt	Tiempo de retención
RWC	Contenido relativo de agua foliar
Ser	Serina
TA	Acidez valorable
Thr	Treonina
Trp	Triptófano
TSS	Sólidos solubles totales
Tyr	Tirosina
UHPLC	Cromatografía líquida de ultra alta presión
UHPLC-MS/MS	Cromatografía líquida de ultra alta presión acoplada a un tándem de masas
Val	Valina

No alfabetizables

(E)C	(epi)catequina
$[M-H]^-$	Ión molecular
*a**	Color rojo-verdoso
*b**	Color azul-amarillento.
*C**	Cromaticidad o croma

g_l	Conductancia foliar
g_{lmd}	Conductancia foliar al mediodía
H^o	Ángulo Hue
L^*	Luminosidad
P_n	Fotosíntesis neta
P_{nmd}	Fotosíntesis neta al mediodía
RWC_a	Contenido relativo de agua apoplástica foliar
RWC_o	Contenido relativo de agua a máxima turgencia foliar
RWC_{tlp}	Contenido relativo de agua en el punto de pérdida de la turgencia foliar
VPD_m	Déficit de presión de vapor medio diario
VPD_m	Déficit de presión de vapor medio diario
ϵ	Módulo de elasticidad foliar aparente
Ψ_l	Potencial hídrico foliar
Ψ_{md}	Potencial hídrico foliar al mediodía
Ψ_{os}	Potencial osmótico foliar a plena turgencia antes del alba
Ψ_{pd}	Potencial hídrico foliar antes del alba
Ψ_{pmd}	Potencial de presión foliar al mediodía
Ψ_{ppd}	Potencial de presión foliar antes del alba
Ψ_{stlp}	Potencial osmótico en el punto de pérdida de turgencia

Ψ_{stem}	Potencial de tallo al mediodía
Ψ_{tlp}	Potencial hídrico foliar en el punto de pérdida de turgencia