XIII Simposio Hispano-Portugués de Relaciones Hídricas en las Plantas

"Aprendiendo a optimizar el uso del agua en las plantas para hacer de nuestro entorno un ambiente más sostenible"

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PROGRAMA

Martes: 18 de Octubre

08:00-09:30 **Registro y entrega de documentación**.

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09:30-09:45 **Apertura** del Simposio

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Jaume Flexas Sans (Universidad de las Islas Baleares, Palma, España).

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11:30-11:50: Transpiración, fijación biológica del nitrógeno y transporte a larga distancia en plantas de soja. *Aldasoro Galán, J., Arrese-Igor C.*

11:50-12:10: Preliminary results on the use of chlorophyll fluorescence and artificial intelligence techniques to automatically characterize plant water status. *Gameiro, C., Pereira S., Figueiredo, A., Bernardes da Silva, A., Matos, A.R., Pires, M.C., Teubig, P., Burnay, N., Moniz, L., Mariano, P., Marques da Silva, J.*

12:10-12:30: Do metabolic and isotopic changes underpin physiological responses to drought in *Medicago sativa* plants? *Molero, G., Roca, R., Mauve, C., Cabrera-Bosquet, Ll., Tcherkez, G., Araus, J.L., Nogués, S., Aranjuelo, I.*

12:30-13:00: **Sesión I.** *Aspectos fisiológicos y moleculares de las relaciones hídricas en plantas* (Visionado y presentación de pósters)

12:30-12:40: Unraveling the root response to drought: functional analysis of the taproot and fibrous roots of *M. truncatula*. *Castañeda, V., Seminario, A., Azcárate, L., de la Peña, M., González, E.M.*

12:40-12:50: Interactive effects of IAA application and arbuscular mycorrhizal symbiosis on root hydraulic conductivity of droughted maize plants. *Erice, G., Quiroga, G., Aroca R., Ruíz-Lozano, JM.*

12:50-13:00: Regulation of aquaporins and root hydraulic properties by SA in arbuscular mycorrhizal maize plants subjected to drought. *Quiroga, G., Erice, G., Ruíz-Lozano, J.*

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17:15-18:15 **IX Premio Ibérico de Investigación en Relaciones Hídricas**: Conferencia del Premiado.

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09:00-10:00 **Conferencia invitada**: *Implicación de las hormonas vegetales y los microorganismos del suelo en la regulación de las características hidráulicas de la raíz.*

Ricardo Aroca Álvarez (Estación Experimental del Zaidín (CSIC), Granada, España).

10:00-10:30 *Descanso: Café*

10:30-12:00 **Sesión II**. *Relaciones hídricas en especies de interés agrícola* (Comunicaciones orales)

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11:10-11:30: Using grapevine fruit-bearing cuttings (Red and White Tempranillo) grown under Temperature Gradient Greenhouses (TGGs) in 2013, 2014 and 2015 to evaluate effects of climate change (elevated CO_2, high temperature, and water deficit). I. Vegetative growth and substrate and plant water status. *Kizildeniz, T., Irigoyen, J.J., Pascual, I., Morales, F.*

11:30-11:50: Cambios en el estado hídrico, fotosíntesis y crecimiento de quinoa en respuesta a la sequía y a la salinidad. *Aguirresarobe, A., Yoldi-Achalandabaso, A., Miranda-Apodaca, J., Mena-Petite, A., Lacuesta, M., Muñoz-Rueda, A., Pérez-López, U.*

11:50-12:10: Modelización y simulación de la disponibilidad de agua en la zona radicular en ensayos de estrés hídrico. *Rodrigo G., Fontanet M., Ferrer F.*

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15:30-15:40: Influence of rootstocks on pistachio (*Pistacia vera* L.) water relations. *Memmi, H., Centeno, A., Moriana, A., Corell, M. Torrecillas, A., Pérez, D.*

15:40-15:50: Effect of water stress in the flowering induction of olive trees (*Olea europaea* L.). *Corell, M., Martín-Palomo, M.J., Girón, Pérez-López, D., Centeno, A., Torrecillas, A., Valverde, F., Moriana, A*.

15:50-16:00: Gestión del estrés hídrico del almendro mediante termografía de infrarrojos. *García-Tejero I.F., Viñuela, I., Hernández, A., Muriel-Fernández, J.L., López-Segura J.G., Durán-Zuazo V.H.*

16:00-16:10: Using grapevine fruit-bearing cuttings (Red and White Tempranillo) grown under Temperature Gradient Greenhouses (TGGs) in 2013, 2014 and 2015 to evaluate effects of climate change (elevated CO_2, high temperature, and water deficit). II. Yield and must quality. *Kizildeniz, T., Irigoyen, J.J., Pascual, I., Morales, F.*

16:10-12:20: Physiological mechanisms induced by the different alternation frequency of partial rootzone drying in *Citrus macrophylla* seedlings. *Pérez-Pérez, J.G., Navarro, J.M., Robles, J.M., Dodd, I.C., Quinto, V.*

16:20-16:30: More cash per drop: Partial rootzone irrigation increased water use efficiency compared with conventional deficit irrigation by enhancing fruit water status in 'Fino 49' lemon trees. *Pérez-Pérez, J.G., Robles, J.M., Botía, P., Dodd, I.C., Quinto, V.*

16:30-17:00 *Descanso: Café*

17:00-18:00 **Sesión II** (Presentación de pósters)

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17:10-17:20: Respuesta fisiológica de nuevos patrones en árboles jóvenes de limonero 'Verna'. *Pérez-Pérez, J.G., Robles, J.M., Navarro, J.M., Lozano, J.L., Quinto, V., Arques, E.M.*

17:20-17:40: Thermal patterns in the vineyard to support management. *Costa J.M., Egipto, R., Lopes C.M., Chaves M.*

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19:00 **Conclusión de la Jornada.** Salida hacia los Alojamientos

21:00-00:00 Cena del Simposio

Jueves 20 de Octubre

9:00-10:00	Conferencia invitada: *Impact of deficit irrigation on berry metabolism*. Olfa Zarrouk (Universidade Nova de Lisboa, Oeiras, Portugal).
10:00-10:30	*Descanso: Café*
10:30-12:00	**Sesión II**. *Relaciones hídricas en especies de interés agrícola* (Comunicaciones orales) 10:30-10:50: Potencial de la medida de reflectancia en el rango de los teraherzios (THz) para el estudio de las relaciones hídricas de las plantas. *Santesteban, L.G., Palacios I., Miranda, C., Iriarte, J.C., Royo, J.B., Torres, V., Gonzalo, R.* 10:50-11:10: Comparación del comportamiento hídrico de las variedades de vid (*Vitis vinifera* L.) "Cabernet Sauvignon", "Graciano" y "Tempranillo". *Insausti, B., Loidi, M., Sagarna, I., Marín, D., Miranda, M., Urrestarazu, J., Royo, J.B., Santesteban, L.G.* 11:10-11:30: Saving irrigation water as a tool to increase pomegranate fruit price and enhance the bioactive compound content. *Galindo, A., Calín-Sánchez, A., Rodríguez, P., Cruz, Z.N., Girón, I.F., Corell, M., Martínez-Font, R., Moriana, A., Carbonell-Barrachina, A.A., Torrecillas, A., Hernández, F.* 11:30-11:50: Metabolic changes in grapevine berry skins of different clones of Tempranillo subjected to regulated deficit irrigation under elevated temperatures. *Torres, N., Goicoechea, N., Antolín, M.C.*
	Sesión III: *Relaciones hídricas en especies no cultivadas y forestales* (Comunicaciones orales) 11:50-12:10: Diferencias en las relaciones hídricas, acumulación de biomasa y de iones Cl y Na en plantas de lentisco bajo condiciones de salinidad y riego deficitario moderado y severo. *Álvarez, S., Broetto, F., Fabricio, D., Sánchez-Blanco, M.J.* 12:10-12:30: Parámetros indicadores de estrés hídrico en plantas micorrizadas de *Cistus albidus* creciendo en un sustrato comercial y otro a base de compost de purines. *Lorente, B., Álvarez, S., Clemente, R., Sánchez-Blanco, M.J., Ortuño., M.F.*
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ÍNDICE

CONFERENCIAS

CONFERENCIA INAUGURAL

Searching for the perfect mesophyll: a trade-off between productivity and stress resistance?

Flexas Sans, J.

Instituto de Investigaciones Agroambientales y de Economía del Agua (INAGEA) – Universitat de les Illes Balears (UIB) jaume.flexas@uib.es

Over the past two decades, our research group has focused on the study of mesophyll conductance to CO2 (gm) as a limiting factor for photosynthesis. There is now ample evidence that gm is as much limiting for photosynthesis and intrinsic water use efficiency as stomatal conductance and, in some organisms like ferns, even more. gm is a dynamic trait, which changes fast in response to environmental variations, which is probably mediated by aquaporins. In addition, the maximum gm of a given species is determined by the specific anatomical arrangements of the mesophyll, with a dominant role of cell wall thickness (CWT) and the fraction of mesophyll cells covered by chloroplasts (Sc/S).

Comparing plants from distant phylogenetic groups, a decreasing trend for CWT and an increasing trend for Sc/S is observed from bryophytes to vascular plants, and among these from ferns to angiosperms. Such trends correspond well with scaling maximum photosynthesis rates in each group. We hypothesize that this trend reflects a trade-off between photosynthetic capacity and desiccation tolerance, which is generalized in bryophytes, common in ferns and rare in higher plants. In our currently ongoing project TOPSTEP (Mechanistic bases for the Trade-Off between Photosynthesis and Stress Tolerance: filling gaps of knowledge for Evolutionary biology and Plant biotechnology; CTM2014-53902-C2-1-P) we aim to confirm the existence of such trade-off, and try to find potential outliers to it.

For this, we have developed a method for fast screening of desiccation tolerance of the photosynthetic tissue, and commenced a series of field campaigns in extreme environments of the world, including hot and cold deserts, like Atacama, salty planes in Los Andes and Antarctica. Preliminary results have revealed that: (1) there is a larger variability of mesophyll arrangements among species than commonly thought; (2) the hypothesized trade-off is confirmed; and (3) a few outliers to this trade-off can be found in particular environments. The outlier species are promising candidates for ‘new generation model species’ in which to study the mechanisms to achieve, simultaneously, stress tolerance and a large photosynthetic capacity, i.e. a high productivity potential.

CONFERENCIA INVITADA

Implicación de las hormonas vegetales y los microorganismos del suelo en la regulación de las características hidráulicas de la raíz.

Aroca Álvarez, R.

Dpto. Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Granada, España. raroca@eez.csic.es

1. Conductividad Hidráulica de la Raíz

El mantenimiento de un estado hídrico óptimo es esencial para el crecimiento y desarrollo de las plantas. Éste estado hídrico óptimo se consigue mediante un equilibrio entre el agua que es absorbida por las raíces y el agua que se pierde mediante la transpiración foliar. Aunque tradicionalmente se ha prestado mayor atención a la regulación estomática, las propiedades hidráulicas de la raíz también juegan un papel preponderante (Aroca et al. 2012). La conductividad hidráulica de la raíz (L) determina en último instante la capacidad de absorber agua de las raíces, siendo L determinada en parte por la actividad de las acuaporinas. Las acuaporinas son proteínas intrínsecas de membrana que son capaces de trasportar agua y otros solutos de pequeño peso molecular, siempre a favor de gradiente. En plantas, la familia de las acuaporinas está dividida en cinco subclases, siendo las de la membrana plasmática (PIPs) las más implicadas en la regulación de L (Aroca et al. 2012).

Al igual que la conductancia estomática, L también responde a diferentes estímulos ambientales incluyendo estreses abióticos, nutrición mineral, hormonas vegetales o microorganismos del suelo (Aroca et al. 2012; Groppa et al. 2012), siendo la respuesta negativa o positiva dependiendo de la planta y de las condiciones ambientales. De entre los factores que regulan L, en este trabajo nos centraremos en las hormonas vegetales y en los microorganismos del suelo [hongos formadores de micorrizas arbusculares (AMF) y rizobacterias promotoras del crecimiento vegetal (PGPR)], y de la interacción entre ambos.

2. Hormonas vegetales y L

Del mismo modo que la apertura estomática está regulada por varias hormonas vegetales incluyendo el ácido abscísico (ABA), el jasmónico (JA), el salicílico (SA) o el indol acético (IAA), L también se ve regulada por estas hormonas (Aroca et al. 2012). De todas ellas, el ABA ha sido el más estudiado, y sólo será objeto de este trabajo tangencialmente. En nuestro grupo de trabajo nos hemos centrado en los últimos años principalmente en el JA. De este modo, descubrimos que el JA exógeno inducía un aumento de L en plantas de alubia, tomate y Arabidopsis, siendo este aumento debido en parte a la señalización por ABA y por calcio (Sánchez-Romera et al. 2014).

3. Hormonas vegetales, AMF y L

En base a los resultados anteriores, y sabiendo que los hongos MA varían tanto los niveles de L como de JA, decidimos realizar experimentos donde se añadió methyl jasmonato (MeJA) a plantas de alubia micorrizadas o no, sometidas a riego óptimo o deficitario. De esta manera, se observó que tanto la simbiosis MA como la adición de MeJA evitaba el descenso de L causado por el tratamiento de sequía. Además se encontró una correlación negativa entre los niveles de SA y L, causando tanto los tratamientos de MeJA y AMF un descenso de los niveles de SA causado por el tratamiento de sequía (Sánchez-Romera et al. 2016). Al mismo tiempo, decidimos usar plantas de tomate deficientes en la síntesis de JA (*def-1*) para determinar como el JA influía en la respuesta de L a la sequía y a la simbiosis MA. De esta manera encontramos que la mutación def-1 impedía que las plantas AM absorbieran más agua durante la sequía que las plantas no inoculadas, relacionado con una menor conductancia estomática. Lo más sorprendente fue encontrar una correlación positiva entre los niveles de MeSA y L. Así en experimentos en condiciones de hidroponía, encontramos que la adición de MeSA aumentaba L, contrario al SA. Además encontramos una correlación positiva entre los niveles de IAA y L.

Finalmente, con estudios de adición exógena de IAA como con plantas deficientes en la síntesis de IAA, descubrimos que el IAA aumenta L, pero sobre todo en plantas no micorrizadas. Este aumento de L por IAA podría estar mediada por una disminución de la síntesis de SA y un aumento de la síntesis de ABA, que se vería disminuida por la inoculación con AMF.

Por lo tanto podemos concluir que L responde a las diferentes concentraciones hormonales, y que hay una interrelación entre ellas, por lo que el estudio del efecto de una hormona vegetal, no se puede separar de lo que ocurre con el resto de hormonas.

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Sánchez-Romera B, Ruiz-Lozano JM, Zamarreño AM, García-Mina JM, Aroca R (2016) Arbuscular mycorrhizal symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity caused by drought. Mycorrhiza 26: 11-126.

CONFERENCIA INVITADA

Impact of deficit irrigation on grape berry metabolism

Zarrouk, O.

Plant Molecular Ecophysiology Lab. Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, Oeiras, Portugal. zolfa@itqb.unl.pt

ABSTRACT

The effect of water stress on grape berry ripening and quality has been extensively investigated during the last decades (Chaves et al., 2010; Lovisolo et al., 2010; Kuhn et al., 2013, Zarrouk et al., 2016). Overall, berry quality benefits from mild to moderate water deficit and the conventional wisdom says that the observed positive effects of water deficits are due to low yield and small berries. But more recently water deficit was shown to profoundly alter berry secondary metabolism, particularly of flavonoids, thus greatly regulating the ripening process. The regulation of genes and proteins of the various metabolic pathways is either the consequence of a direct effect of water shortage and/or indirect via the changing of the light environment around grape clusters due to the impairment of vine vegetative growth.

The matter is far from being conclusively addressed and two major causes can be highlighted: the experimental set-ups adopted in the majority of experiments, namely the water stress timing and conditions (pre- or post-*véraison* water stress); and the diversity of varieties (Zarrouk et al., 2016). Grape berries are non-climacteric fruits wherein abscisic acid (ABA) is central to the ripening process. The differential increase of anthocyanin compounds in berries under pre- or post-*véraison* deficit irrigation is primarily attributed to large differences in abscisic acid (ABA) sensitivity displayed by different varieties as a consequence of large differences in the ability to regulate water losses through chemical (i.e. ABA) signaling. Grape berries thus represent a unique situation in which abiotic stress and developmental signaling intersect via the activities of ABA. In addition, climate condition, namely high temperature along the growing season is considered a putative constraint to the implementation and success of the deficit irrigation regime. Also the interaction elevated temperature – water deficit is considered as the main cause of complexity of field experiments results namely in what concerns the ripening of wine grapes.

During the last ten years, our investigation aimed to comprehend the impact of water stress and heat stress on grape berry ripening, skin secondary metabolism as well as cuticle properties. We have investigated ABA-mediated cross talk between abiotic stress and ripening as it relates to flavonoid biosynthesis in the grape berry skin. We show that the mild

stress imposed by deficit irrigation have a positive impact on berry composition only when high temperature is not a limiting factor. We also show that the biological function of ABA is under berry developmental control and does not correlate with vine water status or berry temperature until a certain water stress threshold. It also appears that ABA catabolism/conjugation processes are implicated in the ripening process and in water and heat stresses responses. In addition, our most recent results in cuticle indicate that water and heat stresses increase berry wax accumulation across berry development, affecting cuticle morphology, composition and transpiration.

The recent data related to metabolic changes and hormonal control of grape berry ripening as well as berry cuticle properties under deficit irrigation will be discussed and limitations in the interpretation of results will be identified.

Key words: berry composition, berry cuticle, deficit irrigation, secondary metabolites, véraison.

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SESIÓN I

Aspectos fisiológicos y moleculares de las relaciones hídricas en plantas

Transpiración, fijación biológica del nitrógeno y transporte a larga distancia en plantas de soja

Aldasoro Galán, J.[1], Arrese-Igor C.[1]

Departamento de Ciencias del Medio Natural. Universidad Pública de Navarra, Campus Arrosadía, sn, 31006, Pamplona (Navarra). e-mail: joseba.aldasoro@unavarra.es

RESUMEN

El descenso de la transpiración provocado por variaciones del déficit de presión de vapor (DPV) de la atmósfera que rodea a la parte aérea de plantas de soja noduladas de cuatro semanas de edad (*Glycine max* (L.) Merr. cv Sumatra) con plena disponibilidad de agua, provocó el descenso del transporte de compuestos nitrogenados de la raíz nodulada a las hojas y de compuestos carbonados de la parte aérea a la raíz. Sin embargo, la fijación biológica de nitrógeno (FBN) aumentó, probablemente debido a la hidrólisis del almidón, que proporcionó energía y esqueletos carbonados al bacteroide para poder llevar a cabo el proceso de fijación.

INTRODUCCIÓN

El cultivo de leguminosas es una herramienta crucial en la lucha contra el cambio climático por ser una fuente renovable de entrada de nitrógeno del suelo. Esto se debe a la capacidad que tienen las leguminosas de fijar el nitrógeno atmosférico mediante la simbiosis con bacterias del suelo, reduciéndose la necesidad de aporte externo a los cultivos mediante el abonado nitrogenado. Así, se reducen las emisiones de CO_2 procedente de la producción de abonos, y se disminuyen las emisiones de óxidos de nitrógeno por la aplicación de fertilizantes. Además, el 25% de la producción mundial de cultivos y más de un tercio de los requerimientos dietéticos de nitrógeno para la humanidad provienen de las leguminosas. Sin embargo, el proceso de FBN es muy sensible a factores abióticos, y cualquier factor que afecte al crecimiento del rizobio o de la planta tiene gran influencia sobre la producción de los cultivos y a la FBN, siendo la sequía el factor más importante. Pero aunque los efectos de la sequía edáfica sobre la FBN se han estudiado de manera muy amplia, existen pocos estudios que analizan los efectos de la sequía atmosférica sobre la FBN en leguminosas, y el transporte a larga distancia de metabolitos.

El objetivo principal de este trabajo es profundizar en el conocimiento de la relación existente entre el déficit de presión de vapor (DPV), la transpiración, el transporte a larga distancia y la FBN en plantas de soja (*Gycine max* (L) Merr.) con plena disponibilidad de agua.

MATERIALES Y METODOS

Plantas de soja (*Glycine max* (L.) Merr. cv Sumatra) de cuatro semanas de edad en simbiosis con *Bradyrizhobium japonicum* fueron sometidas a variaciones de DPV mediante cambios en la humedad relativa (HR) de la atmósfera que rodea a la parte aérea durante 8 horas. Así se obtuvieron tres grupos de plantas con diferente tasa de transpiración: Transpiración $_{\text{ALTA}}$, Transpiración $_{\text{MEDIA}}$, y transpiración $_{\text{BAJA}}$. Al inicio, a las 4 horas y al final del tiempo de exposición de las plantas a los tratamientos se determinó la transpiración, la FBN, y se cosechó y congeló material vegetal (parte aérea y nódulos) para la determinación de compuestos carbonados (carbohidratos solubles y almidón) y nitrogenados (aminoácidos libres totales y ureidos). Para el análisis del transporte de la raíz a la parte aérea de compuestos nitrogenados, se realizó un ensayo incubando las raíces noduladas de las plantas con ^{15}N durante 8 horas de exposición a los tratamientos, y posteriormente se determinó la abundancia de ^{15}N en los diferentes tejidos.

RESULTADOS Y DISCUSIÓN

El descenso de la HR de la atmósfera que rodea a la parte aérea de las plantas provocó el aumento del DPV, y el aumento de la HR de la atmósfera tuvo el efecto contrario. Así, se obtuvieron tres niveles de transpiración en respuesta a los tratamientos (Figura 1A). En cuanto a la FBN (Figura 1B), se observó un aumento inversamente proporcional a la transpiración, aumentando al final del experimento la FBN de las plantas con transpiración media y baja respecto a las plantas con mayor transpiración. Como consecuencia de la exposición a los tratamientos, y al descenso de la transpiración, se observó un descenso en el contenido de los metabolitos analizados en la parte aérea de las plantas (Figuras 2A, 2B, 2C, y 2D), observándose mayores descensos a medida que disminuye la transpiración. Así, los descensos en el contenido en carbohidratos solubles, aminoácidos y ureidos son los más acusados, mientas que la respuesta en el contenido de almidón en la parte aérea no es tan relevante. En el tejido nodular, sin embargo, la respuesta a los tratamientos es más variada (Figuras 2E, 2F, 2G y 2H): A las 4 y 8 horas de exposición a los tratamientos en las plantas con transpiración media y baja, se observa un aumento del contenido de azúcares y un descenso del contenido de almidón, si las comparamos con el grupo de plantas que más transpira (Figuras 2E y 2F). En cuanto a los compuestos nitrogenados analizados, las plantas que más transpiran mantienen constantes su contenido de ureidos y aminoácidos, mientras que en las plantas con transpiración media y baja se observó un descenso en el contenido de ureidos de los nódulos a las cuatro horas de exposición a los tratamientos, recuperando los niveles iniciales al final del tiempo de exposición a los tratamientos (Figura 2G). También se observó un aumento en el contenido de aminoácidos a las 8 horas en las plantas con transpiración media y baja (Figura 2H). En cuanto a la abundancia de ^{15}N, se observó que a las 8 horas, hubo un descenso del marcaje en la parte aérea y un aumento de ^{15}N en los nódulos, observándose mayores efectos cuanto menor era la tasa de transpiración (datos no mostrados).

Como respuesta al aumento del DPV ocasionado por la sequía atmosférica, las plantas cierran los estomas provocando un descenso en la transpiración, minimizando las pérdidas de agua y manteniendo la hidratación de las células. En el caso de las plantas sometidas al descenso del DPV, descendería la demanda de vapor de agua por parte de la atmósfera, lo que explicaría el descenso a casi a la mitad de la transpiración al final del tratamiento. El cierre estomático provoca el descenso de la tasa de difusión del CO_2 al interior de la hoja, y como consecuencia el descenso de la eficiencia de la fijación del carbono y del contenido de azúcares en las hojas. El descenso de la transpiración debido al cierre estomático parece que se debe a la limitación de la conductancia hidráulica en la planta, que restringe el flujo de agua de las raíces a las hojas. Este descenso del flujo del agua sería el responsable de la acumulación de los compuestos nitrogenados en los nódulos. Sin embargo, esta acumulación de compuestos nitrogenados no es suficiente como para inhibir la actividad fijadora de los bacteroides como ocurre en plantas expuestas a sequías edáficas, mediante una regulación *feedback*. Sin embargo, aunque al igual que ocurre en plantas expuestas a sequía edáfica desciende el contenido de compuestos carbonados procedentes de la parte aérea, los bacteroides parece que obtienen la energía y los esqueletos carbonados imprescindibles para la FBN de la hidrólisis del almidón de los nódulos.

AGRADECIMIENTOS

Este trabajo ha sido financiado por el proyecto AGL2014-56561-P del Ministerio Economía y Competitividad. Joseba Aldasoro es beneficiario de un contrato predoctoral de la UPNa (programa FPI).

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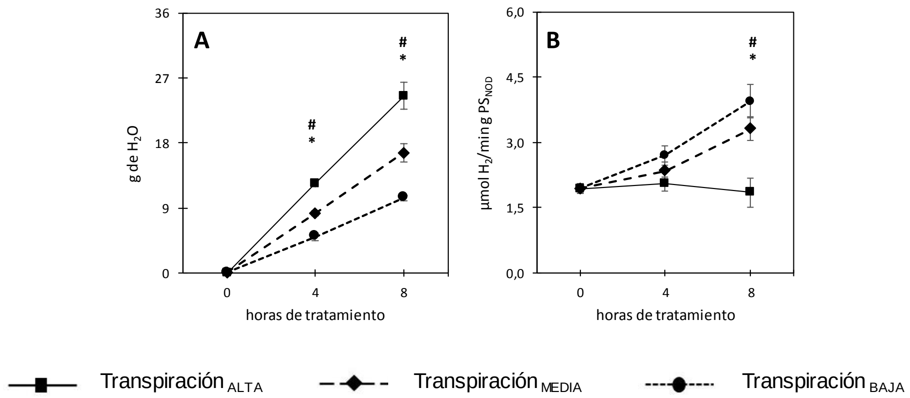

Figura 1: Efectos resultantes de las variaciones de la HR de la atmósfera que rodea a la parte aérea sobre la transpiración (A) y la FBN medida como actividad aparente de la nitrogenasa (B). Los valores representan el promedio ± el error estándar (4<n<6). El asterisco (*) y la almohadilla (#) representan diferencias significativas de las plantas con Transpiración $_{\text{MEDIA}}$ y Transpiración $_{\text{BAJA}}$ respecto a las plantas con Transpiración $_{\text{ALTA}}$ respectivamente (P ≤ 0,05).

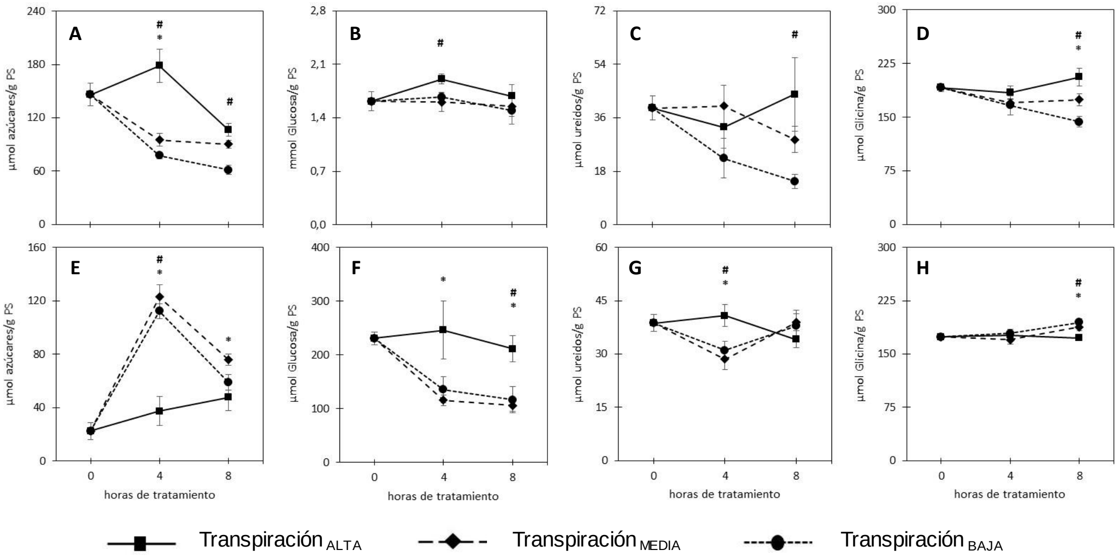

Figura 2: Contenido de azúcares (fructosa+glucosa+sacarosa), almidón, ureidos (ácido alantoico+alantoina) y aminoácidos libres totales en parte aérea (A, B, C D) y nódulos (E, F G y H), respectivamente. Los valores representan el promedio ± el error estándar (4<n<6). El asterisco (*) y la almohadilla (#) representan diferencias significativas de las plantas con Transpiración $_{\text{MEDIA}}$ y Transpiración $_{\text{BAJA}}$ respecto a las plantas con Transpiración $_{\text{ALTA}}$ respectivamente (P ≤ 0,05).

Preliminary results on the use of chlorophyll fluorescence and artificial intelligence techniques to automatically characterize plant water status

Gameiro, C., Pereira S., Figueiredo, A., Bernardes da Silva, A., Matos, A.R., Pires, M.C., Teubig, P., Burnay, N., Moniz, L., Mariano, P., Marques da Silva, J.

University of Lisbon, Faculty of Sciences, BioISI – Biosystems and Integrative Sciences Institute, Campo Grande, 1749-016 Lisboa, jmlsilva@fc.ul.pt

ABSTRACT

The induction curve of leaves' chlorophyll fluorescence in a rapid dark-light transition (Kautsky effect) exhibits a complex pattern that is dependent both of the plant species and the physiological status. The several indexes that can be calculated from the fluorescence induction curves have been extensively used in plant stress physiology. In this study, we opted to use the entire set of data points in each curve and applied artificial intelligence machine learning techniques, namely decision tress, to automatically classify rapidly dehydrated leaf samples into four complementary relative water content classes. The percentage of success (circa 60%) is only modest when compared with the high percentage of success attained (circa 90%) when comparing different horticultural species. The preliminary results suggest, therefore, that the joint use of the chlorophyll fluorescence induction curve and artificial intelligence techniques in the context of high-throughput plant phenotyping is more promising for discriminating between plant genotypes than to classify plant water status.

INTRODUCTION

The dynamics of the induction curve of leaves' chlorophyll fluorescence in a rapid dark-light transition has been observed for the first time by Kautsky and Hirsh in 1931. However, the measurement of the fluorescence induction curve acquired increased potential in photosynthesis research only when continuous fluorescence recording fluorometers equipped with high-time resolution capabilities became available, showing that it exhibits a complex pattern that is dependent both of the plant species and the physiological status. It was the commercial availability of the Plant Efficiency Analyser by the UK-based manufacturer Hansatech that made this technique widely accessible to plant physiologists and plant breeders (Marques da Silva 2016). In Switzerland, Reto Strasser provided the theoretical basis for the interpretation of these signals (Strasser 1986). He and his group at the University of Genève have developed the JIP test (termed after the main inflections in the fast fluorescence rise, called J, I, and P) to analyze the photosystem II (PS II) behaviour (Strasser and Strasser 1995). The several indexes that can be calculated in the JIP test have been extensively used in plant stress physiology. Silvestre et al. (2014) showed that the Performance Index (an integrated index that reflects the events occurring at different phases of the induction curve) is useful to screen for improved drought resistance in legumes. As an alternative to the calculated indexes, the use of the entire data points of the Kautsky curves, aided by the use of artificial intelligence machine learning tools, was first attempted by Tyystjarvi et al. (1999) to automatically identify plant species. In this paper, we used a similar approach to classify *Vitis riparia* plants into different relative water content classes, representing different degrees of water stress. We also used these techniques to automatically identify horticultural species. The percentage of success in the two situations is compared and the results are discussed in the context of high-throughput plant phenotyping.

MATERIALS AND METHODS

Wood cuttings of *Vitis riparia* plants were obtained from Estação Vitivinicola Nacional, at Dois Portos, Torres Vedras, central Portugal. After a vernalization period, they were cultivated in 2.5 L pots in universal substrate under controlled conditions in a climate chamber (Fitoclima 5000 EH, Aralab, Rio de Mouro, Portugal) at 23 / 18 ºC (day / night), relative humidity 60% and a photosynthetic photon flux density of 300 µmol m^{-2} s^{-1}. One month prior to the measurements, pots were transferred to the exterior environment at the campus of the Faculty of Science, University of Lisbon (FCUL), Portugal, and irrigated whenever necessary. At the days of the measurements, 60 leaf discs with 2 cm diameter were obtained from 30 leaves with a leaf cutter. Leaf discs were immediately weighted and assayed for chlorophyll fluorescence. As the leaf discs loose water at the bench, they were periodically weighed and assayed for fluorescence for a period of 24 hours. At the end of the experiment, leaf discs were transferred to an oven at 60 ºC and dry weight was obtained. An additional group of 10 discs was floated in milliQ water for 4 h, weighed and oven dried as previously described. The mean turgid weight / dry weight ratio of that group was used to estimate the turgid weight of the remaining discs. Relative water content (RWC) was calculated. *Brassica oleracea*, *Solanum muricatum* and *Phormium tenax* plants were grown at the HortaFCUL (http://hortafcul.wixsite.com/home), a permaculture demonstration field kept at the FCUL campus by the students. Chlorophyll fluorescence measurements were made in situ in 60 intact leaves of each species. All chlorophyll a (Chl a) fluorescence measurements were made using a Handy Plant Efficiency Analyzer (PEA) – Chlorophyll Fluorimeter (Hansatech Instruments, Kings Lynn, UK). Samples were dark adapted for 10 min by placing light-withholding leaf clips. Samples were then exposed to a saturating light pulse with sufficient intensity (3500 µmol m^{-2} s^{-1}) to ensure closure of all PSII reaction centres (RC) for 1 s in order to obtain the OJIP Chl a fluorescence transient rise. The kinetics of the rapid fluorescence rise was recorded and data was later downloaded for a personal computer into an Excel spreadsheet. Classifiers were built using the high-level programming language Python 3. Decision-tree classifiers from the Sky-Kit learn software were used. Two choosing criteria were tested: gini and entropy. The maximum depth of the trees, as well as the minimum number of samples in a leaf, was changed from 1 to 20 (Mitchell 1997). The maximum number of leaves was changed between 2 and 50. For increased precision tests were repeated 10 times and the mean result is presented. For the data from *Vitis riparia* the total samples (365) were divided in a training group (274) and a test group (91). Each sample had 118 attributes. The four RWC classes were the targets of classification. For the data from HortaFCUL the total samples (198) were divided in a training group (149) and a test group (49). Each sample had 118 attributes. The three plant species were the targets of classification.

RESULTS AND DISCUSSION

The percentage of success on classifying *Vitis riparia* leaves into the four RWC classes was always relatively low, ranging between 50 and 60% (Fig. 1a). This result may be compared with the expected success rate (31%) if the classification were totally random. The impact of the maximum depth of the trees on the percentage of success was very variable (Fig. 1a), impairing the determination of the optimal maximum depth of the trees. In contrast, it was possible to observe that the percentage of success increased with the maximum number of leaves until 10 and afterwards it remained constant (Fig. 1b), showing that the optimal maximum is 10. The gini criterion provided higher percentages of success than the entropy criterion (Fig. 1) and therefore should be adopted in future classifications. The percentage of success on classifying the assayed leaves into three horticultural species was much higher (Fig. 2a and b), averaging 90%. These preliminary results suggest, therefore, that the joint use of the chlorophyll fluorescence induction curve and artificial intelligence techniques in the context of high-throughput plant phenotyping is more promising for discriminating between plant genotypes than to classify plant water status.

ACKNOWLEDGEMENTS

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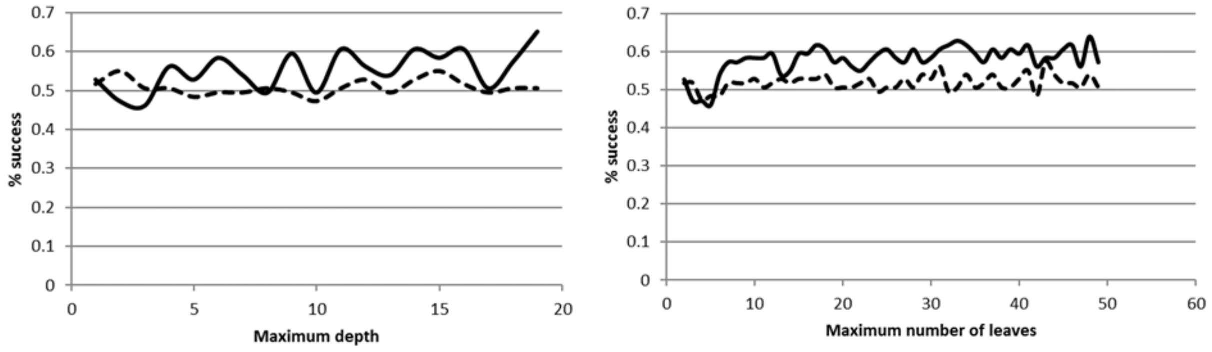

Figure 1. Percentage of success of the classification of *Vitis riparia* leaves in four RWC classes as a function of the maximum depth of the trees (a) and the maximum number of leaves on the trees (b); solid line represents the gini criterion; dashed lines represent the entropy criterion.

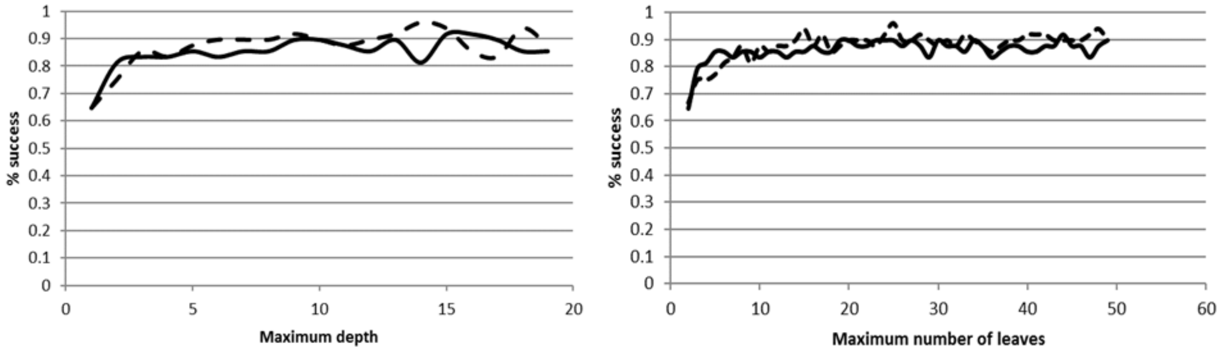

Figure 2. Percentage of success of the classification of assayed leaves into three horticultural species (*B. oleracea*, *S. muricatum* and *P. tenax*) as a function of the maximum depth of the trees (a) and the maximum number of leaves on the trees (b); solid line represents the gini criterion; dashed lines represent the entropy criterion.

Do metabolic changes underpin physiological responses to drought in *Medicago sativa* plants?

Molero, G.[1], Roca, R.[2], Mauve, C.[3], Cabrera-Bosquet, Ll.[4], Tcherkez, G.[3,5], Araus, J.L.[6], Nogués, S.[6], Aranjuelo, I.[7]

[1]International Maize and Wheat Improvement Center, CIMMYT, Int. Apdo. Postal 6-641, 06600 Mexico, DF, Mexico. E-mail address: G.Molero@cgiar.org
[2]Serveis Científico-Tècnics, Universitat de Barcelona, Barcelona, Spain
[3]Plateforme Métabolisme-Métabolome, Université Paris Sud-XI, Orsay, France
[4]UMR LEPSE, INRA, Montpellier SupAgro, 34060, Montpellier, France
[5]Research School of Biology, College of Medicine, Biology and Environment, Australian National University, Canberra, Australian Capital Territory 2601, Australia
[6]Unitat de Fisologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[7]Instituto de Agrobiotecnología (IdAB), Universidad Pública de Navarra-CSIC-Gobierno de Navarra, Campus de Arrosadía, E-31192-Mutilva Baja, Spain.

ABSTRACT

Drought is one of the environmental factors most affecting crop productivity under Mediterranean conditions. The effect of drought on plant performance has been widely described at leaf and nodule level. However, less attention has been addressed to the plant-nodule interaction and its implications in metabolites exchange during a regrowth period. For this purpose, physiological characterization and metabolite profiles of different plant organs and nodules were analyzed. In order to study in more detail how N metabolism was affected by water stress, the plants were labeled with N-enriched isotopic air ($^{15}N_2$) using especially designed chambers. Metabolite profile and isotopic composition analyses revealed that, drought induced major changes in the accumulation of amino acids (Pro, Asn, His, Lys and Cys), carbohydrates (sucrose, xylose and pinitol) and organic acids (fumarate, succinate and maleate) in the nodules in comparison with other organs. The lower ^{15}N-labeling observed in Ser, compared with other amino acids, can be related with its high turnover rate, which in turn, indicates its potential implication in photorespiratory process. Isotopic analysis of amino acids also revealed that Pro synthesis in the nodule was a local response to drought and not associated with a feedback inhibition from the leaves. It is clear that isotopic approaches in combination with metabolic profiling are powerful tools to study the turnover rates of various metabolic intermediates and to predict metabolic origin of the compounds as well as to understand metabolic responses to drought.

INTRODUCTION

The effect of drought at leaf level together with the sensitivity of plant-bacteria symbiosis to low water availability has been extensively studied (Galvez et al. 2005; Aranjuelo et al., 2011; Aranjuelo et al. 2014). However, comparatively less attention has been given to the effect of water availability in plant-nodule interaction and its implications in plant functioning and metabolites exchange. Indeed, some authors reported that the effect of water deficit is more limited by deleterious effect on N_2 fixation rather than by affecting the photosynthetic machinery. In that context, little is known about exchange of carbon (C) and nitrogen (N) metabolism between plant and nodules under stressed conditions (particularly drought). The study of plant metabolites and their fluxes can provide new insights on how these metabolites may confer plant-nodule interaction a better tolerance to water limitation in addition to underpin physiological processes involved. In this experiment, target specific compounds (soluble sugar, organic acids and especially the less characterized amino acids) were investigated together with physiological traits in nodulated alfalfa plants where drought was imposed during regrowth period.

MATERIAL AND METHODS

Plant material and water status and leaf gas exchange

Alfalfa plants (*Medicago sativa* L. cv Demnat) inoculated with *Sinorhizobium meliloti* strain 102F78, were grown in growth chambers at 25/15ºC (day/night) with a photoperiod of 14 hours and at 400 µmol m^{-2} s^{-1} PPFD. After 61 days, $^{15}N_2$ labeling was carried out during 5 days. Immediately after, a subset of control and labeled plants were harvested and frozen in liquid N (T0). The aboveground biomass of the remaining plants was removed and water withholding was imposed during regrowth period. A second harvest was performed 8 days after cutting (T8). Plants were separated into apical shoot (AS), primary root (PR) and nodules (Nod). Plant water status (relative water content, RWC in apical leaves) and osmotic potential (AS, PR and Nod) were determined before harvesting.

Photosynthetic rate of apical leaves was measured at 25ºC and saturating PPFD of 1200 µmol $m^{-2}s^{-1}$ using a LI-6400XT portable gas-exchange photosynthesis system (LI-COR, Lincoln, Nebraska, USA). The gas-exchange response to CO_2 (A/Ci) was measured using different CO_2 concentrations (0, 100, 200, 400, 700, 850, 1000 and 1400 µmol mol^{-1}). Estimation of the maximum carboxylation velocity of Rubisco (V_{cmax}) and the maximum electron transport rate (J_{max}) were calculated using the method of Ethier and Livingston, 2004. Stomatal limitation (l) was calculated according to Farquhar and Sharkey, 1982.

Metabolite analyses, isotope labeling and determination of $\delta^{15}N$ of amino acids

For HPLC analysis of amino acids and GC–TOFMS profiling, extracts were performed as described in (Molero et al., 2011). Metabolite derivatives were identified with MS databases and this enabled detection of 78 different metabolites that were quantified as described in Aranjuelo et al., 2011.

Enriched $^{15}N_2$ ($\delta^{15}N$ ~ 10‰) was injected in the hand-made chamber coupled to the pots using a gas syringe twice a day (two and five hours after the beginning of the daily light period) during 5 days. Non-labeled plants were grown in a second Conviron chamber maintained at ambient N_2 ($\delta^{15}N$ ~ 0.35‰) air conditions. Stable ^{15}N isotope composition ($\delta^{15}N$) of amino acids was determined as detailed in Molero et. al (2011) and analysed by Gas chromatography Isotope Ratio Mass Spectrometry (GC-C-IRMS).

RESULTS AND DISCUSSION

After 6 days of withholding water, even the reduction of RWC, no significant effect on total plant biomass was observed while a significant increase in nodules biomass and marginally significant decrease in primary root was present (Table 1). Gas exchange determinations (Table 1) revealed that drought decreased photosynthetic related traits between 25 to 55% as a consequence of stomatal (as revealed by stomatal limitation data) and non-stomatal processes (confirmed by the diminishment on Vc_{max}, and J_{max}. Comparison of metabolite profiles between WW and WS, showed that 15, 11 and 23 compounds were significantly different for AS, PR and Nod respectively (Figure 1). The lack of differences in leaf osmotic potential (Ψ_s) suggests a low osmoregulatory response to drought supported by the relative low accumulation of metabolites with osmoregulatory role (e.g. Pro, sucrose, myo-inositol). Conversely, in nodules, the significant decrease in Ψ_s with drought was also accompanied by a large increase in Pro and sucrose levels. These results suggest a higher metabolic adjustment in the nodule compared with leaves as a response to drought. In leaves, sucrose levels remained unaltered under drought conditions. In the case of nodules, amino acid caontent decreased with drought: Arg, Asn, Gln, Glu, Gly, Lys, Met, Orn, Ser and Thr.

$^{15}N_2$ labeling provided relevant information on N metabolism. After labeling (T0), PR was the organ containing the most labeled amino acids followed by Nod (Figure 2). At T8 labeling in amino acids was lower under well watered conditions than under water stress. In general, ^{15}N-enrichment was lower in Nod for well water and water stressed plants. Eight days after labeling, under well watered conditions Asp and Asn in Nod and Ser in AS were low labeled in comparison with other amino acids. For water stressed conditions low labeling in Pro was detected in AS and Nod. In general isotopic composition of amino acids between plant and nodule showed different values, only Ala from AS and Nod under well watered conditions presented a similar ^{15}N-enrichment. In general terms, the metabolic and isotopic characterization revealed that roots were the organ where fewer differences were detected associated to water withholding explained by the role of this organ as a N and C compound storage.

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FIGURES AND TABLES

	WS vs. WW
Plant growth	
Total biomass (g DW)	=
Apical shoot (g DW)	=
Primary root (g DW)	↓†
Nodule (g DW)	↑*
Water status	
Leaf RWC (%)	↓*
Apical shoot Ψs (MPa)	=
Primary root Ψs (MPa)	=
Nodule Ψs (MPa)	↓*
Gas exchange	
A (μmol m^{-2} s^{-1})	↓*
Vc_{max}(μmol m^{-2} s^{-1})	↓*
J_{max} (μmol m^{-2} s^{-1})	↓*
l (%)	↑*
g_s (mmol H_2O m^{-2} s^{-1})	↓*

Table 1. Changes in plant growth, water status and leaf gas exchange comparing water stressed (WS) with well watered plants (WW) 6 days after beginning of water withholding.

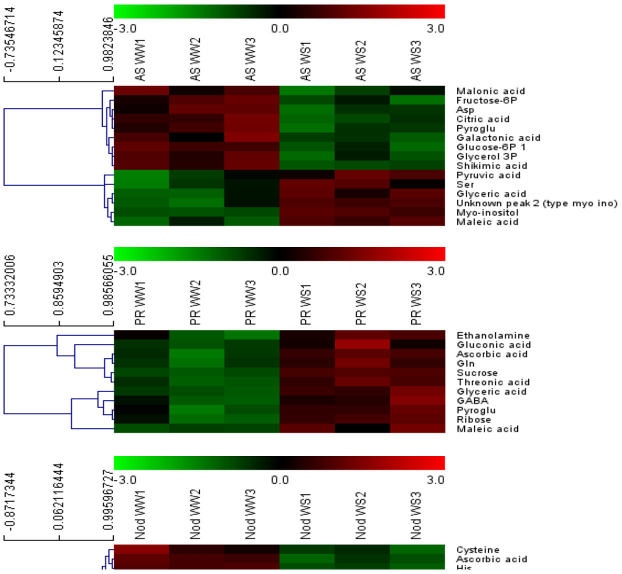

Figure 1. Hierarchically clustered 'heatmaps' of the mean centered-reduced values of metabolites that were found to be significantly different between WW and WS. Intensity of red and green indicates increase and decrease relative to the mean, according to the color scale at the top.

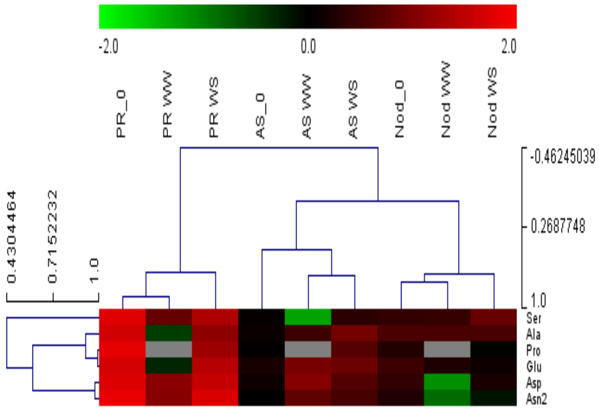

Figure 2. Isotopomic representation of ^{15}N-enrichment in most relevant amino acids.

Unraveling the root response to drought: functional analysis of the taproot and fibrous roots of *M. truncatula*

Verónica Castañeda[1], Amaia Seminario[1], Lidia Azcárate[1], Marlon de la Peña[1] and Esther M. González[1]

[1]Departamento de Ciencias del Medio Natural, Universidad Pública de Navarra, 31006 Pamplona, Spain.
veronica.castaneda@unavarra.es

ABSTRACT

Plant root systems consist of different types of roots, and the understanding of the role and contribution of each root type to the whole root system is essential for explaining its functioning. Comparative physiological, enzymatic, respiratory and amino acid composition studies of the *Medicago truncatula* taproot and fibrous roots revealed physiological and metabolic differences between both root types and in their respective response to drought. In all parameters determined the response of the taproot was less pronounced than that of the fibrous root, with faster and steeper changes in response to drought stress. Water deficit led to a general decline in the respiration rate, protein and starch contents, while the level of soluble sugars and free amino acids increased in both root types. Taking all together, these results indicate a more resistant nature of the taproot than the fibrous root under drought conditions and a more active metabolism of the taproot than the expected for a reservoir organ. These data provide new information on the different role, physiology and metabolism of the taproot and fibrous root and in their distinct performance upon drought, which can be used to improve breeding programs for increasing root efficiency under adverse conditions.

INTRODUCTION

Legumes such as *Medicago truncatula* are characterized by their high nutritional value for humans and livestock and by their ecological value as nitrogen suppliers through their symbiosis with nitrogen-fixing bacteria. These advantages are however compromised by their low ability to adapt to adverse environmental conditions such as drought.

Roots are not only the part of the plant responsible for the absorption of water from the soil but the first organ encountering water-deficit stress. However, most studies regard the root system as a homogeneous organ, without distinguishing between root types. For example, dicotyledoneous plants have a taproot (TapR) from which lateral roots (or fibrous roots (FibR)) branch, being the first ones responsible for the uptake of water and minerals, and the latter ones responsible for the effective distribution of the lateral roots in the soil profile and the transport of water and minerals to the aerial part. One of the various ways to improve plant stress tolerance is the anatomical-morphological adjustment of the plant roots. The aim of this study was to determine the effect of drought on the TapR and FibR of *M. truncatula* and to better describe the physiological and biochemical characteristics of both root types. This information could be used by plant breeders in order to boost plant tolerance to drought stress through roots.

MATERIALS AND METHODS

Medicago truncatula seedlings were grown for six weeks under controlled conditions and irrigated with Evans medium. Drought stress was then imposed by water withdrawal and when the leaf water potential (Ψw) of the drought stressed plants reached the desired value ((for moderate drought (MD) Ψw= -1.50 ± 0.02 MPa) and for severe drought (SD) Ψw= -2.5 ± 0.04 MPa), the plants were harvested. Control plants showed an average Ψw of -0.40 ± 0.01 MPa. Root respiration was measured using a Clark-type O_2 electrode. The amino acid, soluble sugar and starch contents were performed as described (Gil-Quintana et al., 2013; Marino et al., 2006). The protein content was determined by the Bradford assay and enzymatic activities were determined as the oxidation/reduction of NAD(P)(H) nucleotides at 30 ºC and 340 nm for 15 min as previously described (Gibon et al., 2004). Bars in figures represent the mean ± SE (n =10-15) and the statistical differences (Student t-test $p \leq 0.05$) are represented by an asterisk and/or a hash for differences between treatment and control and/or between treatments, respectively.

RESULTS AND DISCUSSION

The taproot and the fibrous root differ both anatomical and physiologically

In *M. truncatula*, the taproot (TapR) and the fibrous roots (FibR) emerging from it are the basic components of its root system. The thicker and rigid TapR had a lower water content than the more delicate and flexible FibR (Fig. 1), the latter having been described as the main responsible for water uptake, with the TapR having a more “connecting” role between the fibrous root and the shoot. However, the TapR metabolic characteristics observed in the current study suggest that this root type plays a more active role in the plant than just as reservoir organ for posterior regrowth or as FibR-shoot connection. The high content of starch and sucrose (Fig. 2) together with the enhancement of sucrose synthase (SS) in this root type suggest a role on carbon partitioning (Fig. 3A). Total respiration rates (Fig. 3B) also indicate that an active carbon metabolism is taking place in this root type.

Both root types exhibit some common responses to drought stress

Even though the FibR reduced its water content to a higher extent than the TapR, the onset of drought stress led to common physiological responses in both root tissues. Thus, total root respiration declined when plants were exposed to water deficit stress (Fig. 3B) as previously reported for other plant species (Hong & Feng, 2005). The root is a major consumer of the carbon fixed through photosynthesis, using it for growth and maintenance. Photosynthesis reduction upon drought stress has been widely shown in different plant systems, so it might limit carbon availability in the root and therefore, plant growth and development (McDowell, 2011). However, the high availability of sucrose observed in the root (Fig. 2A) indicates that the decline in root respiration is a drought stress tolerance mechanism rather than a negative consequence of water deficit stress. In this context, the sharp decline in SS (Fig. 3A) indicates a point of regulation described for the first time in roots, as described for nodules (Gonzalez et al., 1995), which would avoid respiration overloading. The observed amino acid accumulation (Fig. 4B) is a common response towards water deficit stress in various plant species and organs, participating in the osmotic adjustment and thus allowing water to move from the soil to the plant (Rouached et al., 2013).

The taproot is more resilient to drought stress than the fibrous root

Several specific features reveal that the TapR may play a different role in drought stress response. The TapR was able to keep a higher water content than the FibR, and all physiological parameters measured such as carbon and nitrogen metabolite content, respiration rates and enzyme activities (Fig. 1-4) showed a better resilience towards water deficit stress in the TapR. The higher content of compatible solutes (sucrose and amino acids) (Fig. 2A, 4B) also show the better drought stress tolerance ability of the TapR. The accumulation of both soluble sugars and amino acids do not come from *in situ* synthesis but rather from export from other plant tissues such as leaves. Indeed, the amino acid transport from leaves to the roots has been previously described (Aranjuelo et al., 2011). The TapR, being the root type most in contact with the shoot, would make the most out of this nutrient transport to the roots, providing this root type with fast and cheaper weapons to tolerate water deficit stress, while the FibR seems to take less benefit from it.

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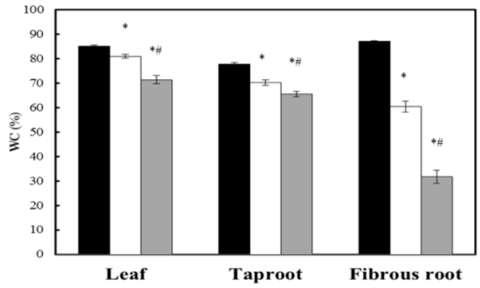


Figure 1. Water content (%) of the leaf, TapR and FibR.

■ **Control conditions**
□ **Moderate drought**
▨ **Severe drought**

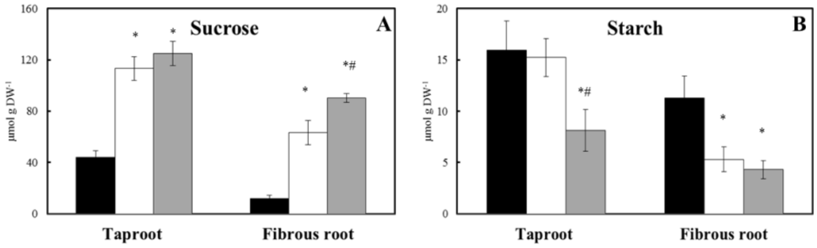
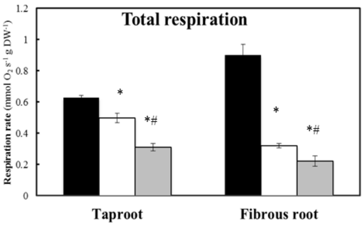


Figure 2. Changes in sucrose (A) and starch (measured as glucose (B) units) (in µmol g DW^{-1}).



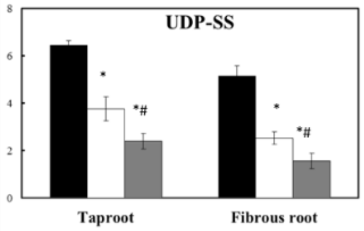
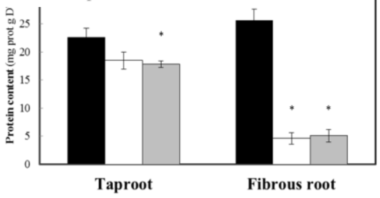


Figure 3. UDP-sucrose synthase (A) and total root respiration (B) measured in the taproot and fibrous



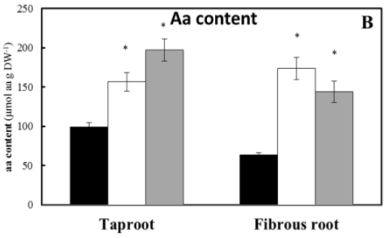


Figure 4. Protein (A) and amino acid (B) content of the taproot and fibrous root.

Interactive effects of IAA application and arbuscular mycorrhizal symbiosis on root hydraulic conductivity of droughted maize plants

Erice, G., Quiroga, G., Aroca R, Ruíz-Lozano, JM.

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Profesor Albareda nº1, 18008 Granada, Spain.

ABSTRACT

The knowledge of drought stress physiology is of increasing interest as water is becoming limiting in a climate change scenario. The present work deals with the relationship between root hydraulic conductivity and aquaporins in maize plants inoculated with arbuscular mycorrhizal (AM) fungi submitted to drought and the interaction with external IAA application. Root hydraulic conductivity was correlated with PIP1 and PIP2 aquaporins abundances. Interestingly droughted plants were more sensitive to IAA treatment but contrasting results were obtained regarding the AM inoculation. Non-inoculated stressed plants showed increased root hydraulic conductivity in the presence of IAA whereas inoculation led to significant decreases. The application of the IAA-synthesis inhibitor, 6-FI, partially reversed this effect.

INTRODUCTION

One of the greatest challenges for the future is the production of sufficient food for a growing population. Larger cultivable areas will be required and climate change scenarios will oblige to optimize resource management, including water. Thus the knowledge or drought physiology to improve stress tolerance is of capital importance. Aquaporins, as membrane intrinsic proteins, facilitate the passive movement of water (Maurel et al., 2008). They comprise a large and diverse family (up to 71 genes) with different isoforms and cell localizations making its study complex. This fact together with species-dependent behavior results on elusive relationship between aquaporins and plant responses to water (Chaumont and Tyerman, 2014). In this sense symbiosis with arbuscular mycorrizhal (AM) fungi has been also described to enhance plant stress tolerance (Ruiz-Lozano et al., 2012) possibly altering root hydraulic properties (Bárzana et al., 2012). Previous work revealed evidences of AM dropping root hydraulic conductivity through auxin modulation (Sánchez-Romera et al., 2014). The aim of the present work is to elucidate if AM inoculated plants have higher flexibility controlling root hydraulic properties via aquaporin regulation and if IAA may modulate this response.

MATERIAL AND METHODS

Maize plants corresponding to a drought sensitive genotype PR34B39 (Pioneer Hi-Bred, Spain) were inoculated or not with the AM fungus *Rhizophagus irregularis* and grown in 1 L pots (soil/sand; 1:9) for 8 weeks. Drought was applied for 2 weeks by irrigating plants with half the water volume of well-watered ones (25 vs. 50 mL). Hormonal treatments were applied 6 hours previous to harvest with 50 mL of water (control), 20 µM AIA, or 75 µM 6-FI.

Osmotic root hydraulic conductivity (Lo) was measured using the free exudation method (Benabdellah et a., 2009). Under these conditions water circulated through root following the osmotic gradient between a bathing solution and the xylem. Water mostly flows through the cell-to-cell pathway (Steudle and Peterson, 1998). Exuded sap was collected for 2 h and osmolality measured for Lo calculation. Hydrostatic root hydraulic conductivity (Lpr) was measured using a Scholander pressure chamber. Stems of maize plants were cut 3 am above the root and the root system placed into the pressure chamber. Pressure was increased gradually at 2-minutes intervals (0.2, 0.3, 0.4 MPa) and the exuded sap was collected and weighted. Sap flow was plotted against pressure, with the slope being the Lpr. Relative apoplastic water flow was calculated using a high-molecular weight dye (Ligth green SF yellowish), which has the ability to move apoplastically but not symplastically (Bárzana et al., 2012). Isolated microsomal membranes were used for aquaporin semiquantification by ELISA using antibodies that recognize several PIP1 and PIP2 proteins (Aroca et al., 2007) and three antibodies that recognize the phosphorilation of PIP2 proteins at their C-terminal region: PIP2A (Ser-280), PIP2B (Ser-283) and PIP2C (Ser-280, Ser-283).

RESULTS AND DISCUSSION

The effects of the different compounds applied were more evident in plants subjected to drought than in well-watered plants. Thus, well-watered plants did not show significant differences in root hydraulic conductivity (osmotic or hydrostatic) by application of IAA or its inhibitor, 6-FI (Figure 1). Among these plants, non AM ones featured higher abundance of PIP1 aquaporins in the presence of IAA or 6-FI and higher PIP2 levels in 6-FI treated plants (Figure 2). When compared the phosphorilated proteins, they showed a trend for higher abundance after hormonal application with significant increases of PIP2A (IAA and 6-FI) and PIP2C (6-FI) (Figure 2). It is also remarkable that the hormonal application did not provoke changes in the percentage of water circulating by the apoplastic route (Figure 1). When well-watered plant were inoculated with the AM fungus, absence of response for root hydraulic conductivity by hormones was confirmed, but the percentage of water circulating by the apoplastic route drop in the presence of 6-FI (Figure 1). This was not related to the amount of the aquaporins measured. In fact, it could be said that the abundance of such protein channels in well-watered inoculated plants were more unresponsive to hormonal treatments than in non-inoculated plants. Only PIP2 abundance significantly decreased after IAA irrigation (Figure 2).

Opposite to these results, root hydraulic conductivity of droughted plants showed to be more sensitive to hormonal treatments, with different behaviour when inoculated with AM. All non-inoculated plants showed significant increase in Lpr despite of the drop of Lo, the osmotic conductivity component, probably linked to the increase of the apoplastic route (Figure 1). The addition of 6-FI helped to recover the steady levels of Lpr (Figure 1) but these changes were not directly associated to individual changes in any of the measured aquaporins (Figure 2). AM plants submitted to drought were able to maintain Lpr after IAA application despite the decrease of Lo and apoplastic pathway. Interestingly the measured plant aquaporin abundance of droughted plants was less altered by the AM inoculation or hormonal treatments (Figure 2). Taking into account all treatments, PIP1 and PIP2 protein abundance was positively correlated with root hydraulic conductivity measurements, Lpr and Lo (Table 1). The percentage of water flowing through the apoplastic route was also directly related to changes in root hydraulic conductivity (Table 2).

In summary, radial water flow was shown to be differently regulated under well-watered or water deficit conditions. Droughted plants were more prone to changes in root hydraulic properties due to IAA levels. It is highlighted that under water stress AM inoculation made plants respond differently to IAA application. Even if there was correlation between aquaporins and root hydraulic conductivity, the abundance of measured plant aquaporins does not fulfil the Lpr results. Further research is needed to confirm the modulation of radial water flow by AM including plant and fungal aquaporin gene expression.

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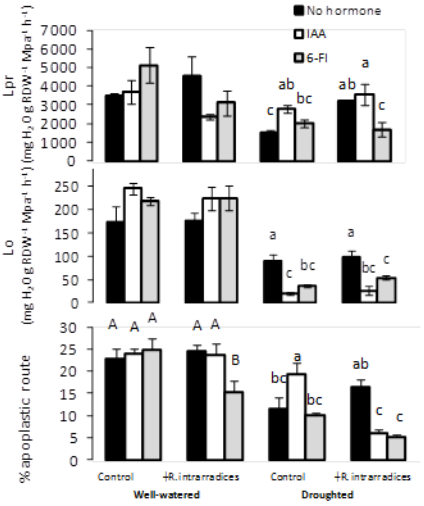

Figure 1. Effect of IAA and 6-FI in Lh, Lo and the percentage of apoplastic route of maize plants inoculated with AM and submitted to drought.

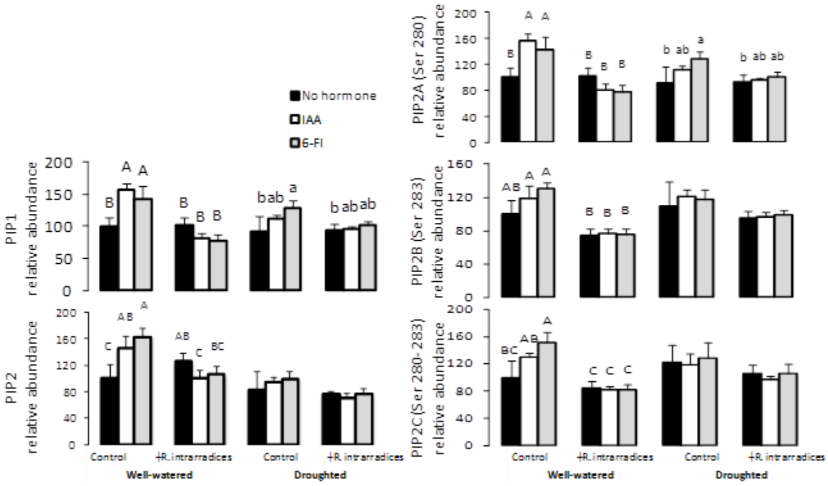

Figure 2. Effect of IAA and 6-FI in PIP1, PIP2, PIP2A, PIP2B and PIP2C relative abundance of maize plants inoculated with AM and submitted to drought.

Table 1. Correlation between Lpr, Lo and % apoplastic route with the abundance of measured aquaporins of maize plants.

		PIP1	**PIP2**	**PIP2A**	**PIP2B**	**PIP2C**
Lpr	Pearson Correlation	0.688*	0.694*	0.375	0.044	0.117
Lo	Pearson Correlation	0.693*	0.717**	0.144	-0.259	-0.111
% apoplastic	Pearson Correlation	0.564	0.74**	0.294	-0.001	0.057

Regulation of aquaporins and root hydraulic properties by SA in arbuscular mycorrhizal maize plants subjected to drought

Quiroga, G., Erice, G., Ruíz-Lozano, J.

Estación Experimental del Zaidín-CSIC, C/Profesor Albareda 1, 18160 Granada; gabriela.quiroga@eez.csic.es

ABSTRACT

Water uptake and transport in plants need to be finely regulated to ensure growth and development when environmental conditions are adverse. During water shortage, some physiological changes occur in order to prevent water loss, and the knowledge of these mechanisms is crucial for improving agriculture in a changing environment. In the present work, we studied root hydraulic properties and aquaporin regulation in maize plants subjected to drought stress and inoculated with an arbuscular mycorrhizal (AM) fungus, as well as, the effect of external application of salicylic acid (SA). As expected, Lpr was affected by drought and by the AM symbiosis. Salicylic acid (SA) decreased root hydraulic conductivity under water stress, and its inhibitor 2-aminoindan-2-phosphonic acid (AIP) recovered Lpr to control levels. In any case, AM plants maintained higher root hydraulic conductivity than non AM plants. The percentage of water flowing by the apoplastic pathway increased after SA application in non-AM plants, while it decreased in AM plants. There was a negative correlation between Lpr and PIP1 protein accumulation. However, measured aquaporin contents alone cannot explain changes in Lpr, and expression patterns of specific aquaporins should be measured.

INTRODUCTION

Cultivable soils are progressively drying worldwide, thus, a better understanding of the mechanisms that help to improve plant water status during water stress would lead into a more efficient agriculture. As an alternative to the use of inorganic fertilizers, arbuscular mycorrhizal (AM) fungi (which establish a mutualistic relationship with most crop plants) have been described to improve water and nutrient uptake, enhancing tolerance to abiotic stresses such as drought (Ruiz-Lozano et al., 2012). This melioration is achieved by allowing the access to distant water from the soil, and by altering root hydraulic properties (Bárzana et al.; 2012).According to the composite model (Steudle & Peterson, 1998), radial water and solute transport occurs through the symplastic (via plasmodesmata), transcellular (across the cell membranes) and apoplastic (via the cell wall continuum) pathways. Aquaporins (AQPs), a highly conserved protein family with 36 isoforms in maize (Chaumont & Tyerman, 2014), play an important regulatory role in the cell-to-cell pathway (symplastic and transcellular pathways). Within this protein family, water channel activity is mainly found in the PIP2 subfamily (Maurel et al.; 2008). Root transport capacity can be estimated by measuring root hydraulic conductivity (Lpr), whose behaviour is regulated partially by aquaporins. Previous work revealed that AM symbiosis could modulate Lpr, aquaporin gene expression and protein accumulation (Bárzana et al. 2014). Moreover, SA was also found to modify Lpr (Calvo-Polanco et al.; 2014, Sánchez-Romera et al.; 2014) and was identified as a possible aquaporin regulator (Boursiac et al.; 2008). To elucidate the role of SA on root water transport in AM maize plants subjected to drought stress, root hydraulic properties were measured and correlated with PIP1 and PIP2 protein abundance after exogenous application of SA.

MATERIAL AND METHODS

Plant material and growth conditions

Zea mays L. seeds from the drought sensitive genotype PR34B39 (Pioneer Hi-Bred, Spain, SL) were inoculated or not with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and grown in 1 L pots with soil/sand (1:9) for 8 weeks. A drought stress treatment was applied for 2 weeks, by irrigating plants with half the water volume of well-watered ones (25 vs. 50 mL). SA 20 μM and AIP 75 μM (an inhibitor of SA activity) were applied 6 hours before harvesting. Dose of the phytohormone and exposure time needed to affect root hydraulic conductivity were established in previous experiments.

Osmotic root hydraulic conductivity (*Lo*): Lo was measured on detached roots exuding under atmospheric pressure by the free exudation method (Benabdellah et al.; 2009). Under these conditions, water is only moving following osmotic gradient. Therefore, the water would be transported trough the cell-to-cell path (Steudle & Peterson, 1998). The exudate was collected after 2 hours and weighed. The osmolarity of the exuded sap and the nutrient solution was determined using a cryoscopic osmometer and used for *Lo* calculation.

Hydrostatic root hydraulic conductivity (Lpr). Measured with a Scholander pressure chamber. A gradual increase of pressure (0.2, 0.3 and 0.4 MPa) was applied at 2-minutes intervals to the detached roots. Sap was collected at the three pressure points. Sap flow was plotted against pressure, with the slope being the *L* value.

Relative apoplastic water flow. Relative changes in apoplastic water flux were estimated using the high-molecular weight dye light green SF yellowish, which has the ability to move apoplastically but not symplastically (Bárzana et al.; 2012). Detopped root systems were immersed in 250 μM light green dye inside the pressure chamber. The concentration of the dye in the whole collected sap was determined at 630nm.

Microsome isolation and ELISA**.** Microsomal fraction isolation and ELISA were performed as described previously by Calvo-Polanco et al.; 2014. We used, as primary antibodies, the two antibodies recognizing most of the PIP1 and PIP2 subfamilies and three antibodies that recognize the phosphorylation of PIP2 proteins in the C-terminal region: PIP2A (Ser-280), PIP2B (Ser-283) and PIP2C (Ser-280/Ser-283).

RESULTS AND DISCUSSION

1. Hydraulic properties

As expected from previous results, the AM symbiosis enhanced Lpr and Lo in plants under drought stress, reaching values similar to well-watered conditions. SA affected both Lpr and Lo under water deficit conditions, reducing their levels in non-inoculated and AM plants, while AIP recovered the effect of SA to control values (Figure 1). However, under well-watered conditions the response of non AM plants to SA was different, producing a sharp increase of Lpr that cannot be explained alone with the results from Lo or % apoplastic route. Thus further research is needed to unravel the mechanisms behind this effect. The effect of SA application on apoplastic water flow was opposite in AM and non-AM plants, decreasing in AM plants but increasing in non AM ones (Figure 1). It was revealed the imbalance between the rise of the percentage of water through the apoplastic pathway and the decrease of Lo. Thus, Lpr resulted unaltered by the hormonal treatment in non-AM plants.

2. PIP1 and PIP2 protein abundance

We measured PIP1 and PIP2 proteins abundance, as well as the PIP2 phosphorylation state in roots, as aquaporin water channel activity depends on this post-translational modification. Hormonal treatment did not significantly affect protein levels in both well-watered and droughted plants, neither the protein phosphorylation (Figure 2). No clear trend in protein accumulation was observed in response to any of the treatments applied. This behaviour could be due to the presence of different aquaporin isoforms in each PIP subgroup, which are accomplishing different functions in roots. Thus, following steps include the study of the expression patterns of each PIP isoform, in order to elucidate their specific role under water shortage. Moreover, inmunolocation of proteins in the cell could also give essential information about protein performance. When comparing hydraulic properties with protein levels, we found that abundance of PIP1 and PIP2 phosphorylated at Ser-280, were negatively correlated with Lpr. Lo was also negatively correlated to PIP1 and PIP2 accumulation (Table 1).

In conclusion, we can say that water transport in plants is differently regulated under well watered and drought stress conditions, with AM symbiosis having a clear effect in the recovery of water status. SA application affects water properties, with a different plant response due to the presence of the fungus. Further research is needed to understand the relationship of aquaporins with the modulation of water flow in roots.

ACKNOWLEDGEMENTS

Technical assistance from Sonia Molina and Francisca López is well appreciated. This work was financially supported by Ministerio de Economía y Competitividad (Project AGL2014-53126-R). G. Quiroga is FPI PhD Grant Holder.

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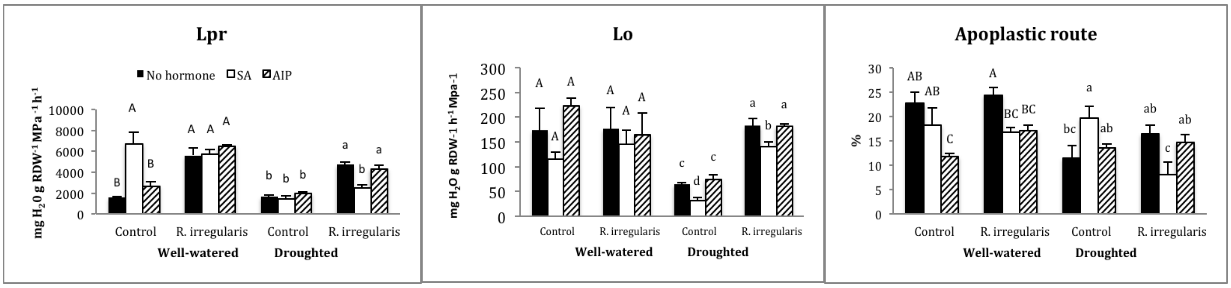

Figure 1. Lpr, Lo and Apoplastic route. Columns represent means +/- standard errors (n=3). Different Letters mean significant differences (p<0,05) inter-treatments (One way ANOVA/LSD test)

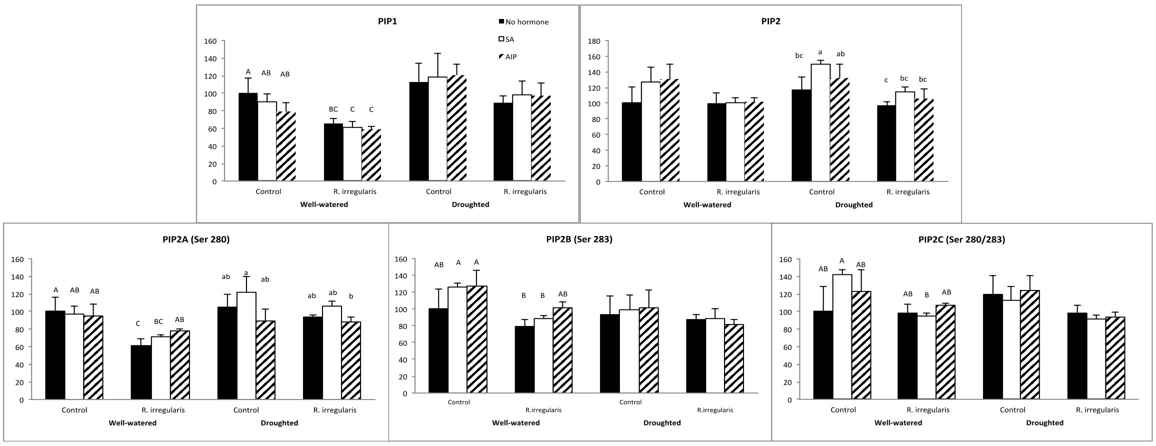

Figure 2. PIP1, PIP2, Ph-PIP2A, Ph-PIP2B and Ph-PIP2C protein abundance in roots of non-mycorrhizal and mycorrhizal maize plants, subjected or not to drought stress. Means (n=5)±SE. Different letters mean significant differences (p<0,05) among treatments (One way ANOVA/LSD test).

Table 1. Pearson’s correlation coefficient between Lpr, Lo and % of apoplastic water with the abundance of PIP aquaporins. * *p*<0.05, ** *p*<0.01

	PIP1	**PIP2**	**PIP2A**	**PIP2B**	**PIP2C**
Lpr	-0,786**	-0,47	-0,681*	-0,01	-0,023
Lo	-0,660*	-0,627*	-0,514	-0,008	-0,381
% apoplastic	-0,281	-0,241	-0,241	-0,126	-0,086

SESIÓN II

Relaciones hídricas en especies de interés agrícola

Precision irrigation or the need to join forces

Fernández, J.E., Padilla-Díaz, C.M., Hernández-Santana, V., Díaz-Espejo, A., Cuevas, M.V.

Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain. jefer@irnase.csic.es

ABSTRACT

Precise irrigation is one of the most promising approaches to manage irrigation in fruit tree orchards, but it is challenging to the user. In this work we use examples of the work carried out in our group (www.irnas.csic.es/rec) to outline and illustrate the need for a combined work of physiologists and agronomists to develop more efficient irrigation strategies and irrigation scheduling methods. We also consider the need of their contribution, together with that of specialists on economics, for a wise choice of the production target.

INTRODUCTION

Precision irrigation (PI) is a holistic approach for a rational use of water in agriculture (Smith and Baillie, 2009). Within the PI framework, the aim is to irrigate each plant with the right amount of water and at the right time. The base for PI is an optimum combination of the irrigation system, the irrigation strategy and the irrigation scheduling method, as well as a correct choice of the production target. The complexity behind a rational use of water in agriculture (Geerts and Raes, 2009, Fernández, 2014a) advices for a joint work of specialists on hydraulics, physiology, agronomy, electronics, data processing and transmission, and economy, among other disciplines (Fernández, 2014b).The aim of this work was to outline the need for a multidisciplinary approach to overcome the challenges inherent to PI.

THE IRRIGATION STRATEGY

A number of irrigation strategies have been developed, from full irrigation to supplementary, or complementary, irrigation. Both sustained deficit irrigation and regulated deficit irrigation are recommended for fruit tree orchards (Ruiz-Sanchez et al., 2010; Fernández, 2014a). At the beginning of 2010 we started a research line focused to irrigation management in hedgerow olive orchards with high plant densities. By that time we made a literature review to design an irrigation strategy suitable for our experimental orchard (cv Arbequina, 1667 trees ha^{-1}). The irrigation strategy was published in a paper in which we showed results from the first three experimental years (Fernández et al., 2013). In the Discussion of that paper we suggested the need for changes in our irrigation strategy, based on recent findings by Hammami et al. (2011), among others, on olive fruit development. Basically, the group of Hammami, led by Prof. Hava Rapoport, studied the influence of water stress on cell division and growth of different olive fruit tissues, which have marked consequences on the fruit size and, consequently, on the net incomes of the grower. The right irrigation strategy for olive is still a matter of debate, since there uncertainties remain on the crop sensitivity to water stress at different stages of the productive cycle (Fernández, 2014a).

IRRIGATION SCHEDULING

Uncertainties

The need of physiological knowledge to understand how plants use water applies to even basic processes, e.g. how plants take up water from the soil. Zimmermann et al. (2004) questioned the cohesion-tension theory (C-T theory) and proposed the multi-force theory of water ascent in trees. Basically, they claimed the tension generated by leaf transpiration is not the only force involved in water lifting from the roots to the leaves. Instead, several forces of physical and chemical origin act together. This proposal by Zimmerman was criticized in a letter to the Editor by 45 top scientists on plant hydraulics (Angeles et al., 2004). Recent evidence, summarized by Bentrup (2016), suggests that the C-T theory does not give a satisfactory explanation of how water travels from the roots to the leaves, and gives credit to the multi-force theory. One of the aspects of Zimmermann's theory is that readings with the Scholander chamber are misleading. He claimed that the actual xylem tension is much lower than that recorded with the Scholander chamber, and that the chamber measures relative changes in turgor pressure, but not absolute values. Strikingly enough, therefore, the information provided by the widely used Scholander chamber is still questioned.

Another key feature of plant hydraulics related to water uptake is vulnerability to cavitation. Here, again, there is a lack of consensus in the scientific community. Xylem cavitation is characterized by vulnerability curves (VC), which relate xylem pressure with the percentage of cavitation. The literature provides two types of VC, "sigmoidal" (s-type) and "exponential" (r-type), the shape depending on the method used to generate it (Cochard et al., 2013). The problem is that there is a lack of consensus on the method that should be used. Exponential curves largely overestimate the vulnerability to cavitation as compared to sigmoidal curves, so there is not a consensus on something as basic as the effect of xylem tension on the loss of hydraulic conductivity. In olive, we compared different methods to generate VC and found that the "sigmoidal" type seems to be more representative for the species (Torres-Ruiz et al., 2014), which is in accordance with its high resistance to cavitation (Fernández, 2014a).

Errors in irrigated agriculture also come from a wrong choice of the production target. Many believe that full irrigation (FI) increases water use efficiency (WUE), i.e. the amount of biomass produced per unit of water consumed by the crop. But the standard curve of WUE vs. g_s, applicable to most crops, shows that the greatest values of yield per unit of water consumed are achieved when the crop water consumption is far below the maximum (Cifre et al., 2005). Another factor often forgotten is the influence of irrigation in quality. Our regulated deficit irrigation (RDI) treatments, in fact, led to oils with greater quality than those from the FI trees. Actually, oils from the RDI treatments had higher contents of pigments and phenolic compounds, a higher oleic/linoleic ratio and greater oxidative stability, among other characteristics affected by irrigation (García et al., 2016). In these cases in which the quality of the marketable product counts, the water productivity (WP), defined as the net income achieved per unit of water consumed by the crop, could be a more appropriate production target than WUE.

Physiology to the rescue

Precise irrigation requires an effective irrigation scheduling method based on the continuous and precise monitoring of the plant water stress. Stomatal conductance (g_s) is considered as one of the most reliable indicators of water stress (Jones, 2007), mainly because of the early response of stomata to stressing conditions and because it regulates both transpiration and carbon uptake, two processes highly related to water productivity. But we are still far from knowing in detail how stomata work (Chaves et al., 2016). In addition, the continuous recording of g_s is not easy under field conditions, which explains why g_s is not widely used to schedule irrigation in commercial orchards. Recently, however, Hernandez-Santana et al. (2016) suggested a method for the assessment of g_s in fruit tree orchards from continuous and automatic records of sap flux density (J_s) in the outer rings of the sapwood.

Other examples on how physiological advances lead to improvements on irrigation scheduling have been provided by researchers working with other plant-based methods to schedule irrigation, such as those based on sap flow, trunk diameter and leaf turgor related measurements. These three methods have a high potential to schedule irrigation in commercial orchards, and are useful for precise irrigation because they allow for continuous and automatic data recording and transmission, and because. In addition, they can be combined with remote imagery, to schedule irrigation in zones within the orchard with different sensitivity to water stress. None of the methods, however, has been widely adopted by the growers, likely because environmental and crop conditions have an influence on the recorded data, such that interpreting the collected information requires training (Fernández 2014b). The most recent of these three methods is that based on leaf turgor potential measurements. Until recently, leaf turgor measurements were possible in the laboratory, but difficult under field conditions. This changed with the Leaf patch clamp pressure probe, named as LPCP or ZIM probe, developed by Zimmermann et al. (2008). Our group was the first to test the ZIM system in a commercial olive orchard, as part of the experiments starting in 2010 (Fernández et al., 2011). Since then, we have published a number of papers, some from a joint work with the group that developed the system. Comparisons with the Scholander chamber and with the cell turgor pressure probe (Fernández et al., 2011; Ehrenberger et al., 2012) allowed us to propose an approach suitable to irrigate high density olive orchards just from a visual analysis of the daily curves recorded by the probes. The approach, tested by Padilla-Díaz et al. (2016), is an example of how the combination of physiological and agronomical studies can provide useful tools for the precise irrigation of commercial fruit tree orchards.

CONCLUSIONS

New irrigation approaches, such as precise irrigation, advices for a joint work of physiologists and agronomists. Advances on both disciplines must be combined to increase the rationale of water use in agriculture. Despite of their high potential, widely accepted tools and methods to monitor water stress and to schedule irrigation still have dark aspects that must be elucidated, both for improving our understanding on how plants use water and to increase their applicability to commercial orcchards.

ACKNOWLEDGMENTS

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Quantifying the photosynthesis limitation imposed by deficit irrigation in olive trees with a novel methodology based on measurements of sap flux density

Hernández-Santana, V., Fernández, J.E., Gutiérrez-Gordillo, S., Montero, A., Pérez-Arcoiza, A., Raya-Sereno, M.D., Díaz-Espejo, A.

Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain. virginiahsa@irnas.csic.es

ABSTRACT

With the ultimate goal of achieving more resilient agricultural systems through deficit irrigation practices managed with a reliable and sensitive indicator of water stress, we conducted an experiment in olive trees with the following specific objectives: (1) to calibrate and validate a method to estimate stomatal conductance (g_s) and photosynthesis (A_N) automatically based on sap flux density measurements (J_s) measurements and (2) to quantify the A_N limitations produced by soil water content and vapor pressure deficit in a super-intensive olive orchard. Results show that g_s can be determined from J_s measurements directly and that the approach is sensitive enough to allow us inferring how stomata respond to soil water content controlled by irrigation and vapor pressure deficit and quantify their limitation on A_N. The main advantages of this method are that it provides absolute values of g_s, with physiological meaning to interpret plant water stress and that A_N limitation imposed by deficit irrigation can easily quantified from the calculated values.

INTRODUCTION

There is an increasing need of developing more *resilient agricultural systems* to achieve the goals of the 2030 Agenda for Sustainable Development of the UN. More resilient systems include the use of deficit irrigation practices as they make crops less dependent on varying rainfall, using a reasonable amount of water. The correct use of deficit irrigation strategies requires both a good understanding of physiological mechanisms involved in the response of plants to water stress and the use of reliable and sensitive indicators of water stress (Fernández, 2014). Stomatal conductance is a good plant-based indicator for irrigation purposes: g_s reflects finely the level of water stress and how much A_N is limited (Flexas et al. 2013) which determines biomass production and, thus, yield. However, its use for irrigation scheduling purposes faces a major limitation from the difficulty of being automatically and continuously monitored. We demonstrated recently (Hernandez-Santana et al. 2016) that g_s can be estimated continuously from J_s values normalized by vapor pressure deficit (D_a). The automated estimation of g_s opens the possibility of using it both as a reliable water stress indicator and in photosynthesis models, which applied to fruit tree orchards can help to estimate yield in advance. Thus, our aims were to (1) to calibrate and validate a method to estimate g_s and A_N automatically based on J_s measurements and (2) to quantify the A_N limitations produced by soil water content and vapor pressure deficit in a super-intensive olive orchard.

MATERIAL AND METHODS

The experiment was conducted in summer of 2016 in an orchard nearby Seville (Spain) (37º 15' N, -5º 48' 102). The area has a Mediterranean climate with hot and dry weather from May to September and mild and wet for the rest of the year. The studied trees were central individuals located in plots with four irrigation treatments: two control treatments where irrigation fulfilled tree water demand, using one (C1L) or two (C2L) dripper lines, and two deficit irrigation treatments in which only 45% of the water added to control was applied along the whole irrigation season using one (WS1L) or two (WS2L) dripper lines. Four trees were instrumented per irrigation treatment with one probe set installed into the stem at the east facing side. The Compensation Heat Pulse (CHP) method (Green et al. 2003) was used to obtain point J_s measurements within the sapwood of the sample trees (Tranzflo NZ Ltd., Palmerston North, New Zealand) at 5 mm below the cambium. Heat pulses (60 J; 60 W over 1 s) were applied once every 30 min and data was stored by a CR1000 datalogger connected to AM25T multiplexer (Campbell, Campbell Scientific Ltd., Shepshed, UK).

Measurements of g_s and A_N to calibrate the method were conducted on clear days from May to July every 30-60 mins from dawn to midday in three sun-exposed current-year leaves per instrumented tree of the canopy facing SE. In addition, two measurements of maximum g_s and A_N ($g_{s,max}$ and $A_{N,max}$) were conducted every other week in every instrumented tree from mid-July to the beginning of September (8:00-9:00 GMT) to validate the method. We used a Licor LI-6400 portable photosynthesis system (LI-COR, Lincoln NE, USA), with a 2 cm × 3 cm standard chamber, at ambient light and CO_2 conditions.

Stomatal conductance was estimated using the procedure described in Hernandez-Santana et al. (2016) and A_N was estimated using the model of Farquhar et al. (Farquhar et al. 1980) and the previously estimated g_s. Specific temperature response for olive were taken from Diaz-Espejo et al. (2006) and the maximum rate of ribulose bisphosphate (RuBP) carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}) were determined from five A_N–C_i response curves measured with a Licor LI-6400 in the instrumented trees of the control and water-stress plots.

Air temperature, air humidity and PAR were recorded every 30 min at canopy height by a standard weather station (Campbell Scientific Ltd., Shepshed, UK) located in the center of the experimental area .

RESULTS AND DISCUSSION

All relationships established between J_s/D_a and g_s were strong (coefficients of determination between 0.44 and 0.84) and significant ($P<0.05$) for all the trees, extending the results observed in Hernandez-Santana et al. (2016). Validation measurements confirmed the uniqueness of the equations established for each tree, regardless the environmental conditions or the time of the day when the measurements were conducted (Fig. 1). The robustness of the J_s/D_a –g_s relationships found for several trees and different environmental conditions are explained by the fact that although empirical, the relationships are physiologically based on the control that stomata can exert on transpiration flux (estimated here from sap flow related measurements in the tree trunk) under conditions of high coupling to the atmosphere. The method is simple, relatively inexpensive, it does not require any upscaling and it does not assume any parameter or the measurement of total leaf area or more environmental variables than D_a. The major limitation of our approach is the empirical nature of the calibration factor relating J_s/D_a with g_s which makes necessary to establish a relationship for every sap flow sensor. The modelled A_N also showed good accordance with measured A_N for every instrumented tree (Fig. 2).

We also calculated the time course of $g_{s,max}$ and $A_{N,max}$ for almost 100 days (Fig. 3) for every instrumented tree. Maximum stomatal conductance was estimated using the previously established relationship of g_s– J_s/D_a and $A_{N,max}$ using the Farquhar et al. model. The seasonal course of the calculated $g_{s,max}$ and $A_{N,max}$ was mainly driven by D_a and the irrigation treatment: $g_{s,max}$ and $A_{N,max}$ decreased as D_a increased and they decreased with soil water content, produced by the deficit irrigation. We also quantified $A_{N,max}$ limitation imposed by deficit irrigation as the difference between the average $A_{N,max}$ reduction in the control plots (67.14%±0.99) and in the water-stress plots (45.13%±0.95). We calculated this reduction considering the absolute maximum $A_{N,max}$ found in control plots and thus, we assumed that this reduction was mainly produced by D_a. The difference between the reduction of control and water-stress plots allowed us to quantify the reduction imposed by soil water deficit, which was on average 21.27%±0.52 for the whole dataset. The reduction by soil water deficit was slightly lower (17.28%) for the lowest D_a (0.5-0.7 kPa) than for the highest D_a (24.39% for 2.4-2.6 kPa). As demonstrated here, this approach is sensitive enough to allow us inferring how stomata respond to soil water content and D_a and quantify their limitation effect on A_N. This approach has two potential advantages compared to other indices and methods used for irrigation scheduling: it could be based on absolute values of $g_{s,max}$, which has a solid physiological meaning of plant water stress and allows to quantify the A_N limitation imposed by deficit irrigation, which is a direct indicator of yield.

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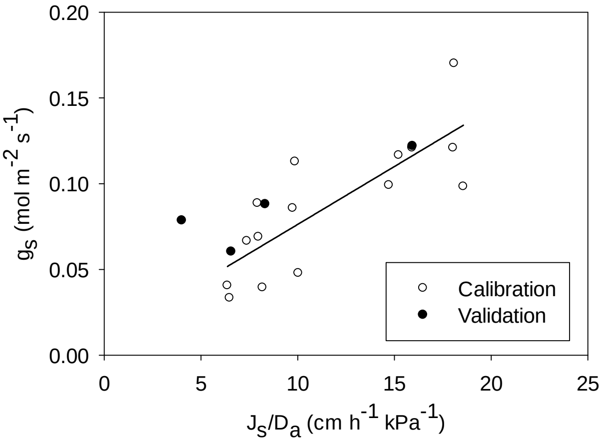

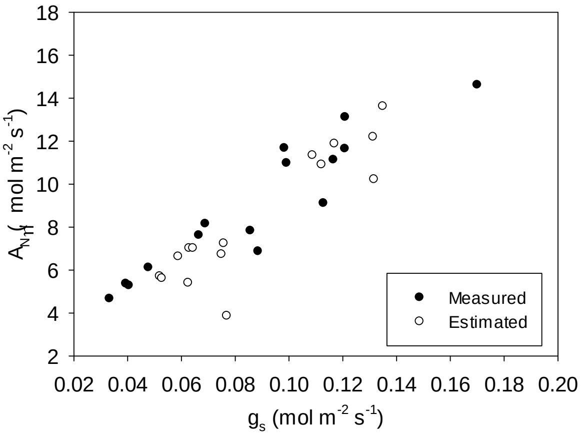

Figure 1. Example of the relationship between sap flux density (J_s) measured at 5 mm below the cambium divided by the air vapor pressure deficit (D_a) and the stomatal conductance (g_s) measured in sun exposed leaves. White circles are calibration measurements conducted in four days and black circles are validation measurements conducted in four different days. The fitted curve was statistically significant ($P < 0.001$).

Figure 2. Example of the relationship between stomatal conductance (g_s) and photosynthesis (A_N) measured (black circles) and modelled (white circles). Modelled g_s was estimated using the relationship established for sap flux density divided by vapor pressure deficit and g_s and A_N was modelled using the modelled g_s and Farquhar model.

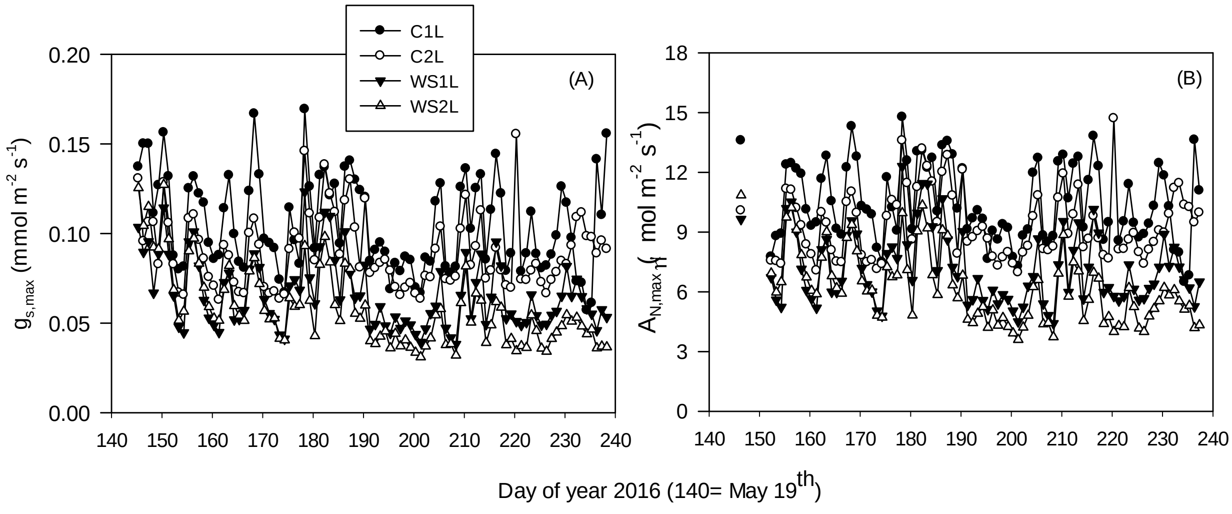

Figure 3. Temporal course of (a) maximum stomatal conductance ($g_{s,max}$) and (b) maximum photosynthesis ($A_{N,max}$), in the four irrigation treatments at Sanabria experimental orchard. Each point is the average of the data of four trees.

Using grapevine fruit-bearing cuttings (Red and White Tempranillo) grown under Temperature Gradient Greenhouses (TGGs) in 2013, 2014 and 2015 to evaluate effects of climate change (elevated CO_2, high temperature, and water deficit). I. Vegetative growth and substrate and plant water status

Kizildeniz, T.[a]*, Irigoyen, J. J.[a], Pascual, I.[a], Morales, F.[b]

[a] Universidad de Navarra, Facultades de Ciencias y Farmacia y Nutrición, Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño), c/ Irunlarrea 1, 31008, Pamplona, Spain.

[b] Estación Experimental de Aula Dei (EEAD). CSIC. Dpto. Nutrición Vegetal. Apdo. 13034. 50080. Zaragoza, Spain

*email: fmorales@eead.csic.es

ABSTRACT

The aim of this study was to analyze plant vegetative growth and plant and substrate water status of *Vitis vinifera* L. cv. Red and White Tempranillo grown under elevated CO_2, high temperature and water deficit in TGGs. Vegetative growth (total vegetative mass and leaf area) was significantly reduced by drought and was more associated with a worse substrate water status. Elevated CO_2 stimulated total vegetative mass but decreased leaf water content. This effect was also year-dependent. In conclusion, the responses of plant vegetative growth were highly variable especially with regard to the elevated CO_2 effects. Drought inhibitory effects were rather consistent the three years (2013, 2014 and 2015), and clearly more dependent on the substrate water status. The year-to-year variability in the response in some treatments can be probably due to the variability of the climate and the interactions among factors defining the climate (temperature, humidity, sunlight, etc.).

INTRODUCTION

Grapevine is a typical Mediterranean species fully adapted to summer drought. In general, grapevine plants respond to the elevated CO_2 by increasing vegetative growth and yield (Bowes, 1993), but high temperature combined with water deficit reduce stomatal conductance, leaf water potential, net photosynthesis and leaf area (Edwards et al., 2011). Previously, we had reported a drastic reduction of vegetative growth induced by drought in Tempranillo grapevine plants (Kizildeniz et al., 2015). Therefore, the aim of this study was to analyze influence of climate change factors, acting individually and/or interacting, on plant vegetative growth (total vegetative mass and leaf area) and plant and substrate water status in fruit-bearing cuttings of two grapevine cultivars (Red and White Tempranillo).

MATERIAL AND METHODS

Climate change scenarios were simulated in four Temperature Gradient Greenhouses (TGGs) located in Pamplona (42º 48’ 9.486”N, 1º 40’ 1.5882”W) Spain during the years 2013, 2014 and 2015 (as three experimental repetitions). Eight different treatments were applied in fruit-bearing cuttings of two grapevine (*Vitis vinifera* L.) cultivars (Red and White Tempranillo) from fruit set to maturity by combining: CO_2 level (400 *vs* 700 µmol mol^{-1}), temperature (ambient *vs* ambient +4ºC), and water availability (full irrigation *vs* cyclic drought). The vegetative growth (total vegetative mass and leaf area) and water status (leaf and substrate water status) were evaluated when berries reached 21-23ºBrix. Substrate water content was monitored from the beginning of treatment application until harvest.

RESULTS AND DISCUSSION

Vegetative growth (total vegetative mass and leaf area) was significantly reduced by drought (Fig. 2A and 2B), as reported previously (Kizildeniz et al., 2015). This inhibitory effect of water deficit treatments in grapevine plants was more associated with lower substrate volumetric water contents (Fig. 1) than with worse leaf water content (Fig. 2C). In this work, elevated CO_2 stimulated growth especially total vegetative mass (Fig. 2A) rather than plant total leaf area (Fig. 2B) in well irrigated and water deficit treatments. This stimulatory effect of the elevated CO_2 was not associated with an improvement of plant water status (Fig. 2C). Usually the elevated CO_2 treatments showed worse leaf water status than ambient CO_2 treatments (Fig. 2C). Finally, the effects of the elevated CO_2 are not consistent showing clear differences in the three years (Fig. 2A). Elevated CO_2 in interaction with high temperature had influence on full irrigated Red Tempranillo substrate volumetric water content (Fig. 1). In an experiment with Thompson Seedless, Williams and Matthews (1990) reported a linear relationship between soil water content and leaf water potential that depended on cultivar.

CONCLUSIONS

In conclusion, plant vegetative growth decreased especially with water deficit treatments, showing a similar pattern the three years (2013, 2014 and 2015). The inhibitory effect of water deficit was more related with substrate water status than with leaf water content. Elevated CO_2 stimulated vegetative growth, reflected better in total vegetative mass than in leaf area. This stimulatory effect of the elevated CO_2 was not associated with a better leaf water status, rather the contrary. The vegetative growth response to the elevated CO_2 was more variable between years than the one to the water deficit, probably due to the variability of the climate and the interactions among factors defining the climate (temperature, humidity, sunlight, etc.).

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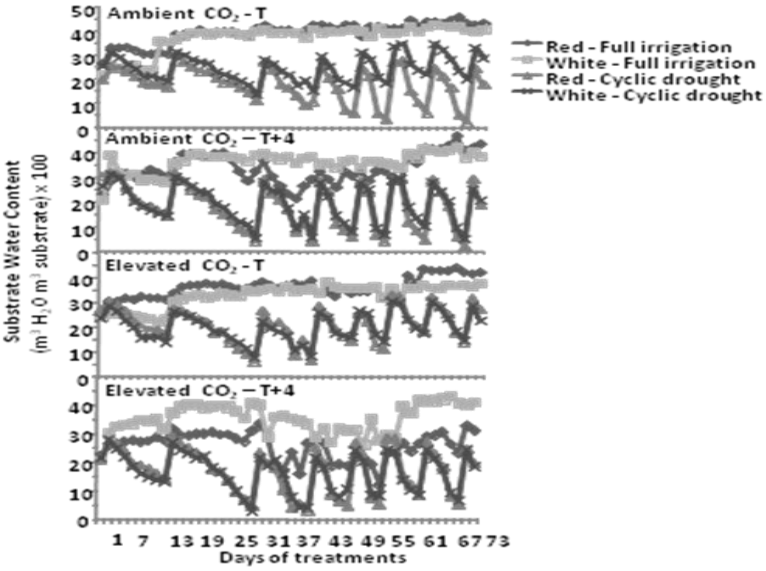

Figure 1. Pot substrate volumetric water content recorded from fruit set to harvest. Values represent the means of n = 3-7.

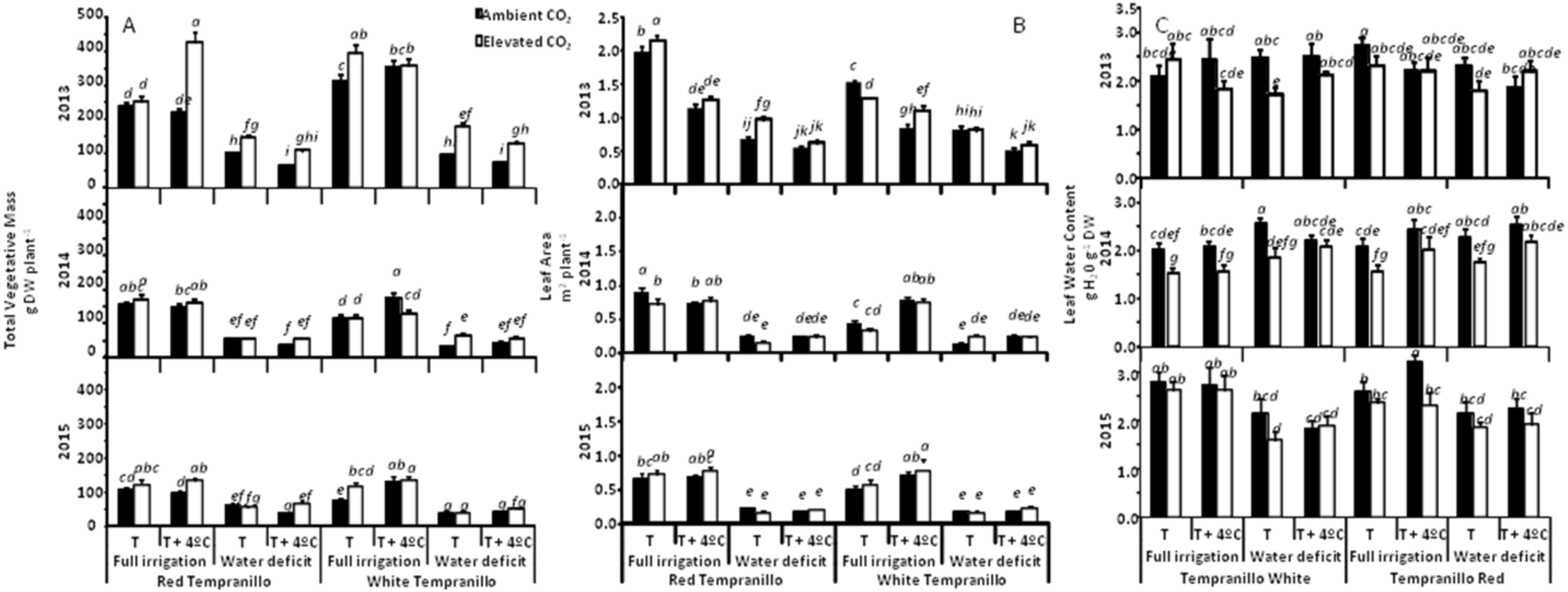

Figure 2. Total vegetative mass (A), leaf area (B) and leaf water content (C) recorded at harvest from fruit-bearing cuttings of Red and White Tempranillo subjected to different CO_2 levels: elevated CO_2 or ambient CO_2, temperature regimes: high (T+4°C) or ambient (T) and irrigation treatments: full irrigation or water deficit during 2013, 2014 and 2015. Values represent means (n = 5-10). Within each parameter and year, means followed by a different letter are significantly different (P<0.05).

Cambios en el estado hídrico, fotosíntesis y crecimiento de quinoa en respuesta a la sequía y a la salinidad

Aguirresarobe, A.[1], Yoldi-Achalandabaso, A.[1], Miranda-Apodaca, J.[1], Mena-Petite, A.[1], Lacuesta, M.[2], Muñoz-Rueda, A.[1], Pérez-López, U.[1]

[1]Universidad del País Vasco/EHU, Bº Sarriena s/n 48940 Leioa, usue.perez@ehu.eus

[2]Universidad del País Vasco/EHU, Paseo de la Universidad, 7, 01006, Vitoria-Gasteiz

RESUMEN

Ante las consecuencias del cambio climático, como el incremento de la sequía y de la salinización de los suelos, la especie *Chenopodium quinoa* puede ser un cultivo alternativo a los cereales por su alta tolerancia a dichas condiciones y por su alto valor nutricional. Por eso, el objetivo de este trabajo ha sido analizar la respuesta de la planta de quinoa en condiciones de sequía y salinidad. Bajo condiciones de sequía y estrés salino más severo (500 mM NaCl) se detectaron descensos muy similares en el contenido hídrico relativo. Sin embargo, las estrategias puestas en marcha en cada condición de estrés y sobretodo su importancia relativa fueron diferentes. Bajo condiciones salinas, la capacidad de ajuste osmótico y estomático fue superior que bajo condiciones de sequía donde se detectó una mayor deshidratación y, además, a pesar de que se produjo una menor reducción de la conductancia estomática, se apreció un mayor efecto no estomático como lo demuestra el hecho de que la concentración interna de CO_2 se viera incrementada. Como resultado, los descensos en la fotosíntesis no fueron tan severos bajo condiciones de salinidad y en estas condiciones la planta pudo mantener unas tasas de crecimiento más altas. Estos resultados plantean la posibilidad de utilizar la especie *Chenopodium quinoa* en suelos con alta salinidad como cultivo alternativo para el futuro, mientras que estaría peor adaptada a suelos con sequía edáfica.

INTRODUCCIÓN

La salinidad y la sequía son los factores ambientales más importantes que limitan el crecimiento y la producción de las plantas (Razzaghi et al., 2011). Se ha estimado que el 20% de las tierras cultivables a nivel mundial sufren de salinidad y cada año 1,6 millones de hectáreas se convierten en salinas, las cuales quedan inservibles para su uso para la agricultura (Eisa et al., 2012). Teniendo en cuenta que los cultivos tradicionales son glicófitos, la pérdida de cosecha se está convirtiendo en un problema habitual a escala mundial como consecuencia del aumento de las áreas salinizadas y las pérdidas de producción que ello comporta (Morales et al., 2011). Tales efectos se deben, por un lado, a que el estrés de salinidad produce estrés osmótico por lo que la planta sufre de sequía fisiológica siendo mermada la absorción de agua y el crecimiento. Por otro lado, la salinidad también puede provocar estrés iónico (Parida y Das 2005) debido a que la elevada concentración de los iones cloro y sodio tomados puede resultar tóxica para la planta. En consecuencia, se ha incrementado el estudio de las plantas capaces de tolerar el estrés de salinidad (Eisa et al., 2012) y el interés en la introducción de nuevos cultivos que como la quinoa (Jensen et al., 2000) están bien adaptados a condiciones de elevada salinidad. El objetivo de este trabajo ha sido analizar la respuesta de la planta de quinoa en condiciones de sequía y salinidad. Esta especie es originaria de la región de los Andes en Sudamérica y gracias a su rica diversidad genética se ha observado que ha logrado adaptarse a distintas zonas agroecológicas, desde el nivel del mar hasta a 4000 metros de altura (Adolf et al., 2013). El éxito de este cultivo se debe, por un lado, a su gran capacidad de tolerar diferentes tipos de estrés abiótico (González et al., 2009), y por otro lado, a su alto valor nutricional (Eisa et al., 2012).

MATERIALES Y MÉTODOS

Semillas de *Chenopodium quinoa* (Willd.) fueron sembradas en macetas con un sustrato de perlita y vermiculita (3:1) y crecidas durante 6 semanas en cámara de cultivo Conviron PGR15 (Conviron, Manitoba, Canadá). Las condiciones de crecimiento fueron: 400 ppm de CO_2, 24/20 ºC, 70%/80% HR día/noche, fotoperiodo de 14 h de luz y radiación fotosintéticamente activa (PAR) 400 µmol fotón m^{-2} s^{-1}. La salinidad se aplicó mediante riego con solución Hoagland suplementada con concentraciones salinas 0 mM (Control), 60 mM, 120 mM, 240 mM y 500 mM NaCl durante los últimos 14 días del ensayo. Para el tratamiento de sequía los tiestos se dejaron de regar, también durante los últimos 14 días del experimento. Se utilizaron 4 réplicas para cada tratamiento. El crecimiento se determinó a través del peso seco de raíces, tallos y hojas. El contenido hídrico relativo (CHR), el potencial hídrico y osmótico (Ψo), la deshidratación y el ajuste osmótico se calcularon según Pérez-López et al. (2009). La prolina se determinó siguiendo el método descrito por Bates et al. (1973) y los parámetros de intercambio gaseoso fueron estimados mediante análisis de gases en el infrarrojo (IRGA). Los resultados se han expresado como el promedio de las cuatro réplicas ± error estándar.

RESULTADOS Y DISCUSIÓN

La sequía redujo de manera notable el CHR. El efecto de la salinidad fue dependiente de la concentración, alcanzando a 500 mM de NaCl un CHR similar al detectado bajo condiciones de sequía (Tabla 1). La reducción del potencial hídrico en el sustrato causado por la sequía y por la salinización provocó que la planta pusiese en marcha distintas estrategias para, por un lado, seguir captando agua por las raíces y, por otro lado, disminuir la pérdida por las hojas (Pérez-López et al., 2009; Razzaghi et al., 2011). Así, se observaron valores significativamente más negativos del potencial osmótico a medida que se incrementaba la concentración salina y en condiciones de sequía. La reducción del potencial osmótico fue consecuencia tanto de deshidratación (DH) como de ajuste osmótico (AO). En el caso de la sequía, la DH fue más importante que el AO; sin embargo, en la salinidad el AO fue el factor más determinante, incrementándose su importancia a medida que la salinidad era más severa (Tabla 1). Para ajustar osmóticamente la quinoa puede sintetizar y almacenar solutos compatibles o puede almacenar iones inorgánicos (Adolf et al., 2013). La concentración de prolina tendió a aumentar a medida que la intensidad del estrés era mayor. Así, en ambos estreses el incremento de la prolina podría explicar, al menos en parte, el aumento del ajuste osmótico. Al objeto de disminuir las pérdidas de agua por las hojas se observó un descenso de la conductancia estomática en condiciones tanto de sequía como de salinidad que se tradujo a su vez en un descenso de la tasa fotosintética por limitación estomática. En concentraciones salinas, la caída de la fotosíntesis se debió, fundamentalmente a cierre estomático; sin embargo, cuando el estrés es más severo pueden aparecer limitaciones a nivel fotoquímico y bioquímico (Pérez-López et al., 2012), como es el caso de la sequía, donde se produjo un efecto no estomático sobre la fotosíntesis como vendría avalado por el aumento de la concentración interna del CO_2 (datos no mostrados).

Como resultado del descenso del CHR y la fotosíntesis, el síntoma más significativo observado en este trabajo fue la reducción en el crecimiento de la planta a causa de la sequía y alta salinidad (Fig. 1). La reducción varió dependiendo del órgano de la planta. La sequía redujo un 52% el peso seco del tallo y un 57% el peso seco de las hojas; la concentración máxima de sal disminuyó la producción de biomasa un 38% y 41% en tallos y hojas, respectivamente. No se observó diferencia entre los tratamientos en el peso seco de las raíces, correspondiéndose este resultado con el hecho de que, por lo general, las plantas dirigen una mayor proporción de carbohidratos a los órganos responsables de la adquisición de los recursos más limitantes, en este caso el agua (González et al., 2009; Ruiz Carrasco et al., 2011).Bajo condiciones salinas de hasta 120 mM NaCl el crecimiento de la quinoa no se vio afectado, lo que corroboraría la hipótesis inicial del buen comportamiento de la quinoa en suelos salinos y su posible utilización como cultivo en unas condiciones climáticas futuras donde la incidencia de la salinidad en las tierras de cultivo será cada vez más grande.

AGRADECIMIENTOS

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Tabla 1. Parámetros hídricos y fotosintéticos en plantas de quinoa tratadas con sequía y salinidad.

Tratamiento	CHR (%)	Ψ_o (MPa)	DH (MPa)	OA (MPa)	Prolina (nmol g^{-1}PS)	A (µmol CO_2 $m^{-2}s^{-1}$)	gs (mol H_2O $m^{-2}s^{-1}$)
Control	93,0±1,1^{c}	-0,66±0,05^{d}	-0,11±0,02^{a}	-	3,62±0,94^{a}	14,5±0,7^{c}	0,21±0,02^{c}
Sequía	78,1±1,6^{a}	-1,81±0,47^{ab}	0,7±0,24^{b}	0,35	6,55±0,42^{ab}	3,15±2,10^{a}	0,07±0,05^{ab}
60 mM NaCl	89,9±1,7^{c}	-0,95±0,03^{cd}	0,05±0,10^{a}	0,14	3,90±0,40^{a}	11,4±2,3^{c}	0,15±0,03^{bc}
120 mM NaCl	84,8±1,7^{b}	-1,05±0,06^{cd}	-0,12±0,05^{a}	0,41	4,24±0,42^{a}	12,9±1,2^{c}	0,14±0,02^{bc}
240 mM NaCl	84,6±1,7^{b}	-1,37±0,10^{bc}	0,13±0,10^{a}	0,48	7,48±0,50^{ab}	10,9±1,1^{bc}	0,11±0,01^{ab}
500 mM NaCl	76,7±1,0^{a}	-2,01±0,14^{a}	0,28±0,12^{a}	0,97	8,74±2,31^{b}	6,4±1,4^{ab}	0,05±0,01^{a}

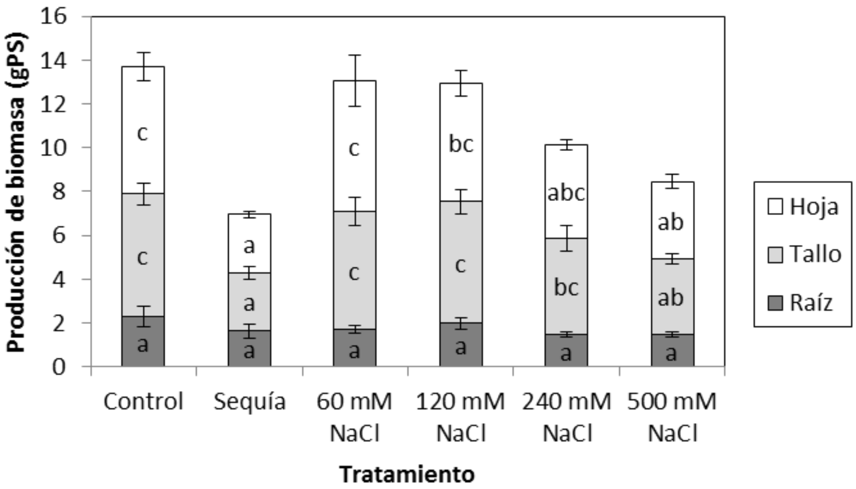

Figure 1. Producción de biomasa en plantas de quinoa tratadas con sequía y salinidad.

Modelización y simulación de la disponibilidad de agua en la zona radicular en ensayos de estrés hídrico

Rodrigo G., Fontanet M., Ferrer F.

LabFerrer, c/Ferran el Catòlic 3, 25200 Cervera · www.lab-ferrer.com · info@lab-ferrer.com

RESUMEN

La conductividad hidráulica y la capacidad de retención de agua del suelo pueden proporcionar información cuantitativa del estado hídrico de las plantas y de la disponibilidad de agua en los ensayos de estrés hídrico en condiciones de campo y laboratorio. Describimos una metodología de cálculo de la conductividad hidráulica y su implementación en modelos de simulación 2D para conocer el movimiento de agua en el suelo y el efecto de las propiedades hidráulicas para poder emplearlas en diferentes escenarios de estrés hídrico.

INTRODUCCIÓN

A menudo los estudios de respuesta al estrés hídrico de las plantas emplean sistemas muy artificiales de imposición del estrés (Pinheiro y Chaves, 2011) que no reflejan las condiciones de campo y las interacciones que se producen (Condon et al., 2004, Tardieu y Simonneau, 1998) y que pueden tener muy poca relevancia en la mejora de la producción agrícola (Jones, 2007). Según Tardieu y Simonneau (1998), las diferencias en los valores de conductividad hidráulica no saturada entre suelos y sustratos es uno de los factores responsables de las diferencias en el comportamiento entre las plantas de los ensayos de campo y de laboratorio. A medida que el suelo o el sustrato se seca, la conductividad hidráulica (K) y la capacidad de retención de agua disminuyen (Campbell y Campbell, 1982) y como consecuencia se ralentiza el movimiento del agua y se limita el agua disponible (O'Meara et al., 2014). Trabajos recientes también revelan que las características hidráulicas del suelo participan en el control de la absorción de agua en suelos y sustratos, cuestionando el valor umbral de -1,5MPa del punto de marchitez permanente (PMP), por lo que cada vez parecen más necesarios enfoques más integradores del sistema suelo-planta-atmósfera (SPA) para poder comprobar el estado hídrico y compara las diferencias de las plantas control y las estresadas, y sus diferencias (Lobet et al., 2014; O'Meara et al., 2014) Durante los últimos años, los modelos matemáticos de dinámica del agua en el suelo se han ampliado para abarcar el sistema SPA, bajo diferentes escenarios ambientales (Condon et al., 2004) aunque estas herramientas parece que permanecen dentro de la ciencia del suelo (Simunek et al., 2011). El objetivo de este estudio es por un lado, comparar las propiedades de retención y movimiento del agua en suelos y sustratos, para a continuación evaluar su efecto sobre la disponibilidad de agua a diferentes profundidades en un ensayo simple y simulado de estrés hídrico, con el fin de contribuir a una mejor definición de los estudios de estrés hídrico.

MATERIAL Y MÉTODOS

Se procesaron dos muestras inalteradas de sustrato: 100% fibra de coco, y 70% fibra de coco 30% chips de coco; y dos muestras inalteradas de suelo: textura franca y franco arcillosa.

Determinación de las propiedades hidráulicas con el Sistema HYPROP: Las muestras se procesaron como describen Schindler et al. (2008). Con la ayuda de un anillo de 250cm^3 se recogieron muestras individuales inalteradas, que a continuación se saturaron por capilaridad y se procesaron con el sistema HYPROP (UMS, Munich, Alemania) que emplea el método evaporativo y la tensiometría en un intervalo de medida entre 0 y -85 kPa.

Determinación del Potencial Hídrico con el Higrómetro WP4C): Con el WP4C (Decagon Devices, Pullman, WA, USA) únicamente se procesaron las muestras de suelo que se tamizaron y humectaron en distinto grado (Campbell et al., 2007). El WP4C proporcionó medidas puntuales del potencial hídrico en el intervalo de -0,1 a -300 MPa.

Ajuste de Modelos (HYPROP Fit): Los datos obtenidos del sistema HyProp y del WP4C se procesaron con el programa HYPROP FIT (versión 2.0). Para generar las curvas características de retención de humedad y de conductividad hidráulica se empleó el modelo van Genuchten bimodal. Para calcular el agua disponible total (TAW) se empleó la metodología descrita en AquaCrop versión 4.0

Modelización con Hydrus 2D: En las simulaciones con Hydrus 2D (versión 2.4.0460) (Simunek et al., 2011) con las muestras ensayadas se empleó una condición de contorno similar a un contendor de dimensiones 40x40x30cm con cuatro puntos de observación a 2, 10, 20, y 30 cm, se aplicó 1mm agua/h durante 24h. Las condiciones ambientales fueron idénticas, con una Eto promedio diaria de 6mm y drenaje libre. La simulación duró 2 semanas y no se validó.

RESULTADOS Y DISCUSIÓN

Los principales parámetros hidráulicos obtenidos se muestran en la Tabla 1, así como los valores de contenido volumétrico de agua para algunos valores de potencial hídrico que pueden asociarse con el agua fácilmente disponible, capacidad de campo (CC) y PMP. La muestra de suelo franco arcillosa fue la que tuvo la conductividad hidráulica saturada (K_s) más baja, hasta 267 veces menor que la muestra con K_s más elevada (100% coco, tabla 1). Las muestras de sustrato mostraron un descenso drástico del contenido de volumétrico de agua en el intervalo de -1 a -5KPa (figura 1 a) al igual que su K (figura 1 b) pudiendo limitar el movimiento de agua hacia las raíces y su disponibilidad para las plantas, como también observaron Campbell y Campbell (1982) y O'Meara et al. (2014), ya que para los valores de contenido de humedad del 27%, las K tienen valores de 0,0012 cmd^{-1}. Las muestras de suelo mostraron una TAW superior de la de los sustratos, siendo a su vez el suelo de textura franca el que presentó la TAW más elevada.

Los resultados de las simulaciones con Hydrus 2D en la muestra de 70% coco y el suelo franco arcilloso se muestran en la figura 2. En todas las muestras, salvo en el suelo franco arcilloso, se observó un comportamiento similar en las 4 profundidades ensayadas. A 2cm se observaron oscilaciones diarias en el contenido de humedad, mientras que a 10, 20 y 30cm se produjo un descenso progresivo y con la misma tendencia de este contenido. En el suelo franco arcilloso, durante los primeros 5 días, frente de humectación alcanzó las distintas profundidades ensayadas y a continuación el contenido de humedad se estabilizó (20-24%) y mantuvo constante hasta el fin de la simulación. Con las condiciones de contorno simuladas, el suelo franco no alcanzó el valor de CC en ninguna de las profundidades (datos no mostrados), por lo que el aporte de agua no fue suficiente y en un ensayo de estrés hídrico las condiciones de partida no hubiesen sido adecuadas. La muestra franco arcillosa tampoco alcanzó CC ni a 20 ni 30cm (figura 2 b). En los sustratos, CC se alcanzó a los 4 días en la muestra 100% Coco y a los 2 días y medio en la de 70% coco. Los resultados también mostraron un comportamiento similar en la evolución del contenido de humedad entre la muestra de coco 70% y la muestra de suelo franco a todas las profundidades ensayadas (figura 2 a). Las curvas características de humedad, la K y simulación con Hydrus 2D permitieron mostrar la variación del contenido de humedad y su relación con la disponibilidad de agua, mostrándose muy útiles para definir las condiciones de un ensayo de estrés hídrico.

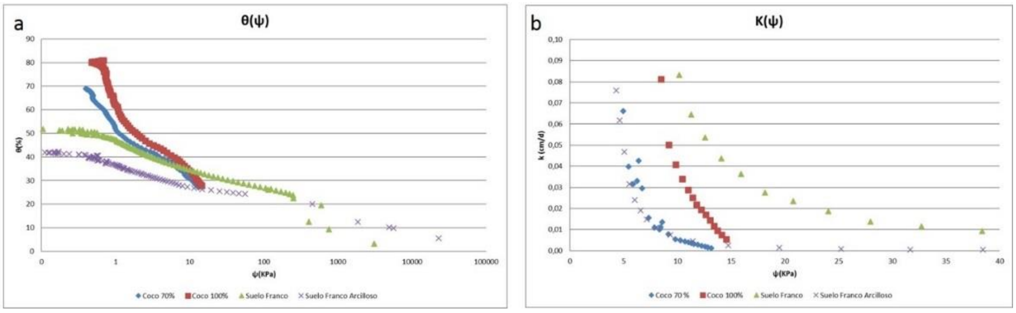

Figura 1 – Curvas características de retención de humedad (a) y representación de la conductividad hidráulica en función del potencial hídrico (KPa) (b) de las muestras ensayadas.

Tabla 1. Parámetros hidráulicos de las muestras ensayadas. K_s conductividad hidráulica saturada, φ porosidad, θ contenido volumétrico de agua en %, Ψ_e potencial de entrada de aire.

Muestra	K_s (cmd^{-1})	Φ (%)	θ (Ψe)	θ (-5kPa)	θ (-10kPa)	θ (-33kPa)	θ (-1500kPa)
Sustrato 70% Coco	462	75,5	75	39	30	17	--
Sustrato 100% Coco	6290	86,5	76	42	33	18	--
Suelo Franco	299	51,6	45	37	34	30	5
Suelo Franco arcilloso	23	42,7	40	29	27	25	14

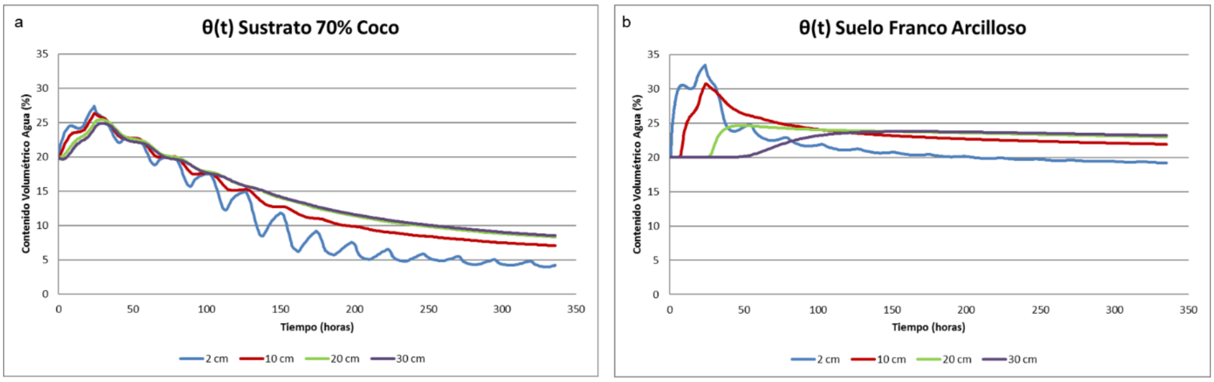

Figura 2 – Resultados de las simulaciones con Hydrus 2D. Evolución del contenido volumétrico de agua a 2, 10, 20 y 30 cm de profundidad en la muestra de sustrato de 70% coco (a) y en la muestra de suelo de textura franco arcillosa (b)

CONCLUSIONES

En los ensayos y simulaciones realizadas ponen de manifiesto la importancia de la conductividad hidráulica en suelos y sustratos poder trabajar con la disponibilidad de agua en ensayos de estrés hídrico. El siguiente objetivo es realizar una validación de estos datos de humedad en condiciones de campo

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Potencial de la medida de reflectancia en el rango de los teraherzios (THz) para el estudio de las relaciones hídricas de las plantas

L.G. Santesteban [1], I. Palacios [2], C. Miranda [1], J.C. Iriarte [2], J.B. Royo [1], V. Torres [2] y R. Gonzalo[2]

[1] Dpto. Producción Agraria, Universidad Pública de Navarra, Campus Arrosadia, 31006 Pamplona (Navarra) e-mail: gonzaga.santesteban@unavarra.es
[2] Dpto. Ingeniería Eléctrica y Electrónica, Universidad Pública de Navarra, Campus Arrosadia 31006 Pamplona, Navarra

RESUMEN

En las últimas décadas se ha generado un notable avance en el desarrollo de métodos que permitan conocer el estado hídrico de las plantas. El objetivo de este trabajo es evaluar la potencialidad de las medidas de reflexión del tronco en la banda de los THz para la estimación del estado hídrico de las plantas, lo que supone un enfoque novedoso, no explorado hasta la fecha. Para ello, se realizó un seguimiento del estado hídrico de una planta de vid mediante dos métodos convencionales (dendrometría y humedad de suelo), observándose que la señal de reflexión en THz-TDS permite detectar perfectamente los ciclos de desecación y la posterior recuperación con el riego. En todo caso, es necesario profundizar en la interpretación de las lecturas, desarrollar sensores *ad hoc* que puedan tener un coste razonable y evaluar la robustez de esta tecnología en condiciones ambientales no controladas para poder valorar su potencial.

Palabras clave: estrés hídrico, sensorización, medidas no destructivas, *Vitis vinifera* L.

INTRODUCCIÓN

La gestión del riego requiere partir de información fiable que nos permita conocer de manera sencilla el estado hídrico de las plantas y, por tanto, estimar sus necesidades. Habitualmente la toma de decisiones de riego se basa en (i) estimar el consumo a partir de datos meteorológicos, o bien en medir (ii) el contenido de agua en el suelo, (iii) el estado hídrico de la planta o (iv) su actividad, siendo las dos últimas fuentes de información las más fiables ya que proceden directamente de la planta.

En las últimas décadas se ha producido un notable avance en el desarrollo de métodos que permiten conocer el estado hídrico de la planta. Por ejemplo, el potencial hídrico, medido con cámara de presión, es considerado como el método de referencia por fisiólogos y agrónomo, ya que proporciona una información relativamente rápida y fácil de interpretar. Desafortunadamente, esta medida es destructiva y no automatizable, por lo que su aplicación a nivel comercial es reducida. Por el contrario, los métodos que son automatizables y no destructivos (p. ej.: flujo de savia, dendrometría) requieren instalaciones relativamente complejas, un mantenimiento experto y frecuentemente los resultados que proporcionan son complejos de interpretar. Por todo lo anterior, existe un gran interés en desarrollar nuevos métodos que permitan estimar de manera asequible y sencilla el estado hídrico de las plantas. En este sentido, la región del espectro electomagnético de los Terahertz (THz) puede ser una fuente de información muy valiosa, dada su elevada sensibilidad a la presencia de agua, poco explorada hasta la fecha. En la última década se han realizado algunos trabajos en plantas con el objetivo de estimar el contenido de agua de hojas in vivo (Breitenstein et al., 2012; Castro-Camus et al., 2013; Born et al., 2014), pero el enfoque de nuestro equipo, en el que las medidas se realizan directamente sobre el tronco presenta, al menos a nivel teórico, grandes ventajas por la capacidad de integración que tiene este órgano y por facilidad de fijación de los sensores que supone. El objetivo de este trabajo es evaluar la potencialidad de las medidas de reflexión del tronco en la banda de los THz para la estimación del estado hídrico de las plantas

MATERIAL Y MÉTODOS

El experimento se realizó empleando una cepa de 14 años de la variedad de vid Tempranillo, que había sido arrancada cuidadosamente dos años antes de un viñedo comercial y había sido trasplantada a una maceta de 26 L rellenada con una mezcla de turba y arena (2:1) y mantenida convenientemente hasta el inicio de los experimentos. Un mes antes del inicio de los experimentos, para garantizar su aclimatación, la planta se trasladó a un fitotrón en el que las condiciones de iluminación fueron de aproximadamente 400 µmol m^{-2} s^{-1}, el fotoperiodo de 14 h (día) 10 h (noche), y la temperatura se fijó en 21ºC para evitar interferencias sobre las medidas.

Durante un periodo de cinco semanas, se realizó un seguimiento del estado hídrico de la cepa mediante dos métodos convencionales (dendrometría y humedad de suelo), que se compararon con las medidas de reflexión en los THz. Estas medidas se hicieron empleando un analizador de redes (VNA, Agilent E3861C), una cabeza emisora/receptora en el rango de frecuencias entre 140 y 220GHz (OML V05VNA2-TR), y un par de lentes plano-convexas, cuya configuración se ha representado en la Fig. 1. En Santesteban et al. (2014) y Torres et. al (2016) pueden encontrarse más detalles sobre el dispositivo experimental.

La planta se sometió a cinco periodos de desecación de aproximadamente una semana, al final de los cuales la planta se volvía regar hasta capacidad de campo. Para evaluar el interés de las medidas de reflexión en THz-TDS, se compararon los dichos valores con las lecturas proporcionadas por la sonda de humedad de suelo y por el dendrómetro.

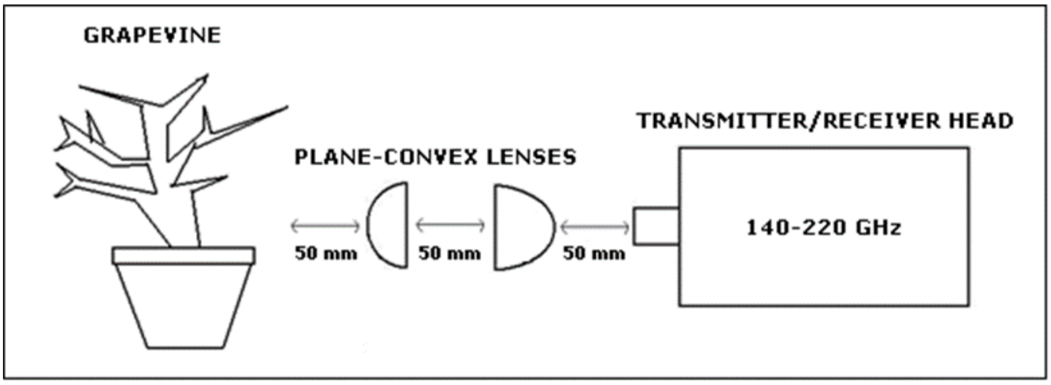

Figura 1. Esquema del dispositivo de medida de la reflexión en THz.

RESULTADOS Y DISCUSIÓN

En la Fig. 2 se representa la evolución a lo largo de las cinco semanas del experimento de la humedad del suelo y del diámetro del tronco, comparados con la señal de reflexión THz-TDS. Se comprueba que la señal de reflexión en THz-TDS permite detectar perfectamente los ciclos de desecación y la posterior recuperación con el riego, siguiendo un patrón muy similar al registrado por las sondas de humedad de suelo. Las medidas del dendrómetro no han sido capaces de mostrar con tanta claridad dichos ciclos, lo que coincide con el hecho ya descrito en la bibliografía de que, a partir de la parada de crecimiento, el comportamiento de los dendrómetros es peor.

En la respuesta de la señal de reflexión en THz-TDS se observa además una serie de patrones día-noche, que podrían estar relacionados con la actividad hídrica o fotosintética de la planta, lo que podría dar un valor adicional a la información aportada.

CONCLUSIONES

La medida de la reflexión THz-TDS a nivel de tronco ha mostrado ser una técnica prometedora de cara a medir en tiempo real y sin contacto el estado hídrico de las plantas. Evidentemente, es necesario profundizar en la interpretación de las medidas, desarrollar sensores ad hoc que puedan tener un coste razonable y evaluar la robustez de la técnica en condiciones ambientales no controladas para poder valorar el potencial de esta tecnología.

AGRADECIMIENTOS

Este trabajo ha sido financiado por el Departamento de Industria del Gobierno de Navarra (VITICS, Ref: IIM14244.RI1, cofinanciado con fondos FEDER de la Unión Europea) y por el Ministerio de Economía y Competitividad (Proyectos BACAVID, Ref: AGL2011-30408-C04-03 y DIDAcTIc, Ref: TEC2013–47753-C3-1-R).

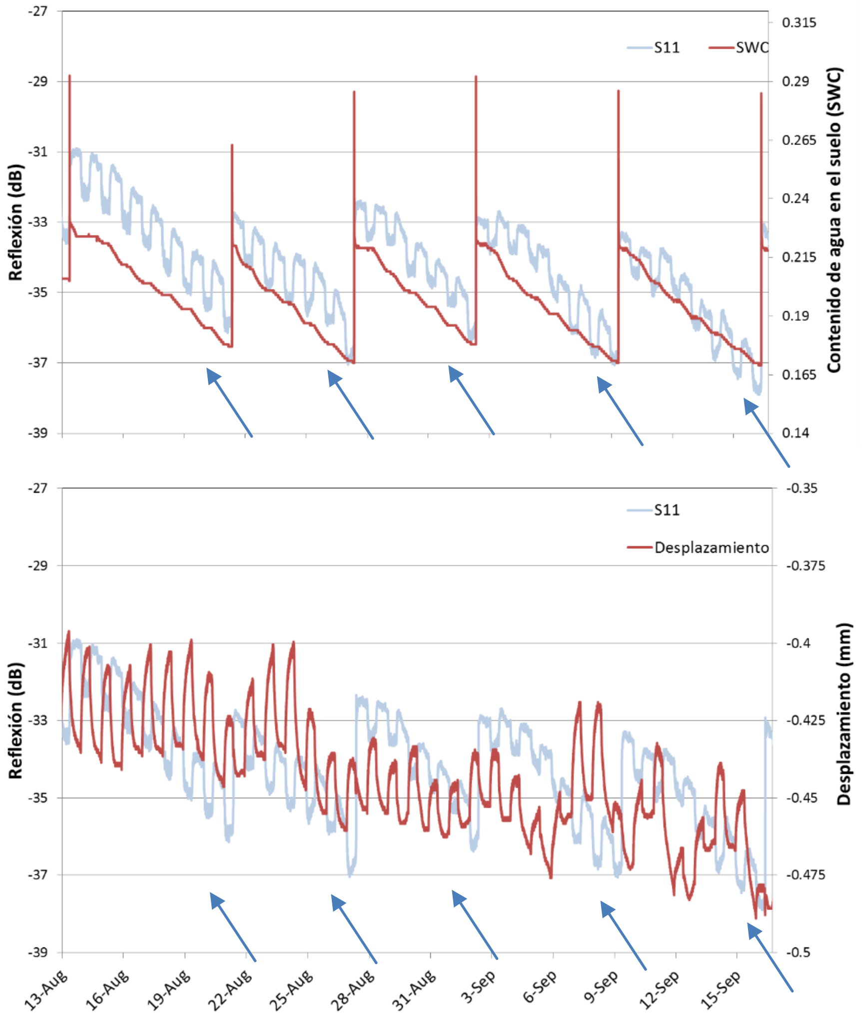

Figura 2. Comparación de la evolución de la señal de reflexión en THz frente al contenido de agua en el suelo (a) y a las variaciones de diámetro de tronco. Las flechas indican los momentos en los que se realizó un riego.

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Comparación del comportamiento hídrico de las variedades de vid (*Vitis vinifera* L.) Cabernet Sauvignon, Graciano y Tempranillo

B. Insausti[1], M. Loidi[1], I. Sagarna[1], D. Marín[1], C. Miranda[1], J. Urrestarazu[1], J.B. Royo[1], y L.G. Santesteban[1]

[1] Dpto. Producción Agraria, Universidad Pública de Navarra, Campus Arrosadia, 31006 Pamplona (Navarra) e-mail: gonzaga.santesteban@unavarra.es

RESUMEN

Palabras clave: estrés hídrico, ratio isotópico de carbono, *Vitis vinifera* L.

El objetivo del presente trabajo es evaluar los aspectos que influyen en el estado hídrico de las variedades de vid Cabernet Sauvignon (CS) y Graciano (GR), comparándolo con la información previa para la variedad Tempranillo (TE). Es estudio se realizó en viñedos situadas en Traibuenas, Navarra, y se basó en la comparación de valores de potencial hídrico, ratio isotópico de carbono y condiciones ambientales obtenidos durante dos campañas consecutivas. Los resultados obtenidos ponen en evidencia diferencias en el comportamiento de las tres variedades, destacando CS por ser la más conservadora del agua, seguida de GR y, por último, destacando TE como la variedad menos conservadora de las tres.

INTRODUCCIÓN

La vid es un cultivo del que se conocen un número muy elevado de variedades, que han mostrado una amplia variabilidad en términos de respuesta fisiológica frente al estrés hídrico (Bota et al., 2015). En un contexto en el que los recursos hídricos son cada vez más escasos, resulta de interés profundizar en el conocimiento de la respuesta hídrica de las variedades que se cultivan en una zona vitícola.

El objetivo del presente trabajo es evaluar los aspectos que influyen en el estado hídrico de las variedades de vid Cabernet Sauvignon (CS) y Graciano (GR), comparándolo con la información obtenida en ensayos anteriores de nuestro grupo de investigación para la variedad Tempranillo (TE)

MATERIAL Y MÉTODOS

Los trabajos se llevaron a cabo en los años 2014 y 2015 en dos viñedos de las variedades Cabernet Sauvignon y Graciano, ubicados en Traibuenas (Navarra). De cara a aumentar el rango de condiciones hídricas en las que se desarrollaban las plantas, se establecieron puntos de control en dos zonas de cara parcela (Figura 1) en la que el suelo fuera diferente y, en cada una, se establecieron tres niveles de riego, R0 (secano), R1 (riego estándar, consistente en un riego semanal de 32 L cepa $^{-1}$ a la semana entre cuajado +15d y vendimia) y R2 (dos riegos de 32 L cepa-1 en el mismo periodo). En estos puntos, con frecuencia semanal, se midió el potencial hídrico antes de amanecer (Ψaa), y el potencial hídrico xilemático a media mañana (Ψ11) y a mediodía (Ψ14) (4:30, 9:00 y 12:00 hora solar respectivamente). Este seguimiento se llevó a cabo entre el 26 de junio y el 10 de septiembre en 2014 y entre el 16 de julio y el 10 de septiembre, empleando cinco hojas por tratamiento. Los detalles de preparación de las hojas y precauciones durante las medidas son las mismas que se describen en Santesteban et al. (2011). En vendimia, se tomaron muestras de 100 bayas de cada una de las situaciones hídricas, se secaron en estufa, se molieron y se analizó su ratio isotópico de carbono ($\delta^{13}C$) siguiendo el procedimiento detallado en Santesteban et al. (2012).

Figura 1. Ubicación de las zonas de medida en (a) Cabernet Sauvignon y (b) Graciano

La caracterización de las condiciones climáticas se hizo a partir de los datos semihorarios recogidos en la estación meteorológica de El Plano (Bardenas), de libre acceso (http://meteo.navarra.es/), y se emplearon para calcular el déficit de presión de vapor (DPV, Eq. 1) y la duración del tiempo de rocío (LWD) según lo detallado en Lulu et al. (2008).

$$DPV = (e_s - e), \qquad \text{(Eq. 1)}$$

siendo

$$e_S = 6.108 * \exp\left(\frac{17.27 * T_S}{T_S + 273.3}\right) \quad y \quad e = e_S * HR \qquad \text{(Eq. 2)}$$

Las comparaciones entre variables se realizaron mediante análisis de regresión lineal simple y múltiple. En este último caso, la relevancia de cada variable se evaluó mediante el método “hacia atrás”, el peso de cada variable significativa mediante el coeficiente estandarizado (β) y la independencia entre las variables mediante el cálculo del factor de inflación de la varianza (VIF). Los análisis se llevaron a cabo mediante el paquete estadístico R y sus librerías QuantPsyc y DAAG.

RESULTADOS Y DISCUSIÓN

En la Figura 2 se enfrentan los valores de potencial observados para cada una de las variedades en los tres momentos de medida. CS y GR tuvieron un comportamiento muy similar, pero diferente al mostrado por TE en Santesteban et al. (2011), especialmente cuando se enfrentan los valores antes de amanecer y de media mañana. En todo caso, de cara a la interpretación de los resultados hay que tener en cuenta que aunque las medidas se hicieron siguiendo el mismo protocolo que en Santesteban (2010), ha podido haber algún factor externo que condicionara estas relaciones.

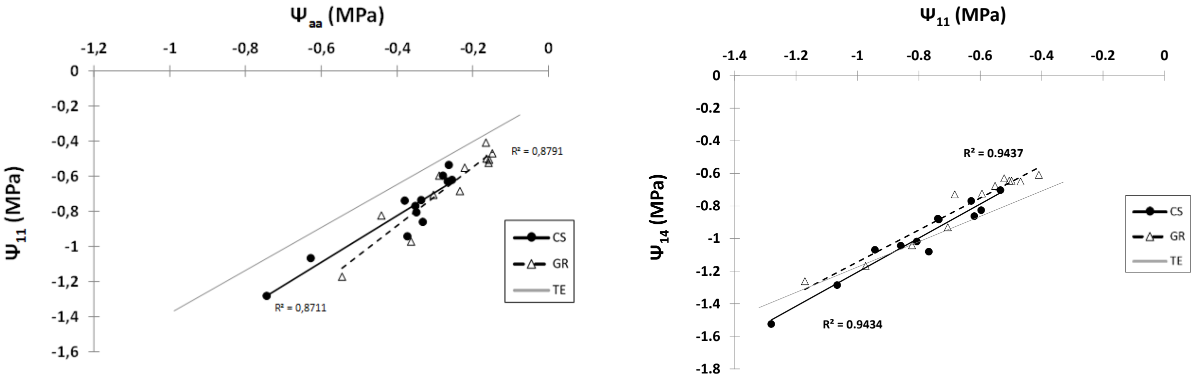

Figura 2. Comparación del potencial hídrico medio observado (a) antes de amanecer (Ψaa) y a media mañana (Ψ11) y (b) a media mañana (Ψ11) y a mediodía (Ψ14) en Cabernet Sauvignon (CS), Graciano (GR) y Tempranillo (TE), datos de Santesteban et al. 2012).

En la Figura 3 se presenta la comparación de potencial hídrico con el ratio isotópico de carbono. De acuerdo a los resultados obtenidos, y de la comparación con el trabajo de referencia de Santesteban (2012), se deduce que para un mismo valor de potencial hídrico, TE presenta los ratios isotópicos más negativos, seguido de GR, mientras que CS presenta los valores más altos Las diferencias en la relación entre el ratio isotópico de carbono y el potencial pueden tener una gran importancia práctica, ya que dan una idea de la eficacia del cierre estomático para un determinado nivel de estrés, y podría estar asociada a diferencias en el control estomático de las distintas variedades. GR y, sobre todo, CS parecen tener un control estomático más efectivo que TE, de forma que tienden a conservar el agua disponible.

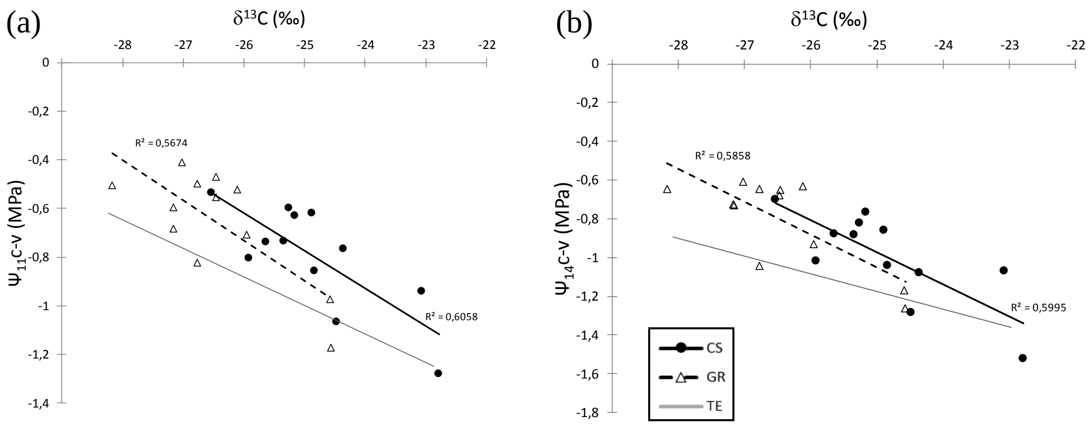

Figura 3. Comparación del (a) potencial hídrico entre y cuajado y vendimia a media mañana (Ψ11) y (b) a mediodía (Ψ14) y el ratio isotópico del carbono ($\delta^{13}C$) en Cabernet Sauvignon (CS), Graciano (GR) y Tempranillo (TE), datos de Santesteban et al. 2012).

´Por último, en la Tabla 1, se presentan las relaciones observadas entre las condiciones ambientales y el potencial hídrico. Por limitaciones de espacio no se presentan los estadísticos de las relaciones, si bien hay que indicar que en todos los casos las variables mantenidas eran significativas y el VIF < 5. La variable con mayor influencia tanto en CS como en GR fue el estado previo de la planta, es decir, en el caso del potencial hídrico antes de amanecer (Ψaa) el leaf wetness duration (LWD) tuvo mayor influencia y en los potenciales de media mañana (Ψ11) y de mediodía (Ψ14).

Tabla 1. Efecto de las condiciones meteorológicas sobre las relaciones entre las medidas de potencial hídrico medido a distintas horas del día

Graciano	**Cabernet Sauvignon**
Ψ_{aa}= -0.012-0.004 HR_{06}+0.016LWD	Ψ_{aa}= -0.089-0.004HR_{aa}+0.022LWD
Ψ_{11}= -0.629+0.005HR_{11}-0.017LWD+1.083 Ψ_{aa}	Ψ_{11}= -1.077+0.012HR_{11}-0.025LWD+1.037Ψ_{aa}
Ψ_{14}= 0.177-0.004HR_{14}-0.009T_{14}+0.433 Ψ_{aa}+0.677 Ψ_{11}	Ψ_{14}= 0.131-0.003HR_{14}-0.008T_{14}+0.404 Ψ_{aa}+0.7914 Ψ_{11}
Ψ_{aa}= -0.33+0.106VPD_{aa}+0.012LWD	Ψ_{aa}= -0.445+0.133VPD_{06}+0.019LWD
Ψ_{11}= -0.186-0.133VPD_{11}-0.015LWD+1.097 Ψ_{aa}	Ψ_{11}= -0.103-0.277VPD_{11}-0.02LWD+1.036 Ψ_{aa}
Ψ_{14}= -0.245+0.408 Ψ_{aa}+0.677 Ψ_{11}	

CONCLUSIONES

CS ha sido la variedad con un comportamiento más conservador del agua, lo que equivale a un comportamiento más isohídrico, mientras que TE se encontraba en el extremo contrario, mostrando GR un comportamiento intermedio.

AGRADECIMIENTOS

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Saving irrigation water as a tool to increase pomegranate fruit price and enhance the bioactive compound content

Galindo, A.[1], Calín-Sánchez, A.[2], Rodríguez, P.[3], Cruz, Z.N.[3], Girón, I.F.[4,5], Corell, M.[5,6], Martínez-Font, R.[7], Moriana, A.[5,6], Carbonell-Barrachina, A.A.[2], Torrecillas, A.[1], Hernández, F.[7]

[1] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC). Department of Irrigation, P.O. Box 164, E-30100 Espinardo, Murcia, Spain (atorreci@cebas.csic.es)
[2] Universidad Miguel Hernández de Elche, Department of Agrofood Technology, Food Quality and Safety Research Group, Ctra. de Beniel, km 3,2. E-03312 Orihuela, Alicante, Spain
[3] Instituto Nacional de Ciencias Agrícolas (INCA). Department of Physiology and Biochemistry, Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba
[4] Instituto de Recursos Naturales y Agrobiología (CSIC), PO Box 1052, E-41080 Sevilla, Spain
[5] Unidad Asociada al CSIC de Uso Sostenible del Suelo y el Agua en la Agricultura (US-IRNAS). Crta de Utrera Km 1, 41013, Sevilla, Spain
[6] University of Sevilla. EUITA, Dept. Ciencias Agroforestales, Carretera de Utrera km 1, E-41013 Sevilla, Spain
[7] Universidad Miguel Hernández de Elche, Department of Plant Sciences and Microbiology, Plant Production and Technology Research Group, Ctra. de Beniel, km 3,2. E-03312 Orihuela, Alicante, Spain.

ABSTRACT

The non-climateric character of pomegranate (*P. granatum*) fruit underlines the importance of determining the optimum harvest time to improve fruit quality. The effect of irrigation withholding during 6, 15, 25 and 36 d before harvest was evaluated in order to clarify whether fruit ripening is critical or non-critical from the yield, fruit characteristics and composition point of view. The results indicated that this phenological period is critical because irrigation is essential during most of this phenological period to achieve maximum yield. However, a 6 d of irrigation restriction at the end of ripening period can be used as a tool to come early harvest time, saves irrigation water, enhances the bioactive compounds (anthocyanins, phenolic compounds, punicalagin and ellagic acid) and increases the price of the fruit without affecting marketable yield and fruit size.

INTRODUCTION

All Mediterranean agrosystems must cope with water scarcity, and any policy involving greater use of the water available is unsustainable. For this, pomegranate (*Punica granatum* L.) farming must be directed towards the use of deficit irrigation strategies, maximizing crop water productivity rather than maximizing the yield per land unit, allowing significant water savings, and the profitable production of high quality fruits. Regulated deficit irrigation (RDI) is an irrigation strategy designed to save water while having a minimum impact on yield and fruit quality (Naor, 2006). This requires precise knowledge of the crop response to drought stress during the different phenological phases in order to identify phenological periods when adverse effects on productivity are minimal (non-critical periods) or maximal (critical periods). Intrigliolo et al. (2013) and Laribi et al. (2013) indicated that the period comprising flowering and pomegranate fruit set could be regarded as non-critical from the yield point of view, that irrigation water restriction during linear fruit growth period increased the concentration of many bioactive compounds in the juice, such as anthocyanins, that could be related to health and taste and that irrigation water restriction during the last part of fruit growth and ripening enhances peel red colour intensity and TSS in the juice

The aim of this research was to evaluate whether water restrictions only during the ripening stage can affect yield and whether water deficit during this period has secondary effects on fruit characteristics, which could be used to improve fruit maturity and come early harvest time.

MATERIALS AND METHODS

Drip irrigated adult own-rooted pomegranate (*P. granatum* L. cv. Mollar de Elche) plants spaced at 3 m x 5 m were irrigated above crop water requirements (control plants, T0). Also, T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277–283, fruit late ripening), 15 (DOY 268–283, second half fruit ripening), 25 (DOY 258–283, fruit ripening) and 36 (DOY 247–283, end fruit growth and ripening) days before harvest (DOY 283), respectively, when irrigation was withheld. The total amount of water received by each treatment during the experimental period (DOY 247–283) was 128, 110, 86, 49 and 0 mm for T0, T1, T2, T3 and T4 treatments, respectively, without considering precipitation (basically the 84 mm rainfall that fell on DOY 271).

Midday leaf conductance (g_{leaf}) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potentials were measured in two leaves similar to those used for g_{leaf} using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA) (Galindo et al., 2014a). Midday fruit water potential (Ψ_{fruit}) was measured following the procedure described by McFadyen et al. (1996) and Galindo et al. (2014a). Water stress integral (SI) was calculated from the g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data according to the expression

$$\mathrm{SI_A} = \left|\sum(\bar{\mathrm{A}} - \mathrm{H})\, n\right|$$

where A can be g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} and $\bar{\mathrm{A}}$ is the average g_{leaf}, Ψ_{leaf}, Ψ_{stem} or Ψ_{fruit} value for any interval, H is the maximum value measured during each interval and n is the number of days in the interval.

Fruit juice total soluble solids (TSS), total titrable acidity (TA), juice colour, total phenolic compounds (TPC), total anthocyanin content (TAC), total antioxidant activity (TAA), punicalagin (isomers α and β) and ellagic acid contents were determined following the procedures described by Galindo et al. (2014b) and Calín-Sánchez et al. (2013).

RESULTS AND DISCUSSION

In spite of the rainfall events, the cumulative water stress tended to increase with the number of days irrigation was withheld, the treatments T1 and T2 producing a similar and moderate water stress level and a more pronounced water stress level being observed in the treatments T3 and T4. SI_{gleaf}, $SI_{\Psi stem}$, $SI_{\Psi leaf}$ and $SI_{\Psi fruit}$ values showed some differences in describing the cumulative water deficit reached by the plants. $SI_{\Psi fruit}$ was the most reliable indicator to detect differences between the treatments at moderate water stress (T0 and T2), while SI_{gleaf} was the only indicator able to detect differences between the treatments at more pronounced water stress levels (T3 and T4) (Table 1).

The decrease in fruit yield in T2 and T3 (Table 2) confirmed the hypothesis that fruit ripening is a critical period from the yield point of view (Laribi et al., 2013; Intrigliolo et al., 2013). However, the fact that plants that were water stressed only at fruit late ripening stage (T1) showed similar marketable yield and fruit size to T0 plants clarifying some aspects of the concept of phenological critical period. In this sense, it is probable that sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Whatever the case, although pomegranate trees are able to withstand severe drought conditions, irrigation was essential during most of the ripening stage to achieve optimum yield.

The first pomegranate fruits reaching the market fetch higher prices and, in this sense, ‘Mollar de Elche’ cultivar is often harvested when the peel has a sufficient red colouratiing. The significant increase in juice colour from T3 and T4 fruits (Table 3) is also very interesting for producers because pomegranate fruit attractiveness is primarily related to colour and taste parameters of the arils and their juice (Borochov-Neori et al., 2009). However, despite the fact that pomegranate colouration is predominantly due to anthocyanins, TAC levels in T3 and T4 fruits were similar to that observed in T0 fruits (Table 4).

The fact that (i) TAC juice levels increased only in T1 fruits, (ii) TAA levels were similar in juices from the different irrigation treatments, and (iii) redness significantly increased only in T3 and T4 fruit juices (Tables 3 and 4), confirmed the view that juice antioxidant capacity is not linearly correlated with the red colour intensity, meaning that the anthocyanins are not major contributors to the antioxidant capacity exhibited by the pomegranates and their juice (Borochov-Neori et al., 2009). Moreover, the fact that withholding water irrigation decreased TPC levels and did not affect TAA levels (Table 4) does not agree with the linear relationship between soluble phenolic levels and antioxidant capacity indicated by Borochov-Neori et al. (2009), who supported the idea that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this sense, further analysis of fatty acids and organic acids must be conducted to fully understand the antioxidant capacity and bioactivity of pomegranate fruits subjected to deficit irrigation strategies (Alcaraz-Mármol et al., 2015; Calín-Sánchez et al., 2013).

It is well known that water stress influences the content of secondary metabolites in plant tissues, having also contradictory results in other crops. For example, Chaves et al. (2007) reported the substantial accumulation of anthocyanins in grape berries under water stress. In contrast, Kennedy et al. (2002) showed that osmotic stress had little or no effect on anthocyanin accumulation in grape berries. This, at first sight, confusing relation between water stress and the production of bioactive compounds could be attributed to the fact that most manuscripts are not meticulous when it comes to recording aspects of plant water stress (precise phenological period at which it takes place, water stress rate of development, duration of maximum water stress, incidence of partial recoveries and other aspects) although such information is essential for the characterisation of experimental water stress conditions. In addition, it is essential to underline that is not possible to establish a linear correlation between water stress and secondary metabolite contents (Gobbo-Neto and Lopes, 2007). For this reason, Horner (1990) proposed a quadratic model to predict the concentration of phenolic compounds as a function of plant water status. So, under a mild water stress, CO_2 assimilation could be maintained and carbon-based secondary metabolites will probably increase when carbohydrates exceed the amount required for growth. Thus, mild osmotic stress may lead to a reduction in plant growth, accompanied by an increasing concentration of non-nitrogenous secondary metabolites. When water stress increases, stomatal regulation takes place and CO_2 assimilation is reduced. In this situation, carbon will be preferentially allocated to the synthesis of primary metabolites to the detriment of the synthesis of secondary metabolites.

CONCLUSION

The results indicated that the SI calculated from g_{leaf}, Ψ_{leaf}, Ψ_{stem} and Ψ_{fruit} data vary as regards their ability to describe the cumulative water deficit reached by plants. $SI_{\Psi fruit}$ was the most feasible indicator for detecting differences between the treatments at moderate water stress levels while SI_{gleaf} was the only indicator able to detect differences between the treatments at higher water stress levels. Fruit ripening is a critical period from the yield point of view because irrigation is essential during most of this phenological period if maximum yields are to be achieved. Nevertheless, the fact that a very short irrigation restriction period (around 6 days) at the end of ripening bring the harvest time forward and so increase pomegranate fruit price, saves irrigation water and enhances the bioactive compound content (anthocyanin, phenolic compounds, punicalagin and ellagic acid) without affecting marketable yield and fruit size suggests that the sensitivity to water stress during a given critical phenological period is not constant and/or it is necessary to exceed a certain level of water stress to achieve adverse effects on productivity during a critical period. Moreover, the increase in fruits colouration as a result of water stress during fruit ripening may be considered as an interesting aspect because the appeal of pomegranate fruit is directly associated with colour. In spite of this, it is important to note that a very short irrigation restriction (around 6 days) at the end of the ripening period advances the harvest time, increases pomegranate fruit price, saves irrigation water and enhances the bioactive compound contents (anthocyanin, phenolic compounds, punicalagin and ellagic acid). Finally, the results confirmed the hypothesis that there is no a linear correlation between pomegranate water stress and secondary metabolite contents, because mild water stress may lead to a reduction in plant growth and a higher concentration of secondary carbon metabolites, whereas under a more pronounced water stress carbon are preferentially allocated to the synthesis of primary metabolites to the detriment of secondary metabolites.

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Table 1. Effect of irrigation treatments on leaf conductance (SI_{gleaf}, mmol m^{-2} s^{-1} x day), stem ($SI_{\Psi stem}$, MPa x day), leaf ($SI_{\Psi leaf}$, MPa x day) and fruit ($SI_{\Psi fruit}$, MPa x day) water stress integral.

Treatment	SI_{gleaf}	$SI_{\Psi stem}$	$SI_{\Psi leaf}$	$SI_{\Psi fruit}$
T0	5434.5c	10.5b	27.6c	8.5c
T1	5911.5c	15.0b	29.1c	15.1bc
T2	6414.0c	21.5b	31.1bc	22.7b
T3	9784.0b	49.5a	49.2ab	43.7a
T4	15215.3a	62.8a	53.5a	52.9a

Table 3. Effect of irrigation treatments on pomegranate peel and juice lightness (CIE *L**), red/greenness (CIE *a**), blue/yellowness (CIE *b**), chroma (*C**) and hue angle (*H*º) values.

	Treatment	*L**	*a**	*b**	*C**	*H*º
Peel	T0	64.2a	26.5b	31.2a	41.6b	50.1a
	T1	60.8ab	30.7a	30.5a	43.9a	45.3ab
	T2	60.1b	32.0a	30.1ab	44.5a	43.7abc
	T3	57.2bc	33.5a	27.6bc	43.9a	40.1bc
	T4	55.1c	34.6a	26.8c	44.3a	38.2c
Juice	T0	32.4b	8.3b	2.3b	8.6b	14.8b
	T1	33.1ab	10.5ab	3.1ab	10.9ab	16.5ab
	T2	33.1ab	9.6ab	2.7ab	9.9ab	15.3ab
	T3	33.9a	11.7a	3.6a	12.3a	17.2ab
	T4	33.7a	11.8a	3.9a	12.5a	18.2a

Means within a column that do not have a common letter are significantly different by $LSD_{0.05}$ test.

Table 2. Effect of irrigation treatments on marketable pomegranate fruit yield (MY, kg $tree^{-1}$), average fruit weight (FW, g), fruit equatorial diameter (ED, mm), and fruit length (FL, mm).

Treatment	MY	FW	ED	FL
T0	56.8a	293a	86.9a	75.0a
T1	55.5a	258ab	80.4b	69.7b
T2	35.2b	252b	81.4b	71.4ab
T3	28.9b	249b	81.6b	69.8b
T4	17.8b	253b	78.1b	67.2b

Table 4. Effect of irrigation treatments on pomegranate juice total polyphenols content (TPC, mg GAE L^{-1}), total anthocyanin content (TAC, mg L^{-1}), total antioxidant activity (TAA, mM Trolox), α- punicalagin, β-punicalagin, and ellagic acid (mg L^{-1}).

Treatment	TPC	TAC	TAA	α-punicalagin	β-punicalagin	Ellagic acid
T0	3133a	69.5b	12.1a	168.7ab	164.5b	19.0b
T1	2681b	123.1a	13.3a	184.2a	174.2a	19.6a
T2	1945c	76.1b	13.3a	169.5ab	172.0a	19.5a
T3	1534c	75.1b	11.9a	162.2b	170.2ab	19.5a
T4	1589c	75.1b	12.0a	157.7b	168.7ab	19.3ab

Means within a column that do not have a common letter are significantly different by $LSD_{0.05}$ test.

Metabolic changes in grapevine berry skins of different clones of Tempranillo subjected to regulated deficit irrigation under elevated temperatures

Torres N, Goicoechea N, Antolín MC.

Universidad de Navarra, Facultades de Ciencias y Farmacia y Nutrición, Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño), c/ Irunlarrea 1, 31008, Pamplona, Spain. cantolin@unav.es

ABSTRACT

This study investigates the impact of pre- and post-veraison deficit irrigation under elevated temperatures in berry skin metabolites from two Tempranillo clones. The study was carried out on fruit-bearing cuttings from two clones of *Vitis vinifera* (L.) cv. Tempranillo (CL-1089 and CL-843) subjected to two temperature regimes (24/14°C and 28/18°C (day/night)) combined with three irrigation regimes during berry ripening. Irrigation treatments were: (i) water deficit from fruit set to onset of veraison (early deficit, ED); (ii) water deficit from onset of veraison to maturity (late deficit, LD); and (iii) fully irrigation (FI). Results showed that at 24/14°C, LD treatment performed better than ED treatment but such differences were attenuated at 28/18°C. Berry primary metabolism was mainly altered by temperature whereas secondary metabolism was more modified by water deficit irrigation, being CL-843 more sensitive to high temperature than CL-1089. Thereby, our data suggest that the adaptation of grapevines for climate change in south Mediterranean Europe might be plausible with the optimization of timing of water deficit and the adequate clonal selection.

INTRODUCTION

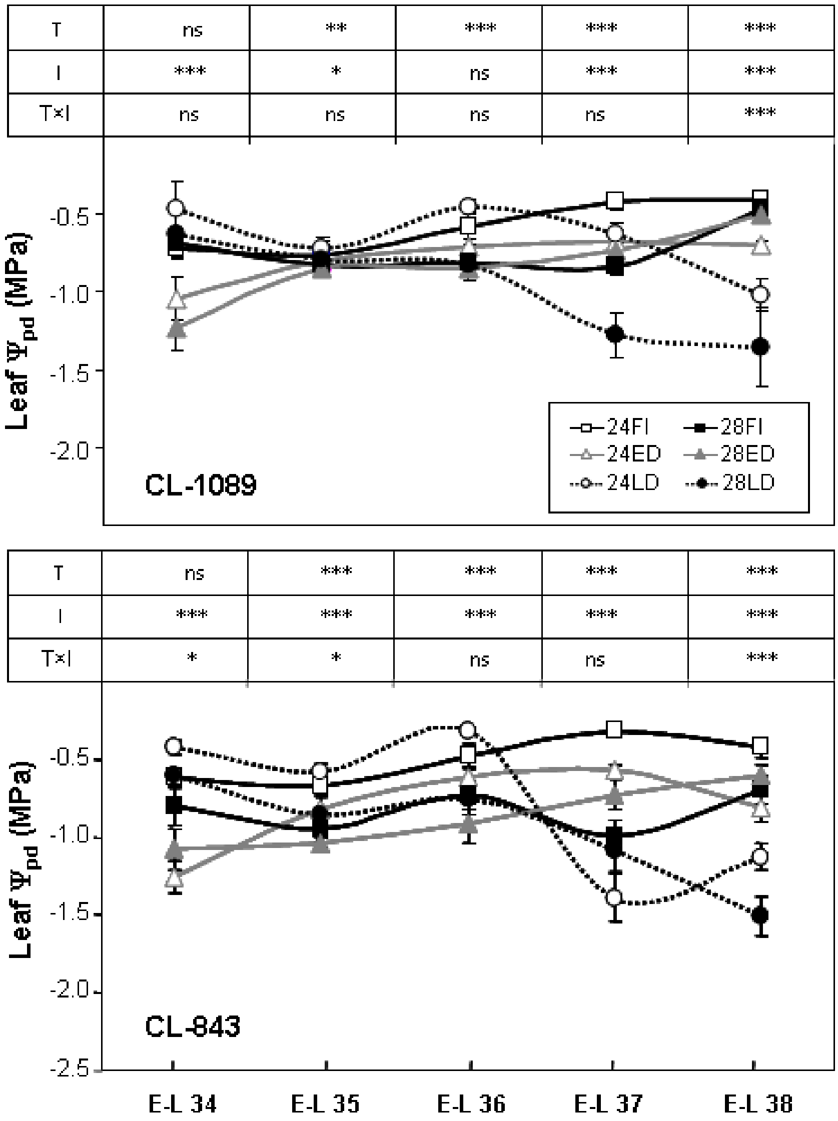
Over south Mediterranean Europe region, the projected warming combined with severe droughts in the growing season is expected to have detrimental impacts on the grape berry ripening (Kuhn et al. 2014). At present, irrigation of vineyards has an increasing trend to control plant water stress (Costa et al. 2016). Therefore, the aim of this study was to investigate the impact of pre- and post-veraison deficit irrigation under elevated temperatures in berry skin metabolites from two Tempranillo clones.

MATERIAL AND METHODS

The study was carried out on fruit-bearing cuttings two clones of *Vitis vinifera* (L.) cv. Tempranillo (CL-1089 and CL-843) subjected to two temperature regimes (24/14°C and 28/18°C (day/night)) combined with three irrigation regimes from fruit set to berry maturity. Irrigation treatments were: (i) water deficit from fruit set to onset of veraison (early deficit, ED); (ii) water deficit from onset of veraison to maturity (late deficit, LD); and (iii) fully irrigation (FI). Plants were grown in greenhouses adapted to simulate climate change conditions (Morales et al. 2014). Predawn leaf water potential (Ψ_{pd}) was measured on fully expanded leaves just prior to irrigation. Primary metabolites (including sugars, organic acids and amino acids) were determined in berry skin extracts following Bobeica et al. (2015) and secondary metabolites (anthocyanins and flavonols) were analysed according to the method described by Martínez-Lüscher et al. (2014).

RESULTS AND DISCUSSION

Temperature and irrigation treatments caused significant differences in grapevine water status throughout fruit ripening, as indicated by the decrease in predawn leaf water potential (Ψ_{pd}) measured in plants subjected to water deficit at pre-veraison (ED) or at post-veraison



T	ns	**	***	***	***
I	***	*	ns	***	***
T×I	ns	ns	ns	ns	***

T	ns	***	***	***	***
I	***	***	***	***	***
T×I	*	*	ns	ns	***

Figure 1. Pre-dawn leaf water potential (Ψ_{pd}) recorded in fruit-bearing cuttings of Tempranillo clones grown either at 24/14°C or 28/18°C (day/ night) temperature regimes. Values represent means ± SE (n = 3). Two-way ANOVA analysis to evaluate temperature (T), irrigation (I) and interaction (T×I) effects was performed. ns, *, **, and *** indicate non-significance or significance at 5%, 1%, and 0.1%

probability levels, respectively. FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation.

(LD) compared with FI plants (Figure 1). At maturity (E-L 38), two-way ANOVA showed significant interaction between factors for plant water status (T×I, P≤0.001). The extent of alteration in grape skin primary metabolism due to temperature was higher than in secondary metabolism, which was mainly affected by water deficit irrigation (Table 1). In general, at 24/14°C LD treatment performed better than ED in terms of anthocyanins and flavonols, but such differences were clearly attenuated at 28/18°C. In addition, temperature and water deficit irrigation modulated berry skin metabolite levels in both clones, CL-843 appearing to be more sensitive to high temperature than CL-1089 (Torres et al. 2016). In this last clone, LD irrigation emerged as an interesting strategy to avoid the effects associated with the warming trends.

CONCLUSION

Data presented here suggest that the adaptation of grapevines for climate change in south Mediterranean Europe might be plausible with the optimization of timing of water deficit and the adequate clonal selection.

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Table 1. Effect of irrigation treatments on main primary and secondary metabolites quantified at maturity in grape skins of fruit-bearing cuttings of Tempranillo CL-1089 grown either at 24/14°C or 28/18°C (day/ night) temperature regimes. Values are means (n = 3). Within each column and clone, means followed by different letter are significantly different ($p < 0.05$) according to Duncan test. ns, *, **, and *** indicate non-significance or significance at 5%, 1% and 0.1% probability levels, respectively. FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation. DM indicates dry matter.

	Glucose ($mg\ g^{-1}$ DM)	Fructose ($mg\ g^{-1}$ DM)	Malic acid ($mg\ g^{-1}$ DM)	Tartaric acid ($mg\ g^{-1}$ DM)	Amino acids ($mg\ g^{-1}$ DM)	Anthocyanins ($mg\ g^{-1}$ DM)	Flavonols ($mg\ g^{-1}$ DM)
CL-1089							
24FI	74.78 b	78.34 c	33.36 ab	41.20 a	81.20 c	31.24 a	0.80 b
24ED	67.76 b	86.70 c	30.32 ab	36.51 ab	90.65 c	8.67 c	0.34 c
24LD	101.32 b	120.44 b	35.00 a	41.93 a	119.32 bc	32.51 a	1.12 ab
28FI	172.63 a	150.00 ab	32.29 ab	29.77 bc	140.88 ab	21.99 ab	0.97 ab
28ED	155.85 a	150.45 ab	25.03 b	24.59 c	114.43 bc	17.90 bc	0.94 ab
28LD	179.54 a	155.86 a	31.91 ab	30.86 bc	177.82 a	27.85 ab	1.26 a
Temperature (T)	***	***	ns	***	**	ns	**
Irrigation (I)	ns	ns	ns	ns	*	**	**
T×I	ns	ns	ns	ns	ns	ns	ns
CL-843							
24FI	125.58 bc	133.18 c	33.94 a	40.31 a	94.83 b	36.37 a	0.72 b
24ED	110.45 c	121.64 c	31.82 a	30.02 bc	102.74 b	12.57 c	0.58 b
24LD	112.52 c	111.84 c	37.16 a	37.87 ab	103.77 b	38.51 a	0.93 ab
28FI	191.00 a	192.48 ab	29.82 a	24.39 c	127.72 b	14.35 bc	1.22 a
28ED	132.43 bc	196.97 a	31.30 a	25.74 c	86.67 b	20.59 b	0.86 ab
28LD	173.86 ab	150.77 bc	36.41 a	28.51 c	287.51 a	17.02 bc	0.88 ab
Temperature (T)	**	***	ns	***	***	***	ns
Irrigation (I)	ns	ns	ns	ns	***	***	ns
T×I	ns	ns	ns	ns	***	***	ns

Influence of rootstocks on pistachio (*Pistacia vera* L.) water relations

Memmi, H.[1], Centeno, A.[2], Moriana, A.[3], Corell, M.[3], Torrecillas, A.[4], Pérez, D.[2]

[1] CA "El Chaparrillo". IRIAF. Ciudad Real.
[2] Universidad Politécnica de Madrid. Av. Complutense s/n. Madrid. ana.centeno@upm.es
[3] Universidad de Sevilla. C/ S. Fernando, 4. Sevilla.
[4] CEBAS-CSIC. Campus Universitario de Espinardo. Espinardo. Murcia.

ABSTRACT

Pistachio potted plants budded on three different rootstocks were submitted to water stress during 28 days with the aim of studying their water relations and physiological responses. Water stress resulted in an accented drop of stem water potential and leaf conductance. Nonetheless, pistachio plants showed a great capacity to contrast drought effects by the recourse to osmotic adjustment mechanisms. Regarding rootstocks, UCB-I results being the less adapted rootstock to conditions of water stress.

INTRODUCTION

The preference of a rootstock against another would be closely related to its drought tolerance which could be defined by its capacity to improve growth in limited water conditions (Kramer and Boyer, 1995).The improvement of growth under water stress seems to be mostly dependent on turgor maintenance (Bradford and Hsiao, 1982). This mechanism is defined as osmotic adjustment. Previous evidence of the occurrence of osmotic adjustment in pistachio has been reported (Gijon et al., 2011) in response to water stress but there is no evidence of the fact that different rootstocks could affect differently the osmotic adjustment at the scion level. Thus the aim of this work was to study the response of pistachio plants to water stress by the means of water relation and to analyze the possible different responses of three widely used rootstocks.

MATERIAL AND METHODS

Site description and experimental design

The experiment was conducted during the summer of 2013 at "La Entresierra" Research Station, Ciudad Real, Spain. Thirty pistachio plants of two-year old (P. vera L. cv. Kerman) budded onto three different rootstocks, P. *atlantica Desf*., *P. terebinthus L.*, and UCB-I were used. The experiment took place from "Day Of the Year" (DOY) 178 until DOY 246 and consisted in the implementation of 28 days of water stress to the half of pots. The experimental design was a completely randomized factorial design with 5 replicates. The main factor was the rootstock and the secondary factor was irrigation. The different combination of the two factors will be named as follows:

P. atlantica-Control (AC); P. atlantica-Stress (AS); P. terebinthus-Control (TC); P. terebinthus-Stress (TS); UCBI-Control (UCB-C) and UCBI-Stress (UCB-S).

Measurements

Soil moisture measurements were taken at 10 and 20 cm depth with a portable capacitance probe (Diviner, 2000, Sentek Pty. Ltd., Australia) placed approximately 15 cm away from the stem.Stem water potential (Ψ_x) measurements were made weekly in all plants. At the same time abaxial leaf conductance (g_l) was measured with a steady-state porometer (LICOR-1600, UK) between 12:00 and 14:00 local time. Pressure–volume (P–V) curves were performed at the end of stress period. The parameters derived from each curve were: osmotic potential at full turgor ($\Psi_{s,100}$), osmotic potential at zero turgor ($\psi_{s,0}$), relative water content at zero turgor (RWC_0), percentage of the symplastic water content (R), tissue elasticity (E_o), osmotic adjustment index (OA_{index}) and the breaking point (BP) (Turner, 2006). An ANOVA was done and means were compared using the test of Tukey, with a significance $P < 0.05$.

RESULTS

Water relations

Figure 1 shows the pattern of Ψ_x throughout the experiment. Ψ_x for stressed plants ranged between -0.79 and -3.4 MPa and the effect of water stress was not detected until DOY 210, ten days after the implementation of stress. From this date until the last day of stress, Ψ_x was significantly different between control plants and stressed plants. Independently from irrigation treatment, PA and UCB-I kept significantly higher Ψ_x trend than PT plants except on DOY 179, 210, 214, 228 and 246 in which a same tendency was preserved but differences were not significant. On DOY 210, AS had the highest values followed by UCB-S and TS being respectively -1.33 MPa, -1.61 MPa, and -1.66 MPa. On DOY 221, no statistical difference was seen between AS and UCB-S but they were significantly different from TS (Figure 1). On the last day of stress, no difference was perceived between rootstocks dropping all to a similar level of Ψ_x and reaching the lowest values during the experiment 28 days after stress implementation. Leaf conductance (g_l) ranged between 36 and 338 mmol $m^{-2}\ s^{-1}$ in stressed plants (Figure 1). Until DOY 214, plants were not grouped into irrigated and stressed plants, and maximum leaf conductance was randomly distributed between irrigated and stressed plants. On DOY 221, UCB-S and AS had a similar gl and were significantly different from TS being respectively 174 mmol $m^{-2}\ s^{-1}$, 149.9 mmol $m^{-2}\ s^{-1}$ and 90.9 mmol $m^{-2}\ s^{-1}$. Water stress decreased RWC_0 in 8 %, $\Psi_{s,100}$ in 0.3 MPa and $\Psi_{s,0}$ in 0.78 MPa. No significant differences were found concerning the rest of parameters.Regarding rootstocks, the three rootstocks showed a significant difference in $\Psi_{s,100}$, E_o and the BP. $\Psi_{s,100}$ and BP were significantly higher in UCB-I and PT showed a significant difference compared to both other rootstocks in the Eo (Table 1).

DISCUSSION

Water stress results in a decrease of stem water potential and a partial closure of stomata (Figure 1). Gijón et al., (2010) applied a stress of 14 days to potted pistachio plants under the same conditions and obtained similar results than the obtained in this work (similar gl values). So, a longer period of stress was not translated in a greater drop of leaf conductance. The maintenance of stomatal opening at lower water potential was reported to be a result of an osmotic adjustment mechanism (Turner and Jones, 1980). A drop of Ψ_x from -0.79 MPa to -3.4 MPa in 28 days was accompanied by a decrease of $\Psi_{s,100}$ by 0.3 MPa and $\Psi_{s,0}$ by 0.78 MPa (Table 1). This mechanism allows the leaves to maintain turgor at lower water potentials, thereby increasing the drought tolerance of the plant. The water potential at turgor loss has been often used to assess physiological drought tolerance and can consequently situate the degree of tolerance of pistachio to water stress among other species. Comparing the present values with other fruit tree species showed that the decrease in $\Psi_{s,0}$ in pistachio is lower than that reported for stressed olive trees (Rieger, 1995) but higher than that reported for citrumelo (Rieger, 1995) and apple (Fanjul and Rocher, 1984) situating then pistachio as less drought tolerant species than olive but more tolerant than the other mentioned ones.

Water stress affected gl in all rootstocks. Nonetheless, PT showed a somewhat higher stomatal control than the other rootstocks. This result seems contradictory with the results obtained from the P-V curves. PT and PA showed a lower $\Psi_{s,100}$ than UCB-I. Considering $\Psi_{s,100}$ as an index of physiological drought tolerance, PT and PA seems to improve their drought tolerance by making their $\Psi_{s,100}$ more negative. These results are in accordance with the previous confirmations that UCB-I is less adapted to water stress than the other mentioned rootstocks (Ferguson et al., 2005). Regarding the difference between PT and PA, the first rootstock was characterized by an increased Eo. Highly elastic cells have been reported as a trait aiding to maintain turgor in some cases. Nevertheless, results are not clear since the increase and the decrease of elasticity have been reported to be linked to drought tolerance (Turner and Jones, 1980).In this work, no difference in the OA_{index} was found but the three rootstocks seem to be able of a full osmotic adjustment regarding BP which is an indicator of drought resistance. UCB-I showed a lower break point than the other rootstocks confirming the previous results (Table 1).UCB-I is known to be the most vigorous rootstock, PA with a median vigor and PT as the smallest (Ferguson et al., 2005). This fact presents contrast with the previous results if osmotic adjustment is considered as the major mechanism driving scion growth. Then, above osmotic adjustment, different mechanisms seem to be operating between rootstock and scion.

CONCLUSIONS

Pistachio plants were able to maintain turgor by different variable emanating from a high osmotic adjustment capacity. Confirming previous findings, UCB-I seems to be the less adapted rootstock to water stress conditions compared to *Pistacia terebinthus* and *Pistacia atlantica*.

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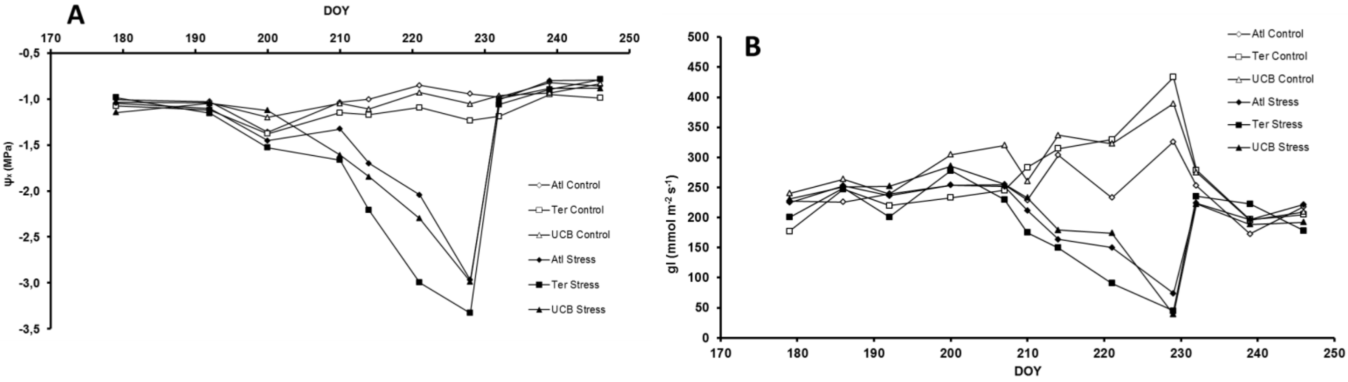

Figure 1. Stem water potential (Ψ_x) (A) and leaf conductance (gl) (B) course during the experiment. Each point is the average of 5 replicates.

Table 1. Pressure–volume curve parameters obtained control, stressed treatment and for each rootstock. Each value is the average of 3 data. Different subscript letters mean significant differences between treatments (Tukey test; $P < 0.05$).

Irrig-trt/rtstk	RWC_0(%)	$\Psi_{s,0}$(MPa)	$\Psi_{s,100}$(MPa)	E_o	R (%)	a	b	BP(MPa)	OA_{index}
Control	88±0**a**	-2.39±0.09**a**	-1.91±0.07**a**	14.90±3.75	34±6	0.39±0.06	-0.59±0.07	-1.49±0.10	0.40±0.06
Stress	80±1**b**	-3.17±0.18**b**	-2.21±0.08**b**	11.93±3.46	34±4	0.52±0.05	-0.66±0.06	-1.72±0.09	0.34±0.05
PT	82±3	-2.98±0.38	-2.15±0.13**a**	25.21±5.11**a**	30±9	0.56±0.08	-0.75±0.08	-1.79±0.13**b**	0.27±0.08
UCB	86±1	-2.44±0.12	-1.82±0.06**b**	7.50±1.51**b**	43±5	0.28±0.06	-0.48±0.07	-1.34±0.11**a**	0.51±0.06
PA	82±1	-2.92±0.13	-2.20±0.09**a**	7.54±2.43**b**	28±3	0.52±0.07**a**	-0.66±0.08	-1.70±0.12**b**	0.33±0.07

Effect of water stress in the flowering induction of olive trees (*Olea europaea* L.).

Corell, M[1,2]., Martín-Palomo, M.J[1,2]., Girón , I[2,3]., Pérez-López, D[4]., Centeno, A[4]., Torrecillas, A[5]., Valverde, F.[6], Moriana, A[1,2].

[1] Dpto. Ciencias Agroforestales, Universidad de Sevilla. Crta de Utrera Km 1. 41013 Sevilla (Spain). amoriana@us.es
[2]Unidad Asociada al CSIC de Uso sostenible del suelo y el agua en la agricultura (US-IRNAS). Crta de Utrera Km 1, 41013, Sevilla, Spain.
[3]Instituto de Recursos Naturales y Agrobiología (CSIC), P.O. Box 1052, E-41080 Sevilla, Spain.
[4]Departamento de Producción Vegetal: Fitotecnia, Escuela Universitaria de Ingeniería Agrícola, Universidad Politecnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain.
[5]Dpto. Riego. Centro de Edafología y Biología Aplicada del Segura (CSIC). P.O. Box 164. E-301000 Espinardo, Murcia, Spain
[6] Instituto de Bioquímica Vegetal y Fotosintésis (CSIC-US). Avda. Americo Vespucio, 49. 41092 Seville (Spain)

ABSTRACT

Olive trees are a traditional fruit in the Mediterranean basin. However, from the 90’s, there has been a great increase in the surface along the world, in traditional and new zones. In some of these new locations such as south of USA, Peru or north of Chile, problems with flowering have been reported. In some fruit trees, such as citrus, drought periods produced a flowering return. Water stress induced for chilling conditions has been reported in different olive cultivars. The aim of this work is to study if water stress could replace the chilling needs of olive trees for flowering. One experiment was performed in growth chamber (24/20ºC, Short day conditions) with 4 years old potted olive trees cv Arbequina which had flowered the previous year. Treatments were: full irrigated, water stress and treatment with AP10 (commercial flowering product). Significant differences in the water relations of full irrigated and AP10 vs water stress trees were found, with a clear reduction in water potential and leaf conductance. Full recovery in around 3-7 days was obtained in stressed trees. Flowering was inhibited in the growth chamber but was promoted when the pots where moved to outdoor conditions. Full irrigated trees presented significant higher number of flower.

INTRODUCTION

The increase of olive surface out of the traditional zones and climate changes could enhance in a near future the problems with flowering in olive orchard (Gabaldón, 2016). Flowering is a biennial process in olive trees with an induction process during winter which needs chilling conditions and a differentiation period strongly links with warm temperatures. Water relations in olive trees during is not widely studied but chilling temperatures induced dehydration in potted olive trees when soil temperature was lower than 10ºC (Pavel and Fereres, 1998) The aim of this work was to study if water stress could replace chilling needs in the flowering of olive trees. The hypothesis of the present work is that the flowering induction process is produced for chilling temperature which induces dehydration in the tree. Therefore, water stress conditions could promote flowering in warm conditions.

MATERIAL AND METHODS

Eight 4-years-old potted trees (50 L) were placed in a growth chamber at the beginning of October 2015. All the trees presented fruits, which were removed before. Chamber conditions were 24/20ºC (day/night) and short day (11/13 hours, day/night) with an average radiation intensity of 240 µmol m^{-2} s^{-1}. All the trees were full irrigated until January 28th 2016 when treatments started. Trees were divided in three blocks of 6 trees and three different treatments were applied from January 28th until March 3rd. Treatments were. Full irrigated control, Stress trees no irrigated until a threshold value of water potential around -3 MPa and AP10 trees. AP10 trees were treated with a commercial product that promotes flowering in vegetables species (AP10). Trees were treated the first day when irrigation was withdrawn and one week later at the growth chamber and 7th and 14th March in outdoor conditions with the same dose ($80*10^{-3}$ g L). When stress treatment were completely recovered, at 7th March, all the trees were moved to outdoor conditions and full irrigated. Water relations were characterized with water potential and leaf conductance. Water potential was measured in a leaf per tree which was covered with aluminium foil at least 2 h before measurement with a pressure chamber (PMS 1000). Leaf conductance was measured in one full expansive and full illuminated leaf per tree with a steady porometer (Decagon).

RESULTS AND DISCUSSION

Fig. 1 presents the pattern of stem water potential during the growth chamber experiment. Stress trees significantly decrease stem water potential values in comparison with Control and AP10 treatments. No significant differences were found between these two latter treatments. Minimum values were near to -3 MPa during 3 consecutive weeks. In this period, partial defoliation of stress trees was observed. After the treatment with AP10, black spot was observed in the AP10 trees but partial defoliation was not detected. AP10 treatment was performed in the first and second week of the drought period (DOY 28 and 35), from this date until the end of the experiment AP10 water potential values were slightly higher than Control. Recovery was completed at the end of the experiment. The pattern of leaf conductance confirms the results of stem water potential. Significant differences were found between Stress and the other two treatments. No significant differences were measured between Control and AP10 during all the experiment in the growth chamber. Recovery of leaf conductance was statistically completed at the end of the experiment. Leaf conductance in the AP10 trees trended to higher values than Control from the date of treatment.

No flowering was observed in the growth chamber. Trees were moved the 7th of March to outdoor conditions and were full irrigated. No significant differences were measured in stem water potential and leaf conductance outdoor (data not shown). Full bloom of the trees was observed at the 6th of June, around two months of delay with the mature trees of the same cultivar which were grown 100 m far. The amount of inflorescences was very low (Fig. 3a) but significant differences were measured between Control with Stress and AP10 trees. This disagreement between number of inflorescence and water status of the trees was related with partial defoliation. The percentage of leaves in the final 20 cm of shoots is presented in Fig 3b. Control trees presented a significantly higher number of leaves than Stress. The partial defoliation in this treatment took places in the growth chamber during the drought cycle. In addition, AP10 trees were an intermediate treatment with no significant differences with Control and Stress. AP10 presented around 10% lower value than Control (85.2% vs 78.9%). This partial defoliation of AP10 occurs in the outdoor after the second treatment.

Flowering was inhibited during the growth chamber experiment. AP10 treatment produces flowering conditions after a short period after the treatment in several vegetables species such as strawberry and tomato. Such lack of results suggests that probably this product needs that the induction process is completed and the treatment alone cannot replace chilling needs. The effect of water stress in theory could replace chilling conditions and, therefore, normal flowering should be obtained outdoor. However, since flowering was delay in all treatments and was very low, also in all the treatments, water stress was not effect in the promotion of flowering. The level of water stress in the growth chamber is moderate according to the references level suggested in the literature (Girón et al., 2015). The low level of flowering was likely related with the chilling needs of the trees which probably they did not were completed. The significant differences between Control and the other two treatments in the number of flower were likely related with the partial defoliation. Rubio (2009) reported that flowering in chamber conditions was inhibited when the leaves besides the budding is eliminated.

CONCLUSIONS

Water stress and AP10 cannot replace chilling needs in olive trees. Further works are needed in order to establish if both could enhance flowering.

ACKNOWLEDGE

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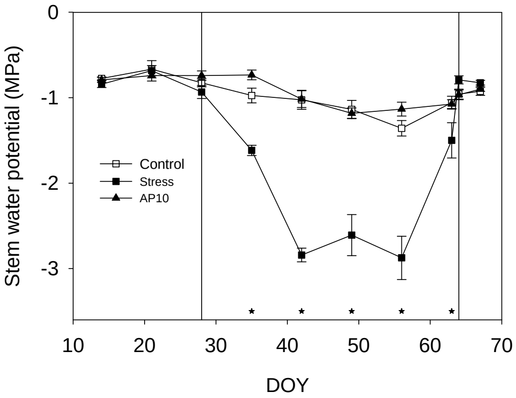

Figure 1. Pattern of stem water potential during the drought cycle (vertical lines) at the growth chamber. Each symbol is the average of 6 data and vertical bars represent standard error. Asterisks indicate the date when significant differences are found (Tukey test, p<0.05).

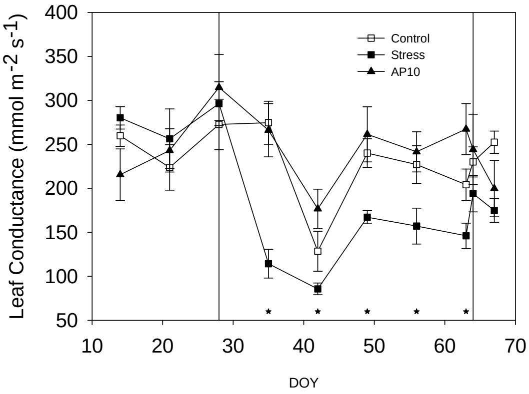

Figure 2. Pattern of leaf conductance during the drought cycle (vertical lines) at the growth chamber. Each symbol is the average of 6 data and vertical bars represent standard error. Asterisks indicate the date when significant differences are found (Tukey test, p<0.05).

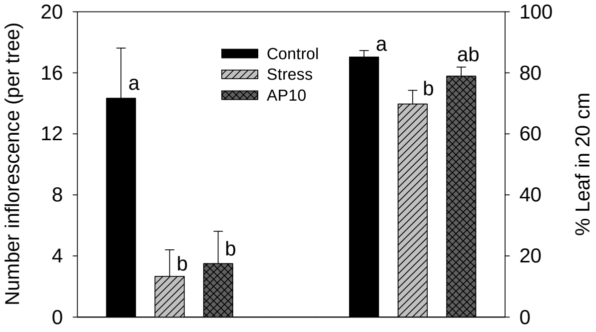

Figure 3. Number of inflorescence per tree (left) and percentage of leaf in shoots (right) of the three treatments. Each column is the average of 6 and 48 data respectively. Vertical bars represent standard error. Different letter in each parameter indicate significant differences (Tukey Test, p<0.05)

Gestión del estrés hídrico del almendro mediante termografía de infrarrojos

García-Tejero I.F.[1], Viñuela, I[1]., Hernández, A[1]., Muriel-Fernández, J.L., López-Segura J.G[1]., Durán-Zuazo V.H[1].

[1]Instituto Andaluz de Investigación y Formación Agraria, Pesquera y de la Producción Ecológica, ivanf.garcia@juntadeandalucia.es.

RESUMEN

El presente trabajo aborda la viabilidad del uso de la termografía de infrarrojos como técnica para el seguimiento del estado hídrico del cultivo del almendro (*Prunnus dulcis* Mill. *cv*. Guara), especialmente cuando éste se ve sometido a situaciones de estrés hídrico. El ensayo experimental se desarrolló en una parcela de almendros jóvenes sometidos a diferentes estrategias de riego deficitario durante el periodo de llenado de grano. Durante dicho periodo, se realizaron tres curvas diarias de potencial hídrico, conductancia estomática y temperatura de copa; entre las 8:30 y 20:00 h. Se definieron las relaciones entre las variables fisiológicas estudiadas y diferentes índices térmicos obtenidos a partir de los valores absolutos de temperatura. De acuerdo con los resultados obtenidos, se pudo concluir que el momento óptimo para la captura de imágenes era entre las 11:30 y 14:30 h, siendo los valores absolutos de temperatura y el CWSI los ofrecían una mayor robustez a la hora de estimar el estado hídrico del cultivo.

INTRODUCCIÓN

El cultivo del almendro en Andalucía ha pasado de tener un carácter marginal, relegado a zonas poco productivas y en condiciones de secano, a ser una clara alternativa a otros cultivos tradicionales en zonas agrarias en regadío. Se caracteriza por su gran capacidad de adaptación para convivir con el déficit hídrico, presentando una respuesta muy positiva a situaciones de escasez de agua. En este sentido, la aplicación de dotaciones hídricas por debajo de las necesidades del cultivo requiere una monitorización del estado hídrico de la planta a modo de evitar situaciones de estrés no permisibles que pudieren provocar efectos negativos en términos de producción y/o desarrollo. La introducción de nuevas herramientas tales como la termografía de infrarrojos está permitiendo desarrollar nuevos estudios relacionados con la eco-fisiología vegetal y las aplicaciones prácticas que dicha técnica tiene en la agricultura y ciencias forestales. Cuenta con la ventaja de ser un método no invasivo, no destructivo, rápido; permitiendo trabajar a diferentes escalas (desde plántulas hasta grandes áreas de cultivo). El objetivo general de este trabajo fue evaluar el potencial de la termografía de infrarrojos como herramienta destinada a la monitorización del estado hídrico en el cultivo del almendro, así como su aplicación para la gestión óptima del riego deficitario.

MATERIAL Y MÉTODOS

El trabajo experimental se desarrolló durante los meses de julio y agosto de 2014, en una parcela de almendros jóvenes (*Prunus dulcis* Mil. *cv*. Guara) de seis años de edad, bajo un marco de plantación de 7 x 6 m, injertados sobre patrón GF677 y localizada en la provincia de Sevilla (37º 30´47¨ N; 5º 58´ 2¨ O).

Se establecieron tres tratamientos de riego: i) un tratamiento control que recibió el 100% de la evapotranspiración del cultivo (ETc), ii) un tratamiento de riego deficitario controlado (RDC), el cual recibió el 50% de la ETc durante el periodo de llenado de grano (finales de junio hasta cosecha), iii) y un tratamiento de riego deficitario de baja frecuencia (RDBF), donde se le aplicó una restricción total del riego, con riegos de apoyo en aquellos momentos donde el cultivo alcanzaba valores de potencial hídrico foliar al medio día por debajo de -2.5 MPa.

Se realizaron tres curvas diarias, con medidas a las 08:30h, 11:30h, 14:30h, 17:30 y 20:00h, durante los días 29 de julio (Curva 1); 5 de agosto (Curva 2); y 27 de agosto (Curva 3); con un seguimiento del potencial hídrico foliar (Ψ), la conductancia estomática (g_s) y la temperatura de copa (T_C) en un total de 12 árboles por tratamiento. El seguimiento de la temperatura foliar se realizó usando una cámara de termografía de infrarrojos (Flir SC660, Flir System, USA, 7-13 µm, 640 x 480 pixels), orientándose el foco de la cámara perpendicularmente a la cara soleada del árbol monitorizado y a una distancia de la copa de 2 m aproximadamente. El análisis de las imágenes se realizó de acuerdo a la metodología propuesta por García-Tejero y col. (2012). Los valores absolutos de temperatura fueron normalizados mediante el cálculo de los índices de estrés CWSI (crop water stress index); I_G (índice de la conductancia estomática relativa) y ΔT (diferencia entre la temperatura foliar menos la temperatura atmosférica en el momento de la captura de la imagen) de acuerdo con Costa y col. (2013).

RESULTADOS Y DISCUSIÓN

La figura 1 muestra la evolución de las variables estudiadas a lo largo del día, observándose que los valores más bajos de Ψ se alcanzaban a las 17:30 h, coincidiendo con el momento de mayor temperatura. En cuanto a g_s, ésta mantenía una tendencia creciente, con descensos en los momentos de máxima demanda atmosférica, seguidos de una recuperación posterior.

En general, Ψ registró las mayores diferencias entre tratamientos, siendo estas diferencias menos patentes en los valores de g_s y T_C. Así, durante la curva 1 y 2 se detectaron diferencias significativas a las 11:30 y 14:30 h entre el control y los tratamientos deficitarios, con un descenso brusco en RDC y RDBF a lo largo del día.

A partir de los valores de temperatura foliar en el control menos los valores de temperatura atmosférica; y teniendo en cuenta los niveles de DPV detectados a lo largo de las 3 curvas y en los cinco momentos de medida (n=15); se calculó la función de referencia como primer paso para la obtención de los índices de estrés CWSI y I_G, tal y como describen Idso y col. (1981) y Jackson (1982). La función obtenida (Fig. 2) mantuvo un comportamiento similar a la registrada para otros cultivos leñosos (Agam y col., 2013; González-Dugo y col., 2014) con un valor de r^2 =0.73 lo suficientemente aceptable como para poder ser usada en la estimación de los índices de estrés anteriormente citados.

En cuanto a las relaciones entre los índices térmicos de estrés (ΔT, CWSI, I_G), y las variables fisiológicas estudiadas (Ψ y g_s), no se observaron relaciones significativas destacables en las medidas realizadas a las 8:30 y 20:00 h (Tabla 1). Especialmente interesantes fueron las relaciones observadas a partir de las medidas tomadas a las 11:30 y 14:30 h, principalmente entre los valores de temperatura absoluta y CWSI frente a Ψ, poniéndose de manifiesto que incrementos en los valores de temperatura foliar se traducían en descensos significativos Ψ.

CONCLUSIONES

En base a los resultados obtenidos se concluye que la termografía de infrarrojos es una alternativa viable para la monitorización del estado hídrico del almendro, especialmente cuando éste se encuentra sometido a situaciones de restricción hídrica. Tanto la temperatura absoluta del cultivo como el CWSI se mostraron como los indicadores térmicos más recomendables, siendo el periodo óptimo del día para la obtención de medidas representativas entre las 11:30h y las 14:30h.

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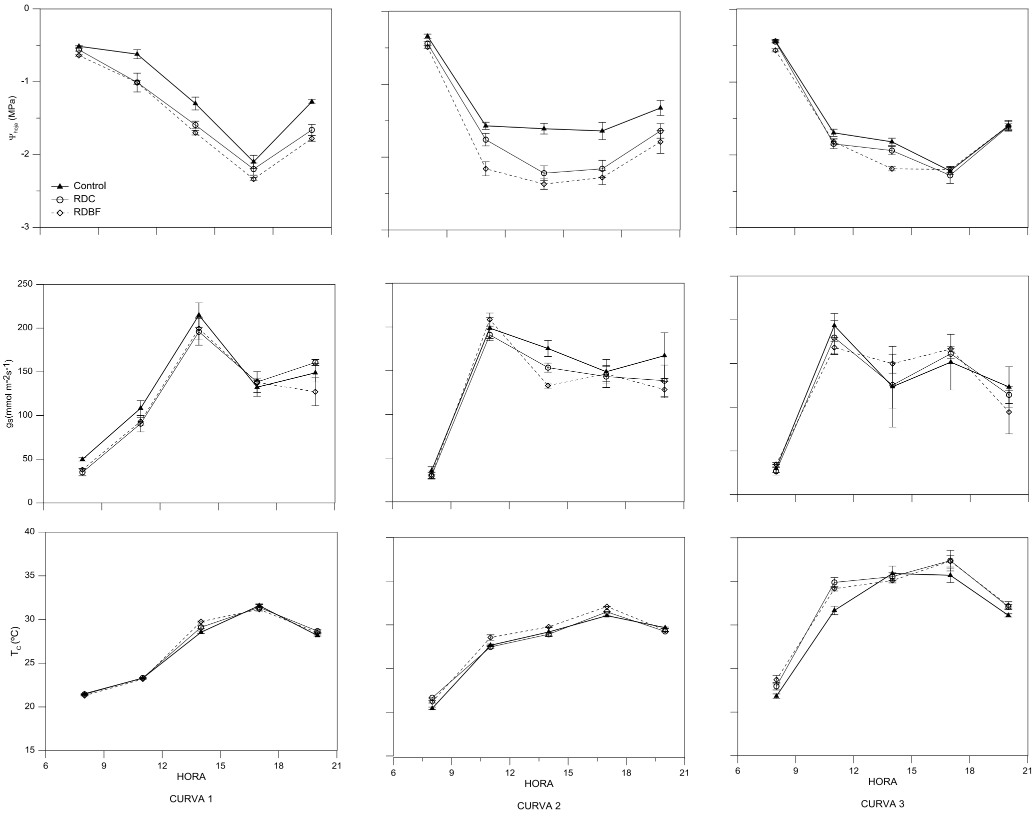

Figura 1. Evolución a lo largo del día del potencial hídrico foliar, la conductancia estomática y temperatura de copa durante las curvas desarrolladas

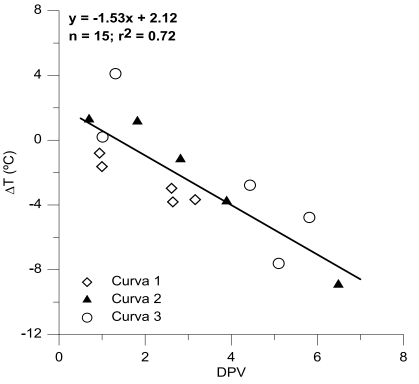

Figura 2. Línea base para la estimación de los índices de estrés CWSI y I_G. DPV, déficit de presión de vapor; ΔT, diferencia entre la temperatura de copa y temperatura del aire

Hora		T_C	ΔT	CWSI	I_G
8:30	g_s	-0.32*	ns	-0.40*	ns
	Ψ_{hoja}	ns	ns	ns	ns
11:30	g_s	ns	ns	ns	ns
	Ψ_{hoja}	-0.85**	-0.69*	-0.85**	-0.95**
14:30	g_s	-0.70*	ns	-0.82**	ns
	Ψ_{hoja}	-0.39*	ns	-0.69*	ns
17:30	g_s	ns	-0.70*	-0.62*	ns
	Ψ_{hoja}	-0.39*	ns	-0.34*	0.74**
20:00	g_s	-0.75*	ns	ns	ns
	Ψ_{hoja}	ns	ns	ns	ns

Tabla 1. Coeficientes de correlación de Pearson entre los indicadores térmicos y las variables fisiológicas.

T_C, temperatura de copa; ΔT, diferencia entre la temperatura de copa menos la temperatura del aire; CWSI, crop-water stress index; I_G, índice de conductancia estomática relativa; Ψ_{hoja}, potential hídrico foliar al mediodía; g_s, conductancia estomática foliar al mediodía.

Using grapevine fruit-bearing cuttings (Red and White Tempranillo) grown under Temperature Gradient Greenhouses (TGGs) in 2013, 2014 and 2015 to evaluate effects of climate change (elevated CO_2, high temperature, and water deficit). II. Yield and must quality

Kizildeniz, T.[a]*, Irigoyen, J. J.[a], Pascual, I.[a], Morales, F.[b]

[a] Universidad de Navarra, Facultades de Ciencias y Farmacia y Nutrición, Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño), c/ Irunlarrea 1, 31008, Pamplona, Spain.

[b] Estación Experimental de Aula Dei (EEAD). CSIC. Dpto. Nutrición Vegetal. Apdo. 13034. 50080. Zaragoza, Spain

*email: tkizildeniz@alumni.unav.es

ABSTRACT

The effects of climate change factors, including elevated CO_2, high temperature and water deficit, acting individually and/or interacting, on reproductive growth and berry quality were investigated on grapevines with 3 experimental repetitions in TGGs. Yield was significantly reduced by drought and was year-dependent, being especially low in 2015 due to eventual heat shocks in the first week of July. In three years of experiments, high temperature and drought significantly and consistently increased must pH, due to the decrease in malic acid. On the contrary, elevated CO_2 decreased pH probably associated with significant increases in tartaric acid concentration. In conclusion, the response of grape quality and reproductive growth was highly variable and depended on the year, probably due to the variability of the climate and the interactions among factors defining the climate (temperature, humidity, sunlight, etc.).

INTRODUCTION

In the Mediterranean area, limitation of crop yield and quality is occurring by climate change that is associated to increased atmospheric CO_2 concentration, elevated temperature and scarce water availability (Tubiello et al., 2000). For this reason, it is urgent to study the effects of climate change scenarios. Previously, we had described a drastic attenuation on vegetative growth by drought (Kizildeniz et al., 2015). In this work, the influence of climate change factors, acting individually and/or interacting, on reproductive growth in fruit-bearing cuttings of two grapevine cultivars was evaluated.

MATERIAL AND METHODS

The climate change scenarios were simulated in experiments (2013, 2014 and 2015, as three experimental repetitions) where eight different treatments from fruit set to maturity on both Red and White Tempranillo (*Vitis vinifera* L.) were applied: CO_2 level (400 *vs* 700 µmol mol^{-1}), temperature (ambient *vs* ambient +4ºC), and water availability (full irrigation *vs* cyclic drought). Experiments were conducted in four temperature gradient greenhouses (TGGs) located in Pamplona (42◦48’9.486”N, 1◦40’1.5882”W) Spain. Yield (bunch fresh weight) and quality (pH, TSS, malic and tartaric acid) were evaluated when berries reached 21-23ºBrix.

RESULTS AND DISCUSSION

Our results agree with the finding that grapevine yield is generally impacted by water stress (Korkutal et al., 2011; Williams and Matthews, 1990). Yield was significantly reduced by drought and year, especially in 2015 due to eventual heat shocks. The high temperatures of the heat shocks (above 35ºC) induced berry burns and browning and finally the loss of 50% of the bunch berries. It is known that water deficit decreases malic acid concentration (López et al., 2007) and must pH (Bahar et al., 2011), which has negative impact on wine quality. In this work, high temperature and drought significantly increased must pH, due to a decrease in malic acid as previously reported in Kizildeniz et al. (2015). In this study, three experimental repetitions showed that this effect is consistent. By the contrary, elevated CO_2 decreased pH probably associated with a significant increase in tartaric acid concentration (Table 1).

CONCLUSIONS

Within the climate change-related factors investigated during 3 years, drought and temperature decreased the grapevine yield of Red and White Tempranillo. The response of grape quality to climate change-related factors was highly variable and depended on the year, probably due to the variability of the climate and the interactions among factors defining the climate (temperature, humidity, sunlight, etc.). This study, which to our best knowledge is the first to address the combined effects of three factors (elevated CO_2, high temperature and drought) linked to climate change on grapevine Red and White Tempranillo berry composition, shows that primary and secondary must metabolites change under elevated CO_2, high temperature and drought conditions, but consecutively repeated experimental simulations are needed in order to minimize the high variability of climate factors.

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Table 1. Yield and fruit characteristics at harvest from fruit-bearing cuttings of Red and White Tempranillo subjected to different CO_2 levels: elevated (E CO_2) or ambient (A CO_2), temperature regimes: high (T+4°C) or ambient (T) and irrigation treatments: full irrigation or water deficit during 2013, 2014 and 2015. Values represent means (n = 5-10). Within each parameters, means followed by a different letter are significantly different (P<0.05).

Treatments				**Years**	**pH**	**TSS** (°Brix)	**Malic Acid** (g L^{-1})	**Tartaric Acid** (g L^{-1})	**Fresh Bunch Weight** (g plant^{-1})
Red Tempranillo	**Full irrigation**	**T**	**A CO_2**	**2013**	4.0 cde	21.8 bcd	4.0 ab	1.22 cdefg	190.3 bcd
				2014	3.9 f	20.7 e	4.0 abc	0.51 ab	193.6 defg
				2015	3.8 d	22.6 abcd	4.1 a	0.25 de	53.6 def
			E CO_2	**2013**	3.9 de	21.1 cdef	4.1 ab	1.26 cde	194.1 bcd
				2014	3.5 g	21.0 de	4.8 a	0.45 abc	252.6 ab
				2015	4.0 abcd	22.7 abcd	3.8 abc	0.39 abcd	96.4 a
		T + 4ºC	**A CO_2**	**2013**	4.1 bcd	21.6 bcd	3.2 cde	1.56 b	164.4 cd
				2014	4.0 cde	21.9 bcde	4.3 abc	0.40 abc	263.6 a
				2015	3.9 bcd	22.5 abcd	3.8 abc	0.34 cd	51.0 efg
			E CO_2	**2013**	4.0 cde	21.3 bcde	3.4 bcde	1.03 gh	173.7 bcd
				2014	3.9 f	21.9 bcde	4.6 ab	0.54 a	249.8 abc
				2015	3.8 cd	23.0 ab	2.8 def	0.55 a	87.4 ab
	Water deficit	**T**	**A CO_2**	**2013**	4.1 bcd	21.8 bcd	3.0 de	0.95 h	187.8 bcd
				2014	4.2 ab	22.0 abcde	4.6 ab	0.35 bc	134.1 h
				2015	3.8 cd	21.8 cd	4.1 a	0.36 bcd	43.2 fg
			E CO_2	**2013**	4.0 cde	24.7 a	3.0 e	1.04 fgh	190.9 bcd
				2014	4.2 ab	23.2 abc	4.4 abc	0.49 abc	218.2 abcde
				2015	4.0 abcd	22.3 abcd	4.0 abc	0.23 de	64.2 cde
		T +	**A CO_2**	**2013**	4.5 a	23.3 ab	3.8 abcd	1.38 bc	138.8 d

		4ºC		**2014**	4.3 a	20.8 de	3.8 abc	0.32 c	181.4 efgh
				2015	4.3 a	23.3 a	2.8 def	0.32 cd	40.5 fg
			E CO_2	**2013**	4.0 cde	20.2 def	3.1 cde	1.80 a	196.5 bcd
				2014	3.9 def	22.7 abcd	4.6 ab	0.44 abc	193.7 defg
				2015	4.0 abcd	22.5 abcd	2.2 fg	0.39 abcd	32.2 g
White Tempranillo	**Full irrigation**	**T**	**A CO_2**	**2013**	4.0 bcde	20.3 def	3.9 abc	1.11 efgh	224.6 abcd
				2014	3.9 ef	22.6 abcd	4.2 abc	0.12 d	211.0 bcde
				2015	4.0 abcd	22.1 bcd	3.2 bcd	0.13 e	84.1 abc
			E CO_2	**2013**	3.9 de	19.0 f	3.5 bcde	1.13 efgh	228.0 abcd
				2014	4.1 cde	23.8 a	4.3 abc	0.51 ab	211.8 bcde
				2015	4.1 abcd	22.0 bcd	3.3 abcd	0.35 cd	86.5 ab
		T + 4ºC	**A CO_2**	**2013**	4.2 b	20.1 def	3.6 bcde	1.14 defgh	204.5 abcd
				2014	3.9 cdef	22.2 abcde	3.4 bc	0.12 d	199.8 cdef
				2015	4.1 abc	21.9 bcd	3.1 cde	0.46 abc	65.5 cde
			E CO_2	**2013**	4.0 cdef	21.0 cdef	3.8 abcd	1.23 cdef	293.3 a
				2014	3.8 f	21.7 bcde	3.5 abc	0.47 abc	243.5 abcd
				2015	3.9 bcd	21.6 d	2.1 fg	0.52 ab	35.7 fg
	Water deficit	**T**	**A CO_2**	**2013**	4.1 bc	19.0 f	4.0 ab	0.99 h	249.7 abc
				2014	4.1 cd	22.3 abcde	3.9 abc	0.12 d	153.0 fgh
				2015	4.0 abcd	21.7 cd	3.2 cde	0.26 de	90.2 ab
			E CO_2	**2013**	3.8 e	23.2 abc	3.4 bcde	1.34 cd	265.7 ab
				2014	4.1 bc	23.5 ab	4.3 abc	0.36 bcd	176.0 efgh
				2015	3.8 cd	22.8 abc	2.1 fg	0.45 abc	78.5 bc
		T + 4ºC	**A CO_2**	**2013**	4.6 a	19.3 ef	4.4 a	0.62 i	190.5 bcd
				2014	4.3 a	20.6 e	4.0 abc	0.47 abc	147.7 gh
				2015	4.2 ab	22.0 bcd	2.4 efg	0.33 cd	81.0 abc
			E CO_2	**2013**	4.1 bcd	21.8 bcd	3.1 cde	0.73 i	203.9 abcd
				2014	4.1 cd	21.5 cde	3.0 c	0.44 abc	172.8 efgh
				2015	4.0 abcd	23.3 a	1.7 g	0.44 abc	72.3 bcd

Physiological mechanisms induced by the different alternation frequency of partial rootzone drying in *Citrus macrophylla* seedlings.

Pérez-Pérez, J.G.[1], Navarro, J.M.[1], Robles, J.M.[1], Dodd, I.C.[2], Quinto, V.[1]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). Departamento de Recursos Naturales, Equipo de Riego y Fisiología del Estrés. c/Mayor s/n 30150 La Alberca (Murcia), Spain. (juang.perez@carm.es)
[2]The Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom.

ABSTRACT

Partial rootzone drying (PRD) establishes discrete wet and dry parts of the rootzone (for example using parallel drip lines on either side of the crop row), and frequently alternates them, thus this study aimed to assess how the alternation frequency affects plant physiological responses. For that, *Citrus macrophylla* Wester seedlings were grown with the root system split in two pots and 5 irrigation treatments were applied: Control, Fixed PRD (where wet and dry parts of the rootzone were not alternated) and three alternated PRD treatments with different alternation timing at 3 (3-PRD), 6 (6-PRD) and 12 days (12-PRD). In control plants, water was equally distributed between both pots, whereas in PRD plants, only one pot was watered while the other was allowed to dry. At the end of the experiment, soil water content (SWC), leaf water potential (LWP), root water potential (RWP), abscisic acid (ABA) concentration in roots ($[ABA]_{root}$), leaves ($[ABA]_{leaf}$) and leaf xylem sap ($[X\text{-}ABA]_{leaf}$), biomass allocation and leaf area were measured. Shorter wetting-drying cycles of PRD (every 3 and 6 days) did not produce significant changes in plant water relations, hormonal response (ABA) and plant biomass allocation. However, increasing the duration of exposure of part of the root system to dry soil (12-PRD and Fixed-PRD) decreased RWP and stimulated root ABA accumulation. Following alternation, root mass on the irrigated side was increased. In addition, in 12-PRD plants, leaf area was significantly reduced by changes in LWP and leaf ABA concentration although xylem leaf ABA concentration was not altered. Since the physiological changes produced by PRD on plant biomass allocation were produced by long wetting-drying cycles, these should be considered when designing future alternated PRD strategies in the field.

INTRODUCTION

PRD is a new irrigation technique initially conceived to alter root-to-shoot chemical signalling by drying part of the rootzone, thereby stimulating root synthesis of ABA and its subsequent transport to the shoot in order to partially close the stomata, thereby increasing leaf water use efficiency (Dodd et al. 2006). In addition to increase the intensity of ABA signalling, PRD can also be involved in other adaptive responses to conserve water such as decreasing canopy area and increasing root biomass (Mingo et al. 2004). However, in some studies the improved water use efficiency of PRD trees has not been related to changes in the root-to-shoot ABA signalling (Pérez-Pérez et al. 2012), so other mechanisms may be involved in this response. The impact of the PRD on the physiology of the plant has been directly related to the exposure time of roots to drying soil. Prolonged exposure of part of the root system to drying soil decreased the effects of chemical signals on stomatal conductance (Dodd et al. 2006), but re-watering the soil after a period of drying promoted new root biomass accumulation (Mingo et al., 2004). Thus, the frequency of alternation is one of the most important factors implicated in the adequate management of PRD. To exploit all the benefits that PRD can provide to the plant, it is necessary to know how different timing of alternation of the drying and rewetting sides affects the physiology of the plant. Thus, the aim of this study was to assess the effect of different alternation frequencies on the physiological parameters of *Citrus macrophylla* seedlings.

MATERIAL AND METHODS

The experiment was carried out in a walk-in controlled environment room (3×6.5 m) at the IMIDA under a 16 h photoperiod (07.00-23.00 h). Day-night variation caused fluctuations in temperature (20-24 ºC) and relative humidity (65-85%). Seeds of *Citrus macrophylla* Wester were germinated in vermiculite over 40 days. Then, the main root was cut to stimulate the development of lateral roots, and seedlings were transferred to 10 L containers (20 seedlings per container) filled with modified Hoagland solution for hydroponic culture. After 8 weeks, roots were pruned maintaining only two main roots and seedlings were transplanted to 2×0.55 L pots (90×90×95 mm) containing silica sand, placing each main root in a different part of the pot. Seedlings were grown for 2 more months before 5 different irrigation treatments were applied: Control, Fixed PRD and three alternated PRD with different alternation timing: at 3 (3-PRD), 6 (6-PRD) and 12 days (12-PRD). The irrigation treatments were maintained for 24 days, watering both sides in Control plants (every 3 days – at 9:00 h) and only the wet side in PRD plants (every 1 and a half days – at 9.00 and/or 21.00 h).

The physiological measurements were done at the end of the experiment. Soil water content of each pot of an individual plant was determined by the gravimetric method. Leaf water potential was measured in a single leaf per plant using a Schölander type pressure chamber, then an overpressure (0.5 MPa) was applied to the leaf to express xylem sap which was collected for later determination of ABA concentration. Root water potential was measured individually in each main root placed in each pot of the plant using a Schölander type pressure chamber. Leaf and root samples for ABA determination were freeze-dried, ground and extracted with deionized water at 1:50 ratio. ABA concentration in leaf xylem sap, leaf and root samples were analysed by a radioimmunoassay. Leaf area of new leaves that appeared during the experiment were measured using a leaf area meter. All leaves, stem and roots from each pot were independently dried and weighed for each plant.

RESULTS AND DISCUSSION

At the end of the experiment (when 12-PRD plants had completed 2 wetting-drying cycles) the application of different alternation frequencies of PRD established distinct soil moisture in the dry side of the root system (side A), whereas the soil water content (SWC) from the wet side of the root system (side B) was maintained higher than 0.18 cm^3 cm^{-3} (Fig. 1A). Analysing the plant water relations, root water potential (RWP) in the side A decreased as soil become dryer, but RWP of Fix-PRD plants was 0.14 MPa higher than in 12-PRD plants (Fig. 1B). Changes in leaf water potential (LWP) were only observed in the 12-PRD treatment, with values 0.35 MPa lower than the other treatments (Fig. 1C).

The different frequency of alternation increased $[ABA]_{leaf}$ only in 12-PRD plants (Fig. 2A), but it was not related to root ABA export, since $[X\text{-}ABA]_{leaf}$ remained unaltered (Fig. 2B). $[ABA]_{root}$ increased in roots exposed to dry soil more than 12 days (12-PRD and Fix-PRD) (Fig. 2C). However, in 12-PRD plants $[ABA]_{root}$ also increased in roots from the wet side, even though RWP remained similar to well watered plants.

Alternated PRD at different frequencies also changed biomass allocation. The root/shoot ratio increased in the irrigation treatments with longer drying cycles (6-PRD, 12-PRD and Fix-PRD) (Table 1). The root mass allocation between sides A and B of the pot was differently altered in 12-PRD and Fix-PRD compared with the control plants. In Fix-PRD, the prolonged heterogeneous soil moisture conditions increased root mass in the wet compartment (side B) (Table 1). However, in 12-PRD plants, root mass was higher in the newly drying side (side A). Based on the response observed in Fix- PRD plants, it is possible that heterogeneous root distribution found in 12-PRD plants, could be partially due to the previous changes in root growth before alternation of the irrigation. In addition, in 12-PRD plants leaf area of new leaves was significantly reduced.

CONCLUSIONS

Correctly choosing the timing of alternation of PRD can offer several agronomic benefits. Greater physiological changes were produced by long wetting-drying cycles of PRD, and were related to the control of vegetative growth by reducing leaf area, and increasing root/shoot ratio, which could improve the capacity of the root to take up water and nutrients.

ACKNOWLEDGEMENTS

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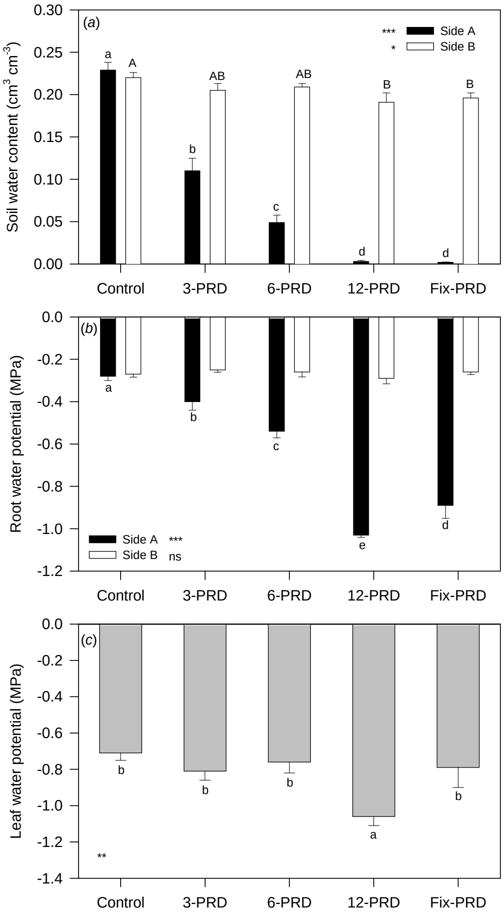

Figure 1. Soil water content (*a*), root water potential (*b*) and leaf water potential (*c*) for each irrigation treatment at the end of the experiment (n=7).

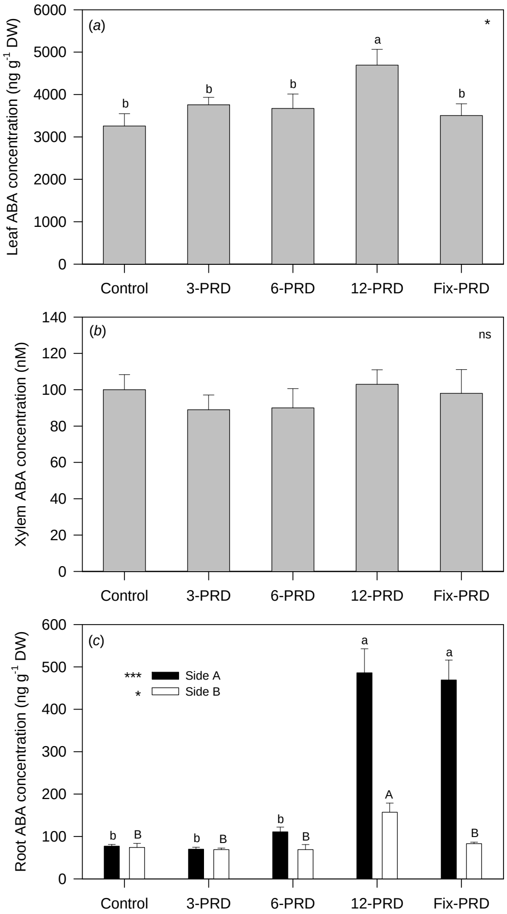

Figure 2. Leaf ABA concentration (*a*), leaf xylem ABA concentration (*b*) and root ABA concentration (*c*) for each irrigation treatment at the end of the experiment (n=7).

Table 1. Root and shoot biomass and leaf area for each irrigation treatment at the end of the experiment.

Treatments	Root mass Side A (g D.W.)	Root mass Side B (g D.W.)	Total root mass (g D.W.)	Shoot mass (g D.W.)	Root/shoot ratio	Leaf area (cm^2)
Control	0.36 b	0.37 ab	0.68	2.59	0.26 b	236 ab
3-PRD	0.46 ab	0.33 b	0.73	2.79	0.26 b	265 a
6-PRD	0.43 ab	0.36 ab	0.73	2.45	0.30 a	212 ab
12-PRD	0.52 a	0.32 b	0.78	2.43	0.32 a	190 b
Fix-PRD	0.34 b	0.47 a	0.74	2.33	0.32 a	212 ab
P-value	*	*	ns	ns	***	*

‘*ns*’, * and *** indicate not significant, $p<0.05$ and $p<0.001$, respectively (n=7). For each column, different letters indicate significant differences at $p \leq 0.05$, by Duncan’s test.

More cash per drop: Partial rootzone irrigation increased water use efficiency compared with conventional deficit irrigation by enhancing fruit water status in 'Fino 49' lemon trees

Pérez-Pérez, J.G.[1], Robles, J.M.[1], Botía, P.[1], Dodd, I.C.[2], Quinto, V.[1]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). Departamento de Recursos Naturales, Equipo de Riego y Fisiología del Estrés. c/Mayor s/n 30150 La Alberca (Murcia), Spain. (juang.perez@carm.es)
[2]The Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom.

ABSTRACT

To determine whether partial rootzone irrigation (PRI) altered water use efficiency and fruit size in lemon (*Citrus limon* (L.) Burm. fil.) compared with regulated deficit irrigation (RDI), a trial was conducted in 2015 on field-grown trees in a semiarid climate. Three irrigation treatments were established: Control (100% ETc), PRI and RDI. In both deficit irrigated treatments the irrigation water volume applied was 75% ETc (from middle of April to the beginning of August) and 100% ETc for the rest of the season. In PRI trees, the wet and dry parts of the rootzone were alternated every 28 days. Stem water potential (Ψ_{stem}), fruit water potential (Ψ_{fruit}), fruit growth and yield parameters were measured. PRI altered the seasonal and diurnal patterns of Ψ_{stem} and Ψ_{fruit}, showing better tree and fruit water status compared with RDI. Compared with RDI, PRI maintained higher fruit growth rates during the deficit irrigation period. Water use efficiency was significantly increased by the PRI strategy mainly due to a higher total yield (25%), without any impact on fruit size compared with control trees. In addition, since more fruits were picked at the first harvest in PRI trees, the economic water use efficiency was increased by 48%.

INTRODUCTION

The lemon crop for fresh fruit market is one of the most important crops in south-east Spain. In this region the availability of water is a major factor limiting production. Thus increasing water use efficiency by using improved irrigation techniques is a priority for citrus growers, to maintain market competitiveness, levels of production and fruit quality. In this sense, new water-saving techniques such as the partial rootzone irrigation (PRI) have been proposed as an agronomic practice for more efficient use of the limited water resources (Stoll et al. 2000). This technique consist in applies alternate drying and wetting cycles to the rootzone, with the aim of supplying sufficient water to prevent or attenuate any leaf water deficit, while exposing some roots to drying soil to stimulate production of chemical signals and their transmission to the shoots to restrict water use and increase crop WUE (Dodd et al., 2006). In comparing PRI with conventional deficit irrigation techniques (which applied the same amount of water), the use of PRI in lemon greatly improved water use efficiency (Pérez-Pérez et al. 2012). However, although the use of PRI throughout the cropping season did not decrease yield, it affected negatively the fruit size (Hutton and Loveys, 2011), suggesting that PRI may only be suitable for producing fruit for juicing rather than fresh fruit. Long drying cycles of PRI increased the root-shoot ratio of citrus (Pérez-Pérez et al. unpublished), which could enhance the supply of water to the fruit and improve the fruit growth. Thus, the aim of this study was to determine whether an optimized PRI strategy (compared with conventional regulated deficit irrigation (RDI)) can improve WUE by minimizing the impact of deficit irrigation on fruit size of lemon. Differences between irrigation treatments in plant and fruit water relations, fruit growth and yield were studied in adult lemon trees grown in a semiarid environment.

MATERIAL AND METHODS

The study was carried out in 2015 in an experimental orchard located in Torre Pacheco (Murcia, Spain) in 22-year-old ‘Fino 49’ lemon trees grafted on *Citrus macrophylla* Wester with a tree-spacing of 8 m x 3 m. The irrigation was applied through two drip-lines separated by 1 m from each side of the trunk, with six self-compensated drippers (3.5 L h^{-1}) per tree, 1 m apart. Three irrigation treatments were applied: Control (100% ETc), regulated deficit irrigation (RDI) and partial rootzone irrigation (PRI); which received 75% ETc during the period of highest root growth (from 17th April to 6th August) and 100% ETc during the rest of the season. Both RDI and PRI treatments received the same water volume. PRI was applied by alternating the wetting and drying irrigation lines every 28 days.The stem (Ψ_{stem}) and fruit (Ψ_{fruit}) water potentials were measured with a pressure chamber (model 3000; Soil Moisture Equipment Corp.). The seasonal measurements of Ψ_{stem} and Ψ_{fruit} were done at noon (12:00-14:00), whereas during the daily measurements were done every 2-3 hours from 6:00 to 21:00 at the end of the deficit irrigation period (6th August) and three weeks after irrigation was fully restored (27th August). For the Ψ_{stem} measurement, leaves were enclosed within foil-covered plastic and aluminium envelopes at least 2 h before the measurement.The fruit growth rate (mm day^{-1}) was calculated as $(D_2\text{-}D_1)/(t_2\text{-}t_1)$, where D is the value of fruit diameter at t_1 and t_2 (mm) and t the time between measurements (days). The individual tree yield (expressed as kg $tree^{-1}$), the number of fruits and the total fruit weight were measured. Yield was assessed in two harvests, with fruits that reached commercial size (fruit diameter > 58 mm) harvested. The yield water use efficiency (YWUE, expressed as kg m^{-3}) was calculated as the ratio of yield to the annual water applied for each irrigation treatment. The economic water use efficiency (EWUE, expressed as € m^{-3}) was calculated as the ratio of gross income (based on mean price of lemon of the last 5 years: 0.40 € kg^{-1} at 1st harvest and 0.21 € kg^{-1} at 2nd harvest) to the annual water applied for each irrigation treatment.

RESULTS AND DISCUSSION

Applying both water saving strategies decreased irrigation volumes by 13% with respect to the control (irrigation water applied: Control=602 mm, PRI=526 mm and RDI=524 mm). The seasonal pattern of the tree water status was differently affected by PRI and RDI strategies during the stress period (75% ETc). The Ψ_{stem} of the RDI trees was lower than of PRI, especially at the end of the fourth wetting-drying cycle (Fig. 1A). The seasonal pattern of Ψ_{fruit} was similar affected by PRI and RDI, showing only significant differences between both treatments at the end of the stress period (Fig. 1B). At this point, the diurnal evolution of Ψ_{stem} and Ψ_{fruit} also revealed a different response between PRI and RDI. During the afternoon, tree and fruit water status of PRI trees recovered faster than RDI trees (Fig. 2). In addition, after the full irrigation dose (100% ETc) was re-established at the beginning of August, tree and fruit water status of PRI trees recovered faster than in RDI trees (Fig. 1).Compared with the RDI treatment, the better tree and fruit water status induced by PRI was reflected in enhanced fruit growth. Trees of PRI maintained higher fruit growth rates than RDI during the deficit irrigated period (Fig. 3). During the recovery period, both PRI and RDI trees had higher fruit growth rates than control trees, indicating that compensatory fruit growth occurred in response to the water stress imposed. RDI and PRI treatments had no negative impacts on yield and precocity of the harvest (Table 1). Indeed, in PRI trees total yield was significantly increased, so the YWUE was significantly improved (44% higher) compared with Control trees (Table 1). From an economic point of view, the use of the PRI strategy increased the EWUE due to the increase of the gross income by the higher number of fruits picked at first harvest (Table 1) compared with the control.

CONCLUSIONS

Based on these results, the use of an optimized PRI strategy, applied only during the first stages of the fruit growth, represents a profitable tool to improve not only the crop water use efficiency, but also the economic profitability of the lemon crop in semi-arid regions.

ACKNOWLEDGEMENTS

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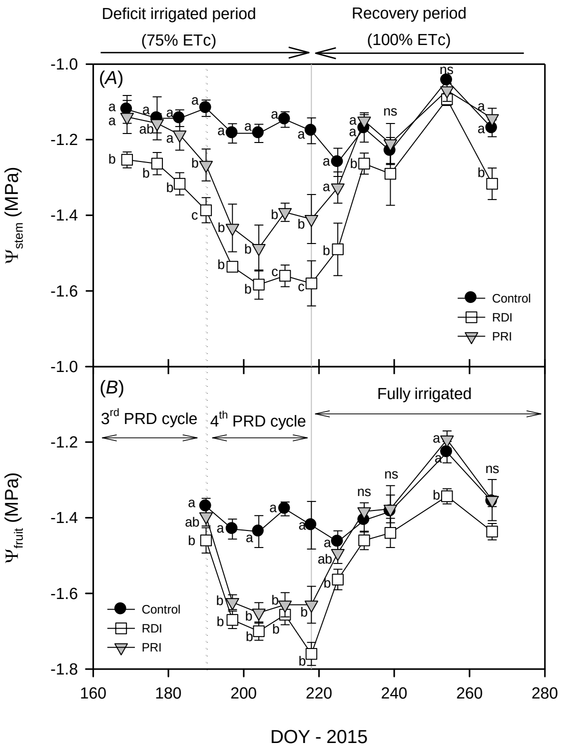

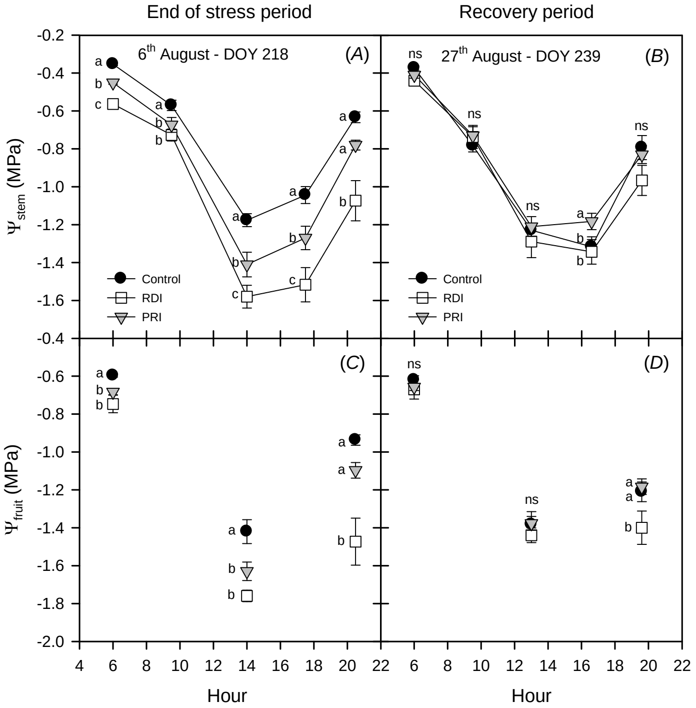

Figure 1. Seasonal values of the stem water potential (Ψ_{stem}) and the fruit water potential (Ψ_{fruit}) at midday (12:30-14:00) for control, regulated deficit irrigation (RDI) and partial rootzone irrigation (PRI) treatments.

Figure 2. Daily values of the stem water potential (Ψ_{stem}) and the fruit water potential (Ψ_{fruit}) for control, regulated deficit irrigation (RDI) and partial rootzone irrigation (PRI) treatments.

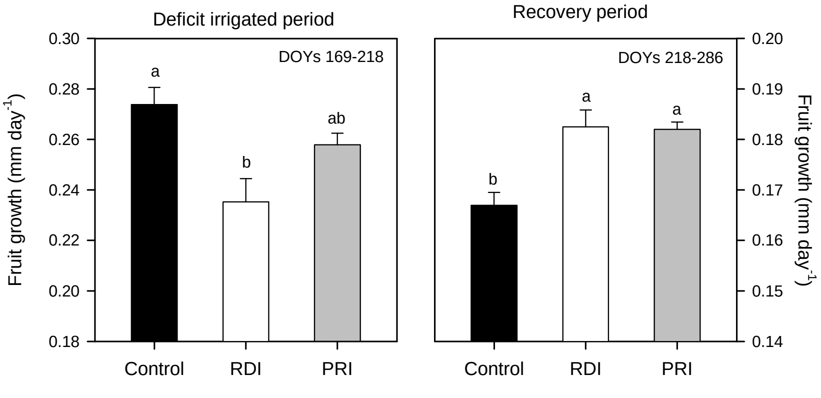

Figure 3. Fruit growth rate for control, regulated deficit irrigation (RDI) and partial rootzone irrigation (PRI) treatments during two representative periods of the study.

Table 1. Yield parameters, yield water use efficiency (YWUE) and economic water use efficiency (EWUE) for control, regulated deficit irrigation (RDI) and partial rootzone irrigation (PRI) treatments.

Treatment	1st harvest (14/10/2015)			2nd harvest (24/11/2015)			TOTAL			YWUE (kg m^{-3})	EWUE (€ m^{-3})
	Yield (kg tree^{-1})	Fruit load	Fruit w. (g)	Yield (kg tree^{-1})	Fruit load	Fruit w. (g)	Yield (kg tree^{-1})	Fruit load	Fruit w. (g)		
Control	42.7	362b	117	85.9	846	102b	128.5b	1208b	107	8.4b	2.3b
RDI	48.3	437ab	110	88.4	838	106ab	136.7ab	1275ab	107	10.1ab	2.9ab
PRI	54.7	468a	117	106.4	979	109a	161.1a	1447a	111	12.1a	3.4a
ANOVA	ns	*	ns	ns	ns	*	*	*	ns	**	**

'*ns*', * and *** indicate not significant, p<0.05 and p<0.01, respectively (n=6). For each column, different letters indicate significant differences at p≤0.05, by Duncan's test.

Evaluación de indicadores fisiológicos de estrés hídrico en limonero ‘Verna’

Robles, J.M.[1], Mira-García, A.B.[1], García-Sánchez, F.[2,3], Quinto, V.[1], Lozano, J.L.[1], Pérez-Pérez, J.G.[1,3]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). Departamento de Recursos Naturales, Equipo de Riego y Fisiología del Estrés. 30150 La Alberca, Murcia. (juang.perez@carm.es)
[2]Centro de Edafología y Biología Aplicada del Segura (CEBAS), CSIC, Campus Universitario de Espinardo, 30100 Murcia.
[3]Unidad Asociada al CSIC de Fertirriego y Calidad Hortofrutícola (IMIDA-CEBAS).

RESUMEN

En este estudio se evalúo la capacidad para detectar el estrés hídrico de diversos indicadores fisiológicos como el potencial hídrico al alba (Ψ_{alba}), el potencial hídrico del tallo a mediodía (Ψ_{tallo}), la máxima contracción diaria del tronco (MCD), y la conductancia estomática (g_s), en árboles adultos de limonero ‘Verna 51’ (*Citrus limon* (L.) Burm. fil.) injertados sobre naranjo amargo (*C. aurantium* L.). Para ello se aplicaron dos tratamientos de riego: Control (100% evapotranspiración del cultivo - ETc) y Sequía (0% ETc durante 49 días y 100% ETc en el periodo de recuperación). A fin de evaluar la capacidad para detectar el estrés de los indicadores se calculó la intensidad de señal (IS), el coeficiente de variación (CV), así como la sensibilidad (S), a diferentes niveles de humedad del suelo. La g_s fue el indicador que mayor IS mostró a lo largo del estudio. Sin embargo, su fiabilidad disminuyó a partir de que el nivel de déficit hídrico del suelo fue moderado, resultado de la gran variabilidad que presentaron sus medidas. La MCD presentó una buena capacidad para detectar el estrés hídrico únicamente cuando el déficit hídrico del suelo fue ligero. Respecto a los indicadores basados en el potencial hídrico, el Ψ_{alba} mostró buena capacidad para detectar el estrés hídrico, a lo largo de todo el experimento, mientras que el Ψ_{tallo} mostró una respuesta similar al Ψ_{alba} hasta que el nivel de déficit hídrico del suelo fue severo, momento en el que la IS dejó de aumentar.

INTRODUCCIÓN

El uso de estrategias de riego deficitario controlado (RDC) tradicionalmente se ha basado en una reducción del porcentaje de agua de riego aplicada en aquellos periodos fenológicos menos críticos para el cultivo. Sin embargo, el grado de estrés hídrico inducido en la planta no ha sido siempre el deseado, provocando en múltiples ocasiones indeseables situaciones severas de estrés que han afectado negativamente tanto a la carga productiva como al tamaño del fruto (Pérez-Pérez et al. 2008). Para evitar estas situaciones, durante los periodos de déficit hídrico, la dosis de riego debe ser ajustada en base al estado hídrico de la planta, consiguiendo así controlar el grado de estrés inducido en estos periodos (Ballester et al. 2011). Existen diversos indicadores que basan su capacidad para detectar cambios en el estado hídrico de la planta en diversos mecanismos fisiológicos sensibles al déficit hídrico.

En cítricos, los indicadores fisiológicos más utilizados son los basados en la medida del potencial hídrico (Ψ_{alba} y Ψ_{tallo}), de la g_s y los derivados de las fluctuaciones diarias del diámetro del tronco (MCD). Por lo tanto, el objetivo de este estudio fue evaluar en árboles adultos de limonero 'Verna' la capacidad para detectar cambios en el estado hídrico de la planta de diversos indicadores fisiológicos de estrés (Ψ_{alba}, Ψ_{tallo}, MCD y g_s), a diferentes niveles de humedad del suelo; ligero (agua útil – AU ≈100-25 %), moderado (AU ≈ 25-10 %), severo (AU ≈ 10-5 %) y recuperación (AU ≈100%).

MATERIALES Y MÉTODOS

El presente estudio fue llevado a cabo entre los meses de mayo y julio de 2016, en una parcela experimental del IMIDA, ubicada en Torre Pacheco (Murcia), en árboles adultos de limonero 'Verna 51' (*Citrus limon* (L.) Burm. fil.) injertados sobre naranjo amargo (*Citrus aurantium* L.). A lo largo del ensayo se aplicaron dos tratamientos de riego, control (100% evapotranspiración del cultivo - ETc) y sequía (0% ETc durante 49 días y 100% ETc en el periodo de recuperación). El Ψ_{alba} y Ψ_{tallo} fueron medidos con la cámara de presión. En el caso del Ψ_{tallo} las hojas fueron introducidas en bolsas de celofán y cubiertas con papel de aluminio, como mínimo 2 horas antes de la medida, siendo ésta entre las 12:00 y 14:00. El contenido de agua del suelo (θ_v) se obtuvo haciendo uso de una sonda de capacitancia (Diviner 2000) y el agua útil se calculó como AU (%) = $(\theta_v-\theta_v^{PM})/(\theta_v^{CC}-\theta_v^{PM})$ x 100, siendo θ_v^{PM} y θ_v^{CC} el contenido de agua en el suelo en el punto de marchitez (PM) y a capacidad de campo (CC). La g_s se determinó a mediodía, entre las 12:00 y 14:00, haciendo uso de un analizador portátil de fotosíntesis (Li-6400). Las fluctuaciones del diámetro del tronco se midieron de manera continúa con dendrómetros (Verdtech, modelo D-5) calculando la máxima contracción diaria (MCD) como la diferencia entre el máximo y el mínimo diámetro diario del tronco. La intensidad de señal (IS) del Ψ_{alba}, del Ψ_{tallo} y de la MCD fue calculada como $IS = \chi_{Sequia} / \overline{\chi}_{Control}$, mientras que en el caso de la g_s fue calculada como $IS = \overline{\chi}_{Control} / \chi_{Sequia}$. El coeficiente de variación (CV) fue calculado como el cociente entre la desviación estándar de las medidas de las plantas sequía en relación a su media ($\sigma / \overline{\chi}$). A partir de la IS y del CV se calculó la sensibilidad (S= IS/CV). Con estos tres parámetros, IS, CV, y S, se evaluó la capacidad que tienen estos indicadores para detectar el estrés hídrico.

RESULTADOS Y DISCUSIÓN

Al inicio del periodo de sequía, cuando el déficit hídrico en el suelo fue ligero (AU entre el 100% y el 25%) (Figura 1A), todos los indicadores presentaron una capacidad similar para detectar estrés hídrico (Tabla 1). Destacar, que la g_s a pesar de presentar una IS ligeramente superior a la del resto de indicadores (1,51), fue el indicador que menor S mostró, consecuencia de un alto CV (≈28%) (Tabla 1). A medida que el contenido de humedad en el suelo descendió por debajo del 25% de AU, la capacidad para detectar el estrés hídrico de los indicadores estudiados fue diferente (Tabla 1). La IS del Ψ_{alba}, del Ψ_{tallo} y de la g_s continuó aumentando gradualmente, mientras que la IS de la MCD empezó a disminuir. En el caso del Ψ_{alba}, el aumento en su IS fue progresivo y uniforme hasta el final del ensayo, mientras que en el caso del Ψ_{tallo} fue progresivo hasta niveles de AU < 10%. (Figura 1F). Esto podría ser consecuencia de un cierre estomático generalizado de la planta, ante condiciones de déficit hídrico del suelo severo, para mantener el estado hídrico de la misma. Por el contrario, la IS de la g_s no aumentó de manera uniforme a partir de un nivel de AU < 20%, mostrando ciertas fluctuaciones (Figura 1F). Dichas fluctuaciones pueden ser debidas principalmente a que la influencia del DPV sobre la g_s fue mayor en los árboles bien regados que en los árboles sequía (Pérez-Pérez et al. 2012). Cuando el déficit hídrico en el suelo fue moderado-severo, la g_s, presentó una mayor IS que el Ψ_{alba} y el Ψ_{tallo}. A pesar de ello, su fiabilidad se vio considerablemente reducida consecuencia de la gran variabilidad que presentaron sus medidas (31%). La MCD fue el indicador que menor sensibilidad presentó en ambos periodos de déficit hídrico en el suelo. (Tabla 1). La falta de sensibilidad de este indicador en condiciones de sequía se debe a la pérdida de plasticidad del tronco ocasionada por la reducción del transporte de agua por el floema. En el periodo de recuperación, el aumento de la humedad del suelo fue paulatino, mostrando ligeras diferencias entre el tratamiento de sequía y el control a los 7 días, mientras que estas diferencias desaparecieron a los 14 días. La capacidad para detectar cambios en el contenido de humedad del suelo durante este periodo fue diferente entre el Ψ_{alba} y el resto de parámetros. La IS del Ψ_{alba} fue significativamente mayor a la del Ψ_{tallo}, de la g_s, y de la MCD (Figura 1F), lo que indica que el Ψ_{alba} posee una mayor capacidad para detectar pequeños cambios en el estado hídrico de la planta ocasionados por pequeñas variaciones en la humedad del suelo.

En base a estos resultados, se recomienda el uso de la MCD, principalmente para la programación del riego, al ser sensible únicamente en condiciones de déficit hídrico del suelo ligero. El uso de la g_s es apropiado cuando el nivel de déficit en el suelo es moderado, pero hay que tener en cuenta la elevada variabilidad que presentan sus medidas. En el caso del Ψ_{tallo}, su capacidad para detectar estrés fue buena hasta un nivel de déficit hídrico en el suelo moderado-severo, mientras que el Ψ_{alba} fue el indicador más versátil, ya que mostró una gran sensibilidad para detectar cambios en el estado hídrico de la planta a un nivel sequedad del suelo muy bajo.

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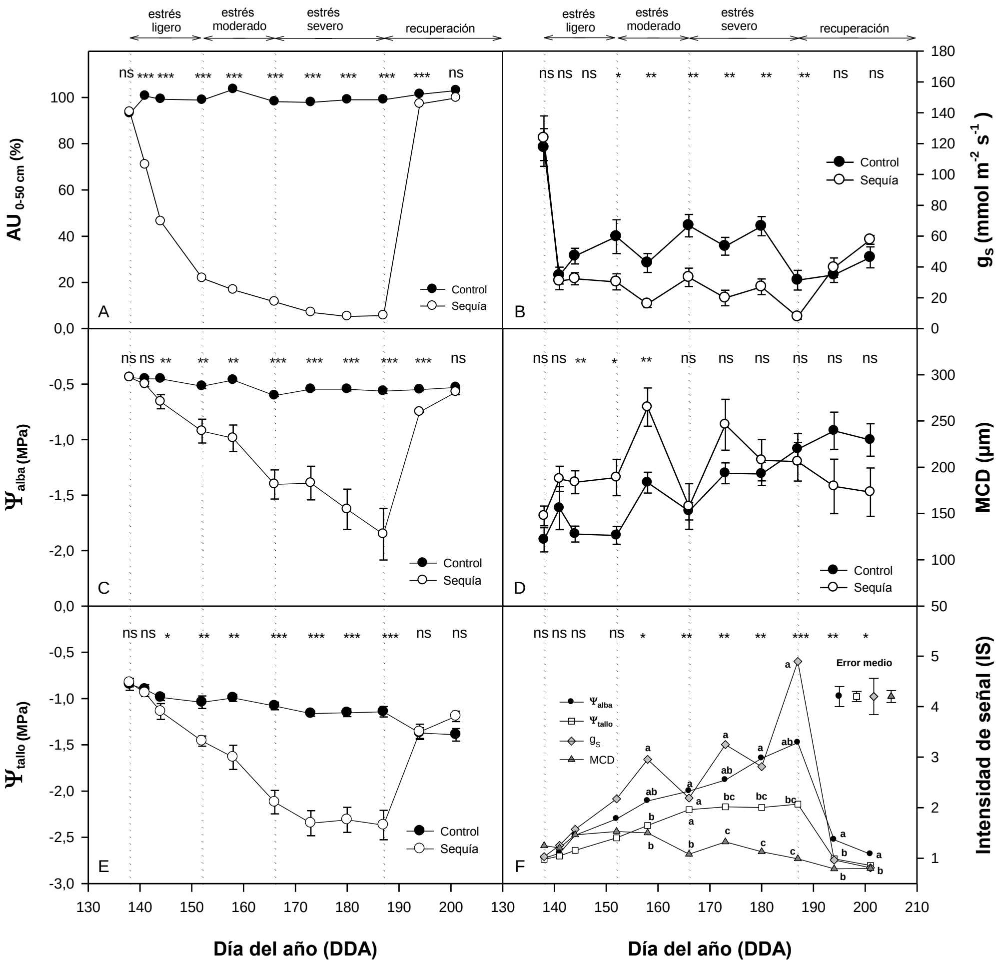

Figura 1. Evolución del porcentaje de agua útil en los primeros 50 cm del suelo ($AU_{0\text{-}50\ cm}$) (A), de la conductancia estomática (g_s) (B), del potencial hídrico al alba (Ψ_{alba}) (C), de la máxima contracción diaria del tronco (MCD) (D), del potencial hídrico del tallo (Ψ_{tallo}) (E), y de la intensidad de señal (F) a lo largo del ensayo. Cada punto corresponde a la media de 6 medidas. Las barras verticales de error muestran el error estándar (± ES). 'ns' indica diferencias no significativas, *, ** y *** diferencias significativas a P< 0.05, P<0.01 y P<0.001 respectivamente.

Tabla 1. Valores medios de la intensidad de señal (IS), del coeficiente de variación (CV) y de la sensibilidad (S) del potencial hídrico al alba (Ψ_{alba}), del potencial hídrico del tallo (Ψ_{tallo}), de la conductancia estomática (g_s) y de la máxima contracción diaria del tronco (MCD) durante diferentes períodos de estrés hídrico.

	Indicador	IS	CV (%)	S
Estrés ligero AU: 100%-25%	Ψ_{alba}	1,33	17,9	7,4
	Ψ_{tallo}	1,15	10,8	10,6
	g_s	1,51	28,0	5,3
	MCD	1,36	16,2	8,4
Estrés moderado AU: 25%-10%	Ψ_{alba}	2,08ab	26,7	7,8
	Ψ_{tallo}	1,67bc	14,8	11,8
	g_s	2,44a	27,7	8,8
	MCD	1,37c	23,3	5,9
Estrés severo AU: 10%-5%	Ψ_{alba}	2,78ab	26,6	10,5
	Ψ_{tallo}	2,01bc	14,0	14,4
	g_s	3,29a	31,2	10,5
	MCD	1,13c	26,4	4,3
Recuperación	Ψ_{alba}	1,22a	4,7	26,2
	Ψ_{tallo}	0,92b	12,6	7,3
	g_s	0,89b	18,8	4,7
	MCD	0,79b	35,1	2,3

En cada columna y en periodo de estrés, letras diferentes en la IS indican diferencias significativas según el método de rango múltiple de Duncan al 95%. (n = 6).

Respuesta fisiológica de nuevos patrones en árboles jóvenes de limonero 'Verna'.

Pérez-Pérez, J.G., Robles, J.M., Navarro, J.M., Lozano, J.L., Quinto, V., Arques, E.M.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). Departamento de Recursos Naturales, Equipo de Riego y Fisiología del Estrés. c/ Mayor s/n 30150 La Alberca (Murcia), (juang.perez@carm.es)

RESUMEN

El objetivo principal de este trabajo fue evaluar la respuesta fisiológica de nuevos patrones en árboles jóvenes de limonero en condiciones de elevada demanda evaporativa. Para ello se llevó a cabo un estudio en árboles de 2 años de edad de la variedad 'Verna 51' injertados sobre tres portainjertos diferentes: *Citrus macrophylla* Wester (CM), Forner-Alcaide 5 (FA-5, híbrido entre mandarino Cleopatra (*C. reshni* Hort. ex Tan.) × *Poncirus. trifoliata*) y Forner-Alcaide 418 (FA-418, híbrido entre citrange Troyer (*C. sinensis* x *P. trifoliata*) × mandarina común (*C. deliciosa* Ten.). Las medidas se realizaron en un día representativo del mes de máxima demanda evaporativa (julio). Comparando la respuesta fisiológica de los nuevos portainjertos FA-5 y FA-418 con CM, los árboles de FA-418 mostraron un vigor bajo, induciendo un menor desarrollo vegetativo, debido principalmente a un descenso de la conductividad hidráulica durante los periodos de máxima demanda y a una baja capacidad para absorber N. Respecto al portainjerto FA-5, el vigor inducido fue similar al mostrado por CM, basado el desarrollo vegetativo y la concentración de N en hoja, pero la limitación hidráulica mostrada durante las horas centrales del día redujo la capacidad fotosintética de la planta, lo cual puede inducir un efecto negativo en el desarrollo vegetativo de la planta en años posteriores.

INTRODUCCION

España es el segundo país más importante a nivel mundial en el cultivo del limón, siendo el sureste español la principal zona productora. El portainjerto tradicionalmente utilizado en limonero ha sido naranjo amargo, pero en los últimos años ha sido reemplazado por el portainjerto *Citrus macrophylla,* ya que no solo resuelve el problema de la incompatibilidad con el portainjerto sino que mejora considerablemente la respuesta productiva respecto a naranjo amargo (Pérez-Pérez et al., 2005). Las condiciones edafoclimáticas del sureste español están caracterizadas por veranos con una elevada demanda evaporativa y una escasez de precipitaciones, lo hace que la disponibilidad de recursos hídricos sea el principal factor limitante de la producción. El cultivo intensivo al cual son sometidas las nuevas plantaciones de limonero para conseguir una rápida entrada en producción ha conllevado a la aplicación de volúmenes de agua y fertilizantes (sobre todo nitrogenados) muy elevados, que en la mayoría de los casos han contribuido a agravar la contaminación de las aguas superficiales y subterráneas por nitratos procedentes de la fertilización, originando uno de los problemas medioambientales más graves a los que se enfrenta la agricultura en la Región de Murcia.

En este sentido, en los últimos años, a través del programa de mejora del IVIA de Valencia se han obtenido nuevos portainjertos con un buen comportamiento en general frente a los diferentes estreses abióticos característicos del sureste español (Forner et al., 2003; Forner-Giner et al., 2014), destacando entre ellos los portainjertos híbridos FA-5 y FA-418. Sin embargo, la información que existe a cerca de la respuesta de estos nuevos portainjertos en limonero en condiciones semiáridas es nula. Por tanto, el objetivo de este estudio fue comparar la respuesta fisiológica de estos nuevos portainjertos (FA-5 y FA-418) frente a *C. macrophylla,* en base a las relaciones hídricas de la planta, intercambio gaseoso, concentración de N en hoja y desarrollo vegetativo.

MATERIAL Y MÉTODOS

En julio de 2016 se llevó a cabo un estudio en la parcela experimental del IMIDA ubicada en Torre Pacheco (Murcia). Para ello se utilizaron árboles de 2 años de edad (plantados en campo en junio de 2015) de la variedad de limonero 'Verna 51' injertados sobre tres portainjertos diferentes: CM, FA-5 y FA-418 con un marco de plantación de 5 × 6 m. El sistema de riego utilizado fue riego por goteo con dos goteros por árbol de 4 L/h y la dosis de riego aplicada fue la adecuada para cubrir las necesidades hídricas del cultivo. El plan de abonado fue 38 UF de N, 10 UF P_2O_5, 14 UF K_2O, 1.4 UF MgO, 1.9 UF CaO y 12 g/árbol de quelato de hierro EDDHA.

Las medidas de potencial hídrico (Ψ_{hoja}), osmótico (Ψ_{π}) y de turgor (Ψ_p) se realizaron al alba (6:00-6:30), a primera hora de la mañana (9:15-9:45) y al mediodía (13:00-14:00). El Ψ_{hoja} se determinó usando una cámara de presión (Soil moisture, mod. 3000), el Ψ_{π} mediante un osmómetro de presión de vapor (Wescor 5520), y Ψ_p se calculó como la diferencia entre Ψ_{hoja} y Ψ_{π}. Los parámetros de intercambio gaseoso se midieron a primera hora de la mañana y al mediodía y para ello se utilizó una consola LICOR 6400. La limitación al mediodía (ΔM) se calculó como el cociente de variación de un parámetro al mediodía respecto a primera hora de la mañana [$1-(M_{13:00}/M_{9:45})$] La conductividad de la planta (Kh_{planta}) se ha calculado mediante el método de flujo comparativo (flujo de transpiración dividido por el gradiente del potencial hídrico ($\Delta\Psi$) entre el potencial del suelo ($\approx\Psi_{hoja}$ al alba) y el Ψ_{hoja} en el momento de la medida).

El desarrollo vegetativo se determinó mediante la medida (en m) de la altura (Alt_{copa}) y el diámetro de la copa (D_{copa}) del árbol. También se calculó el volumen de copa utilizando la siguiente ecuación: $V_{copa}(m^3)=0.5238\times Alt_{copa}\times D_{copa}^2$ (Turrell, 1961). En el material vegetal lavado con agua destilada, secado en estufa a 65 ºC durante 48 horas y triturado, se determinó el N total mediante el proceso de combustión de Dumas utilizando un analizador de nitrógeno Leco FP-428.

RESULTADOS Y DISCUSIÓN

Bajo condiciones no limitantes de riego, la diferente morfología del sistema radicular de los portainjertos estudiados alteró de forma diferente la capacidad de absorción de agua y nutrientes del suelo. Durante el día, el aumento de la demanda evaporativa produjo un descenso del Ψ_{hoja} más rápido en FA-418 y FA-5 que en árboles de CM (Figura 1A), debido a una disminución de la Kh_{planta} (Tabla 1). La baja capacidad de las raíces de ambos portainjertos híbridos para suplir las pérdidas de agua por transpiración produjo un descenso del Ψ_p respecto a portainjertos más vigorosos como CM (Figura 1C). Respecto a los parámetros de intercambio gaseoso, a primera hora de la mañana los portainjertos híbridos mostraron una mayor A_{CO2} respecto a CM, debido principalmente a una mayor g_s (Tabla 1). Sin embargo, la elevada demanda evaporativa durante las horas centrales del día (DPV=2.92 kPa) provocó un mayor descenso de la A_{CO2} y de la E_{hoja} en FA-5 y FA-418 que en CM, ocasionada por una fuerte limitación estomática (Tabla 1). Esta diferente respuesta indica menor sensibilidad de la g_s a cambios en el DPV en CM que en los portainjertos híbridos.

Aunque la dosis de fertilizante aplicada fue similar para todos los árboles del estudio, CM y FA-5 mostraron una concentración de N en hoja significativamente superior a FA-418 (Tabla 2). Tras un año de la plantación en campo, las diferencias en el vigor se hicieron evidentes entre el portainjerto de menor vigor (FA-418) y los portainjertos CM y FA-5 (Tabla 2), induciendo menor desarrollo vegetativo cuando está injertado bajo limonero, típico de patrones con carácter enanizante (Forner-Giner et al., 2014). La falta de vigor de FA-418 se debió principalmente a una mayor limitación de la A_{CO2} debido una limitación de la conductividad hidráulica al mediodía, en condiciones de máxima demanda, y a una baja capacidad para absorber N (Tabla 2). Aunque no se apreciaron diferencias significativas en el volumen de copa entre árboles de FA-5 y de CM, es necesario matizar que el volumen de copa de los árboles de FA-5 fue un 21% inferior a los de CM. Este hecho es debido a una mayor limitación al mediodía de la A_{CO2}, similar a la observada en árboles de FA-418, por lo que se espera que aparezcan diferencias en el desarrollo vegetativo en años posteriores.

Por lo tanto, desde un punto de vista fisiológico y, en base a su desarrollo vegetativo mostrado durante el primer año de plantación, CM fue el portainjerto que mostró un uso más eficiente del agua y de los fertilizantes que los nuevos portainjertos FA-5 y FA-418.

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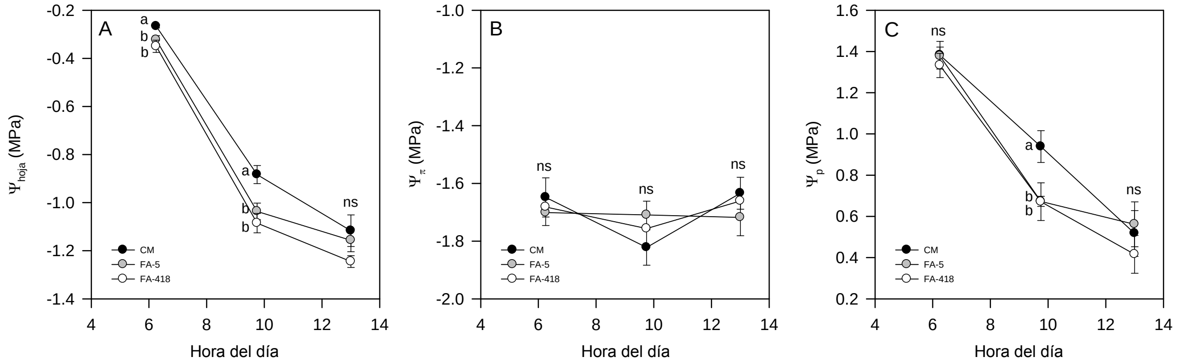

Figura 1. Evolución diaria del potencial hídrico (Ψ_{hoja}) (A), osmótico (Ψ_{π}) (B) y de turgor (Ψ_{p}) (C) en árboles jóvenes de limonero 'Verna 51' injertados sobre tres portainjertos (CM, FA-5 y FA-418). 'ns' indica diferencias no significativas. Letras diferentes indican diferencias significativas al 95% según el test de Duncan.

Tabla 1. Parámetros de intercambio gaseoso y conductividad hidráulica de la planta en árboles jóvenes de limonero 'Verna 51' injertados sobre tres portainjertos (CM, FA-5 y FA-418) en dos momentos del día.

	A_{CO2} *(µmol CO_2 m^{-2} s^{-1})*	g_s *(mol H_2O m^{-2} s^{-1})*	E_{hoja} *(mmol H_2O m^{-2} s^{-1})*	Kh_{planta} *(mmol m^{-2} MPa^{-1} s^{-1})*
Primera hora de la mañana (9:45) – DPV = 1.41 kPa				
CM	9.0 b	0.085 b	3.08	4.98
FA-5	13.0 a	0.125 a	4.17	5.93
FA-418	10.8 ab	0.120 a	3.76	5.18
ANOVA	**	*	ns	ns
Mediodía (13:00) – DPV = 2.92 kPa				
CM	9.0	0.090	3.74 a	4.50
FA-5	7.6	0.069	2.92 b	3.59
FA-418	7.5	0.070	2.96 b	3.31
ANOVA	ns	ns	*	ns
Limitación al mediodía - $\Delta M=1-(M_{13:00}/M_{9:45})$				
CM	-0.05 b	-0.13 b	-0.28 b	0.07 b
FA-5	0.41 a	0.43 a	0.29 a	0.37 a
FA-418	0.30 a	0.41 a	0.22 a	0.36 a
ANOVA	*	*	*	*

'ns' y * indican diferencias no significativas y *P*<0.05 respectivamente. Letras diferentes dentro de cada columna y cada periodo de medida indican diferencias significativas al 95% según el test de Duncan.

Tabla 2. Valores medios de la concentración de nitrógeno en hoja y de los parámetros de desarrollo vegetativo de árboles de limonero 'Verna 51' injertados sobre tres portainjertos (CM, FA-5 y FA-418).

Patrones	Nitrógeno (% peso seco)	Altura de copa (m)	Diámetro de copa (m)	Volumen de copa (m^3)
CM	2.48 a	1.40 a	1.27 a	1.20 a
FA-5	2.44 a	1.36 a	1.15 a	0.95 a
FA-418	2.17 b	1.04 b	0.71 b	0.29 b
ANOVA	*	***	***	***

* y *** indican diferencias significativas a P<0.05 y P<0.001 respectivamente. Letras diferentes dentro de cada columna indican diferencias significativas al 95% según el test de Duncan.

Thermal patterns in the vineyard to support management

Costa J.M. [1,2] Egipto, R.[2], Lopes C.M.[2,] Chaves M. [1]

(1) LEM-ITQBNova, Universidade Nova de Lisboa, Apartado 127, 2780-901 Oeiras, Portugal

(2) LEAF, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

E-mail: miguelc@itqb.pt

ABSTRACT

Irrigated viticulture expanded fast in Southern European countries to optimize yield and quality and to promote vine longevity. However, intensive irrigation practices increase pressure over the already scarce water resources. In addition, row crops such as grapevine, are more vulnerable to heat stress due to the effects of soil heat fluxes which can influence canopy and berry thermal condition. Therefore, a better understanding of grapevine responses (diurnal and seasonal) to environmental factors (air temperature, VPD, soil water) and agronomic practices (deficit irrigation, soil management) are still required. Ground based thermography was used to monitor canopy and soil temperature patterns along the day and season as a tool to assess vine water status and predict risks of heat stress damage. In the frame of the EU-INNOVINE project, field trials were carried in 2014 and 2015 in South Portugal. We examined the diurnal and seasonal response of two *V. vinifera* varieties Aragonez (syn. Tempranillo) and Touriga Nacional subjected to sustained deficit irrigation (SDI), and regulated deficit irrigation (RDI, about 50% of the SDI). Diurnal canopy (T_C), and soil surface (T_{soil}) temperatures were assessed by thermography. Punctual measurements of leaf temperature with thermal couples, leaf water potential and leaf gas exchange were also done. T_C values were above the optimal temperature for leaf photosynthesis during the day light period (11-14h to 17h), especially under high VPD and high Tair conditions and in RDI vines. T_{soil} was on average about 10-15ºC higher than T_C. We found good correlation between T_C retrieved by thermography and eco-physiological parameters in both years. In turn, the good correlation between T_C and thermal couple temperature data supported robustness of the thermal imaging approach.

INTRODUCTION

Extreme air temperatures combined with dry soils, and an increasing frequency of heat waves, represent a major threat to South European Mediterranean viticulture (Medrano et al., 2015; Costa et al, 2016). In addition, row crops such as grapevine, can be more exposed to heat stress due to the effects of soil heat fluxes (latent and sensible). Therefore, studies on drought and heat responses in grapevine together with improved knowledge on heat and water fluxes in the vineyard are highly relevant to minimize risks for the crop due to extreme climate events. Remote sensing is one of the pillars of precision viticulture but it still needs optimization for open-field applications (e.g. crop and soil monitoring, genotype characterization). This applies to thermal remote sensing, which can support characterization of vine's water status (Garcia-Tejero et al., 2016) and detection of energy and moisture fluxes at the land surface (Soliman et al., 2015).

MATERIAL AND METHODS

Field trials were located in South Portugal (38º22' N 7º33' W) under typical Mediterranean climate conditions. We used 11-year old vines of the cvs Touriga Nacional (TOU) and Aragonez (ARA) (syn. Tempranillo) grafted on the 1103-P rootstock (2,200 pl/ha), trained on a vertical shoot positioning and pruned on a bilateral Royat Cordon system. The soil has a silty-clay-loam texture, with pH=7-7.6, low O.M., high P_2O_5 and K_2O. Vines were subjected to two irrigation treatments: 1) Sustained Deficit Irrigation (SDI, control), watered since pea size berry (about 30% ETc, done according to farm's schedule) and 2) Regulated Deficit Irrigation (RDI), subjected to early imposed water deficit (pea size – veraison) and late deficit (ripening) (about 65% of the SDI). Diurnal courses of leaf water potential (Ψ), canopy (T_C) and soil temperature (T_{soil}) and individual leaf gas exchange were implemented. T_C was determined by thermography (B20, Flir Systems, 7-13 µm, ε=0.96). Thermal IR imaging was followed by RGB imaging and individual leaf gas exchange (Licor 6400, Li-cor, USA). Thermal images were analysed with the ThermaCAM Researcher software (Flir Systems, USA). We used a randomized complete block design (2 irrigation treatments with 4 replications/blocks). Pearson correlations between variables (T_C, g_s, ψ_{pd}) were done (Statistix 9.0 software).

RESULTS AND DISCUSSION

Inter-annual climate variation resulted in different irrigation requirements and different T_C patterns in the vineyard. T_C values derived from thermography (sunlit side of the canopy) and individual leaf temperature values were well correlated indicating that ground based thermography provides robust information on T_C, in a faster way and for a larger monitored area. Thermography enabled to assess T_{soil} and T_C variation patterns along the day (and night). T_{soil} often exceeds canopy temperature, on average 10-15ºC, and the largest differences between T_{soil} and T_C occur at 14h, when sun is at its zenithal position and sun beams are more perpendicular to the soil. Results from the two years, indicate that canopy temperature can be a simple but robust thermal indicator to assess vine's performance (Fig. 1). Correlations between gs and Ψ_{pd} and the T_C measured between 14.00h and 17.00h ($T_{C14-17h}$) were particularly strong in 2015 (Fig. 1), when climatic conditions were more stressful (dryer and hotter) than in 2014 (data not shown). Differences between genotypes were not evident under the tested growing conditions. The strong correlation between $T_{C14-17h}$ and major eco-physiological parameters suggest that canopy temperature can be explored as a simple but robust thermal indicator for irrigation management and eventually as a parameter to feed growth models for grapevine.

AKNOWLEDGEMENTS

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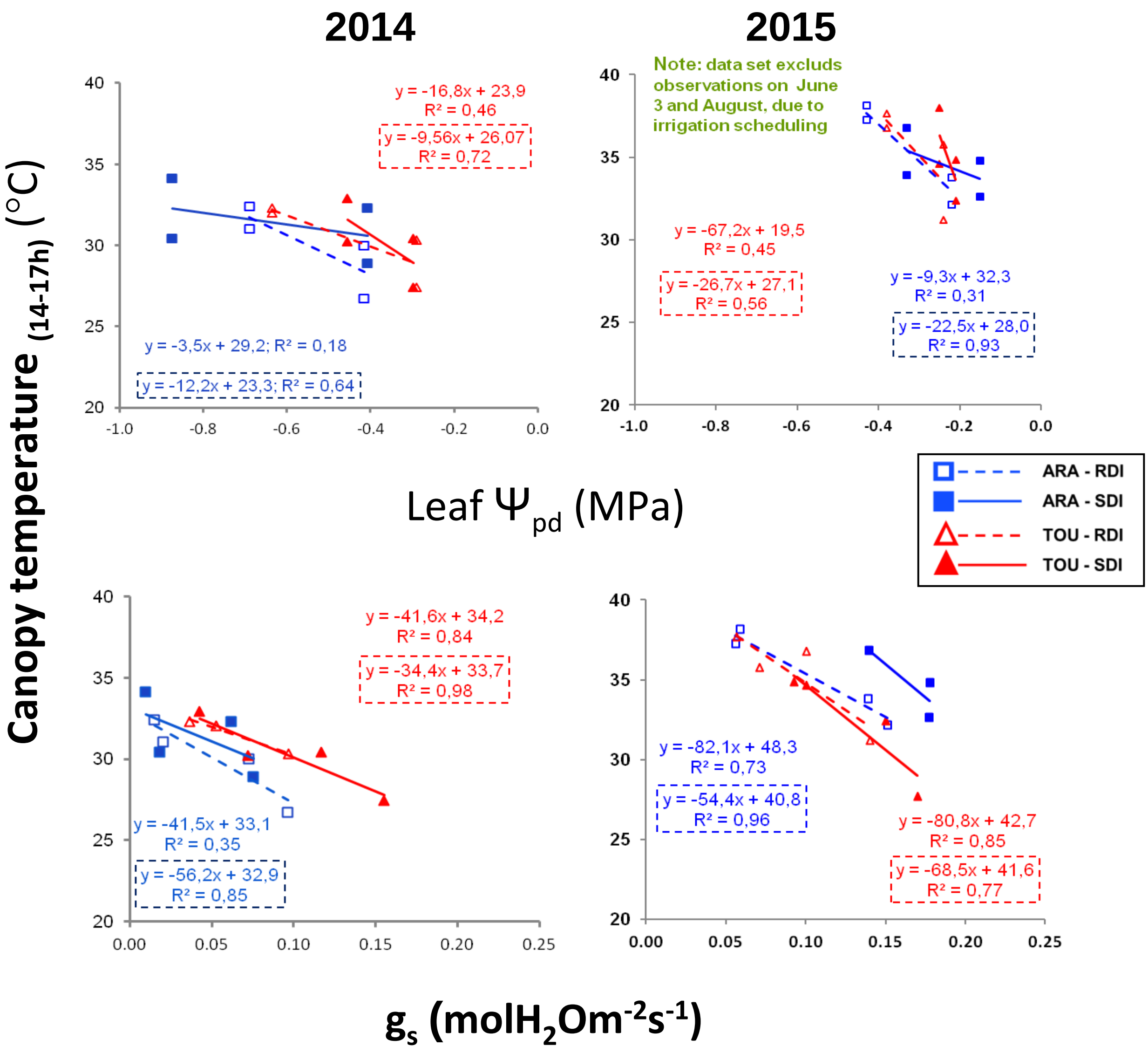

Figure 1. Correlation between sunlit canopy temperature measured between 14 and 17 hours ($T_{C\ 14-17h}$), and two major eco-physiological parameters (leaf water potential at predawn -Ψ_{pd}, and leaf stomatal conductance to water vapour - gs, measured for ARA and TOU vines subjected to two deficit irrigation strategies (RDI e SDI), in the years of 2014 and 2015.

SESIÓN III

Relaciones hídricas en especies no cultivadas y forestales

Diferencias en las relaciones hídricas, acumulación de biomasa y de iones Cl y Na en plantas de lentisco bajo condiciones de salinidad y riego deficitario moderado y severo

Álvarez, S.[1], Broetto, F.[2], Fabricio, D.[2], Sánchez-Blanco, M.J.[1]
[1] Centro de Edafología y Biología del Segura (CEBAS-CSIC). Apartado 164. 30100 Murcia, España. salvarez@eead.csic.es
[2] Instituto de Biociências UNESP, Departamento de Quimica e Bioquimica, Botucatu, Brasil

ABSTRACT

Pistacia lentiscus (mastic) is a wild species that widely grows in the Mediterranean area. However, despite it appears to be particularly resistant to some stressful conditions, drought and salinity may alter its physiological and morphological behavior. Knowledge of the answer to this species at different levels of water and salt stress is interesting for its use in gardening and landscaping projects. *P. lentiscus* plants were grown in nursery conditions and subjected to four irrigation treatments lasting eleven months: control (1 dS m^{-1}, 100% water holding capacity), two deficit treatments: moderate deficit irrigation (RDM) and severe deficit irrigation (RDS), corresponding to 60 and 40% of control, and saline (4 dS m^{-1}, same amount of water supplied as control). Both salinity and water deficit reduced plant height, especially under severe deficit irrigation, while biomass accumulation only was reduced in plants exposed to deficit irrigation. The Na and Cl concentrations in the plants increased with salinity, although the Cl entry through the roots was more restricted than Na entry. Plants submitted to saline stress were not able to retaining Na in the root system and these plants increased their leaf Na concentration sharply. Both stresses produced a reduction on stomata conductance, suggesting an efficient and adaptive stomata control in this species. The decrease in stomatal conductance was more marked in the plants submitted to severe deficit irrigation, which decreased photosynthesis and could delay plant recovery or even cause permanent damage.

INTRODUCCIÓN

Pistacia lentiscus (lentisco) es una especie común de la vegetación de la cuenca mediterránea (Vilagrosa et al. 2003). Debido a que presenta cierto grado de tolerancia al déficit hídrico se ha propuesto como especie adecuada para jardinería y restauración de zonas semiáridas costeras. No obstante, poco se conoce sobre las respuestas en plantas arbustivas ornamentales (Álvarez y Sánchez-Blanco 2015). Por ello, el objetivo de este trabajo ha sido profundizar en el conocimiento de aspectos fisiológicos y morfológicos relacionados con el estado hídrico, intercambio gaseoso, acumulación de iones y crecimiento de plantas de *P. lentiscus* bajo condiciones de salinidad y distintos niveles de riego deficitario.

MATERIALES Y METODOS

En esta experiencia se emplearon 160 plantas de *Pistacia lentiscus*, de 1 año de edad, que fueron trasplantadas a macetas de 4 L de volumen. El sustrato fue una mezcla de fibra de coco, turba negra + turba rubia y perlita (5:4:1) y enriquecida con 2 g L^{-1} con un fertilizante de liberación lenta. Estas macetas fueron trasladadas a un invernadero y a partir de ahí sometidas, durante 11 meses a cuatro tratamientos diferenciales de riego: un control regado diariamente a capacidad de campo (C, CE; 1 dS m^{-1}), dos tratamientos deficitarios (RDM y RDS), que corresponden al 60% y 40% de la cantidad aportada al control, y un tratamiento regado como el control, pero con agua salina (Sal, CE; 4 dS m^{-1}).

Para la evaluación de la acumulación de biomasa se realizó un muestreo destructivo en el que se determinó el peso seco (PS) en 10 plantas por tratamiento al término del ensayo, distinguiéndose hoja, tallo y raíz. Periódicamente, se midió la altura de la planta en 30 plantas por tratamiento. Paralelamente se midió el contenido de solutos inorgánicos. El ión Cl^- se determinó sobre un extracto acuoso mediante un analizador automático (Model 926 Chloride Analyser, Sherwood Scientific Ltd.) y el contenido de Na^+ mediante el analizador ICP-OES IRIS INTREPID II XDL. Al finalizar el ensayo se midió la conductancia estomática (g_s) y la tasa de fotosíntesis neta (P_n) al mediodía solar usando un aparato portátil LI-COR 6400 (LI-COR Inc., Lincoln, NE, USA), así como del contenido relativo de agua (CRA) y el potencial hídrico foliar ($\square_h$), usando una cámara de presión (Model 3000; Soil Moisture Equipment). La significación de los efectos de los tratamientos de riego fue analizada mediante un análisis de varianza simple, usando Statgraphics Plus y las medias de los tratamientos fueron separadas con la Prueba de Rango Múltiple de Duncan ($P \leq 0.05$).

RESULTADOS Y DISCUSIÓN

Al finalizar el ensayo, el riego deficitario redujo el peso seco (PS) del tallo, especialmente en las plantas de que recibían menos agua (RDS), mientras que PS hoja y raíz solo disminuyó en las plantas de RDS (Tabla 1). El efecto de las sales en la acumulación de biomasa al final del ensayo no fue significativo, si comparamos ambos tratamientos control y salino. Tanto el riego deficitario como con agua salina produjo una reducción de la altura de la planta respecto a las plantas control, pero este descenso se produjo antes en las plantas regadas deficitariamente que en las plantas regadas con agua salina (Figura 1). Como ha sido aportado por otros autores, el riego deficitario y la salinidad pueden reducir el crecimiento en plantas ornamentales, aunque el efecto producido es variable y depende de la naturaleza y de la intensidad del estrés (Mugnai et al. 2009).

La concentración de Na y Cl en la planta aumentó con la salinidad, aunque la entrada de cloro por las raíces fue más limitada que la de sodio (Tabla 2). En condiciones salinas controlar la concentración de iones en las hojas, restringiendo su entrada por las raíces o acumulándolo en las partes leñosas, es un mecanismo importante que permite a las plantas crecer en ambientes salinos (Munns, 2002). Las plantas de *P. lentiscus* regadas con sal fueron capaces de limitar el paso de Cl^- a las hojas, sin embargo, Na^+ se acumuló en exceso en las hojas, alcanzando un valor de concentración de Na^+ en hoja 30 veces mayor que en las plantas control, lo que posiblemente contribuyó al descenso de crecimiento observado en estas plantas al final del ensayo (Cassaniti et al. 2009).

El efecto del riego se refleja en los valores de intercambio gaseoso (Figura 2). Los dos tipos de estreses (osmótico y salino) redujeron la conductancia estomática al mediodía, lo que sugiere un eficiente control estomático (Mugnai et al. 2009). Esta reducción resultó más acusada en las plantas sometidas a RDS, provocando una importante disminución de la tasa de fotosíntesis y siendo estas las plantas que presentaros los más bajos valores de CRA. El efecto salino también se reflejó en los valores del potencial hídrico foliar y fotosíntesis, sin embargo, las sales no alteraron significativamente el CRA (Figura 2).

En conclusión, nuestros resultados indican que aunque ambos, riego deficitario y salinidad reducen el crecimiento en *P. lentiscus*, las respuestas fisiológicas y morfológicas son diferentes y dependen de la naturaleza y de la intensidad del estrés. En general, en las plantas de lentisco, el efecto del estrés osmótico debido a la presencia de sales en el medio fue menos acusado que el producido por el déficit de agua. La aplicación de riego deficitario moderado y el uso de agua con un nivel determinado de salinidad (4 dS m^{-1}) podrían usarse de forma satisfactoria en la producción de plantas de *P. lentiscus*, ya que mantienen una buena calidad. Sin embargo el riego deficitario severo no es recomendado, ya que reduce considerable la fotosíntesis y el crecimiento y podría retrasar la recuperación de la planta y causar daños permanentes.

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Tabla 1. Acumulación de biomasa en plantas de *P. lentiscus* sometidas a diferentes tratamientos de riego al final del ensayo.

Peso seco (g pl^{-1})	Control	Sal	RDM	RDS
Hojas	16.5 ± 1.4 b	17.0 ± 2.3 b	16.9 ± 1.1 b	13.0 ± 0.9 a
Tallo	23.1 ± 1.1 c	25.0 ± 3.0 c	16.7 ± 1.4 b	12.5 ± 0.8 a
Raíz	22.2 ± 1.8 b	20.3 ± 2.4 b	19.8 ± 1.3 b	15.8 ± 0.7 a

Tabla 2. Concentración de Na^+ y Cl^- en plantas de *P. lentiscus* sometidas a distintos tratamientos de riego al final del ensayo. Las medias dentro de cada fila que no tienen ninguna letra minúscula en común y las de cada columna que no tienen ninguna letra mayúscula en común son significativamente diferentes según el test de $Duncan_{0.05}$.

(mmol kg^{-1} $_{PS}$)		Control	Sal	RDM	RDS
Na	Hojas	25.0 ± 2.1 aA	736.5 ± 78.8 bB	20.6 ± 2.7 aA	10.4 ± 1.7 aA
	Tallo	22.6 ± 2.5 aA	165.8 ± 16.8 bA	25.0 ± 4.0 aA	10.0 ± 1.3 aA
	Raíz	368.9 ± 33.7 aB	748.5 ± 60.7 bB	297.1 ± 17.8 aB	358.2 ± 27.1 aB
Cl	Hojas	245.0 ± 25.5 aB	490.3 ± 52.6 bB	264.6 ± 32.1 aB	244.0 ± 15.7 aB
	Tallo	157.7 ± 17.5 aA	337.1 ± 29.3 bA	168.0 ± 17.5 aA	173.7 ± 19.3 aA
	Raíz	186.9 ± 16.4 aA	654.6 ± 30.0 bC	192.0 ± 22.7 aA	227.1 ± 19.7 aAB

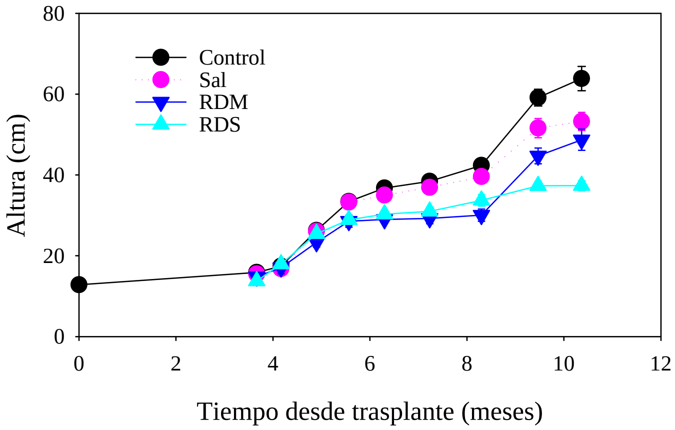

Figura 1. Altura en plantas de *P. lentiscus* sometidas a diferentes tratamientos de riego.

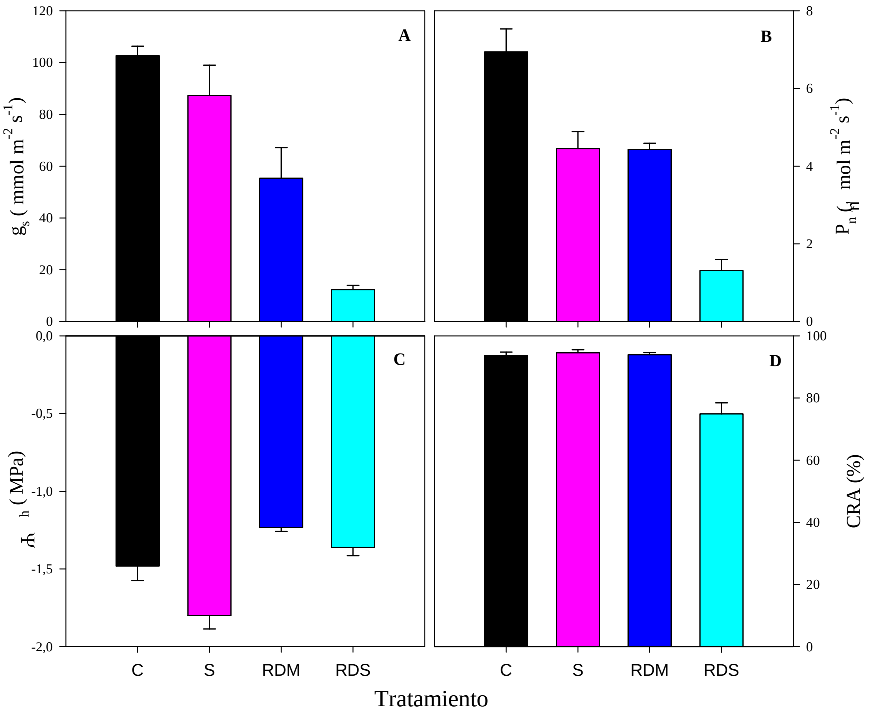

Figura 2. Conductancia estomática (g_s; A), tasa de fotosíntesis neta (P_n; B), potencial hídrico foliar (□$_h$; C) y contenido relativo de agua (CRA; D) al mediodía en plantas de *P. lentiscus* sometidas a distintos tratamientos de riego al final del ensayo.

Parámetros indicadores de estrés hídrico en plantas micorrizadas de *Cistus albidus* creciendo en un sustrato comercial y otro a base de compost de purines

Lorente, B[1]., Álvarez, S.[1], Clemente, R[1]., Sánchez-Blanco, M.J.[1] Ortuño., M.F[1]

[1] Centro de Edafología y Biología del Segura (CEBAS-CSIC). Apartado 164. 30100 Murcia, España. salvarez@eead.csic.es

ABSTRACT

In recent years new research aimed at using various compost as a component of growing media have emerged. In addition, managing a plant with scarce water resources leads us to use biological tools such as mycorrhizal, which can improve the efficiency of water and nutrients, obtaining a good quality plant. The objective of this work was evaluate the use of compost manure as substrate crop production plants in pots in *Cistus albidus*, as well as determining if the fungus *Glomus iranicum var. tenuihypharum*, which it is not frequently used in this genus, could bring benefits, especially when the plant faces water stress. The results showed that plants of *Cistus albidus* could complete their growth cycle in any of the substrates tested, *Glomus iranicum var. tenuihypharum* were compatible with both substrates. Plants growing in the well irrigated substrate with 30% compost help maintain a good nutritional balance. The fungus used increased this effect (minimizing the accumulation of Na and increasing the accumulation of K and P). Water stress treatment enhanced the osmotic effect, limiting proliferation of mycorrhizae and the development of the plant. In addition, it induced a greater osmotic adjustment, while lower gas exchange and higher foliar temperature were observed.

INTRODUCCIÓN

En los últimos años ha surgido un nuevo campo de utilización de diversos compost como componente de sustratos de cultivo (Carmona y Abad 2007). En la producción de plantas ornamentales se utilizan sustratos basados en turba y fibra de coco, componentes costosos y no renovables. Sin embargo, algunos compost pueden llevar elementos dañinos para la calidad de la planta. Por otro lado, aunque se conoce la efectividad de ciertas micorrizas para paliar los efectos del estrés hídrico, no se conocen los efectos cuando las plantas se desarrollan en el medio de cultivo anteriormente citado (Comandini et al. 2006). El presente trabajo profundiza en la respuesta de plantas de *Cistus albidus* sometidas a estrés hídrico, a distintos sustratos e inoculadas con *Glomus iranicus.*

MATERIALES Y METODOS

Se emplearon 80 plantas de *Cistus albidus* L. procedentes del vivero Muzalé (Murcia), cultivadas en bandejas multialveolares. El trasplante se realizó en macetas de polietileno negro, circulares de 14 cm de diámetro y 10 cm de altura con un volumen de aproximadamente 1.2 L y fueron llevadas a una cámara de crecimiento controlado, en la que se simularon los cambios naturales de temperatura (23ºC/18ºC día/noche), luz activa para fotosíntesis (350 μmol m^{-2} s^{-1} de intensidad luminosa), fotoperiodo de 16 horas de luz y 8 de oscuridad, y humedad relativa del 60%. La mitad de las plantas crecieron en un sustrato de cultivo comercial a base de turba Sphagnum, fibra de coco y perlita en proporción 8:7:1 como control (S), y la otra mitad con mezclas de compost de purines, fibra de coco y perlita en proporción 3:6.1 (Cp). Ambos sustratos se fertilizaron con Osmocote. Después de dos semanas, la mitad de las plantas que crecían en cada uno de los sustratos fueron inoculadas con el hongo micorrícico arbuscular *G. iranicum* var. *tenuihypharum* (15 g por 100 ml) y en la mitad de las macetas, tanto inoculadas como no incoculadas, de los distintos sustratos fue suprimido el riego durante un mes. De esta manera quedaron establecidos los 8 tratamientos (10 plantas/trat): Cp (plantas en sustrato compost), CpM (pl. micorrizadas en sustrato Cp), CpE (pl. no regadas en sustrato Cp), CpME (pl. Cp micorrizadas y no regadas), S (plantas en sustrato comercial), SM (pl. micorrizadas en sustrato S), SE (pl. no regadas en sustrato S) y SME (pl. micorrizadas y no regadas en sustrato S). Al final del ensayo se determinó el peso seco (PS) de las 10 plantas por tratamiento, habiendo separado previamente hojas, tallos y raíces. El ión Cl^- en la planta se determinó sobre un extracto acuoso mediante un analizador automático (Model 926 Chloride Analyser, Sherwood Scientific Ltd.) y el contenido de cationes en las mismas mediante el analizador ICP-OES IRIS INTREPID II XDL. A lo largo del ensayo se midió la conductancia estomática (g_s) y la tasa de fotosíntesis neta (P_n) con un LI-COR 6400 (LI-COR Inc., Lincoln, NE, USA) y el potencial hídrico foliar (Ψ_{hoja}), usando una cámara de presión (Model 3000; Soil Moisture Equipment) y el potencial osmótico a máxima saturación (ψ_{100s}) en hojas hidratadas con un osmómetro WESCOR 5520. Las imágenes térmicas se consiguieron con una cámara termográfica (ThermaCam FLIR-e50 System, Inc., UK). La determinación del porcentaje de colonización se realizó por medio de la metodología propuesta por Kormanik y McGraw (1982). La significación de los efectos de los tratamientos fue analizada mediante un análisis de varianza, usando Statgraphics Plus y las medias de los tratamientos fueron separadas con la Prueba de Rango Múltiple de Duncan ($P \leq 0.05$). Las relaciones entre los parámetros de temperatura e intercambio gaseoso se ajustaron a regresiones lineales usando el software SPSS v. 22.0.

RESULTADOS Y DISCUSIÓN

Al finalizar el ensayo, *G. iranicum* var. *tenuihypharum* en las condiciones estudiadas quedó bien establecido en las raíces de Cistus, a pesar de que las plantas crecían en un limitado volumen de sustrato y de que es una planta que generalmente forma ectomicorrizas (Comandini et al. 2006), la falta de agua junto con los componentes del compost, como algunos metales tóxicos y otros elementos dificultaron la proliferación de las micorrizas, presentando valores más bajos de porcentaje de micorrización (33%). El desarrollo de las plantas se vio afectado de forma más significativa por el sustrato utilizado, el compost disminuyó el peso seco de la hoja y de la raíz (Tabla 1). Las plantas micorrizadas que crecían en compost, aunque acumularon más Na^+, las micorrizas minimizaron dicho efecto sobre todo cuando dicho sustrato estaba bien hidratado (Tabla 2) (Talaat y Shawky 2014). El compost también aumentó la acumulación de K^+ lo que demuestra que dicho sustrato no resultó dañino, si no que ayudó a mantener un equilibrio nutricional en las plantas. Adecuados niveles de K^+ son esenciales para el crecimiento de la planta e importantes en los procesos de intercambio gaseoso. En el caso del Zn^{2+} las plantas crecidas en compost y sin micorrizar son las que más acumularon este elemento, para Cu^{2+} las micorrizas tendieron a disminuir el contenido de éste en la planta. Por el contrario, las micorrizas desarrolladas en el sustrato con compost bien regado provocaron que las plantas acumulasen más fósforo (Tabla 2), esto ayudó a un mejor estado hídrico de la planta como lo demuestran los valores menos negativos de potencial hídrico foliar (Figura 1a), sugiriendo que *G. iranicum* pudo aumentar la absorción de agua por las raíces (Vicente-Sánchez et al. 2013).

En el tratamiento de compost en las plantas no regadas se produjo un descenso de $\psi_{100s,}$ (Figura 1b) como resultado de una acumulación activa de solutos. El estrés hídrico disminuyó g_s y P_n (Sánchez-Blanco et al. 2002), y el compost marcó de una forma más acusada este comportamiento (Figuras 1c y d). Existe una estrecha relación entre el cierre estomático y el incremento de la temperatura foliar (Figura 2), haciendo de ésta última una herramienta más precisa para el diagnóstico del estrés hídrico y válida para el tipo de planta utilizada, jara estepeña, arbusto característico de ambientes mediterráneos.

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Tabla 1. Acumulación de biomasa en plantas de *Cistus albidus* sometidas a diferentes tratamientos al final del ensayo. Distintas letras dentro de una misma fila indican diferencias significativas según el test de Duncan$_{0.05}$.

Peso seco (g pl^{-1})	**Tratamientos**							
	S	**SM**	**SE**	**SME**	**Cp**	**CpM**	**CpE**	**CpME**
Hojas	1.3±0.4ab	2.1±0.4a	1.2±0.3ab	2.2±0.3a	0.4±0.1c	0.6±0.2bc	0.6±0.2 bc	0.4±0.2bc
Tallo	2.8±0.3	2.5±0.4	2.4±0.3	2.7±0.5	2.1±0.4	2.7±0.4	2.9±0.4	2.1±0.3
Raíz	2.1±0.5ab	1.9±0.4abc	2.2±0.5ab	2.8±0.3a	1.4±0.3bc	1.4±0.2bc	1.8±0.3abc	0.9±0.2 c

Tabla 2. Concentración mineral en plantas de *Cistus albidus* sometidas a distintos tratamientos al final del ensayo. Distintas letras dentro de una misma fila indican diferencias significativas según el test de Duncan$_{0.05}$.

Solutos (mg kg^{-1} PS)	**Tratamientos**							
	S	**SM**	**SE**	**SME**	**Cp**	**CpM**	**CpE**	**CpME**
Na^+	6699±260bc	6033±511c	5817±709c	5771±382c	8709±735a	6627±355bc	8723±568a	8093±924ab
Cl^-	20320±1818 b	20880±1003 b	19900±1586 b	19200±2111 b	24300±2013 a	26600±2050 a	25066±901 a	25400±2037 a
P	3702±274d	4277±287cd	3311±58d	3860±351d	4966±172bc	7144±664a	5588±181 b	4894±262bc
K^+	19845±549d	20738±1142 cd	19146 ±338 d	21728±1194 bcd	26372±63a	26213±1457 a	23499±681 abc	24633±1765 ab
Zn^{2+}	105.2±2.1bc	108.2±9.0 bc	112.4±4.6 b	92.8±7.6 c	132.9±5.3 a	115.9±4.7 b	128.3±7.5 a	112.9±7.5 b
Cu^{2+}	16.6±1.2 ab	15.5±1.3 ab	14.1±1.1 b	15.6 ±2.1 ab	20.3±2.6 a	13.7±0.9 b	15.2±0.8 ab	13.3±0.6 b

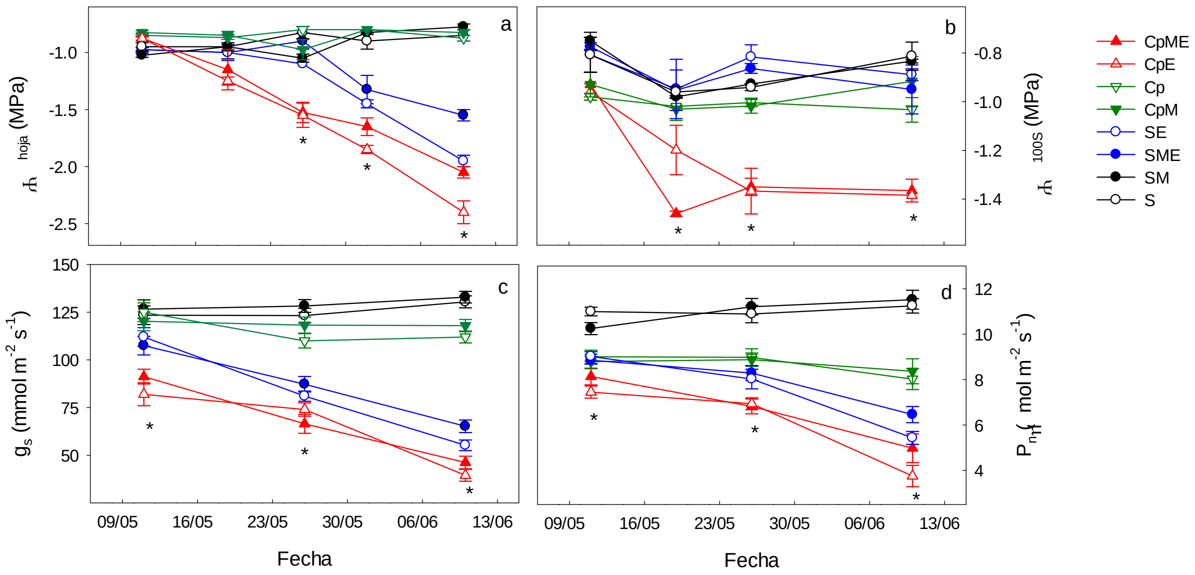

Figura 1. Potencial hídrico foliar (Ψ_{hoja}; a), potencial osmótico a máxima saturación (Ψ_{100S}; b), conductancia estomática (g_s; c) y tasa de fotosíntesis neta (P_n; d) en plantas de *Cistus albidus.* Los asteriscos muestran diferencias significativas entre tratamientos.

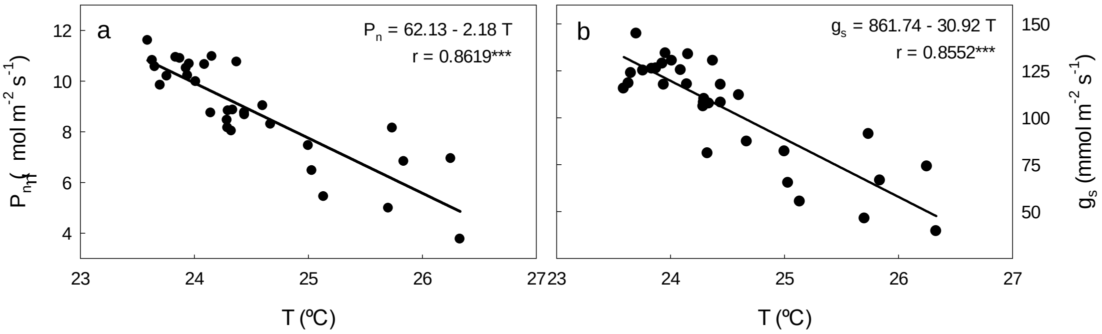

Figura 2. Relación de la temperatura foliar con la tasa de fotosíntesis neta (a) y la conductancia estomática (b) en plantas de *Cistus albidus*.

ÍNDICE DE AUTORES