

SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

Zaragoza 14-16 FEB 2024

PROCEEDINGS BOOK

water us	e efficiency	water deficit		
irrigation	climate chan	ge	droug	nt stress
water stress	crops drou	ght	photosynthes	sis
aquaporins	abscisic acid	water	potential	rootstock
gas excha	ange vapor pre	essure defcit	water re	lations
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1. Program





PROGRAMA

Miércoles, 14 de Febrero de 2024

08:30-09:30: Registro de participantes y entrega de documentación (Hall del Auditorio de Caixaforum)

09:30-10:00: Acto de apertura del Simposio:

María Jesús Lazaro Elorri, Delegada Institucional en Aragón del CSIC

Manuel Matamoros Galindo, Director de la Estación Experimental de Aula Dei del CSIC

Montse Hernández Martín, Jefa de la Oficina de Medio Ambiente, Acción Climática y Salud Pública del Ayuntamiento de Zaragoza

Miguel Gutiérrez López, Director Gerente del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)

10:00-11:00: Conferencia Inaugural

Maurizio Mencuccini (CREAF): Scaling plant hydraulic properties from tissues to biosphere. Can it be done and how?

11:00-11:30: Pausa-Café

11:45-13:00: Sesión I: "Relaciones hídricas en vegetación natural". *Moderador: Juan Pedro Ferrio Díaz*

11:45-12:00: Are you more isohydric than me? Parametrization of the iso/anisohydric behavior of plant species along an altitudinal gradient in Tenerife. <u>A.V. Perera-Castro</u>, L. Díaz-Jiménez, J. Puértolas, B. Fernández-Marín, Á.Mª. González-Rodríguez.

12:00-12:15: A meta-analysis study applied to the characterization of the photosynthetic apparatus performance under changing environmental conditions. <u>M. Ancín</u>, A.L. Gámez, I. Jauregui, J. Galmés, R.E. Sharwood, G. Erice, E.A. Ainsworth, D.T. Tissue, A. Sanz-Sáez, I. Aranjuelo.



12:15-12:30: Ontogenetic plasticity in drought resistance: an underrated factor for predicting the success in seedling recruitment. The case of Hypericum balearicum. <u>M.</u> <u>Carriquí</u>, M. Capó, C. Cardona, T.J. Far, J. Flexas, P.M. Mir, R. Perea, I. Aranda.

12:30-12:45: Scaling relationships between xylem and phloem in oak petioles. <u>R. Martín-Sánchez</u>, D. Sancho-Knapik, J.J. Peguero-Pina, D. Alonso-Forn, J.P. Ferrio Díaz, M. Mencuccini, E. Gil-Pelegrín.

12:45-13:00: Plant community assembly in relation to water and carbon fluxes in a degraded *Mediterranean shrubland*. <u>A. Vilagrosa</u>, L. Morcillo, A. Carrara, R. López, E. Mas, C. Grossiord.

13:30-14:30: *Comida*

15:00-16:00: Sesión I (continuación): "Relaciones hídricas en vegetación natural". *Moderador: Eustaquio Gil Pelegrín*

15:00-15:15: Variability in branch-scale hydraulic capacitance across Europe. <u>S. Hernando</u>, O. Binks, J. Martínez-Vilalta, M. Mencuccini.

15:15-15:30: Physiological and morphological responses of Pinus pinaster Ait. and P. radiata D. Don provenances to imposed drought. <u>C. Pinto</u>, A. Correia, P. Scotti-Campos, J. Semedo, M. Santos, A.P. Rodrigues, R. Moreira, I. Pais, P. Vasilenko, C. Faria, T. Soares David, F. Costa e Silva.

15:30-15:45: *Killing me softly: Key traits determining time to hydraulic failure and risk to drought-induced mortality.* J.M. Torres-Ruiz.

15:45-16:00: Structural and water relations traits determining leaf tolerance to critical dehydration: A case study in California oaks. <u>M. Nadal</u>, E. Barragan, E. Bobich, R. Martín-Sánchez, J.J. Peguero-Pina, D. Sancho-Knapik, C. Scoffoni, E. Gil-Pelegrín.

16:00-16:30: Pausa-Café

16:30-17:30: Visionado de Pósters (sesión 1)

18:00-19:00: Visita cultural al Palacio de la Aljafería



Jueves, 15 de Febrero de 2024

09:00-10:45: Sesión II: "Aspectos genéticos y moleculares de las relaciones hídricas en plantas". *Moderador: Jorge Marques da Silva*

09:00-09:30: The search for genes and gene products to be used as molecular markers in the selection of Quercus ilex elite genotypes resilient to stresses associated to the decline syndrome and climate change. <u>M.D. Rey</u>, M. Labella-Ortega, T. Hernández-Lao, M. Tienda-Parrilla, M. Muñoz-Triviño, A.M. Maldonado-Alconada, M.Á. Castillejo, J.V. Jorrín-Novo.

09:30-09:45: Evaluation of the effect of drought stress on fruit quality of transgenic strawberry plants with cell wall encoding genes down regulated. <u>C. Rodríguez-Hiraldo</u>, S. Aguado, S. Posé, C. Paniagua, R. Blanco-Portales, J. Muñoz-Blanco, M.A. Quesada, A.J. Matas, J.A. Mercado.

09:45-10:00: Involvement of abscisic acid on root hydraulic conductivity and aquaporin regulation by a plant growth-promoting rhizobacteria under drought conditions at two different temperatures. <u>R. Aroca</u>, Á.M. Zamarreño, S. Molina, M.C. Perálvarez, J.M. García-Mina, J.M. Ruiz-Lozano.

10:00-10:15: Rice root-specific E3-ubiquitin ligases in plant-microbe interactions under low water availability: A strategy for a more sustainable production? <u>S. Duarte</u>, T. Lourenço, P. Fareleira, M.M. Oliveira.

10:15-10:30: Vegetative and reproductive hydraulic adjustment in grapevine organs under *deficit irrigation: physiological and molecular aspects.* M. Damásio, C. Pinto, H. Ohlig, T. Soares-David, J. Silvestre, L. Carvalho, <u>O. Zarrouk</u>.

10:30-10:45: Field-Based Transcriptomics Reveals Insights into Drought Tolerance Mechanisms in Tomato Plants: Bridging the Gap Between Greenhouse Experiments and Farmer's Needs. <u>A. Juan-Cabot</u>, M. Fullana-Pericàs, P. Cerdá, G. Romero, J. Medina, J. Galmés, M.À. Conesa.

10:45-11:15: Pausa-Café

11:15-13:15: Sesión III: "Estrés hídrico en cultivos". Moderadora: Olfa Zarrouk

11:15-11:30: Impact of drought stress on yield and seed quality of Pisum sativum *cv. Tom Thumb*. <u>J. Machado</u>, M. Nunes da Silva, M.W. Vasconcelos, C.S. Santos.

11:30-11:45: Effect of Drought Stress on Water Relations in Two Almond Cultivars Grafted onto Different Rootstocks. S. Nasrolahpour-moghadam, <u>J. Montenegro</u>, M. Fattahi, G. Rabiei, B. Shiran, Y. Gogorcena.



11:45-12:00: Biostimulant activity of Galaxaura rugosa seaweed extracts against water deficit stress in tomato seedlings involves activation of ABA signaling. S. Morales-Sierra, J.C. Luis, <u>D. Jiménez-Arias</u>, N.M. Rancel-Rodríguez, A. Coego, P.L. Rodriguez, M. Cueto, A.A. Borges.

12:00-12:15: Use of Amino Acids to Combat Water Stress in Vineyards. <u>F. Lopes de Macedo</u>, J.F. Teixeira Ganança, H. Nóbrega1, J. G.R. de Freitas, M. A.A. Pinheiro de Carvalho.

12:15-12:30: Impact of rhizospheric microorganisms on physiological performance of grapevines under current and future environmental conditions. <u>D. Kozikova</u>, I. Pascual, N. Goicoechea.

12:30-12:45: Magic beans or the miracle of tradition? Resilient bean landraces from Muniesa as a source of drought-adapted, seed-borne biofertilizers. <u>T. Gil</u>, I. Rebelo Romão, C. Mallor, J.I. Vílchez.

12:45-13:00: Enhancing Maize (Zea mays) Resilience to Drought Stress Through Seed-Associated Microbiota. <u>I. Rebelo Romão</u>, T. Gil, L.A. Lopes de Carvalho, J. do Carmo Gomes, A. Sousa, R. Teixeira, F. Kasa, A. Katamadz, O. Vergara-Diaz, R. Vicente, J.I. Vílchez.

13:00-13:15: Estudio del comportamiento hídrico de la higuera, variedad Calabacita, en condiciones de riego en Extremadura. <u>M.J. Moñino</u>, A. Vivas, Á. Caro, P. Redondo, M.H. Prieto.

13:30-14:30: Comida

15:00-15:15: Entrega del Premio Ibérico de Investigación a la Mejor Tesis en Relaciones Hídricas en Plantas

15:15-16:15: Conferencia de la premiada

Nieves Lavado Rodas: Estudio del efecto a corto y medio plazo de la técnica del "forzado de yemas" sobre la productividad y la calidad de la vendimia 'Tempranillo' en Extremadura

16:30-17:00: *Pausa-Café*

17:00-18:00: Visionado de Pósters (sesión 2)



18:00-18:30: *Reunión del Grupo de Relaciones Hídricas de la Sociedad Española de Biología de Plantas*

21:30: Cena del Congreso en el Restaurante "La Lobera de Martín"



Viernes, 16 de Febrero de 2024

09:00-11:00: Sesión IV: "Nuevas metodologías para el estudio de las relaciones hídricas en plantas". *Moderador: Eustaquio Gil Pelegrín*

09:00-09:30: Air-coupled broadband ultrasounds to study water relations in leaves. <u>T.</u> <u>Gómez Álvarez-Arenas</u>, D. Sancho-Knapik, J.J. Peguero-Pina, E. Gil-Pelegrín

09:30-09:45: Determining stomatal response to vapour pressure deficit from gas exchange measurements: conceptual and methodological challenges. J.P. Ferrio, D. Sancho-Knapik, J.J. Peguero-Pina, A. López-Ballesteros, M. Nadal, E. Gil-Pelegrín.

09:45-10:00: Real-time trunk water potential combined with soil water content for precise irrigation in nectarine trees. M.R. Conesa, W. Conejero, A.B. Mira-García, J. Vera, <u>M.C. Ruiz-Sánchez</u>.

10:00-10:15: A new empirical framework to quantify soil and atmospheric forcing on plant water status. <u>M. Mencuccini</u>, O. Binks, K. Novick, A. Konings, W.R.L. Anderegg, R. Poyatos, J. Martínez-Vilalta.

10:15-10:30: Relationship between trunk water potential and other plant-based indicators for continuous monitoring tree water status. <u>V. Blanco</u>, L. Kalcsits.

10:30-10.45: Fostering Low-cost Drought-phenotyping. Part I: Designing and mounting a custom-made multi-image platform. M. Abreu, M. Calejo Pires, G. Evans, J. Soares Augusto, M. Vitorino, P. Correia, J. Marques da Silva.

10:45-11:00: Fostering Low-cost Drought-phenotyping. Part II: Testing a custom-made system with Arabidopsis thaliana and Vitis sp. J. Melo, A. Figueiredo, A.R. Matos, J. Marques da Silva.

11:00-11:30: Pausa-Café

11:30-12:30: Sesión V: "Aspectos relacionados con el movimiento de agua en plantas". *Moderadora: Clara Pinto*

11:30-11:45: Effect of rootstock on xylem embolism vulnerability in grapevine: from leaf to stem. <u>L. Flor</u>, M. Carriquí, G. Toro, I. Buesa, A. Sabater, I. Gómez, J. Bota, H. Medrano, J.M. Escalona.

11:45-12:00: Polyploid trees and water use efficiency: experiments with subtropical fruit crops. N. Blanco-Moure, A. Fonollá, J.I. Hormaza, J.M. Losada.



12:00-12:15: Evaluation of Huber value role in carbon and water relationships across fruit tree species. <u>V. Hernandez-Santana</u>, C.M. Rodriguez-Dominguez, A. Perez-Martin, D. Benzal, F. Rossi, J. Sebastian-Azcona, A. Montero, L.F. Perez-Romero, A. Diaz-Espejo.

12:15-12:30: Plant water relations in wild tomatoes and relatives are better explained by *leaf venation traits than by hydraulics*. <u>M.À. Conesa</u>, J.J. Peguero-Pina, C.D. Muir, A. Molins, E.J. Roldán, E. Gil-Pelegrín, J. Galmés.

12:30-13:00: Acto de Clausura. Foto de Grupo.

Oral Communications Session I: "Relaciones hídricas en vegetación natural"





Are you more isohydric than me? Parametrization of the iso/anisohydric behavior of plant species along an altitudinal gradient in Tenerife

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Abstract: Iso-/anisohydry has played a central role in the study of species-specific differences in water use regulation for decades, occupying a special prominence in the research agendas. The classification of species along the iso/anisohydric continuum is based on the ability to maintain leaf water potential stable during drought periods, which depends on many different plant traits such as stomatal responses to water deficit, hydraulic regulation, root exploration capacity, stem capacitance or leaf functional traits. This complexity makes the determination of metrics that accurately classify species within the iso/anisohydry spectrum elusive. The lineal relationship between seasonal minimum pre-dawn and midday leaf water potentials has been used for quantifying the iso-/anisohydric spectrum by a measurable objective methodology. However, this relationship is far from be linear in some species for the complete range of water potential experienced. We propose a more complete parametrization of the pre-dawn versus midday water potential relationship, as well as of the relation between these water status variables and stomatal responses. This would allow quantifying the components of the iso-/anisohydric behavior and define mathematically plants strategies to face drought. Gas exchange and water status of several species were monitored in Tenerife throughout one year. Measurements were taken across periods with contrasting water availability and included species habiting from arid Euphorbia scrubs to high mountain ecosystems. What determines plant water use strategies? Which mechanisms are the more frequent in arid and semiarid ecosystems?

Keywords: isohydry, anisohydry, water potential, Canary Islands, drought resistance



Zaragoza 14-16 FEB 2024

A meta-analysis study applied to the characterization of the photosynthetic apparatus performance under changing environmental conditions

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⁸ Department of Crop, Soil and Environmental Sciences, Auburn University, 253 Funclass Hall, Auburn, AL 36849, USA.

Abstract: As climate change is affecting plant performance worldwide there is need to better understand the impacts of elevated CO_2 concentration (e[CO_2]) on CO_2 assimilation and the underlying biochemistry of Rubisco, especially in its interaction with a water scarcity scenario. In this regard, the present study compiles 942 data published in 23 manuscripts in which the effects of e[CO_2] and drought on plant photosynthetic machinery were analyzed. From a broad perspective, it was observed that drought did not exacerbate the improvements in photosynthesis driven by increasing atmospheric [CO_2]. In fact, the results showed that the stimulation of net photosynthesis under e[CO_2] was even greater when water stress was considered. Moreover, the negative impact on Rubisco activity was smaller at e[CO_2] x drought than at e[CO_2] alone, although the reduction on Rubisco concentration and total soluble protein was similar. Finally, the

reduction on leaf N content was shown to be higher at e[CO₂] x drought. Overall, this study sheds light on the complex interactions occurring at the plant level within a climate change context.

Keywords: meta-analysis, CO₂, drought, photosynthesis, Rubisco

1. Introduction

Photosynthesis is the central process of all primary production in the biosphere, but it is also a key process to alleviate the accumulation of carbon dioxide (CO₂) in the atmosphere (Baslam *et al.* 2020). Current [CO₂] in the atmosphere generally limits light-saturated photosynthesis in C₃ plants, so greater photosynthetic rates, and consequently greater plant growth could be expected at elevated [CO₂] (e[CO₂]). Photosynthesis under e[CO₂] is also largely influenced by the interaction with other environmental factors, such as water availability or temperature (Ainsworth and Long, 2021). Specifically, moderate drought induces a reduction in stomatal conductance (g_s) to prevent water loss, consequently reducing leaf photosynthetic rates due to a decrease in CO₂ availability in the chloroplast. But also, other non-stomatal limitations could be found linked to the reduced photosynthetic performance of plants under severe drought conditions (Flexas and Medrano, 2002). The interaction between drought stress and e[CO2] could complicate these physiological responses.

In general, the current/future reduced water availability scenario could offset the theoretical benefits of increased atmospheric [CO2] and compromise the efficiency of Rubisco operation. Thus, a careful review of the effects of drought under e[CO2] over the photosynthetic apparatus is necessary to discern if plants grown under drought stress will still benefit from the effects of e[CO2].

2. Materials and Methods

2.1 Data compilation

To assess the impact of e[CO₂] on Rubisco biochemistry and its interaction with drought, we performed a compilation of data presented in the literature on the Web of Science (Thompson-ISI, Philadelphia, USA). To our knowledge, we have considered all the publications that includes at least one parameter related to Rubisco when comparing plant responses to e[CO₂] and drought. Collectively, the 942 data compiled here come from 23 published manuscripts and includes 19 different species. The data compilation was manually performed using the image processing software ImageJ when the data was in the form of graphics.

2.2 Statistical analysis

The effect size metric used in the study to estimate the response of the $e[CO_2]$ treatment on plant photosynthetic performance was the natural logarithm of the response ratio $(ln(r) = response in e[CO_2]/response in a[CO_2])$. This transformation method, based on the statistical studies of Hedges *et al.* (1999), allows to integrate independent studies to perform a meta-analysis using MetaWin v. 3.0.13 as analytical tool. The procedure follows the techniques described by Curtis and Wang (1998), using a mixed-effects model to analyze the data, that is based on the assumption of random variation in effect sizes among studies. The response was reported as the mean percentage change [(ln(r)-1) x 100] at $e[CO_2] \pm 95\%$ confidence interval (CI). The response to $e[CO_2]$ was considered significant when the 95% CI did not overlap with zero. To test significant differences in mean response between categorical variables, between-group heterogeneity (Q_B) for each categorical variable was examined across all data set (Curtis and Wang, 1998).

3. Results and Discussion

3.1 Impact of e[CO₂] and drought on CO₂ diffusion

The exposure of plants to $e[CO_2]$ under well-watered conditions (Fig. 1) supported the theoretical established responses to $e[CO_2]$: enhanced photosynthetic carbon uptake, reduced g_s, E slowdown and stimulation of Ci (Ainsworth and Rogers, 2007). The response of plants to $e[CO_2]$ in combination with drought showed the same behavior (Fig. 1). However, the stimulation of A_n at $e[CO_2]$ x drought resulted higher than the stimulation of plants exposed to $e[CO_2]$ under well-watered conditions (Q_B*; Fig. 1). This is indicative of a maintained stimulation of A_n under $e[CO_2]$ in spite of the imposed stomatal limitations of drought.

There is a well-stablished idea about the photosynthetic rate reduction under moderate drought stress due to diffusive limitations (Flexas and Medrano, 2002). The lack of a higher reduction in g_s under drought could be explained by the effect of $e[CO_2]$ counteracting the g_s reduction or even the limitations in mesophyll diffusion conductance to CO_2 through a higher C_i , that could consequently resulted in a higher $[CO_2]$ in the chloroplast stroma. While stomatal closure limits CO_2 fixation rate, lower g_s might contribute to reduce plant water evapotranspiration. Here, reduction of E under $e[CO_2]$ was observed, and it was similar to that observed at $e[CO_2] x$ drought (Fig. 1). In this line, our results do not support the proposed "water saving effect", although other variables, such as leaf water potential or soil water availability, should be addressed to solidly reject the hypothesis. Overall, we can say that the positive photosynthetic response of plants to $e[CO_2]$ is not exacerbated with the addition of drought. In other words, $e[CO_2]$ mitigates the inhibition of stomatal conductance and photosynthesis under drought conditions.



Fig. 1 Comparative photosynthetic responses of plants subjected to $e[CO_2]$ at well-watered (Ww) conditions vs $e[CO_2] \times drought$. An (net CO₂ assimilation), gs (stomatal conductance), E (transpiration rate), and C_i (intracellular CO₂). The results are presented as the mean percentage change at $e[CO_2]$ relative to $a[CO_2]$. The response to $e[CO_2]$ was considered significant when the 95% CI did not overlap with zero. The error bars represent 95% CI. The number of observations for each response variable is indicated on the left.

3.2 Impact of e[CO₂] and drought on Rubisco biochemistry

Here $V_{c,max}$ decreased by $\approx 10\%$ at both $e[CO_2]$ and at $e[CO_2] \times drought$, but the J_{max} was not significantly reduced (Fig. 2). Regarding the activity of Rubisco, only total activity was shown to be reduced under $e[CO_2]$ (Fig. 2). Interestingly, the addition of drought led to reduce the negative response of Rubisco initial and total activity to $e[CO_2]$ (Q_B^* ; Fig. 2). These results revealed that carboxylation machinery was not significantly impaired when plants grown under $e[CO_2]$ were also subjected to drought.

The Rubisco activation state of plants grown under $e[CO_2] \times drought was stimulated by <math>\approx 13\%$ (Fig. 2), which could be unexpected given the putative diffusive and biochemical limitations imposed by drought. However, lower Rubisco activity but a higher activation state were previously observed, together with a reduction in Rubisco content, as indicative of plant photosynthetic acclimation (Aranjuelo *et al.* 2011). In this line, Rubisco content was reduced by $\approx 20\%$ at $e[CO_2]$, and a similar reduction was detected in combination with drought (Fig. 2) although it was not significant. Similar reductions were observed for total soluble protein concentration (TSP) and leaf N content (Fig. 2). The decreases in leaf N, Rubisco, and TSP content suggest that under $e[CO_2]$ there was a photosynthetic acclimatation of plants, but also in combination with drought. Moreover, the exposure of plants to $e[CO_2] \times drought resulted in a higher reduction of leaf N content compared to plants exposed to <math>e[CO_2]$ under well-watered conditions (Q_B*; Fig. 2), that could be indicative of a greater reallocation of leaf N to sink organs under stressful conditions (Feller *et al.* 2008).



Percentage change in e[CO₂]

Fig. 2 Comparative Rubisco responses of plants subjected to $e[CO_2]$ at well-watered (Ww) conditions vs $e[CO_2]$ x drought. $V_{c,max}$ (maximum carboxylation velocity), J_{max} (maximum electron transport rate), Rub. initial act. (Rubisco initial activity), Rub. total act. (Rubisco total activity), Rub. activation (Rubisco activation state), [Rubisco] (Rubisco content), [TSP] (total soluble protein), Leaf N (leaf nitrogen). Otherwise as for Fig. 1.

4. Conclusion

We confirmed that photosynthetic rates rose and g_s declined under $e[CO_2]$, but $V_{c,max}$, Rubisco activity and content were downregulated. Although it should be highlighted the limited information of Rubisco biochemistry under combination of $e[CO_2]$ and other environmental factors, this study suggest that the addition of drought to $e[CO_2]$ did not result in a different response from $e[CO_2]$ under well-watered conditions, indicating that $e[CO_2]$ could somewhat mitigate the adverse effects of drought, at least under the considered range of stressful conditions.

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Ontogenetic plasticity in drought resistance: an underrated factor for predicting the success in seedling recruitment. The case of *Hypericum* balearicum.

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Abstract: Given the urgent need to predict how plants are likely to respond to climate change, a significant effort is being made to understand the processes underlying plant drought resistance. However, studies focusing on up to one-year-old seedlings, a critical phase for successful regeneration and continuity of populations, are particularly scarce. Therefore, the relevance of potential differences related to ontogeny in the response to drought is largely unknown.

Here, we evaluated the ontogenetic plasticity in drought resistance of *Hypericum balearicum* L., a Mediterranean shrub with limited seedling recruitment at early ages, but negligible adult drought-related mortality. Physiological and anatomical traits associated to drought resistance at seedling and adult stages were evaluated in plants grown under common garden conditions. Additionally, in order to assess whether hydraulic disfunction is a major cause of seedling mortality under natural conditions in this species, we planted 90 seedlings in one of the wettest areas within the species' distribution range and monitored their plant water status and mortality over the course of one year.

Seedlings presented larger photosynthesis but lower intrinsic water use efficiency and drought tolerance, closing stomata at higher water potentials than adults. Once stomata closed, seedlings not only dehydrated faster due to a larger minimum conductance but also presented a surprisingly lower resistance to embolism. Findings were confirmed under natural conditions, in which massive seedling mortality was associated with drought-related vascular hydraulic failure. *H. balearicum* presented a strong ontogenetic shift from a drought-vulnerable strategy in seedlings, to a more slow-growing drought-tolerant strategy in adults. Thus, as seedlings are more prone to die due to hydraulic failure compared to adults in *H. balearicum*, findings highlight that drought resistance from ontogeny is a major -but underrated- factor influencing recruitment success of the species.

Keywords: Seedling recruitment, drought resistance, embolism resistance, mortality



Scaling relationships between xylem and phloem in oak petioles

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Abstract: Petioles act as a bottleneck between source and sink organs in plants. Thus, all water used by a leaf must flow into it through xylem in the petiole. Similarly, the sap produced by photosynthesis must pass by the sieve tubes of the phloem. Therefore, it could be expected that plants produce xylem vessels and sieve tubes accordingly to the optimal transpiration and photosynthesis rates respectively, besides their size.

Nonetheless, the scaling of both, size of the conduits and area of these conductive tissues, is limited by several restrictions. The investment in structural support for those larger leaves is required, for instance. In addition, the production of wider conduits can compromise their own functionality. The risk of embolism by either drought or freezing increases with diameter of the vessels size. Wider sieve tubes in phloem are more vulnerable to aphid attack than narrower ones. Besides, the concentration of sap is also influenced by the size of the sieve tubes, which is critical for the transport of such sap.

Moreover, both conductive tissues are interconnected. Photosynthesis rate, as well as water loading into phloem, depends on the water availability supplied by xylem. For that reason, a scaling relationship between both tissues is also expected.

In our study, we measured xylem vessel and sieve tube diameters and areas, as well as hydraulic conductivity, in petioles of species of Quercus with different leaf habit. We have seen: a) an allometric relationship between xylem and phloem areas, b) a scaling between diameter and size of the tissues with the size of the leaves, c) a correlation between transpiration and xylem on the one hand and photosynthesis net rate and phloem on the other hand.

Keywords: xylem, phloem, hydraulic conductivity, water transport, photosynthesis rate, petiole, Quercus.



Plant community assembly in relation to water and carbon fluxes in a degraded Mediterranean shrubland

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Abstract: Forest hydrology is a key determinant of ecosystem function, which is expected to show essential feedback responses to climate change in the future. In Mediterranean ecosystems, plant communities are composed of different species with contrasting functional strategies regarding water use, carbon fixation, and regeneration after fire or other disturbances. These ecosystems are subject to a changing climate with a combination of different perturbations, such as extreme droughts and recurrent fires, affecting species composition, hydrological patterns and, consequently, ecosystem functions and productivity.

We are investigating the seasonal patterns of drought stress, water use, and carbon fixation in a Mediterranean shrubland community exposed to recurrent fires and droughts. The plant community is composed of seeder (R-), resprouter (R+), and herbaceous (H) species with contrasting functional strategies. The aim is to deepen our understanding of the ecosystem hydrology and carbon cycle and how the different plant functional strategies participate in these global cycles. We measure gas exchange and drought stress at the plant level and relate this to eddy covariance fluxes at the ecosystem level. Preliminary results show that different functional groups (i.e., R+, R-, and H) participate differently to ecosystem water and carbon fluxes each season, depending on their access to soil water. Resprouters were more conservative in water use and carbon fixation in different seasons and water availability. In contrast, seeders were more sensitive to soil water availability seasonal changes and consequently present higher seasonal variability in carbon fluxes may have important implications for biogeochemical cycles at the ecosystem level.

Keywords: water and carbon fluxes, hydrological cycle, Eddy covariance, drought stress, Mediterranean shrubland.



Variability in branch-scale hydraulic capacitance across European trees

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Abstract: Despite its central importance in plant water relations, capacitance is a poorly defined trait, partly because it is not clear on what bases (or units) it should be expressed. To eliminate size-dependency, capacitance is generally normalised by mass, leaf area or water content. However, the impacts of these different definitions have not been systematically investigated. In addition, capacitance can be investigated at different spatial scales, from individual leaves to branches, entire trees or even forests, but the application to different scales raises additional conceptual issues.

The main objective of this study was to predict whole-branch capacitance to identify the relative impacts of allometric scaling versus the functional traits of the individual tissues. For that purpose, we measured whole-branch capacitance as well as total dry mass, its distribution among different tissues (leaves, bark and wood) and functional traits such as P50 (a measure of sensitivity to the loss of xylem hydraulic conductivity), leaf turgor loss point and wood density. All these measurements were conducted on four tree species (*Populus nigra, Fagus sylvatica, Pinus sylvestris* and *Pinus Pinaster*) at several sites across Europe, providing a range of climatic conditions.

Linear mixed models were used to test the hypothesis that variability in branch capacitance can be explained more by variability in allometric scaling than by variability in the tissue-specific traits affecting water storage strategies.

We present results showing how the allometry of branch dry mass of each species influences plant-level capacitance and consequently its water use strategies. Attention needs to be given to the basis on which capacitance is expressed to allow proper upscaling of tissue-level properties to entire trees.

Keywords: capacitance, extensive properties, intensive properties, allometry, biomass, functional traits.

1. Introduction

Hydraulic capacitance is an attribute related with plant water storage strategies. We can describe it as an extensive property with tissue size dependence (e.g., dry mass dependence) or as an intensive property without size dependence. In this study, we use

two distinct definitions of extensive capacitances, as capacity and extensive capacitance. Capacity (C, in gH₂O) is defined as the amount of water that can be extracted (Hartzell et al., 2017). Extensive capacitance (Ce, in gH₂O MPa⁻¹) is capacity per unit of water potential (Ψ) change (Hartzell et al., 2017). Conversely, we also calculate intensive capacitances normalising by dry mass (c_{DM}, in gH₂O g⁻¹ MPa⁻¹), by relative water content (c_{RWC}, in RWC MPa⁻¹) and by leaf area (c_{LA}, gH₂O cm⁻² MPa⁻¹).

The main traits that could be related with predictions of capacitance studied in this research are: 1) Leaf turgor loss point (TLP), that indicates the ability to conserve cell turgor during a dehydration event (Bartlett et al. 2012); 2) Wood density (WD), as an anatomical trait correlated to wood water storage (Ziemińska et al. 2020); and 3) P50, the water potential at 50% loss of xylem hydraulic conductivity (Delzon et al. 2010).

We hypothesised that variability in branch capacitance can be explained more by variability in allometric scaling than by variability in the tissue-specific traits affecting water storage strategies.

2. Materials and Methods

2.1 Study sites and sampling

This study was realized on four tree species (Populus nigra L., Pinus pinaster Ait., Fagus sylvatica L., and Pinus sylvestris L.) at fifteen sites per species across Europe, providing a range of climatic conditions. The sites were selected within in situ forest Gene Conservations Units, which are part of the EUFORGEN programme (de Vries et al. 2015). Trees were sampled during spring, early summer and autumn of 2021 and 2022. At each site, ten individual trees were selected. For these trees, leafy sun exposed branches (40-100 cm long) were collected and stored in fresh conditions before their measurements in the laboratory.

2.2 Laboratory measurements

We determined capacitances using the branch dehydration method, i.e., drying a branch and measuring its fresh weight and water potential at specific desiccation times. Capacitances were calculated in the initial and final, parts and for the entire curve of this branch dehydration curve by plotting the relationship between water loss and water potential. The dry masses and the proportion of each tissue were determined by drying the whole branch while separately measuring bark, wood and leaves.

Wood density (WD) was determined using the Archimede's principle calculating the ratio of dry mass to fresh volume for both bark and wood. Leaf Turgor loss Point (TLP) was obtained using an Osmometer calibrated, following the method describe by Bartlett et al. (2012). Xylem vulnerability curves to cavitation were built using the 'Cavitron' technique (Cochard 2002; Cochard et al. 2005) and following Delzon et al. (2010), we obtained P50 (the potential causing 50 % loss of hydraulic conductance), P12 (the potential causing 12% loss of conductance) and P99 (the water potential causing 99% loss of conductance).

2.3 Statistical analyses

Linear mixed-effects model and theirs marginal and conditional R² were fitted using the lme4 package version 1.1-31 (Bates et al., 2015) for predicting capacitances based on

tissues dry masses and functional traits, following the formula: Capacitance ~ dry mases + traits + (1 | species/population). Capacitances and dry mass branch were natural base log transformed. Models were ranked using Akaike Information Criteria (AIC) and partR2 package (Stoffel et al., 2021) was used to estimate the standardised coefficients (i.e., beta weights) and the inclusive R² by each fixed effect. Significance levels for individual variables were obtained by examining the anova results of the fitted model. All statistical analysis were conducted in R software version 4.2.2 (R Core Team, 2022).

3. Results and Discussion

The relationships between extensive properties (i.e., capacity, and extensive capacitance) and dry mass branch on log-log space were strongly positive for all three types of curves, although with significant intercept and slope differences across species (Fig. 1).



Fig. 1 Relationship between branch dry mass, capacity (C) and extensive capacitance (C_e). Initial, final parts and complete curves.

However, intensive capacitance normalised by dry mass had a strong negative relationship in the initial and complete curves but a lower negative relation in the final part of the curve (Fig. 2). Intensive capacitance normalised by RWC had a negative relationship in the initial part of the curve and not had any relationship in the complete and final part of the curve (Fig. 2). Intensive capacitance normalised by leaf area had a low positive relationship in the initial, final, and complete curves (Fig. 2). Therefore, neither normalisation by dry mass nor normalisation by leaf area eliminated the size dependency expected from intensive measurements. Only normalisation by RWC achieved a substantial size-independence, especially considering the final part of the curve.



Fig. 2 Relationship between branch dry mass and the intensive properties normalised by Dry Mass (CDM), Relative Water Content (CRWC) and Leaf Area (CLA). Initial, final parts and complete curves.

When analysing the prediction of capacitance based on dry masses and functional traits, capacitance was generally more sensitive, more correlated and better explained by dry masses than by traits. Beta weights suggested that capacitance measures were more sensitive to dry mass than to traits with the exception of P50 that displays higher values than the other traits but lower than dry masses. In addition, inclusive R² suggested that capacitance was strongly correlated especially with masses, followed by distribution of masses and with P50.

Dry masses, were in most of the cases significant except when c_{RWC} was explored in the final and complete parts of the curve. This suggests that normalisation by RWC, similarly to what is done in leaves, provides a size-independent estimate of capacitance which can be compared across species and conditions.

WD appeared significant in most predictive models of capacity, and in all the models exploring the complete curve in C_e and c_{DM} , while WD was not significant in c_{RWC} and c_{LA} . When significant, higher WD was always associated with lower capacity and capacitance, as expected from the trade-off between space occupied by cell walls and space available for water storage.

P50 appeared significant in all the predictive models in the final part of the curve, in all predictive models of capacity in the initial part of the curve and in all C_e , c_{DM} , c_{RWC} and c_{LA} in the complete curve. When significant, less negative P50 was almost always associated with higher values of capacitance, as expected from a trade-off between drought vulnerability and use of stored water to support canopy transpiration.

TLP was not significant for any of the capacitance measures in the initial part of the curve; it was however significant for C, C_e , c_{DM} and c_{RWC} in the final part of the curve; it

was also significant in a few models predicting C_e in the complete curve but not significant for the rest of the capacitances. When significant, more negative TLP was almost always associated with higher values of capacitance, suggesting a positive association between turgor maintenance and whole-branch capacitance.

When we explored the explanatory influence of the models, we obtained higher values of marginal and conditional R^2 for the predictive models of C and C_e, as expected from their strong size dependence. Models for c_{LA} and c_{RWC} had lower values of marginal and conditional R^2 . c_{DM} had intermediate values between the other capacitances.

4. Conclusion

Extensive properties have a strong allometric dependence of biomass. On the other hand, intensive properties need to be carefully defined at the branch scale because they can still be allometrically dependent on biomass even after normalising the data. For both intensive and extensive properties, total biomass and tissues proportions are the most important explanatory variables for capacitance compared to the studied traits (P50, TLP and WD). Finally, capacitance normalised by relative water content has the lowest allometric dependency on biomass. Then we can assume that capacitance normalised by relative water content is the closest variable to the concept of a branch level trait.

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Physiological and morphological responses of *Pinus pinaster* Ait. and *Pinus radiata* D. Don provenances to imposed drought

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Abstract: In Portugal, a continuous decrease in *Pinus pinaster* area and timber production has been observed, mainly related to the cumulative impacts of forest fires, pests and diseases, and low site productivity due to inadequate management. To revert this trend, new afforestation options are being tested, considering different species and using pine materials from breeding programs, with known genetic gains (e.g., volume, growth traits, vulnerability to pine wood nematode disease). These actions must be accompanied by a broader knowledge of the physiological responses of these materials to abiotic and biotic stress.

We tested if provenances of two economically important pine species, *Pinus pinaster* and *P. radiata*, differ in physiological and morphological responses to imposed drought. A greenhouse experiment was established, using 2-year-old potted plants (1.7 L) of two provenances per species. Plants were maintained under differing watering regimes: periodical watering to field capacity (control, C); and complete water suppression (water stress, S). After 92 days, S plants were split in two groups: recovery (R, rewatered) and prolonged imposed drought (M). Monitoring included needle water status, transpiration, hydraulic conductance, and plant growth.

In S plants, transpiration rates were below 0.002 gH₂Oh⁻¹cm⁻² in ca. 30 days, but all provenances survived for an additional 62 days without water supply. Comparing to C plants, water stress led to an average reduction of about 60% in mean total height and to an inhibition of new root growth. Under imposed drought, *P. radiata* had higher

biomass growth than *P. pinaster*, reflecting its higher leaf area ratio and hydraulic conductance. Upon rewatering, both *P. radiata* provenances were able to recover needle water status to pre-treatment values. Under prolonged imposed drought, the Portuguese *P. pinaster* provenance survived 15-29 days longer than plants of other provenances because of the more conservative water-use strategy per unit of leaf area.

Keywords: Maritime pine, Monterey pine, water stress, water use strategies, tree survival

1. Introduction

Pinus pinaster Ait. (maritime pine) is a well-adapted Mediterranean tree species, occupying ca. 3 million ha in SW Europe in widely diverse edaphoclimatic conditions (Abad Viñas et al. 2016) reflecting its high level of population differentiation and plasticity (Alía et al. 1995; Oliveira et al. 2000). Being the most widespread conifer in the Iberian Peninsula (DGCONA, 2002; ICNF, 2019), *P. pinaster* has great importance for conservation purposes and soil and coastal protection and is widely used for timber and resin production. However, the species is perceived to be in decline because of the cumulative impacts of multiple stressors, such as poor seedling recruitment, intraspecific competition, wildfires, and pests and diseases (Fernandes and Rigolot 2007; Prieto-Recio et al. 2015; Gea-Izquierdo et al. 2019; Ribeiro et al. 2022).

Although having great economic importance in Portugal, the pine sector represents over 50% of the gross value added of forestry industries (Centro PINUS 2023), between 1995 and 2015, the area occupied by *P. pinaster* is estimated to have decreased approximately by 264,000 hectares (ca. 27%) (ICNF, 2019). This trend, coupled with the low productivity of maritime pine stands due to management practices (Prieto-Recio et al. 2015; Alegria et al. 2021), biotic stressors (Gea-Izquierdo et al. 2019), and recurrent wildfires (Fernandes and Rigolot 2007; Gómez-González et al. 2018), is leading to disinvestment in stands, with negative consequences for timber production, jeopardizing the value chain of the pine sector. An innovative approach to contribute to improving the sustainability and competitiveness of the pine sector in Portugal is the introduction in afforestation actions of new materials (Oliveira et al. 2000), more productive and resilient to biotic and abiotic factors, including tested populations of diverse *Pinus* species, such as *P. pinaster* or *P. radiata*, capable of generating higher income (Wu et al. 2008; Serrano-León et al. 2021; Ivkovich et al. 2023) for forest producers, promoting reinvestment, and reversing the marked decline trend. Hence, the successful

introduction of new species and provenances in such actions requires a broad knowledge of their responses to abiotic (Correia et al. 2008; 2010; Aranda et al. 2010; Corcuera et al. 2010; Espinoza et al. 2014; 2016) and biotic factors (Ribeiro et al. 2012; Carrasquinho et al. 2018), as well as a broader understanding of the influence of the region of origin on seedling performance (Alía et al. 1995).

Recently, under the framework of Project rePLANT, several materials of economically important pine species originated from breeding programs (e.g., Spain, Chile, France) with proven genetic gains (e.g., volume, stem form, resistance to Pine Wilt Disease), were used in experimental afforestation actions aiming to test seedling growth and survival in different soil and climate conditions. However important, the complete evaluation of seedling performance also requires more specific knowledge regarding their physiological responses to biotic and abiotic stresses and comparative studies testing the morphological and physiological responses of pine provenances to drought in field and controlled conditions are still needed. Therefore, we aimed to evaluate the physiological behaviour of *P. pinaster* and *P. radiata* provenances with the potential to be used in afforestation actions under drought conditions. More specifically, we aimed to assess intra- and interspecific differences in seedling growth and survival under imposed drought. Results support the ongoing quest of identifying genetic materials suitable for afforestation with solid physiology-based knowledge and contributing to the broader goal of assuring the sustainability of the Portuguese pine sector.

2. Materials and Methods

2.1 Plant Material and experimental set up

The study was conducted at the National Institute for Agricultural and Veterinary Research, I. P. (INIAV), Oeiras, Portugal. *Pinus pinaster* Ait. and *P. radiata* D. Don. seeds of different origins were sown in July 2020 at the Furadouro Nursery (ALTRI Florestal). In July 2022, two provenances of each species were selected: *P. pinaster* - Portugal (Pp-Pt), obtained from qualified material from the seed orchard of Escaroupim (mixture of 17 families), and *P. pinaster* - France (Villemorin, France; Pp-Fr), and *P. radiata* - Spain (Xunta de Galicia; Pr-Es) and *P. radiata* - France (Villemorin, France; Pr-Fr). Plants were transplanted to 1.7 l pots (50 per provenance) containing a 3:1 mixture of sand and peat inside a plastic bag to prevent evaporation and left growing for ca. six weeks before the trial. In the greenhouse, plants were randomly distributed in four blocks, each including plants periodically watered to field capacity ("C", control) and plants kept under total irrigation suppression ("S", water stress). After 92 days, a group of S plants was allowed

to recover after irrigation for ca. 30 days, the remaining being kept without irrigation until permanent wilting ("M" plants).

2.2 Measurements

Unless stated differently, plant sampling was done in eight plants per treatment (S and C) and provenance, at the beginning of the trial and after drought imposition (92 days) and recovery (120 days) phases.

Biometric characterization (total height, cm; stem diameter at base, mm) was done in all plants using steel tapes and digital callipers (Mitutoyo, Japan), respectively. Total biomass investment (g DW), and partitioning into components – needles, stems, and roots was assessed by destructive sampling and used to calculate growth allometric parameters, e.g., root/shoot ratio, leaf area ratio (leaf area/total biomass), white root/brown root ratio, and new leaf/total leaf biomass ratio.

Water loss through transpiration (g H₂O hour⁻¹plant⁻¹) was monitored by weighing pots, twice a week, and normalizing by leaf area (T, g H₂O hour⁻¹cm⁻²) based on leaf area/dry leaf biomass ratio (SLA).

Predawn leaf water potential (Ψ_{pd} , MPa) was measured between 4-6 a.m. in fully expanded needles using a pressure chamber (PMS 1000, PMS Instrument Co, USA). Relative water content (RWC), needle water amount relative to the maximum (turgid weight), was assessed in needles collected early morning, based on needles fresh, turgid and dry weights (Barrs 1968). Stomatal conductance to water vapour (relative stomatal conductance index, Ig) was assessed indirectly based on canopy temperature measurements by infrared thermography (GF300, FLIR Systems, USA). Images were corrected for spatial calibration (Grant et al. 2007), and determinations based on the temperature of reference leaves and actual leaf temperature. Ψ_{pd} , RWC and Ig monitoring was done on six dates throughout the trial.

Specific hydraulic conductivity (K_{LA} , kg s⁻¹m⁻²MPa⁻¹) was measured in stem segments using a high-precision flow meters (XYL'EM, Bronkhorst, NL) and normalized by the corresponding leaf area. Measurements were done before and after drought imposition.

2.3 Biochemical determinations

Fully expanded needles were collected from the middle third of the crown and used for biochemical determinations. Total phenolic content (mg EAG g DW⁻¹) and antioxidant activity (mg EAG g DW⁻¹) were estimated following Vieira et al. (2009) after adapting the Folin-Ciocalteu's method for *Pinus* material. Total chlorophyll (Chl, μ g Chl mg DW⁻¹) and

carotenoid (Car) contents (μ g Car mg DW⁻¹) were extracted according to Scotti-Campos et al. (2015) and quantified using Lichtenthaler (1987) equations.

2.4 Statistical analysis

A two-way ANOVA (p < 0.05) was applied, using Sigmaplot V13 software (Inpixon, USA), followed by Kruskal-Wallis (H) and Dunn's tests for the comparison of means (95% confidence level).

3. Results

Results show species-specific biomass investment strategies, with plants of *P. radiata* provenances exhibiting higher root/shoot Ratio (*p*=0.002) and leaf area ratio (*p*=0.002) in relation to *P. pinaster*. Not surprisingly, the increase in height of C and S plants of both *P. radiata* provenances at the end of the drought imposition phase (day 92) was higher than the observed for *P. pinaster*. *P. pinaster* provenances significantly differed in height and biomass allocation (Fig. 1), with Pp-Pt having higher total biomass allocation regardless of treatment or sampling time.

Total biomass allocation patterns after 92 days did not reflect the contrasting watering regimes, as no significant differences were observed between control (Fig. 1a) and drought-stress (Fig. 1b) plants of each provenance. Although water suppression affected the production of new roots and shoots (Fig. 1), new root production after rewatering was higher in drought-stressed plants of *P. radiata* provenances and Pp-Fr than in control plants (*p*<0.001) (data not shown).



Figure 1. Biomass partitioning by components (g Dry Weight) for *P. pinaster* and *P. radiata* provenances in C plants (a) and S plants (b) at the end of the drought imposition period (day 92) (n = 8).

Results show that S plants of all provenances responded to the first 35 days without watering with a sharp stomatal closure, which resulted in a nearly ten-fold decrease in T (to below 0.002 gH₂Oh⁻¹cm⁻²) (Fig. 2a). However, during the initial four weeks *P. pinaster*
provenances differed in their responses to drought imposition, with Pp-Fr maintaining significantly higher transpiration rates and Pp-Pt showing a more conservative water use strategy. The intensification of water stress between days 35 and 92, was accompanied by further reductions in transpiration (to ca. 0.0002 gH₂0h⁻¹cm⁻²).

After rehydration, Pp-Fr showed a faster transpiration recovery, with average values at day 120 17.6% above that of C plants. For the other provenances transpiration did not fully recover, remaining between 68% (Pp-Pt) and ca. 81% (*P. radiata*) of the T of control plants at day 120. The pattern of incomplete drought recovery was confirmed by stomatal conductance assessment (Ig) for all provenances, except Pp-Fr (Fig 2b).

Due to their larger leaf area, the total transpiration per plant was higher in *P. radiata* provenances, justifying the lower Ψ_{pd} values (Fig. 3), both in C and S plants. At the end of the drought imposition phase, average Ψ_{pd} values were -2.4 MPa in *P. pinaster* and -4.2 MPa in *P. radiata*, respectively (Fig. 3a).



Figure 2. Variation of transpiration per unit leaf area (g H₂O h⁻¹ cm⁻²) (a) and relative stomatal conductance index (b) throughout the drought imposition and recovery phases for *P. pinaster* and *P. radiata* provenances. Solid lines represent S plants, dashed lines represent C plants. Error bars represent the standard error (n = 8).

After rehydration, all provenances recovered Ψ_{pd} to control values, except Pr-Fr. RWC showed similar patterns to the observed for Ψ_{pd} (Fig. 3b). Overall, *P. pinaster* provenances maintained higher RWC values, but tended to have a higher difference between C and S plants. This pattern was reflecting the lower (p<0.001) RWC value of *P. radiata* C plants, and not a better response to drought imposition by S plants. K_{LA} results also showed different water transport strategies between species, with *P. radiata* (5.5 - 6.4 x10⁻⁴ kgs⁻¹m⁻¹MPa⁻¹) showing significantly higher (p<0.001) water transport capacity than *P. pinaster* (1.7 - 2.4 x10⁻⁴ kgs⁻¹m⁻¹MPa⁻¹), both in control and drought stress plants.

In control plants, pigments content (total chlorophyll and carotenoids) was significantly different (p<0.02) between species (results not shown), with P. pinaster plants presenting higher values. Results suggest that drought imposition negatively affected the levels of chlorophylls and carotenoids in the Pp-Fr (Table 1), where a significant decrease was observed compared to control plants (p=0.041). A reduction of

chlorophyll a/b ratio was also observed except in Pp-Pt which maintained stable values. No significant differences in total phenolics content were observed among the four provenances in response to drought (Table 1). However, the French provenances of both species tended to have lower phenolic levels, which may be related to a genetic adaptation to the milder climate conditions of their region of origin. Pp-Pt showed tendentially higher levels of total phenolics (not significant difference) in the S plants. As with phenolic content, a similar trend was observed for antioxidant activity for the French provenances. In response to drought, the Spanish *P. radiata* provenance significantly increased antioxidant activity (p=0.007).



Figure 3. Pre-dawn leaf water potential (Ψ_{pd} , MPa) (a) and Relative Water Content (RWC, %) (b) throughout the drought imposition and recovery phases, for *P. pinaster* and *P. radiata* provenances. Solid lines represent S plants, dashed lines represent C plants. Error bars represent the standard error (n = 8).

Table 1. Total needle content of chlorophyll (µg Chl mg DW⁻¹), carotenoid (µg Car mg DW⁻¹), phenolics (mg EAG g DW⁻¹), and antioxidant activity (mg TROLOX g DW⁻¹) for S plants of all provenances at three trial phases: Start (before drought imposition), Drought (92 days under water suppression), and Recovery (four weeks after resuming watering). Standard error values are presented in grey.

Chlorophyll <i>a</i>	1.84	1.63	1.72	1.47	1.74	2.13	1.81	1.44	1.94	1.83	1.49	1.81
(µg Chl mg DW ⁻¹)	0.22	0.16	0.14	0.10	0.21	0.12	0.14	0.15	0.29	0.12	0.12	0.15
Chlorophyll <i>b</i>	0.65	0.53	0.81	0.51	0.67	0.99	0.69	0.55	1.15	0.61	0.71	1.10
(µg Chl mg DW ⁻¹)	0.06	0.05	0.13	0.07	0.08	0.13	0.05	0.05	0.16	0.05	0.08	0.12
Chlorophyll (a/b)	2.82	3.24	2.38	3.09	2.61	2.32	2.65	2.61	1.98	3.18	2.26	1.87
(µg Chl mg DW ⁻¹)	0.17	0.48	0.28	0.40	0.17	0.22	0.15	0.11	0.40	0.36	0.22	0.34
Carotenoid	0.52	0.54	0.46	0.42	0.44	0.49	0.53	0.47	0.36	0.47	0.43	0.33
(µg Car mg DW ⁻¹)	0.06	0.07	0.04	0.03	0.09	0.05	0.05	0.05	0.09	0.04	0.06	0.08
Phenolics	763.85	1254.08	533.48	893.58	874.28	835.50	1121.24	1038.63	1057.10	1203.88	1217.80	1013.82
(mg EAG g DW ⁻¹)	70.65	149.43	32.23	95.87	67.32	78.36	62.09	65.15	32.38	75.43	75.53	92.16
Antioxidant Activity (mg TROLOX g DW ⁻¹)	556.33 83.21	705.89 57.10	1117.37 91.28	528.81 68.84	572.41 88.88	397.06 30.14	584.94 38.55	623.88 41.75	450.79 23.88	642.29 67.50	847.13 53.21	477.61 10.80

The different water conservation strategies of the species and provenances were reflected in their varying abilities to survive drought imposition, with M plants of the different provenances reaching 100% of mortality 177 (Pp-Fr) to 206 (Pp-Pt) days after watering.

4. Discussion

Results showed species-specific biomass investment strategies, with *P. radiata* seedlings having higher root/shoot and leaf area ratios which resulted in higher growth capacity regardless of water availability. *P. pinaster* has shown contrasting water-use strategies under drought according to the region of origin, the Portuguese provenance tending to maintain lower transpiration rates and less negative Ψ_{pd} , whilst the French provenance showed higher water-use and faster recovery from drought imposition, with consequences for long-term survival under water suppression. Biochemical analyses showed that French provenances of both species may be better adapted to the milder drought conditions of their region of origin.

Growth and biomass allocation patterns

The contrasting watering regimes did not result in significant differences in biomass allocation patterns between control (Fig. 1a) and drought-stress (Fig. 1b) seedlings of all provenances. This result may reflect both species phenological rhythm as the trial progressed from summer into autumn, with the reduction of daily light hours and average temperature contributing to slowed growth patterns and, thus, the expression of the effects of drought on tree growth and biomass allocation.

Drought has been shown to significantly increase the fraction of root biomass, although the intensity of the process is less pronounced in woody plants (Eziz et al. 2017). In seedlings of *P. pinaster*, root biomass partitioning has been shown to remain unaffected by mild water stress (Aranda et al. 2010). Our results show that whilst the different watering regimes did not affect significantly whole plant biomass allocation patterns, new root growth was significantly reduced during the drought imposition phase in S plants (Fig. 1). However, after rewatering for 30 days, S plants of all provenances (except Pp-Pt) invested significantly more (5 to 10 fold) in new roots than C plants, revealing a clear priority towards below-ground investment following a severe drought.

P. radiata seedlings were able to invest more in new apical shoots, both C and S plants, than *P. pinaster*. However, for S plants growth was restricted to the first weeks of the trial when water availability and light conditions were not yet limiting, reflecting the higher growth capacity conferred by their higher leaf area ratio. Different growth capacities between the two pine species have been previously observed, with *P. radiata* growing faster than *P. pinaster* by 12–21% for volume and 36–59% for height (Lombardero et al. 2016). The higher growth and total biomass observed for Pp-Pt compared to Pp-Fr, agrees with previous studies showing that *P. pinaster* seedlings

originated in Western Iberia display superior growth in relation to provenances originating from colder/northern regions (Correia et al. 2008). Additionally, *P. pinaster* plants with origin in Landes, France (as Pp-Fr) have been shown to decrease the potential for biomass production under limited water availability (Fernández et al. 1999).

Physiological responses to drought

Drought imposition resulted in decreased transpiration and increased stomatal closure (Fig. 2), and sharp reductions of Ψ_{pd} and RWC (Fig. 3). These results suggest that both species exhibit isohydric behaviour, closing stomata and limiting transpiration to avoid hydraulic failure under drying soil conditions (De Miguel et al. 2011; Espinoza et al. 2016; Matías et al. 2017). The greater decrease in Ψ_{pd} in S plants of *P. radiata* (Fig. 2a) may indicate osmotic adjustment through the accumulation of organic solutes, such as soluble sugars, and increased osmotic potential, a mechanism that allows maintaining leaf turgor (maintaining RWC) in situations of water deficit (Fernández et al. 1999).

P. pinaster has been shown to have different strategies of response to drought according to the region of origin (Aranda et al. 2010). Provenances from hotter origins exhibited better or faster responses to water stress (e.g. RWC, osmotic adjustment) and strategies to save water, such as lower SLA (Fernández et al. 1999). Our results also show contrasting water-use strategies under drought, with Pp-Fr showing a faster recovery of Ψ_{pd} , T and RWC after rewatering (Figs. 1 and 2). However, after four weeks of rewatering stomatal conductance of Pp-Fr plants remained affected by drought (Fig. 1b). This result is in line with the lower levels of chlorophylls and carotenoids observed in needles of S plants of the French *P. pinaster* provenance (Table 1), that may be reflecting damages to chloroplast membranes with consequent limitations on photosynthetic activity and caroton assimilation, which could result in biomass losses.

In response to drought, the Spanish *P. radiata* provenance significantly increased antioxidant activity (*p*=0.007), which could be an indicator of effective antioxidant mechanisms providing greater cellular protection by eliminating oxygen free radicals and avoiding cellular damage. On the contrary, French provenances of both species tended to have lower phenolic levels and antioxidant activity (Table 1), which may be related to a genetic adaptation to the milder drought conditions of their region of origin.

Seedling survival under prolonged drought

In our study, seedlings of all provenances survived over 100 days without watering and under almost complete stomatal closure, especially after the initial 35 days (Fig. 2). The ability to withstand prolonged limitations to photosynthesis has been shown for several tree species (Weber et al. 2018), with conifer seedlings surviving several weeks with very low non-structural carbohydrate reserves, likely relying on alternative C sources like lipids, proteins or hemicelluloses. Significant differences in survival have been previously observed for *P. pinaster* and *P. radiata*, with higher survival under dry conditions observed for populations originating from lower or warmer origins (Alía et al. 1995; Correia et al. 2008; Espinoza et al. 2014; Matías et al. 2017). P. radiata seedlings with more inland origin showed increased survival under drought (Espinoza et al. 2014), whilst for Atlantic provenances of P. pinaster, such as Pp-Fr, survival is lower in sites with typical Mediterranean climate (Alía et al. 1995), likely because of their lower water use efficiency. Agreeingly, plants from the Portuguese P. pinaster provenance, with a tendency for more conservative water consumption per unit leaf area (Fig. 2a), reached 100% mortality with a delay of 29 days compared to plants from the French P. pinaster provenance, which maintained higher transpiration rates per unit of leaf area. Furthermore, Pp-Pt maintained low levels of plant mortality (ca. 20%) until day 169 while for all other provenances mortality varied between 80-100%. For Pinus species, smaller seedlings have lower establishment probability under hotter drought conditions (Matías et al. 2017), confirming the observed in our study for Pp-Fr.

5. Conclusion

The decision on planting locations, based on water stress conditions, should consider the different susceptibilities to drought, installing provenances less tolerant to drought but with higher growth capacity (like *P. radiata*) in milder sites and more drought-tolerant provenances, like the Portuguese *P. pinaster* provenance, in drier sites. Under Mediterranean field conditions, where precipitation and the length of drought periods are subject to unpredictability, resilience to very low soil availabilities is crucial to the outcome of a plantation. The ability to survive 20-30 days longer, as observed for Pp-Pt, can have a significant difference in planting success. A careful choice of reproductive forest material (RFM) and forestry allocation decisions are mandatory and should be supported by reliable knowledge of plant physiological and growth responses to drought.

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Killing me softly: Key traits determining time to hydraulic failure and risk to drought-induced mortality.

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Abstract: Hydraulic failure is considered a ubiquitous factor in drought-induced tree mortality. It occurs when, under drought conditions, the accumulation of cavitated xylem vessels reduces the tree's water transport capacity, leading to the progressive dehydration of downstream tissues and organs. This has made xylem resistance to cavitation to be directly linked to plant resistance to drought and, therefore, the risk of hydraulic failure and drought-induced mortality. Nevertheless, while cavitation resistance is a fundamental trait in drought resistance, there are other traits that play a crucial role in a tree's ability to withstand drought. This is the case for the plant residual conductance (gres) that represents the sum of cuticular permeability and residual stomatal conductance. Gres have an important role in determining the time it takes for a plant to reach water potential values that induce xylem hydraulic failure. Therefore, considering the variation of these traits not only within and across species but also under different temperatures conditions, is essential for assessing the risk of drought-induced tree mortality among different species and varieties. This information is highly valuable for improving forest and orchard management practices, especially considering the expected increase in temperature and drought intensity in many areas, such as the Mediterranean basin, due to climate change. This contribution will provide a general overview of the relevance of these traits and their variation for a proper determination of the time to hydraulic failure and, therefore, the risk to drought-induced tree mortality.

Keywords: Drought, hydraulic failure, plant residual conductance, cavitation.



XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Structural and water relations traits determining leaf tolerance to critical dehydration: A case study in California oaks

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Abstract: In recent years there has been increasing research on the processes leading to leaf mortality due to water deficit. Embolism in the xylem precedes damage in the surrounding tissue, which leads to the reduction of photochemical activity and irreversible loss of leaf function. However, there is significant interspecific variation in the water content thresholds for critical damage. Here, we investigated the structural and water-related traits that could determine the variability in leaf tolerance to dehydration. To this end, the relative water content (RWC) at which significant reductions in maximum quantum yield of PSII (F_v/F_m) occurred was assessed in 12 Quercus species native to California (4 deciduous and 8 brevideciduous/evergreen). The bench-dehydration method was used in excised leaves to determine the relative water content (RWC) at which critical reduction of F_v/F_m occurred and the rehydration capacity of leaves. Leaf mass per area (LMA), leaf dry matter content (LDMC) and pressure-volume (P-V) parameters such as osmotic potential at turgor loss point (π_{tip}), osmotic potential at full turgor and modulus of elasticity were measured to investigate structure-function relationships behind species' dehydration tolerance. Species differed in their dehydration tolerance, with RWC at which F_v/F_m declined by 50% (RWC_{F50}) ranging from 16 to 40%, with deciduous species presenting lower RWC_{F50} than evergreens. Leaf rehydration capacity significantly decreased at RWC ranging from 40 to 71% RWC, and was strongly related to RWC_{F50} . Both indicators of leaf dehydration tolerance were correlated with LMA: leaves with higher LMA (evergreens) present in turn higher RWC_{F50} and thus lower dehydration tolerance in terms of critical RWC thresholds. An even stronger relationship was found between water content thresholds of F_v/F_m decline and LDMC. On the other hand, among P-V parameters, only π_{tlp} was positively correlated with RWCF50, and to a lesser extent than either LMA or LDMC. These results

suggest a relevant link between leaf structure and tolerance to dehydration, and a potential key role of constitutive leaf water content in determining such tolerance.

Keywords: Chlorophyll fluorescence, dehydration tolerance, leaf structure, *Quercus*, leaf rehydration capacity, water relations, water stress

1. Introduction

Understanding the causes of irreversible damage at the leaf level is key in helping predict and prevent events of plant mortality, which are likely to become more widespread in the current climate change scenario. Chlorophyll fluorescence, and more specifically, assessment of the maximum quantum yield of PSII (F_v/F_m), provides a reliable measurement of damage in leaves that can help in determining the critical thresholds of leaf dehydration tolerance across different species. There is a significant correlation between changes in F_v/F_m and other indicators of leaf damage, such as electrolyte leakage (Guadagno et al. 2017). Moreover, F_v/F_m can be measured relatively rapidly and is a good proxy of cellular or membrane damage (rather than chlorophyll degradation) when measuring detached leaves under laboratory conditions (Trueba et al. 2019). Decline in F_v/F_m occurs at the last stages of dehydration, after complete cessation of photosynthesis, possibly concomitant with local embolism events which lead to the critical dehydration of the surrounding tissue (Trueba et al. 2019; Brodribb et al 2021; Mantova et al. 2023). Despite significant progress in understanding the processes leading to leaf death, the interspecific variability in the 'timing' of leaf death (i.e., the water content at which irreversible damage occurs) is still poorly understood. The main objective of the present study is to discern the traits determining the interspecific variability in the tolerance to critical dehydration.

2. Materials and Methods

2.1 Plant material

We studied 12 *Quercus* species from the California bioregion (**Table 1**). Individuals were located at the California Botanical Garden (Claremont, CA). Average summer temperature in the location is 25 °C, average winter temperature is 10 °C, and average annual precipitation is 467 mm. Plants were irrigated throughout the dry season. Measurements were made in August and September 2022. Branches were collected from different mature individuals, from the sun-facing part of the canopy and rehydrated overnight in the lab for subsequent measurements.

Hipp et al. (2018).						
Species	Section	Leaf habit	Bioregions			
Q. lobata	Quercus	Deciduous	California			
Q. garryana	Quercus	Deciduous	California			
Q. kelloggii	Lobatae	Deciduous	California			
Q. pacifica	Quercus	Brevideciduous	California			
Q. douglasii	Quercus	Deciduous	California			
Q. durata	Quercus	Evergreen	California			
Q. agrifolia	Lobatae	Evergreen	California			
Q. engelmannii	Quercus	Brevideciduous	California, Mexico			
Q. berberidifolia	Quercus	Brevideciduous	California			

Table 1. List of the studied *Quercus* species. Section, leaf habit and bioregion according to Hipp et al. (2018).

Q. wislizeni	Lobatae	Evergreen	California
Q. john-tuckeri	Quercus	Evergreen	California
Q. chrysolepis	Protobalanus	Evergreen	California, Mexico

2.2 Dehydration and rehydration assay

Leaf dehydration tolerance was assessed by measuring chlorophyll fluorescence in a set of leaves (c. 30 leaves per species) from different individuals. F_v/F_m was measured using a MultispeQ V2.0 (PhotosynQ Inc., East Landing MI, USA) in dark-adapted leaves. Leaves were excised, embedded in moist paper, and hydrated through the petiole in 50 ml Falcon tubes for 12 h to obtain the initial hydrated weight (HW). Then, leaves were dehydrated in the lab under low light conditions for a variable period to obtain a wide range of relative water content (RWC) and leaf water potential (Ψ). At this 'dehydrated' stage, leaf fresh weight (FW), Ψ and F_v/F_m were measured. Then, leaves were rehydrated using the aforementioned method to complete the final F_v/F_m measurement at the 'rehydrated' stage. Finally, leaves were dried at 70 °C to obtain the dry weight (DW). RWC during dehydration was calculated as RWC = (FW – DW)/(HW – DW).

2.3 Leaf bulk structure

Leaf area (LA) and thickness (LT) were measured at the hydrated stage in all leaves used for the dehydration-rehydration assay. Leaf pictures on a flat surface were analyzed using ImageJ (Schneider et al., 2012) to obtain LA. LT was obtained as the average from six measurements per leaf using a digital caliper. Leaf mass per area (LMA) and leaf dry matter content (LDMC) were calculated as LMA = DW/LA and LDMC = DW/HW, respectively.

2.4 Pressure-volume curves

Pressure-volume (P-V) curves were conducted in 4-5 leaves from different individuals per species in rehydrated branches using the bench dry method (Sack et al. 2011). The following parameters were calculated: osmotic potential and relative water content at turgor loss point (π_{tlp} and RWC_{tlp}, respectively), osmotic potential at full turgor (π_o) and bulk modulus of elasticity (ϵ). ϵ was calculated from the symplast water content as described in Bartlett et al. (2012).

2.5 Statistical analysis

The *geom_smooth* function ('ggplot2' package) was used to fit "loess" regressions to obtain the RWC at which F_v/F_m declined by 50 % (RWC_{F50}) for each species. Linear regression analysis (*Im* function from 'stats' package) was performed to test the relationship between RWC_{F50} and leaf structural and P-V parameters. All analyses were performed using R statistical software.

3. Results

LMA varied 2-fold across the studied species, from 111.1 g m⁻² in *Q. lobata* to 227.8 g m⁻² in *Q. chrysolepis* (**Fig. 1A**). LT varied 1.6-fold across species, ranging from 0.209 mm in *Q. durata* to 0.328 mm in *Q. chrysolepis* (**Fig. 1B**). LDMC showed only 1.2-fold variation across species, from 0.438 g g⁻¹ in *Q. kelloggii* to 0.547 g g⁻¹ in *Q. chrysolepis* (**Fig. 1C**).

All species presented a similar response to dehydration in terms of F_v/F_m (**Fig. 2**). Minimal F_v/F_m decline occurred during the first stages of dehydration (100 to 70 % RWC); then, F_v/F_m declined steeply with decreasing RWC. F_v/F_m values below 0.3 were reached at 20-30 % RWC. Mean

RWC_{F50} was 47.0 %, ranging from 28.2 % in *Q. lobata* to 63.1 % in *Q. chrysolepis*. Leaf Ψ was measured simultaneously with F_v/F_m and RWC during the first stages of dehydration (Fig. 2).



Leaf Ψ strongly declined with initial RWC decrease, reaching -3 MPa at RWC of 80-90 % in all species, prior to any significant F_v/F_m decline. Water potential measurements below -4 MPa became increasingly difficult for some species due to leaf rupture. Nonetheless, in most cases no major F_v/F_m decline was observed even in the -4 to -8 MPa range.

Recovery of F_v/F_m after rehydration strongly depended on the degree of dehydration reached prior to leaf rewatering (**Fig. 3**). No leaf was able to achieve higher F_v/F_m values than those during dehydration. On the contrary, rehydrated F_v/F_m was significantly lower than that of dehydration in many cases, specially at dehydration F_v/F_m between 0.3 and 0.6.



Fig. 2 Maximum quantum yield of PSII (F_v/F_m) in response to dehydration. Circles represent measured leaves at different relative water content (RWC), where F_v/F_m (white) and leaf water potential (Ψ , blue) were measured simultaneously. 'Loess' regression was used for F_v/F_m (light blue) and Ψ (dark blue) fittings; shaded area corresponds to the 95 % confidence interval. Red dashed lines represent the F_v/F_m 50 % threshold, solid lines represent RWC_{F50}.



relationship. Dashed lines represent the F_v/F_m 50 % threshold.

Among P-V parameters, only π_{tlp} showed a significant relationship with RWC_{F50} (Fig. 4). On the other hand, both LMA and LDMC were significantly correlated with RWC_{F50} (Fig. 5). Among all structural and P-V parameters, LDMC showed the strongest relationship with RWC_{F50} ($R^2 = 0.90$); in contrast, the relationship with π_{tlp} was relatively weaker ($R^2 = 0.39$).



Fig. 4 Relationship between relative water content at which F_v/F_m decreased by 50 % at dehydration (RWC_{F50}) and water relations parameters. Lines denote significant linear regression; shaded area corresponds to the 95 % confidence interval. (A) osmotic potential at turgor loss point (π_{tlp} ; $R^2 = 0.39$, P = 0.017). (B) RWC at turgor loss point (RWC_{tlp}; $R^2 = 0.05$, P = 0.242). (C) Osmotic potential at full turgor (π_0 ; $R^2 = 0.14$, P = 0.122). (D) Bulk modulus of elasticity (ϵ ; $R^2 = -0.05$, P = 0.500).



Fig. 5 Relationship between relative water content at which F_v/F_m decreased by 50 % at dehydration (RWC_{F50}) and leaf structure. Lines denote significant linear regression; shaded area corresponds to the 95 % confidence interval. (A) leaf mass per area (LMA; $R^2 = 0.74$, P < 0.001). (B) leaf thickness (LT; $R^2 = 0.18$, P = 0.098). (C) leaf dry matter content (LDMC; $R^2 = 0.90$, P < 0.001).

4. Discussion

Although the present results do not necessarily imply greater drought tolerance for species with low RWC_{F50} (e.g., *Q. lobata* and *Q. kelloggii* vs more 'sclerophyllous' species such as *Q.* chrysolepis), assessing dehydration tolerance with this method can provide interesting insights into the overall water stress tolerance of the plant (Trifilò et al. 2023). Despite the relatively narrow range of LDMC variation observed in the studied oak species when compared to larger datasets (see Hodgson et al. 2011), LDMC was the parameter most associated with RWC_{F50}. This contrasts with the relatively poor relations with parameters derived from P-V curves, which are usually considered as proxies to drought tolerance (Bartlett et al. 2012). The strong relationship between RWC_{F50} and LDMC points towards a key role of the initial water content in determining the 'timing' of critical damage. We found that absolute water content (albeit normalized by dry weight), rather than relative water content (or leaf water potential), is a better predictor of leaf damage. Abate et al. (2021) reached a similar conclusion on two Salvia species with very low LDMC (leaf density of 0.07 and 0.13 g cm⁻³, a proxy of LDMC); in contrast, oaks present much higher density (from 0.5 to 0.8 g cm⁻³ for our species). The characterization of a greater number of species, with a wider range of LDMC, will help in establishing the role of water content as a key trait in determining leaf tolerance to dehydration.

5. Conclusion

Variability in the leaf water content at which critical damage occurs during dehydration is strongly associated with leaf structure in terms of leaf dry matter content (LDMC) among oak species. *Absolute* water content is key in determining the 'timing' of leaf death rather than other indices of drought such as osmotic potential or modulus of elasticity.

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 Oral Communications
Session II: "Aspectos genéticos y moleculares de las relaciones hídricas en plantas"





XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

The search for genes and gene products to be used as molecular markers in the selection of *Quercus ilex* elite genotypes resilient to stresses associated to the decline syndrome and climate change.

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Abstract: The holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.) is the predominant species of the Mediterranean basin and the agrosilvopastoral system "dehesa" and considered as a key element of biodiversity. To date, most of the published works carried out in this forest species have focused on aspects of botany, silviculture, and ecophysiology. Although considered an orphan-species of molecular studies, interest has increased significantly in recent years. As an allogamous and non-domesticated species, large phenotypic morphometric, physiological, and molecular variability ranging from individuals to populations has been observed (Maldonado-Alconada et al. 2022). At the molecular level, we have developed the first draft of the holm oak genome (Rey et al. 2022). Following the Central Dogma of Molecular Biology, we have deepened into the effect and response to adverse stress conditions from a transcriptomic, proteomic and metabolomic point of view (Maldonado-Alconada et al. 2022). In addition, and considering the high variability observed in the holm oak, we are determining the contribution of epigenetic mechanisms to such variability.

To date, we have a list of putative genetic, epigenetic, transcript, protein and metabolite markers related to inter- and intra-population variability and stress response. We have identified genes and gene products that are indicators of variability and involved in the response to stresses. For example, some of them have been proposed as putative markers of drought tolerance such as *FtSH6* (a chloroplast ATP-dependent zinc metalloprotease), CLPB1 and CLPB3 (CLPB chaperon family), HSP22 (22.0 kDa heat shock protein) and some enzymes involved in the flavonoid pathways (chalcone synthase, anthocyanidin synthase and anthocyanidin reductase).

Keywords: Holm oak, climate change, biodiversity, molecular marker, genomics, integrated omics analysis.

1. Introduction

Holm oak (Quercus ilex subsp. ballota [Desf.] Samp.) is the predominant species in the Mediterranean basin and the agrosilvopastoral system dehesa, as well as a key element of biodiversity (Pulido et al. 2016). It plays a key role in our environment and economy, especially in rural areas (Leroy et al. 2020). While holm oak has been extensively studied in botanical, silvicultural, and ecophysiological domains, there remains a notable lack of molecular investigations (Maldonado-Alconada et al. 2022). Interest in this species has grown substantially in recent years due to its well-adaptation to arid and semiarid regions, where it plays a key biological role against desertification (Quero et al. 2006). However, it is an established fact that the holm oak, and therefore the dehesa, are facing serious problems that jeopardize their conservation and use. The species faces threats from both anthropogenic factors and a convergence of biotic and abiotic stresses. The manifestation of oak decline syndrome is a consequence of these combined stressors. Specifically, in holm oak, the primary cause of decline stems from the synergy of drought episodes and the presence of the oomycete *Phytophthora cinnamomi* (Brasier 1996; Ruiz-Gomez et al. 2018; San-Eufrasio et al. 2021a). Therefore, there is an urgent need to implement conservation, management, and reforestation strategies, in which biotechnology can make important contributions. The most realistic and plausible biotechnological strategy is the exploitation of biodiversity and the selection of elite or plus genotypes, assisted by molecular markers, for subsequent clonal propagation.

2. Biotechnology and Molecular-Assisted Breeding Programs

Holm oak is an allogamous, non-domesticated species that exhibits broad phenotypic, morphometric, physiological, and molecular variability within and between populations (Maldonado-Alconada et al. 2022). To date, the number of scientific works focused on the molecular aspects of holm oak is limited, due to its biological characteristic (allogamy, long-live cycle) and the recalcitrant property typic of woody species, making molecular research challenging. However, despite the difficulty that this forest species presents from an experimental point of view, the existing biodiversity can be characterized following the Central Dogma of Molecular Biology, as well as the omics cascade that explain the flow of genetic information from DNA (genomics, epigenomics and DNA-based markers) to metabolites (metabolomics), passing through mRNA (transcriptomics) and proteins (proteomics).

Our understanding of holm oak at the molecular level has followed a reverse trajectory considering the genetic information flow. Initial investigations delved into proteomic studies, as documented by Jorge et al. in 2005 and 2006. So far, our proteomics-based research has focused on descriptive and comparative proteomics areas describing the proteome of seeds, pollen, roots, and leaves, both in adult individuals and seedlings, as well as identifying differences in protein profiles among populations (Maldonado-Alconada et al. 2022). Our research has been focused on studying plant responses to

abiotic and biotic stresses related to decline syndrome (drought and P. cinnamomi stresses) combining proteomics, morphometry, and physiological analysis (San-Eufrasio et al. 2021a, 2021b). A panel of 30 proteins and 46 derived proteotypic peptides (viz., subtilisin and chaperone GrpE protein) were proposed as markers of drought tolerance to be used for the selection of elite tolerant genotypes (San-Eufrasio et al. 2021b). In 2015, other aspects of the flow of genetic information were covered in the molecular biology of the holm oak, passed by performing the transcriptomic and metabolomic research (Guerrero-Sánchez et al. 2021; Tienda-Parrilla et al. 2022). Our group has been mainly focused on the generation of RNA-Seq datasets to explore gene expression related to environmental stresses. A high number of transcripts were associated with stress response, of which 46 were involved in drought stress such as FtSH6 (a chloroplast ATP-dependent zinc metalloprotease), CLPB1 and CLPB3 (CLPB chaperon family) and HSP22 (22.0 kDa heat shock protein) (Guerrero-Sánchez et al. 2021). The possible mechanisms and molecular markers of drought tolerance in the seedling leaf metabolome has been recently published (Tienda-Parrilla et al. 2022), describing different compound (epigallocatechin, ellagic acid, pulegone, and indole-3-acrylic acid) that were up-accumulated under drought conditions. In an integrated multi-omics analysis of phenolic compounds and related enzymes, it revealed that some enzymes involved in the flavonoid pathways (chalcone synthase, anthocyanidin synthase and anthocyanidin reductase) were up-accumulated in non-irrigated seedlings (Tienda-Parrilla et al. 2022).

A pivotal milestone in holm oak research occurred with the recent release of the first draft of its genome (Rey et al. 2023). The completion of genome sequencing and the examination of gene sequences in holm oak pave the way for discussions, speculations, and the formulation of hypotheses regarding the origin of mutations and evolutionary shifts in long-lived organisms. It also sheds light on how these mechanisms contribute to adaptation in the face of environmental changes and offers insights into predicting dynamics within the context of climate change scenarios. The holm oak is a long-lived tree facing a twofold challenge, namely: adapting its development to environmental shifts and preserving the flexibility required for successful growth and maturation under varying environmental conditions. Epigenetic mechanisms play a pivotal role in enabling these adaptations by establishing transient, reversible marks. Insight into epigenetic variation, and its correlation with phenotypic plasticity, is expected to improve our understanding of adaptive responses with a view to more accurately assessing the potential risks associated with short- and long-term environmental fluctuations. In holm oak, natural variation in epigenetic marks and their relationship to phenotypic traits remain largely unexplored. Unravelling the role of epigenetics in shaping tree phenotypes might allow us to identify key elements in the control of growth traits and improve our understanding of the evolutionary capacity of this forest species.

3. Conclusions

Holm oak could be considered as a genetically straightforward but experimentally challenging species. To date, significant progress has been achieved at the molecular level, ranging from genome sequencing to the identification of genes and gene products related to variability, biodiversity, and responses to biotic and abiotic stresses associated with the decline syndrome and climate change. However, molecular interpretation in holm oak has been a challenge due to observed high molecular variability. Defining reference phenotypes will aid in better understanding the molecular basis of processes related to adaptation to adverse biotic and abiotic stresses. It will also contribute to the success of genetic improvement and reforestation programs in this forest species. Following genome sequencing, the aim is to advance in the molecular biology of holm oak through a Genome-wide Association Study. This study will identify specific genetic variations, such as Single Nucleotide Polymorphisms (SNPs), associated with tolerance to biotic and abiotic factors. On the epigenetic level, the next step involves the identification and characterization of methylated genes in response to stresses will be identified using whole-genome bisulfite sequencing (WGBS). Moreover, there will be advancements in targeted transcriptomic studies (RT-qPCR), allowing analysis across various sampling times, tissues, developmental stages, and a higher number of biological replicates due to its cost-effectiveness compared to RNA-Seq. In terms of proteomics, progress will be made in the use of the Data Independent Acquisition (DIA) technique, which exhibits superior identification and reproducibility compared to conventional Data Dependent Acquisition (DDA). Directed quantitative proteomic techniques like Parallel Reaction Monitoring (PRM) will enable quick and highly precise identification and quantification of peptides and proteins of interest in extensive datasets. In metabolomics, the focus should persist on searching for bioactive compounds with antioxidant, antiproliferative, anti-inflammatory, and/or antimicrobial activities, adding significant value to holm oak.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Evaluation of the effect of drought stress on fruit quality of transgenic strawberry plants with cell wall encoding genes down-regulated

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Abstract: Strawberry is an important crop in our country. One of the main challenges of this crop is to produce high-quality fruits with a prolonged post-harvest shelf life. In addition, strawberries are particularly susceptible to water stress so climate change will pose a significant challenge for quality fruits production. This study aimed to characterize, under water restriction conditions, the performance of several transgenic lines of strawberry (Fragaria × ananassa, Duch.) with genes encoding cell wall enzymes involved in fruit softening down-regulated. The lines used were: PG29 and 18A13, modified in the polygalacturonase gene *FaPG1*; βGal28, with the β-galactosidase gene FaBGal4 silenced; RGli26, with the ramnogalacturonan lyase gene FaRGLyase1 silenced. All these lines produced ripe fruits significantly firmer than the control. The plants were grown in a greenhouse under sufficient (3.3 L·m⁻²·dia⁻¹) or deficient (0.5 L·m⁻² ·dia⁻¹) irrigation conditions. The vegetative growth of the plants under stress conditions decreased significantly in the same proportion in all lines tested. Likewise, a similar reduction in fruit weight and size was observed in all genotypes tested, as well as in the number of fruits produced per plant. In terms of fruit quality, all transgenic lines produced firmer fruits than the control under sufficient water conditions. In the water stress treatment, the two lines with the modified *FaPG1* gene produced fruits firmer than the control. However, the RGli26 and β Gal28 lines showed a decrease in fruit firmness, obtaining values similar to the non-transformed control. No significant variations in colour and soluble solids were detected in any of the analysed lines. These results indicate that FaPG1 gene manipulation is a viable alternative to increase the postharvest shelf life of strawberry plants cultivated under water restriction; nevertheless, the increase in firmness obtained by β -galactosidase or RGlyase silencing is reversed under water stress.

Keywords: cell wall, drought stress, strawberry, fruit firmness, fruit softening, *Fragaria*, transgenic plants

1. Introduction

Climate change projects greater water limitations for agriculture in Mediterranean regions. This poses a significant challenge for food production, especially in the context of population growth (Kang et al., 2009). Therefore, a profound understanding of plant water relations is crucial for the

success of agricultural production in the future (Iglesias & Garrote, 2015). Strawberries (*Fragaria* × *ananassa* Duch.), a key crop with high socio-economic and nutritional value, are vulnerable to water deficit (Cordoba-Novoa et al. 2022). Spain stands out as the sixth global producer and the main European producer of strawberries, with 97% of this cultivation concentrated in the province of Huelva (FAOSTAT, 2021; Romero-Gámez & Suárez-Rey, 2020). Strawberry plants are highly susceptible to water deficit due to their shallow root system, extensive leaf area, and highwater content in the fruits. For this reason, water scarcity has a direct impact on productivity, ripening, and organoleptic quality of strawberries (Cordoba-Novoa et al., 2022).

On the other hand, one of the main challenges in this cultivation is to produce high-quality fruits with an extended post-harvest shelf life. The strawberry fruit undergoes rapid softening during ripening, which has been associated with the dismantling of the cell wall, especially pectins, the dissolution of the middle lamella and consequent loss of cell adhesion, and a decrease in turgor pressure. Three main classes of pectin have been identified: homogalacturonan (HG), consisting of a main chain of galacturonic acid residues; rhamnogalacturonan I (RG-I), which is formed by a skeleton of alternating galacturonic acid residues with rhamnose, and side chains of galactose and arabinose; and rhamnogalacturonan II (RG-II), which is less abundant than the other two pectins, with a complex composition and highly conserved among species. The degradation of pectin polymers during ripening occurs as a result of the action of pectin-metabolizing enzymes (Wang et al., 2018).

In our group, transgenic strawberry lines have been obtained with low levels of expression of genes related to pectin dismantling, producing firmer fruits with better post-harvest characteristics. Specifically, plants with the polygalacturonase (PG) gene *FaPG1* silenced by antisense (Quesada et al., 2009) or edited by CRISPR/Cas9 (López-Casado et al., 2023), plants with the galactosidase (β Gal) gene *Fa\betaGal4* silenced by antisense (Paniagua et al., 2016), as well as plants with the *FaRGLyase1* gene encoding a rhamnogalacturonan lyase (RGli) silenced by RNAi (Ric-Varas et al., 2024) have been obtained. PG enzymes degrade demethylated HG, while RGli and β Gal act on the skeleton and side chains of galactose in RG-I, respectively. Although all selected lines produce firmer fruits than the non-transformed control, the increase in firmness is greater in lines with low levels of PG than in the rest of the genotypes. The objective of this work was to characterize, under water restriction conditions, the behaviour of these transgenic strawberry lines with silenced wall genes.

2. Materials and Methods

2.1 Plant material.

For the development of the experimental study, strawberry plants (*Fragaria* × *ananassa*, Duch.) cv. Chandler were used. The transgenic lines included PG29 (Quesada et al., 2009) and 18A13 (López-Casado et al., 2023), modified in the PG gene *FaPG1*; β Gal28, with the *Fa* β Gal4 β -galactosidase gene silenced (Paniagua et al., 2016); and RGli26, with the *Fa*RGLyase1 gene encoding a rhamnogalacturonan lyase (RGli) silenced (Ric-Varas et al., 2024).

2.2 Experimental conditions.

Eighteen plants per genotype were grown in 6-liter pots and kept in a confinement greenhouse at the IFAPA Centre in Málaga during the production months (April to July) with a natural photoperiod. Two irrigation conditions were employed: watering 6 days a week (equivalent to 3.3 $L \cdot m^{-2} \cdot day^{-1}$, control treatment) and watering two days a week (equivalent to 0.5 $L \cdot m^{-2} \cdot day^{-1}$, deficit

irrigation treatment). The temperature ranged from a minimum of 18°C to a maximum of 31°C, while the ambient relative humidity remained between 50% and 81%.

2.3 Vegetative growth analysis.

Zenithal projection photographs were taken of 6 plants from each line and treatment, and area values were obtained using ImageJ image analysis software. Additionally, leaf area was determined by scanning 9 replicas of complete leaves from different plants of each line and treatment. Furthermore, the water content per dry weight of the leaves was determined.

2.4 Production and fruit phenotyping analysis.

Fruits at the ripe red stage were harvested, and the total weight and number of fruits produced by each plant during the experiment were recorded. For fruit phenotyping, they were weighed and measured using a precision scale and a digital calliper. Additionally, firmness, soluble solids content, and colour were determined using a penetrometer, refractometer, and colorimeter, respectively.

3. Results

3.1 Vegetative growth.

The vegetative growth of the plants under stress conditions, as well as the leaf water content, decreased significantly in approximately the same proportion in all evaluated transgenic genotypes (*Fig. 1*).



Fig. 1 Vegetative growth. (A) Zenithal projection, (B) Leaf area, and (C) Water Content per unit of dry weight (WC/mg dw). Representative photograph of the zenithal projection of the β Gal28 line (D) and leaf area of the 18A13 line (E). Data represent the mean ± SD. Means with different letters indicate significant differences by Tukey's test at P=0.05.

3.2 Production and fruit phenotyping.

A significant reduction in fruit production, as well as in their weight and size, was observed when cultivating plants under deficit irrigation. The variation in these parameters was similar across all tested genotypes (*Fig. 2*).



Fig. 2 Fruit production and size. (A) Number of fruits produced per plant, (B) Weight, (C) Length, and (D) Width of the fruit. Data represent the mean ± SD of 30 to 120 fruits from each genotype and treatment. Means with different letters indicate significant differences by Tukey's test at P=0.05.

In terms of fruit quality, all transgenic lines produced firmer fruits than the control under sufficient irrigation conditions. In the water stress treatment, the two lines with the modified FaPG1 gene produced firmer fruits than the control. However, the RGli26 and β Gal28 lines showed a decrease in fruit firmness, obtaining values similar to the control (*Fig. 3*).



Fig. 3 Fruit firmness. Data represent the mean ± SD of 30 to 120 fruits from each genotype and treatment. Means with different letters indicate significant differences by Tukey's test at P=0.05.

No significant variations were detected in soluble solids in any of the analysed lines, but differences were observed in some colour parameters (*Fig. 4*).



Fig. 4 (A) Soluble solids. (B) Colour parameter L*, representing luminosity. (C) Colour parameter a*, representing a tendency toward red colour compared to green when decreasing. Data represent the mean \pm SD of 30 to 120 fruits from each genotype and treatment. Means with different letters indicate significant differences by Tukey's test at P=0.05.

4. Discussion

The strawberry is highly susceptible to water scarcity due to its shallow root system, extensive leaf surface, and high-water content in the fruits, all of which require a significant amount of water. In water scarcity situations, a reduction in overall plant growth has been observed, directly impacting productivity, ripening rate, and fruit organoleptic quality (Cordoba-Novoa et al., 2022; Deaquiz et al., 2014). This study has confirmed these effects, showing a significant decrease in parameters such as zenithal projection or overall plant development, leaf surface, and water content per unit of leaf weight under water stress conditions, both in the non-transformed Chandler and in the transgenic study lines.

Concerning plants under sufficient irrigation conditions, the RGIi26 line showed a higher value than 18A13 in zenithal projection, while it was significantly lower in leaf area. The greater leaf surface in the RGIi26 genotype could be due to the formation of a greater number of leaves. On the other hand, the line that produced the highest amount of fruits/plant under stress conditions was RGIi26, almost reaching the same number as the PG29 and 18A13 lines under adequate irrigation. The fruits of β Gal28 were the lightest and the smallest, as observed compared to Chandler in the study by Paniagua et al. (2016), and this situation was repeated under deficit irrigation.

Regarding fruit quality parameters, no significant differences were observed in soluble solids in any of the evaluated genotypes. Deaquiz et al. (2014) found a reduction in the soluble solids content of strawberry fruits as irrigation decreased; on the contrary, an increase in this variable has been described in other fruits such as tomatoes (Wang et al., 2011). This result suggests that osmotic adjustment does not occur in deficit irrigation in the Chandler cultivar. In terms of colour, the 'L*' parameter representing fruit luminosity showed that, both under sufficient and deficit irrigation conditions, the β Gal line outperformed the control, while 18A13 did not reach the values of Chandler. At the same time, the fruits of the RGli26 line were brighter than the controls under water deficit conditions. The 'a*' parameter, describing the tendency towards red compared to green, revealed that the β Gal fruits were redder than Chandler under both irrigation conditions, although to a lesser extent under water restriction.

Finally, all transgenic lines produced firmer fruits than the control under sufficient water conditions, as expected due to their modifications in enzymes that degrade the cell wall during ripening. Additionally, in the water stress treatment, the two lines with the modified FaPG1 gene produced firmer fruits than the control. However, the RGli26 and β Gal28 lines showed a decrease

in fruit firmness, obtaining values similar to non-transformed Chandler fruits. It has been suggested that arabinan side chains of rhamnogalacturonan I play a key role in maintaining cell wall flexibility under dehydration stress (Moore et al., 2008). Arabinans would act as regulators of the proximity of polysaccharides, preventing the formation of tight bonds, such as Ca^{2+} bridges, between HG domains, which stiffen the cell wall. The decrease in firmness in RGli26 and β Gal28 fruits could be due to changes in the cell wall structure related to RG-I induced by water stress. Studies on cell wall properties in these transgenic lines under water limitation conditions are currently underway.

5. Conclusion

The results obtained suggest that modifying the *FaPG1* gene provides a viable option to extend post-harvest conservation duration in strawberry plants cultivated under water scarcity conditions. However, it is important to note that the increase in strawberry firmness achieved by decreasing the expression of genes encoding β -galactosidases or RG lyases is reversed when plants experience water stress.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Involvement of Abscisic Acid on Root Hydraulic Conductivity and Aquaporin Regulation by a Plant Growth-Promoting Rhizobacteria under Drought Conditions at Two Different Temperatures

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Abstract: In the last years, drought episodes are rising, diminishing crop yields. In this context, regulation of root hydraulic properties by aquaporins is crucial. Abcisic acid (ABA) is a plant hormone that among other functions regulates root hydraulic properties. Plant growth-promoting rhizobacteria (PGPR) enhance abiotic stress tolerance (including drought) of plants, and also they may modulate root hydraulic properties. However, there is no information about how ABA is involved in the regulation of root hydraulic properties by PGPR. Since temperature affects plant response to drought, two different temperatures were tested (24°C and 28°C). Therefore, tomato plants (wild type (WT) and ABA-deficient mutant, *sitiens*) were inoculated with a PGPR (*Bacillus megaterium*) and subjected to drought at the corresponding temperature after two weeks of sowing during 1 further week.

Inoculation with *B. megaterium* increased plant biomass in WT plants under all environmental conditions, however the opposite happened in inoculated *sitiens* plants. At 24°C, independently of watering treatment, inoculation increased root hydraulic conductivity (L) in WT plants, but decreased it in *sitiens* ones. But at 28°C, inoculation increased L in *sitiens* ones and had barely effects on WT ones. Aquaporin expression and abundance upon inoculation was depended on temperature and tomato genotype. Inoculation had no effect on stomatal conductance in WT plants, but decreased it on *sitiens* ones. At the root level, PGPR inoculation increased abscisic acid (ABA) levels under drought conditions at 24°C in both plant genotypes, obviously *sitiens* plants having less than 10% values than WT plants. Also, PGPR inoculation raised salicylic acid (SA) contents in roots under all conditions, except in *sitiens* plants under drought and 28°C.

In fact, drought treatment increased root SA contents in non-inoculated plants, but the opposite happened in inoculated ones in both genotypes. Drought treatment raised roots jasmonic acid (JA) contents, which is inhibited by PGPR inoculation, except in *sitiens* plants under 24°C. Therefore, ABA is involved in the response of L to PGPR inoculation, mostly via modifying other hormonal signal pathways and aquaporin activity.

Keywords: Abscisic acid, Aquaporins, Drought, Plant growth-promoting rhizobacteria, Temperature, Tomato

1. Introduction

In the last decades, land zones exposed to drought episodes have increased, risking agricultural production. One environmental friendly solution is the use of beneficial soil microorganisms like plant growth-promoting rhizobacteria (PGPR) (Ruzzi and Aroca 2015). It is known that PGPR are able to increase plant abiotic stress tolerance like drought by several mechanisms including hormonal regulation and improved water relation parameters (Ruzzi and Aroca 2015). In this sense, PGPR action is mediated by several hormonal signaling pathways (Kudoyarova et al. 2024).

One mechanism plants employ to tolerate drought stress is the regulation of L (Aroca et al., 2012). L is regulated by both PGPRs and hormones (Kudoyarova et al., 2024). However, there is no information about how ABA is involved in the regulation of L by PGPRs. To address this gap, we took advantage of tomato ABA deficient mutants (*sitiens*; Porcel et al., 2014). Since climate change is causing both drought and rising temperatures, we explored two temperatures during the experiments.

2. Materials and Methods

2.1. Biological material and experimental design

Seeds of tomato (*Solanum lycopersicum* L.) ABA deficient mutants *sitiens* and the corresponding wild-type (cv Rheinlands Ruhm) were surface disinfected and germinated as described by Porcel et al. (2014). Plants were grown, and drought was applied as described by Aroca et al. (2008), with the addition of testing two temperatures (24°C and 28°C).

2.2. Determinations

Plant growth determined as dry weights, root hydraulic conductivity (L), plant hormones (ABA, JA and SA) and aquaporin abundance were determined as described previously (Calvo-Polanco et al. 2014; Sánchez-Romera et al. 2018).

3. Results

At harvest, bacterial inoculation improved plant shoot dry weight in wild-type plants at both temperatures, especially under drought conditions. However, in *sitiens* plants, bacterial inoculation did not enhance growth; instead, a slight decline was observed in all growing conditions (Fig. 1).



Fig. 1 Shoot dry weight of wild-type (WT) or *sitiens* (Sit) plants well-watered (WW) or droughted (D) at 24 or 28°C, inoculated (B) or not (NI) with *Bacillus megaterium*.

Root hydraulic conductivity (L) increased by inoculation in WT plants almost in all conditions. In contrast, in sitiens plants L decreased or barely changed by inoculation at 24°C, but increased at 28°C (Fig. 2). Sitiens plants always showed lower values than WT ones, except when subjected to drought at 24°C (Fig. 2). The combination of drought and 28°C caused a drastic reduction of L in all treatments (Fig. 2).



Fig. 2 Root hydraulic conductivity (L) of wild-type (WT) or *sitiens* (Sit) plants well-watered (WW) or droughted (D) at 24 or 28°C, inoculated (B) or not (NI) with *Bacillus megaterium*.

Root ABA was always higher in WT plants than in *sitiens* ones. Bacterial inoculation enhanced root ABA accumulation under drought at 24°C in both genotypes. Drought enhanced root ABA accumulation mostly at 24°C (Fig. 3). Sitiens plants showed higher levels of JA than WT ones, specially under drought conditions at 24°C when the PGPR was present (Fig. 3). Similar trend was observed for SA (Fig. 3).



Fig. 3 ABA, JA and SA of wild-type (WT) or *sitiens* (Sit) roots well-watered (WW) or droughted (D) at 24 or 28°C, inoculated (B) or not (NI) with *Bacillus megaterium*.

Amount of plasma membrane intrinsic proteins (PIPs) proteins in roots was quantified by ELISA (Fig. 4). Amount of PIP1 proteins behave in a different way in WT and *sitiens* plants. While in WT plants PIP1 amount decreased by drought, in *sitiens* raised. The effects of bacterial inoculation were specific for each genotype and environmental conditions. Similar trend was observed for PIP2 amount. The highest value observed in *sitiens* plants at 28°C under drought conditions, and the lowest in the same genotype, under drought at 24°C. Regarding phosphorylated PIP2, they were always lower in *sitiens* plants compared to WT. Under well-watered conditions, bacterial inoculation decreased its amount in WT plants and had no effect in *sitiens* plants. Under drought conditions, at 24°C, bacterial inoculation increased PhPIP2 amount in both genotypes, but at 28°C, it decreased or had no effects in WT and sitiens plants, respectively.



Fig. 4 PIP1, PIP2 and PhPIP2 aquaporins in wild-type (WT) or *sitiens* (Sit) roots well-watered (WW) or droughted (D) at 24 or 28°C, inoculated (B) or not (NI) with *Bacillus megaterium*.

4. Discussion

As mentioned earlier, the role of ABA in the effects of PGPRs has been scarcely studied. Porcel et al. (2014) found that *B. megaterium* did not act as a PGPR in *sitiens* plants, mostly because these plants have higher levels of ethylene. In the present study, we observed the same lack of effect on plant growth in *sitiens* plants, accompanied by higher levels of JA and SA. Both hormones are considered stress hormones, and when present in excess, they typically cause growth retardation (Yu et al., 2020). This could explain the lack of effect of *B. megaterium* in *sitiens* plants.

Regarding the effects of *B. megaterium* on L, the response depends on temperature. At 24°C, bacterial inoculation increases L in WT plants, but decreases it or does not change it in *sitiens*. This pattern is accompanied again by higher levels of JA and SA that could reduce L (Boursiac et al. 2008; Lee and Zwiazek 2019). In general, sitiens plants have less aquaporin amount than WT plants, explaining lower L values.

5. Conclusion

We can conclude that the enhanced effect of L by B. megaterium in tomato plants is dependent on ABA at 24°C, because of the overaccumulation of JA and SA in the absence of ABA. However, at 28°C, it is independent of ABA, although no relation to hormonal content or aquaporin amount was found. This could be explained by the different subcellular localization of aquaporins (Calvo-Polanco et al., 2014).

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Rice root-specific E3-ubiquitin ligases in plant-microbe interactions under low water availability: A strategy for a more sustainable production?

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Abstract: Global environmental changes and population growth pose challenges for sustainability, with drought and limited freshwater availability being a major threat to agriculture. Understanding the molecular mechanisms underlying crop resilience to water limitations is crucial. Rice (Oryza sativa L.), a staple food for more than half of the world's population, serves as the model organism for this study. The importance of rice as a critical food source underscores the urgency of this research. The ubiquitinproteasome system (UPS), a posttranslational modification pathway, plays a role in plant abiotic stress responses, but its involvement in water limitation is not well-explored. Roots are essential for plant anchoring, and water and nutrient uptake, which makes the identification of molecular mechanisms governing root traits under water scarcity crucial for sustainable agriculture. Under water-limiting conditions, some plants undergo physiological adaptations such as changes in root architecture and root exudate composition, impacting the rhizosphere microbiota community to aid in drought mitigation. Recently, we identified a root-specific RING-family E3-ubiquitin ligase that responds to drought. In our lab at ITQB, we developed overexpression and silencing lines to functionally characterize this gene under stress conditions. The developed rice lines exhibit contrasting root architectures possibly related to drought tolerance. Our project aims to comprehensively analyze the role of this root-specific E3-ubiquitin ligase by studying the root architecture, transcriptomic and metabolomic profile in drought responses. Furthermore, since the root-associated microbiome can impact drought responses, we also aim to characterize the microbiome associated with these plants in control vs. water-limiting conditions. This research aims to identify key molecular pathways modulated by the root-specific E3-ubiquitin ligase that may be important for plant-microbe interactions under water-limitations, thus contributing to sustainable rice production.

Keywords: *Oryza sativa* L, drought, E3-ubiquitin ligase, rhizosphere, beneficial plantmicrobe interactions



Vegetative and reproductive hydraulic adjustment in grapevine organs under deficit irrigation: physiological and molecular aspects

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Abstract: Water stress induces functional sectoring in grapevine affecting resource distribution among plant organs. However, integrative information on the effects of water availability on xylem functionality in each grapevine organ is limited. This study aims to understand the impact of irrigation regimes on hydraulic adjustments in leaves and berry clusters of "Touriga Nacional" variety along seasonal development. Field plants were subjected to long-term irrigation treatments over five consecutive years: full irrigation (FI, 100% ETC); deficit irrigation (DI, 50% FI); no-irrigation (NI, rain-fed). Stomatal conductance (gs), predawn leaf water potential (ψ_{pd}), and hydraulic conductance (leaf (K_{leaf}), petiole ($K_{petiole}$), berry cluster ($K_{rachis+pedicel}$ and K_{rachis})), were monitored at four phenological stages during 2022: peppercorn-size (PCS), pea-size (PS), veraison (VER) and full-maturation (FM). Additionally, the expression of ten aquaporins (AQPs) and five sugar transporters was assessed in leaf, rachis, pedicel, and berries (skin and pulp).

Results show that *K* is a function of grapevine organ and phenology. K_{leaf} was highest at VER with NI showing the lowest values. A 70% decrease in $K_{cluster}$ was observed after PCS. After VER, $K_{cluster}$ increased significantly in all treatments equally, despite the significant differences in ψ_{pd} . $g_{\underline{S}}$ values were lowest in NI from PCS, when no differences were observed in ψ_{pd} , suggesting a priming effect of water stress. AQPs were differentially modulated among treatments, organs and phenology. In general, water stress induced the up-regulation of *VviTIPs* in leaves. At berry cluster, AQPs were differentially regulated depending on the tissue. Overall, most differences among treatments were observed at VER and FM. Sugar transporters were also modulated by irrigation at leaf and berry clusters. Interestingly, in leaves and berries, *VviSWEET14* was up-regulated in DI and NI
from PCS, while other hexose transporters were mostly down-regulated. The ecophysiological consequences of the differential hydraulic and molecular adjustment on vineyard management will be further discussed.

Keywords: Aquaporins, Berry, Hydraulic conductivity, Stomatal conductance, Sugar transporters, *Vitis vinifera*

1. Introduction

The growth and ripening of grape berries (*Vitis vinifera* L.) are tightly governed by vascular transport and cell expansion (Matthews and Shackel, 2005). Over the past two decades, there has been considerable debate regarding the water supply and flow from the roots to the berry, with a focus on the contributions of the xylem and phloem vascular systems along the berry phenology. Whilst before veraison xylem is the main water supplier to the berries, at the onset of ripening, berry, rachis, and pedicel's hydraulic resistance increases, with a gradual decline in the water flow through the xylem relatively to the phloem (Rogiers et al., 2001; Tyerman et al., 2004; Choat et al., 2009; Knipfer et al., 2015; Scharwies and Tyerman, 2017). After veraison, the phloem becomes the primary conduit for supplying water to support berry growth and transpiration, with a transition from symplastic to apoplastic unloading of sugars within the berries (Zhang et al., 2006). Recent evidence highlighted, however, the functional role of xylem in the berry pedicel during ripening (Knipfer et al., 2015; Scharwies and Tyerman, 2017) and its involvement in the phloem-derived water recycling after veraison (water backflow) (Zhang and Keller, 2017).

The involvement of aquaporins (AQPs) in these processes (e.g., cluster components' water transport) has also been suggested (Fouquet et al., 2008; Choat et al., 2009). AQPs have been associated either with changes in xylem hydraulic resistance along berry maturation to enhance membrane permeability (Choat et al., 2009) or with the accumulation of sugars at ripening (Coetzee et al., 2019). Wong et al. (2018) reported the phenology dependence of AQPs expressions with a specific set of AQPs more closely linked to young green berries and another set more associated with maturation stages. Additionally, other studies (Schlosser et al. 2008; Choat et al. 2009; Wong et al. 2018) showed the dependence of AQP expression to berry tissues, as well as to the genotype (Noronha et al. 2014).

Global warming is having a high negative impact in viticulture, particularly in Mediterranean regions. Extending drought periods and increasing heat wave frequency, particularly during berry ripening, increase the transpiration demand and, consequently, crop water needs. These changing meteorological patterns are highly impacting on the soil-plant-atmosphere continuum and plant water relations. Water stress has been reported to decrease hydraulic conductance in grapevine (Zarrouk et al., 2016; Damásio et al., 2023) and affect grape berry aquaporins' expression (Grimplet et al. 2007; Noronha et al. 2014). In fact, it has been widely reported that AQPs expression in plant vegetative organs is highly controlled by environmental stress factors (Tyerman et al. 2002; Sabir et al. 2021; Damásio et al. 2023). However, despite the increasing attention given to this topic in the last decades (Knipfer et al. 2015; Grimm et al. 2017; Scharwies and Tyerman, 2017), the hydraulic patterns of the fruits, the main target of grapevine production, remain mainly unknown. This study aimed to unravel the impact of vine water status and phenological stage on water movement in berry clusters of Touriga Nacional grapevine variety, through the study of cluster hydraulic responses, as well as the expression of aquaporins, in several tissue compartments. Understanding the physiological and molecular mechanisms that control berry/cluster water movement and relationships at both the cellular/tissue level is key to refine irrigation strategies.

2. Materials and Methods

2.1 Plant Material and Experimental Site

The study was conducted during the growing season of 2022 at a private ampelographic collection from Esporão commercial vineyard located in the Alentejo region, Portugal (38.380098, -7.560724). Eleven-year-old plants of Touriga Nacional variety, grafted on 1103 Paulsen with 1.5 m x 3.0 m (N/S oriented) spacing, spur-pruned and trained on a bilateral vertical shoot positioned system, were used. The plants had been subjected to three irrigation treatments since 2018: full irrigation (FI, 100% ETc); deficit irrigation (DI, 50% of FI); no-irrigation (NI, rain-fed). Plant sampling and monitoring was done at four phenological stages: peppercorn-size (PCS), pea-size (PS), veraison (VER) and full-maturation (FM).

2.2 Grapevine Water Status and stomatal conductance

Grapevine water status was monitored along the experiment by measuring predawn leaf water potential (ψ_{pd}), with a Scholander pressure chamber (Manofrígido, S.A., Lisboa, Portugal), in five plants per treatment, using at least two leaves per vine.

Stomatal conductance (g_s) was measured with a Steady State Porometer (LI-600, LI-COR Inc, Lincoln, NE, USA), between 11 and 12 am, in two leaves per vine, from 10 plants per treatment.

2.4 Leaf and berry cluster hydraulic conductance

Hydraulic conductance (K, kg s⁻¹ MPa⁻¹) was measured with a high precision flow meter (XYL'EM, Bronkhorst, Montigny-lès-Cormeilles, France) in two leaves and two clusters per vine, from five plants per treatment. Leaf (K_{leaf}), petiole (K_{petiole}), rachis with pedicel (K_{pedicel+rachis} = K_{cluster}) and rachis after pedicel excision (K_{rachis}) hydraulic conductivity was obtained by normalizing K measurements to the segment length and to leaf area in the case of leaf (kg s⁻¹ MPa⁻¹ m⁻¹), and to the length of the cluster and to sectional area (kg s⁻¹ MPa⁻¹ m⁻¹).

2.5 RNA Extraction and RT-qPCR

Grapevine leaves, rachis, pedicel, skin and pulp were analyzed for the gene expression of 5 sugar transporters (*SWEET14, GIN1, HT2, TMT2* and *HT6*) and 10 aquaporins (*VviPIP1;1, VviPIP1;2, VviPIP1;3, VviPIP2;1, VviPIP2;2, VviPIP2;3, VviTIP1;1, VviTIP1;2, VviTIP2;1, VviTIP2;2*), previously reported to transport water *in vitro* (Sabir et al., 2021). Samples were harvested at 11 am, immediately frozen in liquid nitrogen, and stored at -80°C. Total RNA extraction, cDNA synthesis and qPCR were performed following the protocol described in Damásio et al. (2023).

2.6 Statistical analysis

The experimental design consisted of a single factor analysis for each variety, considering the irrigation treatment as factor, with three levels: FI, DI, and NI. An exploratory and descriptive analysis was made of all physiological and molecular measurements, followed by a one-way variance analysis (ANOVA; SPSS 15.0 statistical package; SPSS, Chicago, IL, USA) with Duncan test for mean separation (p < 0.05).

3. Results

Results show that *K* is a function of grapevine organ and depends on vine phenology. K_{leaf} peaks at VER in all treatments, with NI showing the lowest values when compared to FI and DI (Table 1). The lower values in NI were maintained at FM. NI also presented the lowest $K_{petiole}$ from PS onward. $K_{cluster}$ and K_{rachis} showed the same evolution trend in all treatments. Both $K_{cluster}$ and K_{rachis} showed highest values at PCS. At PS, a 70% decrease was observed in all treatments (Table 1). After VER, $K_{cluster}$ increased significantly in all treatments. No differences among treataments were observed in $K_{cluster}$ along the season, despite the significant differences in ψ_{pd} (Fig. 1A).

Table 1. Specific hydraulic conductivity (Kg s⁻¹ MPa⁻¹ m⁻¹) in leaf and petiole (normalized to petiole length), cluster (rachis+pedicel) and rachis (normalized to cluster length and sectional area). Data are means \pm SE (n=5-8).

		PCS		PS				VER		FM		
	FI	DI	NI	FI	DI	NI	FI	DI	NI	FI	DI	NI
K _{leaf} (Kg s ⁻¹ m MPa ⁻¹)	2,25E-07 ± 2E-08	2,15E-07 ± 2E-08	2,74E-07 ± 2E-08	2,31E-07 ± 1E-08	2,45E-07 ± 2E-08	2,29E-07 ± 2E-08	5,85E-07 ± 8E-08	3,93E-07 ± 4,7E-08	2,71E-07 ± 2,9E-08	4,17E-07 ± 4,7E-08	2,93E-07 ± 1,8E-08	2,50E-07 ± 1,7E-08
K _{petiole} (Kg s ⁻¹ m MPa ⁻¹)	6,84E-07 ± 3E-07	7,25E-07 ± 3E-07	7,95E-07 ± 3E-07	2,26E-06 ± 3E-07	2,43E-06 ± 2E-07	1,82E-06 ± 1E-07	3,84E-06 ± 4E-07	3,12E-06 ± 2,5E-07	1,78E-06 ± 1,5E-07	2,78E-06 ± 2,6E-07	2,61E-06 ± 2,1E-07	1,19E-06 ± 2,0E-0
K _{rachis+pedicel} (Kg s ⁻¹ m ⁻¹ MPa ⁻¹)	3,58E-02 ± 9E-04	4,06E-02 ± 1E-03	4,29E-02 ± 3E-03	6,50E-07 ± 9E-08	6,42E-07 ± 4E-08	6,55E-07 ± 5E-08	1,03E-01 ± 2E-02	2 1,04E-01 ± 4,7E-03	8,46E-02 ± 1,0E-02	1,37E-01 ± 2,3E-02	1,71E-01 ± 2,9E-02	1,95E-01 ± 4,8E-02
K _{rachis} (Kg s ⁻¹ m ⁻¹ MPa ⁻¹)	3,92E-02 ± 9E-04	4,58E-02 ± 2E-03	4,77E-02 ± 3E-03	7,14E-07 ± 9E-08	6,88E-07 ± 4E-08	7,04E-07 ± 5E-08	1,11E-01 ± 2E-02	2 1,12E-01 ± 5,5E-03	9,80E-02 ± 1,1E-02	2 1,46E-01 ± 2,5E-02	1,89E-01 ± 2,6E-02	2,07E-01 ± 4,9E-02

Differences in g_s were observed since PCS among treatments when no differences were observed in ψ_{pd} between FI and DI (Figure 1A,B). Comparing to FI, g_s was 25 to 40% lower in DI and 55 to 85% lower in NI (Figure 1).



Fig. 1- (A) Predawn leaf water potential (Ψ_{pd} , MPa) and (B) Leaf stomatal conductance (gs, mol m⁻² s⁻¹) in FI, DI and NI treatments measured at pepper-corn size (PCS), pea size (PS), veraison (VER) and full maturation (FM) stages in Touriga Nacional variety. Values are means ± SE (n=30).

At the molecular level, AQPs were differentially modulated among treatments, organs, and phenology. In general, water stress induced the up-regulation of *VviTIPs* in leaves and slight changes in *VviPIPs* (Fig. 2). At berry cluster, AQPs were differentially regulated depending on the tissue (Fig. 2). Interestingly, in the rachis, moderate and severe water stress (DI and NI) up-regulated AQPs at PS, while at veraison AQPs were only up-regulated in NI. In the pedicel, AQPs were in general up-regulated in DI and NI since PCS. In pulp, all AQPs were in general up-regulated under water stress conditions, but the up-regulation was more pronounced in DI than in NI. In skin, *VviPIP1s* were up-regulated in NI while *VviTIP1s* were up-regulated in DI and NI.

Sugar transporters were also modulated by irrigation in leaf and berry clusters (Fig. 2). Interestingly, *VviSWEET14* was up-regulated in NI from PCS in leaves, and in VER and FM in DI and NI. Other hexose transporters were mostly down-regulated (*data not shown*). These results are in accordance with sugar accumulation curves along the phenological

stages (*data not shown*). In cluster organs, both *VviSWEET14* and *VviGIN1* were mostly upregulated under stress conditions since PS in the rachis, and in the other organs/tissues earlier at PCS.



Fig. 2- Aquaporin and sugar transporters gene expression (log2(fold change)) of *VviPIP1s, VviPIP2s, VviTIP1s, VviTIP2s, VviSWEET14* and *VviGIN1* along the grapevine phenology in leaf, rachis, pedicel, pulp, and skin of Touriga Nacional variety. Relative values for the treatments DI and NI are expressed in comparison to FI.

4. Discussion

Despite the low Ψ_{pd} in NI since PCS, K_{leaf} and $K_{petiole}$ did not show differences among treatments until PS. AQPs have been suggested to contribute to rapid and reversible regulation of cells' in several organs by adjusting the membrane water permeability and maintaining water and ion homeostasis of vines (Fouquet et al. 2008; Vandeleur et al. 2009; Choat et al. 2009; Zarrouk et al. 2016). In this regard, our results showed that the up-regulation of *VviPIP1s* and *VviTIP2s* in NI leaves at PCS could mediate the maintenance of high K_{leaf} in this treatment. In contrast with K_{leaf} , gs was significantly lower in NI and DI since PCS. Abscisic acid (ABA) has been related to stomatal closure in grapevine under water stress conditions (Chaves et al. 2010). In the previous season, we observed an increase of ABA in Touriga Nacional leaves in DI and NI, which could explain the observed decline in gs (Salguero et al. 2023). The detected decoupling between K_{leaf} and g_s could be related to the anisohydric behavior of Touriga Nacional. Coupel-Ledru et al. (2017) reported no response of K_{leaf} to increasing ABA in anisohydric genotypes. In

addition, the molecular regulation of membrane permeability through the interaction between AQPs and ABA (Vitali et al. 2016) should also be considered. Conversely, the low g_s in DI compared with FI at PCS, when no differences were observed in ψ_{pd} , might suggest a priming effect of water stress, caused by cumulative years of more intense soil water deficit.

At cluster level, the increase of *K* in the cluster and rachis after PS confirms the functional xylem role during ripening (Knipfer et al. 2005; Scharwies and Tyerman, 2017). At PS stage, the up-regulation of AQPs in the rachis, concomitant with the decrease in K_{rachis} , suggests a likely shift from apoplastic to symplastic water movement. In water stress treatments, the up-regulation of AQPs in the pedicel since PCS, suggests their involvement in berry solute accumulation homeostasis along maturation (water, sugars among others) as part of the developmental process. AQPs increase cell permeability, movement blockage preventing water along ripening, facilitating sugar accumulation/movement. More recently, Zhang et al. (2022), showed the loss of apoplastic sugar from grape berries and its retrieval in pedicels and suggested that sugars may move symplastically to the pedicel parenchyma (for local use or for storage), or to the phloem to be recycled to the berry. The high up-regulation of VviGIN1 (important for sucrose degradation) observed in DI and NI pedicels until FM and concomitant with upregulation of PIPs and TIPs, could support the later hypothesis. Overall, data suggest the impact of water stress in grape berry water and sugar inflow/outflow along ripening.

Contrasting with leaves, the hydraulic conductivity in the grape cluster components (cluster and rachis), does not differ among treatments despite the significant differences in ψ_{pd} , indicating the importance of berries as a strong sink (Patono et al. 2022). Thereby, the stability of cluster hydraulic conductivity is assured by several mechanisms, including AQPs. In addition, the up-regulation of sugar transporters *VviSWEET14* (among other transporters, *data not shown*) and sugar invertase *VviGIN1* under DI and NI, suggests greater carbohydrate allocation to berries under water stress treatments. These results could be controversial, considering the reduction of g_s and *K* at the leaf level, with likely penalties in assimilates production (photosynthesis) of these plants. However, recent studies by Patono et al. (2022) showed the highest allocation of newly photosynthesized carbonaceous resources in water stress grapevines to the berry when compared to well-watered grapevines.

5. Conclusion

The data gathered herein contribute to shed light on water movement mechanisms in berry clusters under different levels of water stress. We show that hydraulic traits are

seasonally dynamic and are responsive to the impacts of cumulative long-term water stress. Results indicate also that the modulation of AQP expression is organ/tissue and phenology dependent, being up-regulated by water stress.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Field-Based Transcriptomics Reveals Insights into Drought Tolerance Mechanisms in Tomato Plants: Bridging the Gap Between Greenhouse Experiments and Farmer's Needs

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Abstract: Climate change poses a significant threat to global agriculture, making the development of drought-tolerant crop varieties an urgent task. While greenhouse experiments in controlled conditions have been pivotal in understanding the molecular basis of drought tolerance in plants, the translation of these findings into practical solutions for farmers necessitates a shift towards open-field transcriptomics. This new approach aims to uncover gene expression patterns in tomato plants under field conditions, where environmental variability can profoundly influence gene regulation. We aim to highlight the dynamic nature of gene expression in response to drought stress, emphasizing the need to bridge the gap between controlled environments and the unpredictable field conditions where farmers cultivate their crops.

In this research, we selected drought tolerant Mediterranean tomato landraces and control, non-tolerant genotypes, and conducted an in-depth RNA analysis of plants grown in open field trials during the Mediterranean summer, under a control well-watered (WW) and a water-stress (WS) treatments. Phenotypic traits measured included total production, fruit quality, leaf photosynthesis and gas-exchange parameters. Results may highlight genetic regulation of drought tolerance in tomato landraces and may allow to correlate it with key phenotypic traits.

The findings of this study serve as a robust foundation for the development of droughttolerant tomato varieties tailored to field conditions across the Mediterranean region. Our ongoing work aims to further elucidate these mechanisms, ultimately contributing to global food security and sustainable agriculture through the creation of field-ready, drought-tolerant tomato plants. **Keywords:** *Solanum lycopersicum* L. (tomato), Open-field Transcriptomics, Mediterranean Landraces, Drought Tolerance, Abiotic Stress, Climate Change

1. Introduction

Climate change-induced shifts in precipitation patterns and rising temperatures heighten the vulnerability of agricultural regions to drought stress globally (FAO 2021; Wang et al. 2005). Changing climate conditions are leading to prolonged and more severe water deficits, and pose a direct threat to crop productivity, leading to food insecurity. Recognizing the necessity for crop resilience in the context of a changing climate underscores the importance of unraveling the genetic mechanisms that govern drought tolerance. Understanding how crops navigate water scarcity is central not only to ensuring food security but also aligns with broader climate resilience efforts (Craufurd and Wheeler 2009; Minoli et al. 2022).

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide, with a global production of over 189.1 million tons just in 2021 (FAO 2021). Additionally, tomato serves as a model crop owing to its compact genome (c. 900 Mb), short life cycle, well characterized diploid genetics and a vast availability of 'omics' data (Chen et al., 2020; The Tomato Genome Consortium, 2012). As such, many transcriptomics studies on this crop's water tolerance have been conducted, yet most of them have been conducted under controlled greenhouse conditions and short-term water deprivation stresses (Ashrafi-Dehkordi et al. 2018).

For this reason, the transition to open-field transcriptomics represents a pivotal advance. Open-field experiments authentically replicate the multifaceted conditions of real-world agriculture (Jing et al. 2022), providing a realistic representation of the challenges agronomic crops face under drought stress. This approach offers essential insights for developing drought-tolerant varieties tailored to a changing climate.

This study explores the transcriptomic responses of four different genotypes of tomato to water deprivation. Emphasizing diversity, we included two commercial cultivars, and two local Mediterranean landraces from geographically diverse origins.

2. Materials and Methods

2.1 Plant materials and Treatments

Four tomato genotypes were selected due to their outstanding phenotypical response to drought in past studies (Fullana-Pericàs et al. 2019; 2022), including 'Ailsa Craig' (AC) and 'Moneymaker' (MM) as controls, one Balearic landrace 'Ramellet' (RAM) and one Italian landrace 'Lucariello' (LUC). Plants were grown in an open field in Porreres (Mallorca, Spain) during the spring-summer season in 2021 (from April to October). The plants were separated into two blocks, one control block irrigated to cover 100% of the potential evapotranspiration (ETP) (WW treatment), and the stress block, irrigated at c. 40% of ETP (WS treatment). For each genotype, 5 plants were grown in the WW block and 8 in the WS block. The order of genotypes within each block was randomized.

2.2 Phenotypic measurements

Plant production was assessed every week by counting, weighing, and assessing the fruit's overall quality. Leaf gas-exchange was measured on the youngest fully expanded leaf of the plant with an open infrared gas-exchange analyser (Li-6400-40, Li-Cor Inc., Lincoln, USA). Measurements were performed from 9:00 to 19:00 h.

2.3 Transcriptomic analysis

Transcriptomic analyses were carried out on young leaves that were formed entirely under drought stress. RNA was extracted with the phenol-chloroform protocol from Oñate-Sánchez et al. (2008). Samples were then sequenced via the BGISEQ platform, and the clean reads were aligned to the reference genome using HISAT. Differential Gene Expression Analysis was carried out with Deseq2 in R (version 1.34).

3. Results

3.1 Phenotype analysis

The observed values for total production and gas-exchange parameters such as net photosynthesis (A_N), stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i) are shown in **Table 1**.

Table 1. Impact of drought on Ailsa Craig (AC), Money-maker (MM), Lucariello (LUC) and 'Ramellet' (RAM) tomato genotypes based on total fruit production and gas exchange parameters. Results are shown for the water stress (WS) and well-watered (WW) irrigation treatments. A_N refers to net photosynthesis, g_s to stomatal conductance and WUE_i to intrinsic water use efficiency (A_N/g_s). Letters denote statistically significant differences among genotypes within each treatment, and asterisks in the WS treatment denote statistically significant differences between treatments for each genotype and parameter at $p \le 0.1$ (·), $p \le 0.05$ (*), $p \le 0.005$ (**) and $p \le 0.0005$ (***) by means of Tukey test.

		Water	r Stress		Well-Watered			
Genotype	AC	ММ	LUC	RAM	AC	ММ	LUC	RAM
Total fruit production	$2.54 \pm$	2.12 ±	$2.10 \pm$	$2.78 \pm$	$6.7 \pm$	$6.05 \pm$	5.16 ±	$5.78 \pm$
[<i>K</i> g]	0.61 ^{a**}	0.39 ^a ·	0.21ª*	0.33 ^a *	0.79 ^a	1.48 ^a	1.42 ^a	0.99ª
A _N	$9.47 \pm$	5.52 ±	$9.97 \pm$	4.35 ±	$17.54 \pm$	$20.08~\pm$	$14.34 \pm$	$20.71 \pm$
$[\mu mol(CO_2)m^{-2}s^{-1}]$	1.08 ^{a**}	0.85 ^b **	0.70^{a**}	0.95 ^b ***	1.72ª	1.07 ^{ab}	1.04 ^c	0.86^{ab}
gs	$0.14 \pm$	0.11 ±	$0.23 \pm$	$0.07 \pm$	$0.45 \pm$	$0.48 \pm$	$0.46 \pm$	$0.48 \pm$
$[mol(H_2O)m^{-2}s^{-1}]$	0.03 ^b *	0.01 ^{b***}	0.03 ^a *	0.01 ^{a***}	0.10 ^a	0.06 ^a	0.06 ^a	0.07^{a}
WUEi	$82.84 \pm$	$53.32 \pm$	$44.54 \pm$	$59.89 \pm$	$42.46 \pm$	$43.54 \pm$	$32.92 \pm$	$45.97 \pm$
$[\mu mol(CO_2)mol^{-1}(H_2O)]$	20.4ª.	7.94 ^a	3.25ª.	9.39ª	4.45 ^a	4.07 ^a	4.31 ^a	7.11 ^a

3.2 Transcriptome variability

Up to 20.895 (60,28% of the *S. lycopersicum* total Coding Sequence, or CDS) genes with expression levels above background noise were detected in leaf tissue from open-field samples. The transformed and normalized read counts were subjected to principal component analysis (PCA), which revealed distinct sample clusters based on genotypes and treatments (**Fig. 1**).



Fig. 1 Principal component analysis of the normalized read counts in leaf samples of 'Ailsa Craig' (AC), 'Moneymaker' (MM), 'Lucariello' (LUC) and 'Ramellet' (RAM) genotypes grown under water stress (WS) and well-watered (WW) open-field conditions. The grey ellipses represent the clustering of samples based on their response to different treatments.

3.3 DEGs in response to water stress

A total of 2.884 and 2.718 genes were up- and down-regulated respectively when considering all four genotypes. The total number of differentially expressed genes (DEGs) per genotype is shown in **Fig. 2**. Of those, most of them were exclusive of control genotypes AC and MM (**Fig. 3**).



Fig. 2 Number of differentially expressed genes (DEGs) per genotype. AC is 'Ailsa Craig', MM is 'Moneymaker', LUC is 'Lucariello' and RAM is 'Ramellet'.



Fig. 3 Venn diagrams of all differentially expressed genes (DEGs) at p-val < 0.05 and |LFC| > 1 detected in Ailsa Craig (AC), Moneymaker (MM), Lucariello (LUC) and 'de Ramellet' (RAM) tomato genotypes in drought stress compared to irrigated conditions in leaves.

4. Discussion

To discuss the outcomes of our study, it is important to underscore the significance of conducting long-term open-field trials as opposed to short-term controlled conditions, given that open-field trials are subject to many uncontrollable variables that directly impact the plant's physiology (Muñoz et al. 2008), and therefore its underlying gene expression patterns. It is, however, a direct representation of the crops' behavior in open-field and thus the obtained results are tightly correlated with farmers' needs. Moreover, our emphasis on long-term water deprivation and subsequent sampling of young leaves formed under stress aims to fully characterize the underlying molecular determinants of both morphological and physiological adaptations to water deprivation.

In our results we observed a significant impact of drought on both fruit production per plant as well as gas-exchange parameters, certifying the effectiveness of the water stress treatment applied to the plants and in accordance with other studies in openfield conditions (Fullana-Pericàs et al. 2019). Interestingly, as opposed to well-watered plants, when analyzing net photosynthesis under drought stress, we observe a clear tendency where Balearic 'de Ramellet' plants exhibit a similar behavior to Moneymaker, while Italian 'da Serbo' behave more similarly to Ailsa Craig.

The WS treatment induced significant variability at the transcriptome across all four genotypes, a total of 60,28% of *S. lycopersicum* CDS was detected above background noise levels. Of the four, 'de Ramellet' expressed the least difference in expression pattern between treatments (**Fig. 1**), which is consistent with phenotypic variation. When assessing the total number of up- and down-regulated genes, local landraces 'de

Ramellet' and 'da Serbo' showed a significantly smaller amount of DEGs as opposed to control genotypes Ailsa Craig and Moneymaker (1.208 and 1.184 versus 4.400 and 2.419) (**Fig. 2**). This could be a result of efficient physio-morphological adaptations against drought stress in local landraces (Fullana-Pericàs et al. 2017), potentially leading to significantly reduced alteration at the transcript level as compared to control genotypes.

Interestingly, most DEGs are exclusive to one of the four genotypes, showcasing a large amount of specificity in the response to drought (**Fig. 3**). Those adaptation mechanisms will be further studied through gene ontology enrichment analyses.

5. Preliminary conclusions

To summarize, our study underscores the relevance of long-term open-field trials, examining the impact of water stress on crop responses over a period of months. Local landraces exhibit more efficient adaptations to drought and a less severe impact on their productivity and physiology, while still showing significantly different gene expression patterns. Transcriptome analysis prompts further exploration of adaptation mechanisms through gene ontology enrichment analyses. The insights gained from this study not only advance our understanding of plant responses to drought but also offer critical information for sustainable agricultural practices amidst the challenges imposed by a dynamically changing climate.

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2. Oral Communications Session III: "Estrés hídrico en cultivos"





XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

Zaragoza 14-16 FEB 2024

Impact of Drought Stress on Yield and Seed Quality of *Pisum sativum* cv. Tom Thumb

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Abstract: The pea (*P. sativum*) cv. Tom Thumb is characterised by a fast growth cycle, short stature, and high yield, making it an ideal model legume for the study of plant abiotic stresses. This study focused on characterising the impact of drought stress on pea plants yield and nutritional quality. Climate change is leading to shifts in weather patterns, resulting in increased occurrences of intense droughts, that reduce crop yields and nutritional value. Legumes, commonly cultivated in drought-prone areas like arid and semi-arid regions, are particularly vulnerable to these changing conditions. While several studies have focused on the effect of drought on pea agronomic traits, there is still a gap in the understanding the changes in nutritional quality beyond protein content. Herein, one assay was conducted with plants grown in a climatic chamber (photoperiod: 16h light; photosynthetic photon flux density: 150 μ mol m⁻² s⁻¹; temperature: 22°C; relative humidity: 60%) under control (80%) Field Capacity) or drought stress (40% Field Capacity) conditions. At harvest, the grains were analysed for their moisture, content of minerals, protein and amino acids, total phenolics and flavonoids, antinutrients (saponins, tannins and phytic acid) and total antioxidant activity. Soil samples were collected for microbial diversity analysis. Plants were significantly smaller and stress biomarkers, such as antioxidant activity, total phenolics, flavonoids and saponins were altered by drought stress. The nutritional quality of the seeds, such as protein and amino acids content, macro and micro minerals as well as phytic acid concentration, was not significantly affected. Additionally, the effect of drought stress on rhizospheric microorganisms was evident with notable shifts in the dominant genera, when compared to the control condition. Overall, results suggest that Tom Thumb cv. might be an important genetic resource to face upcoming climatic events and secure food production.

Keywords: amino acid profile, antinutrients, antioxidant activity, legumes, phenolic compounds



XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Effect of Drought Stress on Water Relations in Two Almond Cultivars Grafted onto Different Rootstocks

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Abstract: In this study the responses of eight almond rootstock/scion combinations under three water regimes were investigated using a factorial completely randomized design. Two almond cultivars, 'Mamaei' and 'Rabie', were grafted onto four rooted cuttings of Prunus rootstocks (Cadaman, Myrobalan 29C, GF677 and GN15). These plants were grown in five-liter pots filled with a soil and sand mixture for four months under regular irrigation. Then, drought stress treatment (100%, 75% and 50% of Field Capacity) was applied for 40 days. After the stress period, three key parameters were assessed: Relative water content (RWC), water use efficiency (WUE) and electrolyte leakage (EL). The results indicated that the RWC was higher in the Mamaei cultivar than the Rabie cv when both were grafted onto Cadaman or GF677 rootstocks. However, no difference was observed when both almond cultivars were grafted onto the other two rootstocks. Comparing rootstocks, we found that the highest WUE was in both, GF677 and GN15, and the lowest in the Myrobalan 29C. WUE was significantly higher in plants irrigated with 75% or 50% levels of field capacity than those fully irrigated. Mamai cv. showed 11.7% higher WUE than the Rabie cv. Concerning membrane integrity, Myrobalan 29C resulted in the highest % of electrolyte leakage which resulted in increases in membrane permeability, whereas the Cadaman, GF677 and GN15 rootstocks exhibited the lowest EL and did not show differences among them. In conclusion, 'Mamaei' demonstrated greater resistance to dehydration than 'Rabie', and the GF677, GN15 and Cadaman rootstocks exhibited superior performance to cope with water stress compared to Myrobalan 29C.

Keywords: Almond, Electrolyte Leakage, Relative Water Content, Rootstocks, Scion

1. Introduction

Drought stress poses a significant threat to global agricultural productivity, impacting crop yields and food security worldwide (Fujimori et al., 2019). With climate change exacerbating water scarcity and erratic weather patterns, there is an urgent need for innovative strategies to mitigate the detrimental effects of drought on crop production. In this context, grafting has become a promising approach to enhance drought resilience because rootstocks influence the response of grafted cultivars to drought stress (Nawaz et al., 2016). Previous research in almonds has demonstrated the effectiveness of rootstocks in mitigating the effects of water deficit on the scion, enhancing performance and productivity through various approaches (Álvarez et al., 2020; Ranjbar et al., 2022; Yadolahi et al., 2011).

Plants can alter their morphological, physiological, and metabolism-related responses at both organ and cellular levels to mitigate the severity of drought. Several studies have explored the physiological, biochemical, and molecular responses in *Prunus* rootstocks under water stress conditions to understand the mechanisms involved in rootstock/variety interactions and their enhanced adaptability to water-limited conditions (Álvarez et al., 2020; Bielsa et al., 2018; Jiménez et al., 2020, Martínez-García et al., 2020). These studies assess various stress response strategies among different rootstocks, seeking those with high osmotic adjustment, greater water-use efficiency (WUE), chlorophyll (Chl) content, antioxidant capacity, and stronger protective mechanisms, while minimizing reductions in relative water content (RWC), growth capacity, and photosynthetic capability of leaves (Jiménez et al., 2013, and references therein; Nasrolahpour-moghadam et al., 2019). Additionally, other physiological factors such as cell membrane stability and reduced electrolyte leakage (EL) are influenced by the specific combination of variety and rootstock (Ranjbar et al., 2022).

The importance of this study arises from the negative impact that water scarcity already has on plant growth, which will worsen with climate change. In this context, understanding the plasticity of *Prunus* rootstocks becomes crucial for almond cultivation and production. Our goal is to investigate whether grafting onto different *Prunus* rootstocks alters responses to drought stress in almond varieties. To achieve this, we will assess the influence of the rootstock on the water relations of eight almond rootstock/scion combinations, with the aim of identifying the optimal combination for drought tolerance.

2. Materials and Methods

To investigate the responses of eight almond rootstock/scion combinations to drought stress, we conducted a factorial experiment employing a completely randomized design. Four *Prunus* rootstocks, Cadaman, Myrobalan 29C, GF677, and GN15 (Garnem), with different genetic background were cultivated in five-liter pots filled with a soil and sand mixture for four months under regular irrigation and greenhouse conditions. Cuttings

from healthy tree branches of the commercial cultivars Rabie and Mamaei were acclimated in the greenhouse for one month. Buds of both Rabie and Mamaei scions were grafted onto all rootstocks. Subsequently, irrigation treatments (100%, 75%, and 50% Field Capacity, FC) were imposed for forty days. After the stress period, we measured relative water content (RWC), water use efficiency (WUE), and electrolyte leakage (EL) as described previously (Álvarez et al., 2020; Bastam et al., 2012).

3. Results and Discussion

As illustrated in Fig. 1A, RWC was higher in the Mamaei cultivar than the Rabie cultivar when grafted onto Cadaman and GF677 rootstocks. No significant difference in RWC were found between both almond cultivars when grafted onto the remaining rootstocks (Fig. 1A). Both levels of drought stress led to a progressively reduction in RWC compared to the control (Fig. 1B). Karimi et al. (2015) noted that drought stress decreased by up to 23% leaf relative water content in almond genotypes grafted onto GF677 rootstock. plants that maintain RWC under drought stress conditions have better performance (Jiménez et al., 2013).



Fig. 1 (A) Relative Water Content (RWC). Interactional effects of Cadaman, Myrobalan29C, GF677 and GN15 (Garnem) rootstocks grafted with Rabie and Mamaei scions. (B) RWC at different levels of drought stress (100%, 75% and 50% FC).

Regarding WUE, we found that rootstock, drought, and scion were significant factors. When comparing different rootstocks. It was found that both the GF677 and GN15 rootstocks exhibited the highest WUE, while the Myrobalan29C rootstock showed the lowest (Fig. 2A). When plants were subjected to 75% and 50% FC of drought stress, WUE increased significantly (Fig. 2B). In the Mamaei cultivar, WUE resulted in 11.7% higher than in the Rabie cultivar (Fig. 2C). Eichi et al., (2014) reported a strong correlation between WUE and various almond genotypes, indicating that genetic variation among almond genotypes plays a significant role in water relations.



Fig. 2 (A) Water-Use Efficiency (WUE) in Cadaman, Myrobalan29C, GF677 and GN15 (Garnem) rootstocks. (B) WUE at different levels of drought stress (100%, 75% and 50% FC) and (C) in the Rabie and Mamaei cultivars.

The Myrobalan29C rootstock exhibited the highest percentage of electrolyte leakage (EL) compared to the other rootstocks, Cadaman, GF677, and GN15 (Fig. 3A). Drought stress led to an increase in EL (Fig. 3B), with Rabie being the most affected cultivar (Fig. 3C). Consistent with these findings, Karimi et al. (2015) reported an increase of up to 43% in EL in almond cultivars under drought stress. An elevation in EL indicates an increase in cell membrane permeability, suggesting that stress tolerance is associated with maintaining membrane integrity (Bolat et al., 2016). Based on the results, it was determined that Rabie is more susceptible to dehydration than the Mamai cultivar, while the GF677, GN15, and Cadaman rootstocks exhibit greater ability to withstand water stress than the Myrobalan29C rootstock. The capacity of rootstocks to influence scion desiccation tolerance has been demonstrated in other woody species such as grapevines (lacono et al., 1998), or peaches (Ksouri et al., 2016; Jiménez et al., 2020).



Fig. 3 (A) Electrolyte leakage (EL) in Cadaman, Myrobalan29C, GF677 and GN15 (Garnem) rootstocks. (B) EL at different levels of drought stress (100%, 75% and 50% FC) and (C) in the Rabie and Mamaei cultivars.

4. Conclusion

In conclusion, enhancing drought tolerance in almonds can be achieved by grafting commercial varieties onto rootstocks known for their drought resilience. The interaction between rootstock and scion appears to play a crucial role in conferring resistance to environmental stresses such as drought. Among the evaluated rootstocks-scion combinations, Mamaei emerges as the superior scion, exhibiting higher RWC and WUE, along with lower EL than the Rabie cultivar. Additionally, the rootstocks GF677 and GN15 (Garnem) demonstrated superior performance, showing higher RWC and WUE, and lower EL percentages. Cadaman displayed intermediate values in these parameters, while Myrobalan 29C performed the poorest. These findings underscore the importance of selecting appropriate rootstock-scion combinations to enhance almond drought tolerance.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Biostimulant activity of Galaxaura rugosa seaweed extracts against water deficit stress in tomato seedlings involves activation of ABA signaling

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Abstract: Water scarcity is a serious constraint for agriculture, and global warming and climate change can exacerbate it in many areas. Therefore, sustainable approaches must be implemented to deal with current and future water scarcity scenarios. Genetic and chemical approaches are being applied to manage this limitation and maintain crop yields. In particular, biostimulants obtained from natural sources such as marine algae are promising aids for coping with water deficit stress in agriculture. Here we present a bioprospection study of extracts of the macroalgae Bonnemaisonia hamifera, Galaxaura rugosa, Dasycladus vermicularis, Ulva clathrata, Cystoseira foeniculacea, Cystoseira humilis, Lobophora dagamae, Colpomenia sinuosa and Halopteris scoparia from the north coast of Tenerife, in the Canary Islands. The aqueous extracts of Bonnemaisonia hamifera, Galaxaura rugosa, Dasycladus vermicularis and Cystoseira humilis show biostimulant activity against water deficit stress in tomato (Solanum lycopersicum) seedlings in pot-based experiments under controlled conditions, providing higher tolerance than the mock-treated control. The Galaxaura rugosa extract showed the highest biostimulant activity against water deficit stress. We demonstrate that this positive effect involves the activation of the abscisic acid (ABA) pathway in Arabidopsis thaliana transgenic line in which the ABA-responsive MAPKKK18 promoter was fused to the LUC reporter. In addition, in tomato the application of G. rugosa extract to the root system, induced two ABA-responsive gene as SI02g084850 (SIRAB18) and SI06g067980 (SILEA). Finally, tomato seedlings subjected to water deficit leads to improved CO2 assimilation and water use efficiency (WUEp), compared to mock-treated plants. These results highlight a new potential seaweed source of substances with osmoprotectant properties, useful for biostimulant development. Future studies may provide further insight into which components of the seaweed extract induce activation of the ABA pathway.

Keywords: biostimulants for agriculture, water deficit, drought, seaweed, bioactive natural products, absicic acid signaling



Use of Amino Acids to Combat Water Stress in Vineyards

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Abstract: Crops will increasingly have to undergo severe adaptation processes because climate change, which was once just a prediction, is now part of our daily lives. The process of maximizing production using less and less water is a line of research that is gaining scientific prominence and will certainly be one of the major solutions to food shortages in the future. This study aimed to test the action of two amino acids (pyroglutamic acid and pipecolic acid) as bioprotectants to combat water stress in grapevines. The trials were carried out on a farm on Madeira Island, Portugal. The trial consisted of two foliar applications of the products, the first before flowering and a booster one month and fifteen days after the first. During the trial, a drone equipped with a spectral camera flew over the crop, from which some vegetation indices were calculated to assess the health of the plants in the face of stress. From the first application, irrigation water was completely cut off in the study area. Various parameters relating to plant production were analyzed. At the end of the trial, there were significant differences between the Number of Bunches and the Total Weight of Bunches. The treatment that used irrigation throughout the crop cycle was the one with the highest averages. Pyroglutamic acid was the second treatment with the highest averages, but it did not differ from the others. Based on the results obtained in this initial trial, a second cycle with the application of the products will be carried out again this year, to confirm or not the propositions presented in this previous work. The other agronomic parameters analyzed did not differ statistically from one another.

Keywords: Water Stress, Climate Change, Remote Sensing, Bioprotectants

1. Introduction

The latest data on world viticulture, released by the Organisation Internationale de la Vigne et du Vin (OIV), indicates that the global grapevine cultivation area reached approximately 7.3 million hectares in 2022, with a total production of 258 million

hectoliters (OIV, 2022). Among all countries, Spain, France, China, Italy, and Turkey accounted for half of this cultivation area.

This culture was traditionally cultivated in rainfed conditions, particularly in Europe, where quality was associated with non, low-yield grapevines (Zarrouk et al., 2016). However, the increasing instances of water scarcity in South Europe and the New World viticulture have changed this approach. As a result, there has been a significant rise in the number of irrigated vineyards over the past two decades (Zarrouk et al., 2016).

Nevertheless, maintaining a high-quality wine necessitates finding a suitable balance between vegetative and fruit growth. This balance can be achieved through deficit irrigation techniques, wherein water supply is deliberately kept below the full crop evapotranspiration (Zarrouk et al., 2016). In other words, mild to moderate water deficits are intentionally imposed to regulate vegetative growth within desired limits. The specific intensity, duration, and timing of implementing these water deficits should be adjusted according to the unique 'terroir,' seasonal conditions, grapevine variety, and desired wine type preferred by the grower (Lopes et al., 2008).

Drought is recognized as a significant abiotic stress that profoundly influences plant growth and productivity, as highlighted by Carvalho et al. (2021). The stress induced by drought leads to physiological, biochemical, and morphological alterations in plants. Furthermore, drought adversely impacts various essential processes, including respiration, photosynthesis, enzyme activities, redox homeostasis, and chloroplast metabolism, as elucidated by AL-Quraan et al. (2021). The impact of drought stress on tree growth and its potential to diminish yield and fruit quality have been documented (Min et al., 2019; Sun et al., 2020). Consequently, it is crucial to explore strategies to address this challenge. The intricate physiological reaction of grapevines to environmental stress, such as drought and heat stress, is contingent upon various factors. This response is notably influenced by the genotype and is largely shaped by traits of both the root system (rootstock) and the shoot (Pavlousek, 2011; Tramontini et al., 2013).

In recent studies, certain biostimulants have been identified for their ability to function as inducers, simulating stress conditions, as reported by Ertani et al. (2013), Lachhab et al. (2014), and Colla et al. (2014). Typically administered in low doses to plants or soils, biostimulants aim to regulate or enhance various physiological processes in plants. This application is designed to boost crop vigor, improve yield and quality, and mitigate stress conditions, as outlined by the European Biostimulants Industry Council in 2012 and Gutiérrez-Gamboa et al. (2019).

The utilization of biostimulants is emerging as a novel and cost-effective approach to enhance tree drought tolerance, recognized for its efficacy and high performance (Bulgari et al., 2019; Drobek et al., 2019). These plant biostimulants consist of diverse organic and inorganic substances or microorganisms that have the potential to augment plant growth, improve crop quality, enhance nutrient uptake, and bolster tolerance to both biotic and abiotic stress, as highlighted by Bulgari et al. (2019).

The newly introduced unmanned aerial vehicles (UAVs) serve as valuable aerial platforms for such applications. Unlike other platforms like satellites and manned aircraft, UAVs enable temporal and spatial remote sensing at a resolution suitable for capturing the highly dynamic relationship between vegetation and its environment (Gago et al., 2014, Araus et al., 2014).

Numerous groundbreaking studies have showcased the utility of UAVs by establishing noteworthy correlations between ground-level plant-truth data, recorded at the leaf level, and information derived from aerial remote sensing imagery. These insights have been instrumental in creating field stress maps that prove valuable for decision-making within the precision agriculture framework (Zarco-Tejada et al., 2012; Gonzalez-Dugo et al., 2013). This technology opens novel possibilities for precision agriculture management, primarily because the conventional measurement solutions are not only time-consuming but also entail high costs, especially when examining a single canopy (Gonzalez-Dugo et al., 2012).

This study aimed to test the action of two amino acids (pyroglutamic acid and pipecolic acid) as bioprotectants to combat water stress in grapevines on Madeira Island.

2. Materials and Methods

2.1 Study area

This study was carried out at Quinta das Vinhas, located in Estreito da Calheta – Madeira (Figure 1). The total area of the property is 2.37 ha, at an altitude ranging between 305 and 347 m above sea level (Pinheiro de Carvalho et al., 2022). According to the Köppen-Geiger classification, the study area can be classified as Csb - Temperate, with hot, dry summers.



Fig. 1 Location of the study area and treatments.

2.2 Climate data

To verify the climatic variations that occurred during the growth/development cycle of the vine crop in the year 2023 in Madeira, local data on average, maximum, and minimum temperature, as well as precipitation between the study months (January - August), is presented below (Figure 2).



Fig. 2 Climatic variations at Quinta das Vinhas – Madeira.

2.3 Preparation of solutions

5 L solutions of both pyroglutamic acid and pipecolic acid 5 mM, dissolved in distilled water, were prepared fresh before use.

2.4 Data Acquisition

Three flights were performed over the vineyard canopy, the first was carried out before flowering (05/02/2023) at the stage of separated bunches (G), the second was carried out on 06/19/2023 (Pea Berry - K), and finally at harvest (08/25/2023 - N). The letters shown represent the phenological scale proposed by Baggiolini (1952). A flight was made on 20/07/2023, but due to the poor quality of the images obtained it was not possible to use them, so they were discarded. In this study, five vegetation indices were tested to see if they correlated with the data obtained during the harvest. These were the Crop Water Stress Index Simplified (CWSIsi), Green Leaf Index (GLI), Green Normalized VegetationIndex (GNDVI), Normalized Difference Red Edge (NDRE), Normalized Difference VegetationIndex (NDVI), and Normalized Green Red Difference Index (NGRDI).

2.5 Statistical Analysis

The statistical design used in this study was a randomized block design with 4 treatments, 4 blocks, and 3 replicates. Treatment 0 - no application of amino acids and no irrigation; 1 - pyroglutamic acid; 2 - pipecolic acid; 3 - no application of amino acids and conventional irrigation.

The statistical analysis was performed using Jamovi computer software version 2.3.16 (The Jamovi Project, 2022). Once the values for the variables to be analyzed had been obtained, the data was analyzed to check whether the samples obtained were normal or not. The Shapiro–Wilk normality test was performed, aiming to verify the normality of a random sample, i.e., whether it comes from a normal or non-normal distribution Shapiro–Wilk, test. From this test, there was no evidence to reject the null hypothesis, and the values came from a normal distribution, with a significance of 1% probability.

2.6 Agronomic parameters analyzed

At the end of the crop cycle, the following agronomic parameters were analyzed: Number of bunches, Total weight of bunches, Weight of 3 bunches, Length of bunches, and Diameter of berries.

3. Results and Discussion

3.1 Effect of amino acids

Analyzing the agronomic parameters concerning the treatments tested in this study revealed significant differences at the 5% probability level (Table 1).

	Table 1. Effect of al	mino acids on agro	phomic parameters	of grapevine crops		
Treatments	s Number of	Total weight of	Weight of 3	Length of bunches	Diameter of	
	Bunches	bunches (kg)	bunches (g)	(cm)	berries (mm)	
0	$9.13\pm4.34~b$	$1\;888 \pm 1\;318\;b$	246.74 ± 60.82 a	15.22 ± 1.72 a	17.11 ± 1.28 a	
1	12.26 ± 8.24 b	$2714\pm2040b$	251.08 ± 124.89 a	13.98 ± 4.33 a	16.04 ± 4.47 a	
2	$10.33 \pm 7.69 \text{ b}$	$1\ 967 \pm 1\ 794\ b$	237.50 ± 104.97 a	14.59 ± 4.33 a	16.46 ± 1.53 a	
3	19.46 ± 10.04 a	$3\ 867 \pm 1\ 736\ a$	253.33 ± 54.85 a	15.10 ± 1.15 a	17.43 ± 1.41 a	
	A 11 .					

Table 1 Effect of amine acids on agreenemic perspectates of granowing grans

Note: Means followed by the same letter do not differ by the Scott–Knott test at a 5% probability level of error. Average ± standard deviation.

For the number of bunches and the total weight of bunches, it was found that Treatment 3 (T3) was the one with the highest averages, it should be noted that this was the treatment that used the producer's irrigation system all the time, not experiencing any kind of stress throughout the growth and development of the crop, however, for the same 2 parameters, pyroglutamic acid (T1) was the treatment with the second highest average for the same parameters, no showing differences between the other treatments, but showing a certain tendency to at least reach the averages obtained by treatment T3 in the next crop cycles, thus showing a great advantage both from an environmental/economic point of view, due to the low need to use water resources. However, it is necessary to apply pyroglutamic acid before the crop blooms.

Based on the results obtained in this initial trial, a second cycle with the application of the products will be carried out again this year to confirm or not the propositions presented in this previous work. The other agronomic parameters analyzed did not differ statistically from one another.

3.2 Correlation analysis

The correlation analysis between the vegetation indices and the agronomic parameters showed no correlation between the data. There were many correlations between the indices and CWSIsi, but these will not be analyzed in this study and will be analyzed in future work. A strong correlation was found between Total Bunch Weight and Number of Bunches (0.884, p < 0.001) according to the classification proposed by Devore (2006). Based on the strong correlation obtained, a linear equation was drawn up to check how the yield estimate behaves from a numerical and spatial point of view (Table 2).

Equation	\mathbb{R}^2	RMSE (g)				
$TWB = 90.7 + 197 \ x \ NB$	0.822	801				
TWB - Total weight of hunches: NB - Number of Bunches						

Table 2. Estimating	vine crop yield
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TWB = Total weight of bunches; NB = Number of Bunches

Table 3. Numerical comparison between verified and estimated yield

Treatments	Verified yield	Estimated yield		
	(kg)	(kg)		
0	5 665,64	5 669,32		
1	8 144,14	7 520,93		
2	5 930,8	6 378,45		
3	11 603,88	11 775,70		

The numerical results (Table 3) obtained show that in treatments 0, 2, and 3 the values were slightly higher than those verified in the field, while for treatment 1 the opposite

results were obtained. Even with these variations, both upwards and downwards, the results obtained are very close to reality.

Analyzing the spatialization of yield (Figure 3) according to the linear equation obtained in general, the estimated results overestimate the results for almost the entire trial. However, as this is an analysis of just one cycle, it is to be hoped that in the next cycle, with more data, this modeling will be more in line with reality.



Fig. 3 Spatialization of Verified and Estimated Yield for Vineyards

4. Conclusions

Treatment T3, which used the farmer's irrigation system, had the highest averages for most of the agronomic parameters analyzed.

For the number of bunches and total weight of bunches, variables intrinsically related to crop productivity, treatment T3 was the one with the highest averages compared to the other treatments analyzed.

Even though treatment T2 (use of pyroglutamic acid) did not show any significant difference from the other treatments, it was the one with the averages just behind T3. The use of pyroglutamic acid shows a certain tendency to at least reach the averages obtained by treatment T3 in the next crop cycles, thus showing a great advantage from an environmental/economic point of view, due to the low need to use water resources. However, it is necessary to apply pyroglutamic acid before the crop blooms.

Based on the results obtained in this initial trial, a second cycle with the application of the products will be carried out again this year to confirm or not the propositions presented in this previous work. The other agronomic parameters analyzed did not differ statistically from one another.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Impact of rhizospheric microorganisms on physiological performance of grapevines under current and future environmental conditions

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Abstract: Climate change (CC) will impose warmer and dryer conditions, especially in the Mediterranean region, thus threatening viticulture. In this context, rhizospheric microorganisms (plant growth promoting rhizobacteria, PGPR, and arbuscular mycorrhizal fungi, AMF, among them) may be crucial for adapting crops to CC scenarios. Our objective was to assess if the association of two varieties of grapevine with AMF and PGPR increases their resilience against environmental conditions that combine enhanced atmospheric CO_2 , increased air temperatures and water deficit. Tempranillo (T) and Cabernet Sauvignon (CS) grafted onto R110 rootstocks, and inoculated (+M) or not (-M) with a consortium of five AMF and four PGPR, were cultivated under either current CO_2 + ambient air temperature (summer 2023, CATA) or predicted CO₂ (700 ppm) + air temperature (ambient + 4°C, CETE) for the end of the century. From fruit veraison until maturity, within each environmental condition, half of the plants of every variety and inoculation treatment were divided into two homogeneous groups and subjected to two levels of water availability: maintained full irrigation (WW) (90-100% substrate field capacity, FC) or restricted irrigation (D) (cycles from 100-90% till 20-30% FC). The results showed that the variety of grapevine modulates plant hydraulic conductivity, water relations and physiological performance under water deficit. An Increase of 300 ppm in the atmospheric CO_2 combined with an increase of 4°C in the air temperature in relation to the current environmental conditions modify the physiological performance of T and CS. The interaction of the rootstock with rhizospheric microorganisms appeared as a key factor for the grapevine acclimation to water deficit under both current and future environmental conditions.

Keywords: mycorrhizal fungi, Cabernet Sauvignon, climate change, gas exchange, rhizobacteria, Tempranillo, water relations

1. Introduction

Europe, and specially the Mediterranean area, will be strongly affected by climate change (CC) in the 21st century (IPCC 2022). Drought, linked to increased air

temperatures, will become prevalent in most Mediterranean areas. Phenology, vegetative development, physiological and biochemical performance, yield, as well as fruit and wine quality of grapevines are highly sensitive to weather extremes (Droulia and Charalampopoulos 2021). In this context, the interactions between grapevine rootstock and soil microbiome can be crucial for a resilient viticulture facing CC (Darriaut et al. 2022). Three of the main aspects involved in the hydraulic properties of rootstocks are (i) the development of roots, (ii) the control of water transport from roots to shoots and (iii) the presence of aquaporins for the inter and intracellular water transport (Simonneau et al. 2017). The symbiotic association of roots with arbuscular mycorrhizal fungi (AMF) can modulate these three aspects since mycorrhized roots can (i) explore higher volume of soil thus improving the uptake of water and minerals and mycorrhizal symbiosis (Smith and Read 2008); (ii) enhance soil-plant hydraulic conductance (Abdalla and Ahmed 2021) and (iii) down-regulate the expression of genes encoding aquaporins thus anticipating its down-regulation in plants subjected to drought (Porcel et al. 2006). Since the association of AMF with Plant Growth Promoting Rhizobacteria (PGPR) can enhance the beneficial effect of AMF on the host plants, our objective was to assess if the interaction of two varieties of grapevine (Tempranillo, T, and Cabernet Sauvignon, CS) with a consortium of AMF and PGPR increases their resilience against environmental conditions that combine enhanced atmospheric CO₂, increased air temperatures and water deficit.

2. Materials and Methods

2.1 Plant material and growth conditions

Two years-old plants of T and CS grafted onto R110 rootstocks, and inoculated (+M) or not (-M) with a consortium of five AMF (*Rhizophagus irregularis, Funneliformis mosseae, Septoglomus deserticola, Claroideoglomus claroideum* and *C. etunicatum*) and four PGPR (*Bacillus subtilis, B. megaterium, B. altitudinis* and *B. licheniformis*), were cultivated, from fruit set to maturity in temperature gradient greenhouses under either current CO_2 + ambient air temperature (summer 2023, CATA) or predicted CO_2 (700 ppm) + air temperature (ambient + 4°C, CETE) for the end of the century. From veraison until maturity, within each environmental condition, half of the plants of every variety and inoculation treatment were divided into two homogeneous groups and subjected to two levels of water availability: maintained full irrigation (WW) (90-100% substrate field capacity, FC) or restricted irrigation (D) (cycles from 90-100% till 20-30% FC)(Fig. 1). The experiment included 4 plants per variety and treatment. Plants were pruned regularly, irrigated with water alternated with nutrient solution (Ollat et al. 1998).

2.2 Determinations

Both pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials were measured 7 and 14 days after veraison. Ψ_{pd} was also recorded at fruit maturity. Data were collected in young fully expanded leaves using a pressure chamber. Photosynthesis (An), leaf conductance (gs) and transpiration rates (E) were measured 7 and 14 days after veraison with a portable photosynthesis system ADC-LCi in fully developed young leaves from 10.00 to 12.00 h. Plant hydraulic conductivity (Kh) was estimated as Kh=E/($\Psi_{pd} - \Psi_{md}$). Proline and total soluble sugars in leaves were determined as
described by Goicoechea et al. (2023). Leaf dry weight (DW) was calculated after drying plant material in oven at 70°C until reaching constant weight. Leaf water content (WC) was calculated as (leaf fresh weight- leaf dry weight)/ leaf dry weight.

2.3 Statistical analysis

Data were analyzed by Excel program (Microsoft office 2010). The Student's t test was applied to each two samples assuming unequal variances. The degree of significance was set at $p \le 0.05$. The Bonferroni correction (0.05/ n) was then applied, being n = total number of comparisons made with Student's t test.



Fig. 1 Evolution of drought cycles from fruit veraison until maturity. Soil water content in drought treatment (dashed line) has been expressed as percentage of soil water content in well-watered treatment (100%, continuous line).

3. Results and Discussion

Drought decreased Ψ_{pd} in leaves of T in a higher extent than in CS under CATA conditions, being the decrease especially marked in –M plants 7 days after imposing drought and in +M plants at fruit harvest compared to their respective WW controls. In CS the strongest reduction in Ψ_{pd} was observed in +M plants 14 days after the onset of water deficit and at fruit maturity (Fig. 2).



Fig. 2 Predawn water potential (Ψ_{pd}) 7 and 14 days after imposing the first drought cycle (at veraison) and at fruit maturity (harvest)

Under CATA conditions, Kh decreased 7 days after applying the first cycle of water deficit only in +M T plants (achieving 60% of their WW control) (Fig. 3). Non-inoculated plants (-M) and +M CS reduced their Kh one week later (reaching values ranging from 60% to 80% of their respective WW controls). Under CETE conditions, Kh decreased the first week after imposing drought in +M CS and seven days later in +M T.

These results contrast with the idea that AMF can enhance soil-plant hydraulic conductance under drought (Abdalla and Ahmed 2021). However, Bárzana et al. (2014) concluded that AMF can up or down regulate genes related to aquaporin expression in roots of their host plants depending on water conditions and the severity and duration

of water deficit periods thus modulating root hydraulic conductance and plant water status. Higher hydraulic resistance may confer advantage to grapevine when undergoing water deficit because it can avoid fast soil water depletion (de Herralde et al. 2006). Hochberg et al. (2017) also observed down-regulation of leaf hydraulic conductance in grapevine under water deficit acclimation and hypothesized that it was not associated with embolism but with leaf turgor and membrane permeability. Changes in Kh did not affect the amount of water in leaves except for -M CS grapevine under CETE, the only treatment in which the water content (WC) achieved values of around 70% of those measured in WW plants (Fig. 4).



Fig. 3 Plant hydraulic conductivity (Kh) in T and CS, inoculated (+M) or not (-M) with rhizospheric microorganisms, cultivated under either ambient CO₂ and temperature (CATA) or elevated $\mathrm{CO}_{_{\rm 2}}$ and temperature (CETE) and subjected to drought. Data were recorded on days 7 (white bars) and 14 (black bars) from veraison (the onset of drought cycles) and have been expressed as percentages of values measured in the respective WW controls. Bars represent means (n= 4 plants) ± SD. Within each grapevine cultivar, different lower case and capital letters indicate, respectively, significant differences ($p \le 0.05$) between treatments on days 7 or 14. Asterisks indicate significant differences at 5% (*), 1% (**) or 0.1% (**) between days 7 and 14 within each treatment.

Fig. 4 Water content (WC) in leaves of T and CS, inoculated (+M) or not (-M) with rhizospheric microorganisms, cultivated under either ambient CO_2 and temperature (CATA) or elevated CO_2 and temperature (CETE) and subjected to drought. The rest as in Figure 3.



Fig. 5 Concentrations of proline in leaves of T and CS, inoculated (+M) or not (-M) with rhizospheric microorganisms, cultivated under either ambient CO₂ and temperature (CATA) or elevated CO₂ and temperature (CETE) and subjected to drought. Within each graph, different lower case and capital letters indicate, respectively, significant differences (p \leq 0.05) between treatments on days 7 or 14. The rest as in Figure 3.

The maintenance of WC in leaves under water deficit may be explained, at least partially, by the accumulation of solutes, which is quite usual in grapevine (Hochberg et al. 2013). We found increased concentrations of proline in CS under CATA and CETE conditions 7 and 14 days, respectively, after imposing the first drought cycle (Fig. 5) and also enhanced soluble sugars levels in leaves of T under CATA conditions 7 days after beginning the water deficit (Fig. 6). In all these cases, accumulation of solutes was only observed in +M plants. In T, the decreased ψ_{pd} in -M plants under both CATA and CETE conditions may be dependent on initial osmolarity of the cell sap, cell wall elasticity and/or a redistribution of the water into symplastic and apoplastic compartments rather than upon leaf dehydration or osmotic adjustment. CS did not showed marked differences in the ψ_{pd} during the first 14 days after beginning drought. The drought tolerance of CS is not very high (Lakatos and Mitre 2023), but acclimation improves the resistance of CS facing recurrent water deficit (Zamorano et al. 2021). Our results suggest that the association of CS with beneficial microorganisms could confer stronger resilience to plants in their process of acclimation to water deficit under CC.



Fig. 6 Concentrations of total soluble sugars in leaves of T and CS, inoculated (+M) or not (-M) with rhizospheric microorganisms, cultivated under either ambient CO_2 and temperature (CATA) or elevated CO_2 and temperature (CETE) and subjected to drought. Within each graph, different lower case and capital letters indicate, respectively, significant differences ($p \le 0.05$) between treatments on days 7 or 14. The rest as in Figure 3.

In general terms, the intrinsic water use efficiency (*iWUE*, An to gs ratio) increased in plants subjected to drought (Fig. 7). In T this was more evident in +M plants 7 days after imposing water deficit under CATA conditions and 14 days after beginning drought cycles under CETE conditions. Such increase was parallel to stomatal closure (data not shown). This same behavior was observed in CS, but in this variety even -M plants enhanced their An/gs ratio when exposed to drought. In both varieties the highest value of An/gs corresponded to +M plants grown under CETE conditions 14 days after imposing drought and this coincided with the lowest Ci to Ca ratio (Fig. 8). Kelly et al. (2016) postulated the 'low Ci effect' as a mechanism by which elevated CO_2 in the air can diminish drought impacts on woody plants: the stomata closing under water deficit reduces the intercellular CO_2 concentration (Ci), resulting in a larger relative enhancement of the photosynthesis under elevated atmospheric CO_2 (Ca).



Fig. 7 (left) and **Fig. 8** (right). Intrinsic water use efficiency (*WUE*, An/gs) (Fig. 7) and intercellular CO₂ concentration (Ci) to ambient CO₂ concentration (Ca) ratio (Fig. 8) in leaves of T and CS. The rest as in Figure 3.

4. Conclusion

The variety of grapevine grafted onto a given rootstock modulates plant hydraulic conductivity, water relations and physiological performance under water deficit. Increases of 300 ppm in the atmospheric CO_2 combined with increases of 4°C in the air temperature above the current environmental conditions modify the physiological performance of T and CS. The interaction of rootstock with rhizospheric microorganisms appears as a decisive factor for the acclimation of grapevine to water deficit under both current and future environmental conditions.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Magic beans or the miracle of tradition? Resilient bean landrace from Muniesa as a source of drought-adapted, seed-borne biofertilizers

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Abstract: Landrace beans from Muniesa, Teruel, Spain, have demonstrated remarkable adaptability to local drought conditions and harsh environments, often thriving in challenging soils. These landraces consistently yield high production rates even in years with extremely low rainfall. In fact, when compared to the Spanish Food Composition Database (BEDCA), these landraces exhibit similar nutritional profiles, while boasting even higher protein content. In contrast, modern varieties are typically bred for increased harvest volume, often at the expense of essential traits such as stress tolerance, defense against pathogens and pests, and the establishment of beneficial microbial associations. Beyond their distinct genetic makeup, we determined that the types of beneficial associations between these landraces and microorganisms play a pivotal role in their ability to thrive in adverse planting and growing conditions. In our study, we identified microbiota association patterns within the seeds of various commercial and landrace beans. These findings were then compared to discern meaningful insights. Our ultimate aim was to identify strains that can potentially be harnessed as bioinoculants in future applications, so we analyzed their features under stressing conditions as screening approach. Notably, certain strains isolated from Muniesa landraces, including Bacillus bombysepticus B1, Bacillus amyloliquefaciens B3, Bacillus cereus M1 and Paenibacillus pabuli M2, have been positively tested as biofertilizers and droughttolerant enhancers for commercially grown beans sensitive to stressful conditions. The results were highly promising, demonstrating significant protection against stress in the early stages of development, which may enhance their performance in later phases. This approach not only deepened our understanding of the impact of the domestication process on microbial association patterns but also yielded a new set of bioinoculants capable of bolstering bean crops under water-deficient conditions. Finally, this serves as an exemplary model for how landraces can contribute to the revitalization of depopulated areas in Spain and Portugal, facilitating the restoration of local biodiversity and establishment of sustainable, high-value crops.

Keywords: seedborne microbiota, drought mitigation, bean landraces, legumes-microbiota interactions

1. Introduction

The agricultural sector is facing increasing challenges, such as drought and high temperatures, which are negatively impacting production. In the case of legumes, these conditions can compromise biological nitrogen fixation by reducing the number of nodules, where this process occurs (Dovrat et al., 2018). These challenges are further compounded by the fact that breeding programs have primarily focused on production factors, rather than stress-tolerant traits (Singh and van der Knaap, 2022). Therefore, it is imperative to find treatments that can ensure early-stage development under restricted water regimes. Microbes are emerging as a promising alternative for enhancing plant tolerance to stress. It has been reported that the most effective treatments based on microorganisms are those that are isolated from the same environment and plant they are intended to be used with (Kumar et al., 2022). However, many commercial varieties of plants available today have limited interactions with beneficial microbiota due to the domestication breeding process (Gutierrez and Grillo, 2022). This can greatly limit the type and number of strains that can potentially serve as a stress-alleviating treatment. Interestingly, the solution may lie in the preservation of landraces, which are better adapted to harsh conditions. These landraces represent a middle stage between wild ancestors and modern varieties, and may preserve beneficial interactions that are worth exploring (Johnston-Monje and Raizada, 2011). Recently, there has been a description of the characterization of seed microbiota to improve the selection and efficiency of candidates for biotreatments, by adding a new layer of complexity. This approach includes parental selection of inherited strains and pre-adaptation to the plant species for future biotreatments (Nelson, 2018). In this sense, parental plants growing under adverse conditions tend to select more suitable strains to face such events. Studies have shown that this type of microbiota in legumes provides impressive results, reducing screening processes and enhancing the adequacy of future microbe-based products (Niza-Costa et al., 2022). The aim of the present study was to describe the seed microbiota from Muniesa (Teruel, Spain) landrace beans, which are known for their high production level under harsh soils and water-restrictive conditions, as candidates for enhancing the response and tolerance of commercial beans to water scarcity processes.

2. Materials and Methods

2.1 Plant material and seedborne microbiota characterization

Landrace (Blanca, Pinta and Color de Caña) and commercial (Carrila, Verdina, Caparron, Amarella, Catarino and Canellini) beans (*Phaseolus vulgaris* L.) were provided by Casa Yus (Muniesa, Spain) and acquired in markets around Zaragoza (Spain), respectively. About 50 seeds were surface-sterilized with 70% ethanol for 5 minutes, and washed with sterile, double distilled water (Vílchez et al., 2021). They were then germinated in Magenta boxes in darkness at 25 °C, and then three 3-cm length roots were grounded, diluted in sterile NaCl 0.45% solution and dispersed in LB agar plates to quantify the culturable populations. Distinguishable colonies were isolated and pure cultures were identified by PCR amplification of the 16S rRNA gene, as previously optimized in our laboratory for seedborne microbiota (Niza-Costa et al., 2022). The core, resilient microbiota was described and the isolates were glycerol-preserved at -80 °C. The strains were evaluated under regular conditions and supplemented by 15% polyethylene glycol (PEG) as drought-mimicking agent. Thereafter,

they were characterized as able to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, biofilm, auxins, antioxidants, proline and siderophores, as well as to fix nitrogen, and solubilize phosphate, calcium, silica, magnesium, zinc and potassium, as previously optimized and tested in our lab for high-throughput, spectrophotometric tests (Niza Costa et al., 2023). Finally, the colonization ratio of the candidates in the commercial variety 'Carrila', was assessed by *in vitro* tests, according to Vilchez and collaborators (Vílchez et al., 2021).

2.3 Evaluation of candidates as drought-tolerance enhancers in seedlings

The best candidates were prepared as liquid inoculants (10⁸ colony forming units/mL in 0.45% NaCl solution) to treat 2-days-old seedlings in 0.5 L pots filled with turf and vermiculite (3:1, v:v). After treatment, irrigation regimes of 100% and 25% were applied (Vílchez et al., 2016). Then, 14 days after treatment (DAT), height, root length, dry weight (DW) and photosystem II quantum yield were evaluated (FluorPen FP110).

3. Results

The various bean varieties displayed diverse microbial profiles and persistent core microbiota, even among landrace varieties from the same region. The main component of the core microbiota across the varieties was Bacillaceae family strains (as depicted in Fig. 1). While some strains, such as *Bacillus subtilis, Bacillus cereus,* and *Paenibacillus polymyxa,* were commonly isolated in most varieties, others were unique to specific varieties. For instance, *Bacillus amyloliquefaciens* and *Bacillus bombysepticus,* as well as *Paenibacillus pabuli,* were only isolated from the 'Blanca' and 'Color de Caña' landrace varieties, respectively, while *Priestia megaterium* was only detected in landrace varieties 'Pinta' and in commercial variety 'Carrila.' In contrast, strains such as *Bacillus atrophaeus* and *Bacillus anthracis* were restricted to commercial varieties.

We selected a series of strains, including B. bombysepticus B1, Bacillus velezensis B2, B. amyloliquefaciens B3, B. subtilis P1, P. megaterium P2, B. cereus P3, P. polymyxa P4, B. cereus M1, and P. pabuli M2, which were isolated from landrace varieties, but some also present in commercial verities. We then evaluated their potential to benefit plants under stressful conditions, and our results indicated that all strains displayed noteworthy levels of the parameters we assessed. Among the most significant findings, we observed that the strains maintained their levels of auxins and biofilm production under 15% PEG treatment (Fig. 2a,b). Additionally, the P1 and P2 strains displayed notable growth in ACC medium under stress conditions (Fig. 2c), while the P3, B1, M1, and M2 strains stood out for their antioxidant production (Fig. 2d). On the other hand, proline production increased under stress for the P1 and M1 strains (Fig. 2e), where only P4 was able to maintain its nitrogen fixation level (Fig. 2f). Furthermore, all strains, except M1, were able to increase the amount of solubilized phosphorous under stress (Fig. 2g), and the same result was recorded for strains P1, B2, and B3 in the case of potassium solubilization (Fig. 2h). The production of siderophores increased under drought-mimicking conditions for strains B1, B2, B3, M1, and M2 (Fig. 2i). Lastly, the colonization ratio was consistent for most strains under stress conditions, but strains B3 and M1 were able to increase their colonization ratio (Fig. 2j). It should be noted that strains P1, B3, M1, and M2 were able to solubilize calcium, and P3, B1, B3, M1, and M2 were able to solubilize manganese, although these skills were not that consistently reported.



Fig. 1 Characterization of the seedborne population in maize. The staked columns chart shows the relative abundance of isolated strains in different landraces and commercial bean seeds.

Due to these results, we decided to perform biotreatment test with B1, B3, M1 and M2 in commercial 'Carrila' beans seedlings to evaluate drought-tolerance enhancing potential from landrace-isolated strains. They were chosen after stand out results in several test and/or consistency top performance for most of the tests previously reported. After 7 days of treatment, the early results indicate all treatments improved the size of the seedlings under full irrigation conditions (100%) respect to the control set, standing out the treatment with M1 and M2. In the case of water depravation (25%), again, all treatments showed bigger and healthier seedlings, with the sets treated with M1 and B1 showing similar results respect to the non-stressing conditions.



Fig. 2 Strains characterization. The graph bars show the performance of the candidate strains in regular (blue) and stressing conditions (+15%PEG; green) for the production of auxin equivalents (a), biofilm structures (b), growth in ACC medium (c), production of antioxidants (d) and proline (e), fixation of nitrogen (f), solubilization of phosphate (g) and potassium (h), production of siderophores (i) and the colonization ratio (j). The * stands for significative difference at least p < 0.05 respect to regular conditions.



Fig. 3 Inoculation effect on early-stage seedlings. The picture shows the phenotype registered 7 days after inoculations with the candidate strains under full irrigation (100%) and water depravation (25%). Here 'Mock' stands for control set; 'M1', for *B. cereus* M1; 'M2', for *P. pabuli* M2; 'B1', for *B. bombysepticus* B2; and 'B3', for *B. amyloliquefaciens* B3.

4. Discussion

The study of seed microbial communities has the potential to introduce a novel approach for safeguarding plants and enhancing their performance under adverse circumstances. While additional research is necessary and the variability is considerable, the core microbiota we have identified in this study aligns with previous findings in the field and with other seed studies in legume plants. The significant presence of the Bacillaceae family, including Bacillus, Paenibacillus, and Stenotrophomonas, was also observed in the seeds of Medicago sativa and Bituminaria bituminosa wild plants (Niza-Costa et al., 2022). Notably, other studies have reported that this seed microbiota serves as a powerful biotreatment for legumes, exhibiting a higher proportion of beneficial strains compared to conventional sources (Laranjeira et al., 2022). Our study revealed that, in most instances, these characteristics were maintained by the strains under stressful conditions, which is advantageous for practical application and ensures the efficacy of biotreatments when water supply is uncertain or restricted. Here, the landrace varieties showed unique and shared strains, which could suggest the consequences of domestication on population interaction and heredity (Gutierrez and Grillo, 2022). However, further studies are required to stablish a solid pattern.

The results of the main candidates *B. cereus* M1, *P. pabuli* M2 and *B. amyloliquefaciens* B3 indicates that seedborne insolated strains from Muniesa landraces were not only able to promote the growth in modern varieties, but also efficiently protect them against intense water depravation. All these strains have been reported as beneficial bacteria for plants (mainly as biocontrollers), some even in legumes, but the specificity seems to be playing a role, as they were not frequently reported as biotreatment for beans (Kulkova, et al., 2023; Trinh et al., 2018; Mokrani et al., 2018). In the case of *B. bombysepticus* B2, as far as we know, this is the first reported case of beneficial effect on plants for this strain These results open an interesting perspective to study further use of landrace sourced strains as new biotreatments for modern varieties of beans.

5. Conclusions

The microbial community within seeds exhibits significant diversity among different varieties, with some containing exclusive species. The microbial population present in landrace seeds was found to possess a number characteristics with a notable performance, suggesting that seed microbiota possesses a promising potential for more effective biological treatments. All the strains improved the size of the seedlings in early stage, both under full irrigation and stressing conditions. The strain *B. cereus* M1 was especially consistent in both conditions. This is the first report of a *B. bombysepticus* as plant beneficial bacteria. Hence, seedborne isolated from Muniesa landraces showed to be an effective treatment enhancing modern varieties to resist the drought.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Enhancing Maize (*Zea mays*) Resilience to Drought Stress Through Seed-Associated Microbiota

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Abstract: Drought stress poses a significant challenge for global maize production, with farreaching impacts on livelihood and economy. As climate change intensifies, the frequency of unpredictable weather events is expected to rise, with the need of adaptative strategies. Endophytic microorganisms are selectively recruited by the plant to cope with a diversity of stresses and conditions, being able to enter the plant through vertical inheritance from the parental line. Our project focuses on the selection of novel bioinoculants, with an innovative approach directed towards seed-associated microbiota. The rationale behind this approach lies in the capacity of seed microbiota to be influenced and selected by parental lines, thereby shaping the performance of future generations. Using the widely-grown SY Carioca maize variety, we isolated, identified and characterized its seedborne microbiota by in vitro tests, and under greenhouse conditions as inoculants. The strains were applied as single treatment and different irrigation percentages (25%, 50% and 100%) were applied. The greenhouse results indicate that seedborne bacteria are able to promote the growth of maize seedlings, and two of them, Burkholderia ubonensis JC and Pseudomonas fluvi MB showed a high protection level of the seedlings under water-restrictive conditions. After this screening, the strain Pseudomonas fluvi MB was selected as the best candidate to proceed with a future characterization as biotreatment on to field trials. These results offer a promising path towards the development of more eco-friendly and efficient solutions to enhance maize resilience against drought stress, ultimately contributing to sustainable agriculture in an era of evolving climate challenges.

Keywords: seedborne microbiota, drought mitigation, corn productivity, abiotic stress

1. Introduction

Maize (*Zea mays* L.) is one of the most relevant crops, but the production is affected by high temperatures and droughts (Khatibi et al., 2022). These phenomena are increasingly observed, causing 20-40% of losses in the Iberian Peninsula (Toreti et al., 2023). Despite de breeding

programs and the efficient use of water, the climatic perspective is requiring a broader perspective to alleviate negative effects. Consequently, the implementation of treatments based on microorganisms represents alternative to improve the drought tolerance in corps (Mubeen et al., 2021). These microorganisms show capabilities as 1-aminocyclopropane-1-carboxylate deaminase, biofilms or auxin production (Gamalero and Glick, 2022). Most of them has been isolated in the rhizosphere, but more recently seed microbiota emerged as a more efficient alternative. This population reflects how the parental line has adapted to environmental stresses, so they could suppose a pre-selected kind of drought treatment (Mascot-Gómez et al., 2021). This work seeks to explore the potential use of maize seedborne bacteria as a new treatment against drought effects. For this, we have evaluated their key features under regular and stressful conditions to enhance the integration of these treatments as biotechnological applications.

2. Materials and Methods

2.1 Plant material and seedborne microbiota characterization

Maize seeds (*Zea mays* L.) of the variety SY Carioca were kindly borrowed by Anpromis Association (Portugal). About 50 seeds were surface-sterilized with 70% ethanol for 5 minutes, and washed with sterile, double distilled water (Vílchez et al., 2021). They were then germinated in Magenta boxes in darkness at 28 °C, and then three 3-cm length roots were grounded, diluted in sterile NaCl 0.45% solution and dispersed in LB agar plates to quantify the culturable populations. Distinguishable colonies were isolated and pure cultures were identified by PCR amplification of the 16S rRNA gene, as previously optimized in our laboratory for seedborne microbiota (Niza-Costa et al., 2022). The core, resilient microbiota was described and the isolates were glycerol-preserved at -80 °C. The strains were evaluated under regular conditions and supplemented by 15% polyethylene glycol (PEG) as drought-mimicking agent. Thereafter, they were characterized as able to grow in 1-aminocyclopropane-1-carboxylate (ACC) medium as indication of ACC deaminase production, and to produce biofilm and auxins, as previously optimized and tested in our lab for high-throughput, spectrophotometric tests (Niza Costa et al., 2023). Finally, the colonization rate of the candidates was assessed by *in vitro* tests, according to Vilchez and collaborators (Vílchez et al., 2021).

2.3 Greenhouse test

The best candidates were prepared as liquid inoculants (10⁸ colony forming units/mL in 0.45% NaCl solution) to treat 2-days-old seedlings in 0.5 L pots filled with turf and vermiculite (3:1, v:v). After treatment, irrigation regimes of 100%, 50% and 25% were applied (Vílchez et al., 2016). Then, 14 days after treatment (DAT), height, root length, dry weight (DW) and photosystem II quantum yield were evaluated (FluorPen FP110).

3. Results

The most resilient population (core microbiota) was composed by *Paenibacillus endophyticus* JA (24.2%), *Pseudomonas fulva* MB (21.9%), *Paenibacillus silvae* JB (17.2%), *Stenotrophomonas indicatrix* MD (14.8%), *Stenotrophomonas maltophilia* MC (9.1%), *Pseudomonas koreensis* MA (4.9%) and *Burkholderia ubonensis* JC (3.1%). Other bacteria less represented (<3%) were not considered for further testing (Fig. 1a). On the other hand, the growth of the strains JA, JC, MA and MB in ACC medium under stress was maintained (Fig. 1b). In the case of biofilm production (Fig. 1c), under stressful conditions, strains JA, JC and MC kept the performance, and the strain MB even increased by 15%. Finally, JC and MC produced similar amount of auxin under stress, and the strain MB increased by 68% (Fig. d).



Fig. 1 Characterization of the seedborne population in maize. The pie chart (a) shows the relative abundance of isolated strains. The bar graphs show the ACCd (b), biofilm (c) and auxins (d) production. Graphs in green and red indicate regular and +15%PEG conditions, respectively. The * stands for significative difference at least *p* < 0.05 respect to regular conditions. The codenames in the graphs stand for *P. endophyticus* JA, *P. sylvae* JB, *B. ubonensis* JC, *P. koreensis* MA, *P. fulva* MB, *S. maltophilia* MC, *S. indicatrix* MD, *Bacillus pumilis* A, *B. safensis* WC, *B. zhangzhouensis* C, *Stenotrophomonas chelatiphaga* YC, *Pseudomonas oryzihabitans* CC, *Paenibacillus humicus* WT, *B. amyloliquefaciens* B, *P. polymyxa* D, and Chryseobacterium sp. E.

Considering the test on seedlings, the colonization ratio was generally reduced under stress, but with the strains MC and MD this was a moderate 15-25%. In the case of the strain MB, no significant reduction was recorded (Fig. 2a). Thus, the strains JA, JC and MB were selected as the best candidates for the greenhouse inoculation test. Here, all the treatment improved plant status under 100% irrigation, but only the one with JC and MB improved seedlings resistance to limited irrigation regimes (Fig. 2b). This tendency is well represented in height, dry weight and photosystem II quantum yield, where a more and more restrictive water regime decreased the parameters in general, maintaining JC an MB treatment better values in physiological and seedling health parameters, respectively. The strain JA showed no difference respect to the mock set under restrictive irrigation regimes, with same clear visual and photosystem II quantum yield wilting status. Interestingly, mid irrigation regime increased root length in the all seedlings treated with the candidate strains. However, under no irrigation, the seedlings treated



with JA showed a critical reduction in this parameter. The strain MB stood out over JC in most of the parameters evaluated.

Fig. 2 Plant testing of candidate strains. The graph bar (a) shows the colonization ratio in regular (green) and stressing conditions (+15%PEG; red). relative abundance of isolated strains. The * stands for significative difference at least *p* < 0.05 respect to regular conditions. The pictures (b) show the status of the seedling after inoculation with the candidate strains under different water regimes. Finally, the bar graphs in the lower panels show the height (c), root length (d), dry weight (e) and photosystem I quantum yield (f) registered for the greenhouse inoculation experiment. Here, columns in green, yellow and red indicate 100%, 50% and 25% regular and +15% PEG conditions, respectively. The codenames in the graphs stand for *P. endophyticus* JA, *P. sylvae* JB, *B. ubonensis* JC, *P. koreensis* MA, *P. fulva* MB, *S. maltophilia* MC, *S. indicatrix* MD.

4. Discussion

Nowadays, the agriculture is facing the challenge of extreme climate conditions, as well as the necessity to reduce fertilizers and other inputs. The use of new biotreatments may suppose a sustainable alternative to improve plant growth and response to stresses as drought (Kumar et al., 2022). Here, the use of seed microbiota emerges as a tailor-made and more efficient approach (Abdelfattah et al., 2023). Many new studies are addressing this option, this still a very unexplored field, which still requires to understand core populations, mechanism or application reliability. In our study, despite several other *Pseudomonas* has been characterized as plant growth-promoting bacteria, to our knowledge, we have characterized for the first time the of a seedborne *Pseudomonas fulva* strain as efficient biotreatment as a plant drought-tolerance enhancer (Yasmin et al., 2022). This strains has been used previously in maize a consortium to improve biocontrol effect on *Fusarium* sp. (Adeniji and Babalola, 2022). The utilization of seed microbiota create new alternatives for the biotreatments use as they provide pre-adapted strains that could consist in better results, especially if we consider the local-sourced and climate-adapted ones (contextualized use) (Bziuk et al., 2021). However, aspects as consortia use, minimal core microbiota by species/genotypes to consider or stress-specific

inheritance need to be discerned to guarantee the future design of products (Abdelfattah et al., 2023). Finally, our model in maize was promising, but it's necessary to check under field conditions, as well as the putative interspecific use of the strains here characterized in order to better understand these types of interactions and potential industrial applications (Simonin et al., 2022).

5. Conclusion

The maize seed population evaluate has high variation, but hold a core microbiota consisting in species from the genera *Paenibacillus, Burkholderia, Stenotrophomonas* and *Pseudomonas*. Most of them were able to produce auxins, biofilms and ACC deaminase, even under stressing conditions. The strains *Paenibacillus endophyticus* JA, *Burkholderia ubonensis* JC, and *Pseudomonas fulva* MB improved plant growth, but only JC and MB enhanced maize seedlings response to water restriction. *Pseudomonas fulva* MB stood out and may suppose a new seed-isolated strain employed as field biotreatment against drought effects.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Estudio del comportamiento hídrico de la higuera, variedad Calabacita, en condiciones de riego en Extremadura

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Resumen: Extremadura es la región con mayor superficie dedicada al cultivo de la higuera, tradicionalmente en secano y para producción de higo seco. En los últimos años se está incrementando considerablemente la superficie en riego. Aunque en las condiciones semiáridas de Extremadura el riego incrementa de forma notable las producciones, existe poca información sobre el comportamiento del árbol en condiciones de alta disponibilidad hídrica. El objetivo de este trabajo es cuantificar las necesidades hídricas del árbol a lo largo de todo el ciclo vegetativo y la respuesta frente a las variables meteorológicas, en los diferentes estados fenológicos. En la finca La Orden se estableció en el año 2018 una plantación de higuera de la variedad Calabacita con un lisímetro de pesada en el centro de la misma y riego localizado por goteo. La zona humedecida por el goteo está permanentemente sombreada por una malla antihierba. Sobre el árbol situado en el lisímetro se realizaron medidas de contenido de agua en el suelo, de estado hídrico y desarrollo vegetativo. Se dispone además de datos meteorológicos de una estación de la red REDAREX situada a 100 m de la parcela. Los resultados muestran que el consumo de agua de la higuera fue creciente desde la brotación hasta junio que se estabilizó y empezó a descender a finales de agosto, con valores medios durante mayor demanda entre 3-4 mm en 2022 y entre 4-5 mm en 2023. El consumo diario de la planta siguió la misma pauta que la radiación.

Palabras clave: Ficus carica, lisímetro, radiación, transpiración, humedad suelo, DPV

1. Introducción

La higuera (*Ficus carica* L.) es una de las primeras plantas cultivadas destacando sus características de rusticidad, facilidad de multiplicación, adaptación y resistencia a la sequía. La producción de higo a nivel mundial supera el millón de toneladas, ocupando España el sexto puesto en producción con 43.500 t (FAO, 2023). Extremadura es la región

española con mayor superficie de higueras, en su mayoría de secano y para producción de higo seco (MAPA, 2022). Aunque se trata de un frutal más tolerante al estrés hídrico que otros frutales (Flaishman et al. 2008), las nuevas plantaciones de higuera se están estableciendo en zonas de regadío debido a la positiva respuesta del cultivo al riego y a las buenas perspectivas de mercado, con interés tanto en el higo seco como fresco (Pereira et al. 2017). Al mismo tiempo, elimina la dependencia del crecimiento y la producción de la higuera cultivada en secano, del agua de lluvia. Sin embargo, la información sobre las necesidades hídricas del cultivo de la higuera es escasa (Flaishman et al. 2008) y con resultados contradictorios al aporte de agua de riego (Abdolahipour et al. 2018; Flaishman et al. 2008). En cualquier caso, se trata de un cultivo emergente, posicionándose como una alternativa viable a otros frutales de mayor demanda hídrica en zonas semiáridas de regadío. El objetivo de este trabajo es cuantificar las necesidades hídricas de la higuera cultiva es estados fenológicos, en los diferentes estados fenológicos.

2. Materiales y Métodos

2.1 Plantación

El ensayo se realizó durante 2 años en una plantación joven de higuera, de 1,2 ha, situada en el Centro de Investigaciones Científicas y Tecnológicas de Extremadura CICYTEX (Guadajira, Badajoz). Los árboles, plantados en 2018, eran del cv. Calabacita, para producción de higo seco. El suelo era franco-arcilloso, siendo la capacidad de campo 0,28 m³/m³ y el punto de descenso tolerable 0,16 m³/m³. El marco de plantación de 4 x 5 m. A lo largo de la fila de árboles disponía de malla antihierba negra. El sistema de riego consistió en dos líneas portagoteros por cada fila de árboles, con goteros integrados de 3,5 l/h separados 1 m. En el centro de la parcela estaba situado un lisímetro de pesada, de 2,67 m x 2,25 m x 1,5 m (Yrisarry y Naveso, 2000; Picón-Toro et al. 2012). La brotación de los árboles fue a finales de marzo y, la caída de hojas a finales de noviembre.

2.2 Consumo de agua

Los valores del coeficiente de cultivo, kc, se calcularon como kc = ETc/ETo, dónde la evapotranspiración del cultivo, ETc, se obtuvo directamente del lisímetro como la diferencia de peso entre dos medidas consecutivas, y la evapotranspiración de referencia, ETo, de una estación agroclimática según el método de FAO 56 de Penman-Monteith (Allen et al. 1998; Allen et al. 2006), situada a 100 m de la parcela.

2.3 Medidas

La conductancia estomática foliar se midió con un porómetro (Li-Cor LI 600, mol⁺¹m⁻²s⁻¹) en el árbol del lisímetro. La evolución del contenido de humedad del suelo se midió con sondas de humedad (TEROS 11 METER Group AG, m³ agua/m³ suelo) a 30 cm, 60 cm y

90 cm de profundidad y la temperatura de la cubierta vegetal con un radiómetro de infrarrojos (Apogee instruments, ºC).

3. Resultados y Discusión

3.1 Climatología y consumo de agua

Durante los 2 años de ensayo, los meses más calurosos fueron julio y agosto, y los más fríos enero y febrero (Fig. 1). La precipitación fue de 420 mm en 2022 y 391 mm en 2023, si bien la distribución fue muy diferente entre ambos años.



Fig. 1 Temperatura máxima, media y mínima diaria (°C) y precipitación diaria (mm) durante los años 2022 y 2023 en el ensayo.

Así, el arranque de campaña en 2022 estuvo directamente influido por las lluvias primaverales, disminuyendo el número de medidas en el lisímetro (Fig. 2a). Las curvas estacionales de evapotranspiración de la higuera (ETc) registradas en el lisímetro siguieron la misma pauta que las de evapotranspiración de referencia (ETo) (Fig. 2a y b). El consumo de agua de la higuera fue creciente desde la brotación hasta junio que se estabilizó y empezó a descender a finales de agosto, con valores medios durante mayor demanda entre 3-4 mm en 2022 y entre 4-5 mm en 2023 (Fig. 2c). El consumo diario de la planta siguió la misma pauta que la radiación (Fig. 2d). En la Fig. 2e se muestra la gráfica anual del coeficiente de cultivo (kc) desde la plantación. En las etapas iniciales de desarrollo de la cubierta vegetal y hasta la parada del crecimiento vegetativo (principios de julio), los valores se mantuvieron constantes y, de media, ligeramente inferiores a 0,5. Posteriormente y hasta la última recolección (mediados de septiembre) el valor de kc aumentó hasta 0,72. Desde ahí hasta caída de hojas, el kc se incrementó rápidamente hasta superar el valor de 1 en los meses de octubre y noviembre. Un aspecto importante que considerar es la presencia de la malla antihierba que ha debido reducir la evaporación de forma considerable y que será necesario evaluar para hacer estos datos extrapolables a condiciones de suelo desnudo o con otro mantenimiento.



Fig. 2 Evolución de la evapotranspiración de referencia (ETo, mm) con la evapotranspiración (ETc, mm) y precipitación diaria (mm) durante **a**) año 2022 y **b**) año 2023 para cada día del año (dda). **c**) consumo de agua anual del lisímetro (ETc, mm) y radiación solar (MJ/m²día) en 2022 y 2023, **d**) consumo diario (mm/h) y radiación (MJ/m²día) el 206 dda y **e**) evolución del coeficiente de cultivo (kc) desde 2018 a 2023.

La evolución del contenido de humedad del suelo recogida por los sensores mostró un patrón recurrente: una subida brusca tras el riego seguido de un descenso paulatino como respuesta a la absorción de las raíces y la distribución del agua en el suelo (Fig. 3). Los días sin riego se observó un rápido descenso del contenido de agua hasta los 90 cm. Con las lluvias de septiembre, el contenido de humedad del suelo se elevó hasta capacidad de campo.



Fig. 3 Evolución estacional del contenido de humedad del suelo a 30, 60 y 90 cm de profundidad (m³ agua/m³ suelo), precipitación (mm) y riego (mm) durante 2023.

La conductancia estomática se incrementó coincidiendo con el crecimiento de los frutos, disminuyendo a medida que se alcanzaba la madurez de los mismos (Fig. 4).



Fig. 4 Conductancia estomática foliar (mol⁺¹m⁻²s⁻¹) y radiación fotosintéticamente activa (μ mol⁺¹m⁻²s⁻¹) en 2023.

Conclusiones

Los resultados obtenidos en este trabajo demuestran el interés de la información obtenida en el lisímetro tanto para cuantificar el consumo de agua como para evaluar el efecto de las variables meteorológicas. Se han obtenido unos coeficientes de cultivo aplicables a los primeros años de establecimiento de la plantación, que muestran un comportamiento similar a otros árboles mediterráneos tradicionalmente de secano, como es el olivo, con un valor reducido en el periodo de mayor ETo.

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2. Oral Communications

Session IV: "Nuevas metodologías para el estudio de las relaciones hídricas en plantas





XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Air-coupled broadband ultrasounds to study water relations in leaves.

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Abstract: This work reviews the origin, development and use of ultrasonic air-coupled techniques for the study of plant leaves tissues and their water relations. This includes the two techniques proposed so far: the leaf resonance spectroscopy and the non-resonant time domain transmittance. The first one has been used to determine water potential, turgor loss point and differential behavior of the different tissues in the leaves, for different species and both in vivo and ex vivo; while the second has been recently proposed as a universal technique that can be applied to all plant leaves regardless of the possibility to excite thickness resonances.

Keywords: air-coupled ultrasound, plant leaves, water relations, thickness resonances, time domain transmittance.

1. Introduction

The possibility of using air-coupled ultrasound signals to test plant leaves was first proposed by Gil-Pelegrín (CITA) and Alvarez-Arenas (CSIC) in 2007 when broadband air-coupled ultrasonic pulses (center frequency 0.65 MHz) were transmitted through poplar leaves with the intention to extract information about the leaf (Álvarez-Arenas et al. 2009). This revealed that it is possible to transmit wide-band air-coupled ultrasonic pulses through some plant leaves with good signal to noise ratio (SNR). Moreover, the analysis of the spectrum of the transmission coefficient revealed the presence of a clear thickness resonance. In addition, it was also observed a clear relation between relative water content and leaf ultrasonic response. After that, the work focused on the understanding of theses resonances and how are they affected by leaf properties like water content, water potential, leaf structure, cell geometry, cell wall, presence of voids, etc. This research leaded to a sound knowledge of the response of plant tissues to

ultrasonic waves, and a powerful technique to study plant leaves properties and water relations from a completely different point of view.

2. The discovery of the possibility to excite and sense thickness resonances in plant leaves using air-coupled ultrasound.

2.1 Technique description and evolution.

This technique is based on the analysis of the transmission of air-coupled ultrasonic pulses (typically in the frequency range 0.2-1.0 MHz) through plant leaves. To this end an ultrasonic transmitter and a receiver are used and the leaf is placed between them. There is no need to touch the leaf, so the ultrasonic transmitter launches the signal to the air, it propagates through the air-gap that separated transducer and leaf and arrives to the leave. Part of the signal crosses the leaf, and is transmitted again to the air where it is eventually collected by the receiver transducer. Therefore, we call this an "air-coupled ultrasonic technique." This is extremely important for the case of plant leaves, because in this way it is possible to fully preserve the state of the leaf, that is, the technique is completely contactless and fully non-invasive. Moreover, this allow us to implement the technique "in-vivo". Previous application of ultrasonic techniques to plant leaves (Fukuhara, Okushima, y Matsuo 2005), (Wilson y Dunton 2009) were developed using conventional ultrasound, that is, using coupling fluids.

Figure 1 shows the main elements of the experimental set-up and their evolution over the years. These main elements are i) the ultrasonic transducers: transmitter (Tx) and receiver (Rx), ii) the excitation and reception electronics, iii) the signal display, iv) transducers and leaf holders. Initially, Fig. 1.a, (Domingo Sancho-Knapik et al. 2010) a conventional ultrasonic pulser-receiver, P/R, (Olympus), a digital oscilloscope (Tektronix) a pair of general purpose air-coupled transducers (CSIC) and conventional lab holders were used, Fig. 1.b (D Sancho-Knapik et al. 2011). Then, a special holder for both transducers and leaf to facilitate the measurement and portability of the system were designed, Fig. 1.c (Álvarez-Arenas et al. 2016), transducers were also redesigned to fit into this system. Finally, the P/R and the oscilloscope were replaced by a digital P/R and a laptop, Fig. 1.d, (Domingo Sancho-Knapik et al. 2016). Figure 1.e (Álvarez-Arenas et al. 2016) shows the typical location of the measured section in some type of leaves (*Platanus x hispanica, Coffea arabiga*, Vitis vinifera, and *Aesculus hippocastannum*).

2.2 Specific features of this technique

In addition to be completely non-invasive and contactless, there are another interesting and specific features of this technique. They are: i) Unlike other techniques the deformation is extremely small, with displacements of the order of few nanometers and wavelengths of the order of 0.3 – 3 mm (Blahovec 1988; Ehrenberger et al. 2012; Hayot et al. 2012; Lintilhac et al. 2000; Onoda, Schieving, y Anten 2015; Radotić et al. 2012; Vincent 1982). ii) the type of deformation is specially simple as this is pure longitudinal and along the thickness direction, in addition, plant cells are subjected to homogeneous pressure as the wave length is much larger that cell size, iii) finally, the deformation rate is so fast (0.2-1.0 MHz) that deformation time is well below the poroelastic relaxation time, so that the deformation of the plant tissue cells take place at constant fluid content.



Fig. 1 Up left: schematic representation of the early experimental set-up. Up-centre: early transducers and leaf positioning. Up-right: transducers embedded in a special holder. Bottom-left: portable experimental system. Bottom-right: location of the measurement point(s) in different species.

2.2 Early results.



Fig. 2 Transmission coefficient spectra (magnitude and phase) of *P. laurocerasus* and *P x euroamericana*, (measured data and model calculations: solid line) showing a thickness resonance.

Figure 2 shows the measured spectra of the transmission coefficient (magnitude and phase) of *P. laurocerasus* and *P x euroamericana*. In the frequency range 0.15-1.2 MHz (Gómez Álvarez-Arenas et al. 2009). Both types of leaves present a clear thickness resonance that is characterized by a maximum in the transmission coefficient magnitude spectrum and a step in the phase spectrum. Resonances for *P. laurocerasus* and *P x euroamericana* are observed at 0.25 and 0.59 MHz, respectively. In addition, this resonances can be modeled using a simple model base on the ultrasound transmission through a homogeneous and isotropic flat layer (see solid line with model predictions).

3. Influence of leaf turgor and relative water content on the leaf thickness resonances.

After this initial work, the efforts focused on the study of how the leaf transmission coefficient changes with the water content (Domingo Sancho-Knapik et al. 2010; Domingo Sancho-Knapik, Peguero-Pina, Fariñas, et al. 2013; Domingo Sancho-Knapik, Peguero-Pina, Medrano, et al. 2013; Sancho-Knapik et al. 2011). It was found that, in all of the studied plant species, the resonant frequency shifts towards lower values following a sigmoid curve when the relative water content (RWC) decreases. In addition, the lower the RWC, the larger the attenuation of the ultrasonic. See results in Fig. 3 for two different species: *Platanus hispanica* and *Viburnum tinus*.



Fig. 3 Left *Platanus hispanica* transmission coefficient spectra showing the thickness resonance at different RWC values, a: 1.00, b: 0.97, c: 0.995, d: 0.94, e: 0.92, f: 0.85, g: 0.77, h: 0.72. Right: *Viburnum tinus*: variation of the resonant frequency with the RWC and fitting with a sigmoid. The point of inflection indicated the turgor loss point.

Moreover, see fig. 4 (Álvarez-Arenas et al. 2016), the comparison of leaf ultrasonic resonant frequency vs RWC with water potential vs RWC (obtained using the pressure chamber), revealed that the point of inflection of the curve of resonant frequency vs RWC takes place at the turgor loss point. So this demonstrated the close relationship between water potential and ultrasonic response and validates the use of this technique to determine the turgor loss point. For *Quercus muehlenbergii* leaves a detailed study of the relation between water potential (pressure chamber), ultrasonic response and cell deformation (CryoSEM) revealed the role of cell deformation with the decrease of RWC in the modification of the ultrasonic response: in particular, the role of the onset of cell buckling. See Fig. 5



Fig. 4 Water potential vs resonant frequency and relative water content (RWC) versus resonant frequency for V. vinifera cv. Grenache leaves.



Fig. 5 *Quercus muehlenbergii* leaves: Left: Water potential vs RWC; Centre: ultrasonic resonant frequency vs RWC and Right: Cryo-SEM micrographs of the palisade parenchyma cells at full turgor and at RWC = 0.72. White arrow indicates the cell buckling phenomenon.

Vitis vinifera leaves were the subject of several studies. Figure 6.a shows the evolution of the leaf resonant frequency in the period July-October for three different varieties, while figure 6.b show the variation of both water potential and resonant frequency at predawn and midday during a water stress cycle of 20 days.



Fig. 6 Left: Evolution of the ultrasonic resonant frequency in the period July-October, Right: evolution of water potential and resonant frequency at predawn and midday along a water strees test.

4. Analysis of multiple resonances and determination of properties of the different layers in the leaf (mainly palisade and spongy parenchyma).

The analysis of the transmission coefficient of some plant leaves revealed that in some cases it is possible to excite several orders of the thickness resonances. In this cases, it is possible to extract information about the different layers of tissue in the leaves (Álvarez-Arenas et al. 2020). Figure 7 shows the example of different resonances in *L. lucidum* leaves with different relative thickness of palisade and spongy parenchyma.



Fig. 7 Left: Thickness resonances in *L. lucidum leaves* with different thickness. Increase in the thickness is due to the increase of the layer of palisade parenchyma thickness see CryoSEM images (right)

This made possible to study the variations in both parenchymas with RWC. Figure 8 shows that for V. vinifera both change in a similar way, but this is not the case for L.

lucidum or *V. Tinus*. In the case of *V. tinus* this was confirmed by CryoSEM observations. See Fig. 8



Fig. 8 Top. Variation in the surface density of both parenchymas for: Left: *V. vinifera*, Centre: *L. lucidum*, Right: *V. tinus*. Bottom: Cryo-SEM images of Viburnum tinus leaves cross section at full turgor (left) and at water potential of -3.5 MPa (right).

5. Recent advances. Non-resonant techniques: towards a universal ultrasonic technique.

More recently (María Dolores Fariñas et al. 2022), a non-resonant technique has been proposed. This has the advantage that can be applied to any plant leaf regardless of the possibility of exciting thickness resonances. The technique is based on the analysis of the transmitted signal through the leaf in the time domain. In particular, both amplitude and time of flight.

Conclusion

The use of air-coupled ultrasound for the study of plant leaves has been reviewed. The technique initially proposed extract leaf information from the analysis of the leaf thickness resonances. The technique permits to extract information about water relations, tissue elastic modulus, cell shape and cell wall contribution, the layered tissue structure, differential information about the different component layers and response to environmental stimuli in real time (Fariñas et al. 2014)). The technique can be applied in-vivo and is fully non-invasive and contactless. Extraction of the water potential or relative water content can be performed through modelling of the leaf response of by applying deep learning algorithms (Fariñas et al. 2019). This technique depends on the capability to excite thickness resonances in plant leaves, however, in some cases, this is not possible, so the technique has been extended to make possible to apply it to any type of leaf, regardless of the possibility of exciting thickness resonances, this is obtained by the analysis of the ultrasonic leaf transmittance in the time domain.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Determining stomatal response to vapour pressure deficit from gas exchange measurements: conceptual and methodological challenges

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Abstract: Plants typically respond to increasing VPD with a decrease in stomatal conductance (gs) at a pace that varies with the water-use strategy of each species. Inferring these responses from field-based measurements is complex due the interaction of VPD with multiple factors, such as soil water content or light. Alternatively, manipulative experiments using gas exchange devices represent a simple approach to isolate the response of stomatal conductance (gs) to VPD from other environmental variables. Here, we built empirical models of stomatal response to leaf-to-air VPD in four oak species with contrasting water-use response and leaf morphology. These models were used in combination with leaf energy-balance models to assess the response of gs to varying conditions in the cuvette (temperature, light) and in the input air (temperature, humidity), in order to define the optimal set-up to assess stomatal response to VPD for different species. We also compared stomatal response to air temperature and humidity under cuvette and open-air conditions, to assess to what extent the differences in radiation and leaf boundary layer in the cuvette and open-air modify plant response to ambient VPD. Our results indicate that for water-spender species it can be difficult to achieve steady-state conditions in the cuvette, particularly under low to moderate mass flow, and with high input air temperatures. We also evidenced that the response to ambient VPD in open air conditions may differ strongly from the response under cuvette conditions, particularly in species with large leaves, and under low to moderate wind speed (<6 m s⁻¹) conditions.

Keywords: Vapor Pressure Deficit, Stomatal Conductance, Infrared Gas Analyzer, Leaf Temperature, Manipulative Experiments, *Quercus*

1. Introduction

The worldwide increase in air temperature implies an exponential increase in atmospheric vapour pressure deficit (VPD). In this context, we are facing an increasing

interest in understanding plants' response to VPD (Grossiord et al. 2020). Although landsurface and vegetation models account for changes in VPD, e.g. as one of the factors determining the water balance, little attention has been paid to the physiological response to VPD. Plants typically respond to increasing VPD with a decrease in stomatal conductance (gs) at a pace that varies with the water-use strategy of each species (Oren et al. 1999, Mediavilla & Escudero 2003). Inferring these responses from field-based measurements is complex due the interaction of VPD with multiple factors, such as soil water content or light (Mediavilla & Escudero 2003, Sancho-Knapik et al. 2022). In this context, manipulative experiments using gas exchange devices constitute a simple approach to isolate the response of stomatal conductance (gs) to VPD from other environmental variables, but also face some methodological limitations. Conditions inside the leaf cuvette are modulated by the leaf, with transpiration partially compensating for the increase in VPD (e.g. in response to varying cuvette temperature or input air humidity). This may cause a negative feedback on cuvette VPD under intermediate VPD conditions, which can be particularly strong in species with high transpiration rates, leading to unstable measuring conditions. Hence, although current gas exchange systems allow to set cuvette conditions, the probability to attain steadystate conditions depends on the species response, as well as on the input air conditions (temperature, humidity), and volumetric flow inside the chamber. On top of that, physiological response of plants is driven by the leaf-to-air VPD, which also depends on leaf temperature, and hence may differ substantially from atmospheric VPD due to the energy balance of the leaf (Peguero-Pina et al. 2020; Sancho-Knapik et al. 2022). In this regard, conditions in a gas exchange cuvette do not fully mimic open-air conditions, particularly due to the response of leaf temperature to different factors, such as the light quality or the boundary layer, which are different inside the cuvette and in open air conditions. Cuvette conditions are characterized by a fixed exposed leaf area and forced air convection, whereas in open air the size and shape of the leaf, together with other environmental conditions (wind speed, radiation) modulate leaf temperature, and hence the response to VPD (Peguero-Pina et al. 2020).

Despite the increasing number of studies addressing plant response to VPD, little attention has been paid to the methodological issues in determining these response (Sancho-Knapik et al. 2022). For gas exchange systems, we hypothesize that achieving high flow rates may be critical to build VPD response curves, particularly for species with high transpiration rates. We also postulate that under open-air conditions the divergence between leaf-to-air and ambient VPD will be exacerbated when leaf boundary layer is large, e.g. under low wind speed or for species with large leaves. To test these hypotheses, we will follow a modelling approach combining empirical models of stomatal response to leaf-to-air VPD with energy balance models, applied to four oak species with contrasting water-use response and leaf morphology (size and lobation).

2. Materials and Methods

2.1 Empirical stomatal response models

All analyses were performed in *R* version 4.2.1. We built empirical models of stomatal conductance to leaf-to-air VPD in four oak species (*Quercus ilex* subsp. *rotundifolia* (Lam.) O.Schwarz ex Tab.Morais; *Quercus pyrenaica* Willd.; *Quercus robur* L.; *Quercus suber* L.), using data compiled from the literature (Mediavilla and Escudero 2003; Morecroft & Roberts 2002; Sancho-Knapik et al. 2022), plus own unpublished data. We fitted a three-parameters logistic curve to account for different thresholds for stomatal response (Sancho-Knapik et al. 2022). Since we aimed to model maximum *gs* for a given VPD, we used a quantile regression (tau=0.95), using the package *quantreg* in *R*.

2.2 Modelling leaf response under gas exchange cuvette

To model leaf response to VPD under gas exchange cuvette, we tested for each species 1944 scenarios resulting from six input air temperatures (from 20 to 45°C, in 5°C steps), six input air relative humidity (RH from 0 to 50%, in 10% steps), three mass flow rates $(250, 500 \text{ and } 1000 \text{ cm}^{-3} \text{ min}^{-1})$, three levels of photon flux density (500, 1000 and 2000) µmol m-2 s-1), and six cuvette temperatures (from 20 to 45°C, in 5°C steps). For each scenario, we used the empirical models above to determine gs under initial cuvette conditions, and the von Caemmerer & Farguhar (1981) model to determine the corresponding net transpiration rate, which in turn will modify the cuvette conditions and leaf temperature (hence leaf-to-air VPD). This was performed iteratively, until reaching convergence in gs and leaf temperature (to the 9th and 4th decimal, respectively). If convergence was not reached after 1000 iterations, or yielded negative VPD, we flagged the scenario as 'not converged'. To determine leaf temperature, we used two alternative energy balance models (Parkinson 1983; Barbour et al. 2000). We found the lowest proportion of not-converged scenarios with the Barbour model, so this was chosen for subsequent calculations. Boundary layer resistance to water vapour (rb) was fixed to a typical factory-calculated value for a 18 x 25 mm cuvette window (rb=0.4 m² s mol⁻¹; CIRAS3 PLC3 Universal Cuvette).

2.3 Modelling leaf response under open-air conditions

For open-air conditions, we first tested different approaches to determine *rb* across a wide range of wind speed: from leaf area for an elliptical leaf (Ball et al. 1988), and from the characteristic dimension (*d*, after Jones 1992), accounting for leaf lobation (Parkhurst et al. 1972), the latter assuming either free (still air) or forced convection. For subsequent calculations, we choose the most responsive method (*d* under forced convection), as it reflects the effect of leaf morphology, and is valid across a wide range of wind speed We calculated leaf-to-air VPD and *gs* in open air following the same approach as for the gas exchange cuvette, but in this case accounting for variations in ambient air temperature (from 20 to 45°C, in 5°C steps), ambient RH (RH from 10 to 70%, in 10% steps), incoming photon flux density (500, 1000 and 2000 μ mol m-2 s-1), and an exponential scale with eleven levels of wind speed (from 0.1 to 19 m s⁻¹), i.e. overall 1386 scenarios.

3. Results and Discussion

As expected, stomatal response to leaf-to-air VPD differed substantially among the four oaks species (Fig. 1), with *Q. robur* showing the steepest response (Fig. 1C), followed by
Q. pyrenaica (Fig. 1B), but in this case with a much higher VPD threshold and higher maximum gs. Conversely, *Q. suber* showed the least sensitive response, but with more conservative maximum gs values (Fig. 1C), whereas *Q. ilex* was also conservative, but more sensitive than *Q. suber* (Fig. 1A).



Fig. 1 Fitted models for the response of stomatal conductance (gs) to leaf to air vapour pressure deficit (VPDleaf) for A) *Quercus ilex* subsp. *rotundifolia*, B) *Quercus pyrenaica*, C) *Quercus robur* and D) *Quercus suber*.

Applying these models to gas-exchange cuvettes, we found that 'failure' scenarios (nonconverging or with high cuvette humidity, >75%) were most frequent under low flow rates and high input air temperatures (Fig. 2). The most sensitive species was the water spender *Q. pyrenaica*, with the conservative *Q. ilex* being the least sensitive (Fig. 2).



Fig. 2 Probability of non-converging or high humidity scenarios (%) using gas exchange systems to assess stomatal response to water pressure deficit (VPD), for the four species considered, and within each combination of input air temperature, input RH and mass flow rate (cm³ min⁻¹). Each combination includes 18 scenarios (six levels of cuvette temperature x three levels of photon flux density).

Even after excluding not-converged and high humidity scenarios, high maximum *gs*, together with a fast response of gs to VPD, made particularly difficult to build a response curve for *Q. pyrenaica* under low to intermediate flow rates, shifting rapidly from high *gs* to near zero values (Fig. 3, upper and middle panes). In all cases, measurements under high input air temperature may be problematic (Fig. 3, right panes).



Fig. 3 Built response curves of gs as a function of leaf-to-air VPD for *Q. pyrenaica*, within each combination of input air temperature, and mass flow rate (cm³ min⁻¹). Incomplete curves denote a higher sensitivity to changes in two key parameters used to build the curves, i.e. input relative humidity and cuvette temperature. Colour scale indicates cuvette temperature.

Under open air conditions, we found that only with a moderately strong wind speed (>6 m s⁻¹), the *rb* reaches values comparable to those inside a gas exchange cuvette (Fig. 4). Species with smaller leaves (*Q. ilex, Q. suber*) had lower and less sensitive *rb*, while the highly lobed *Q. pyrenaica* had lower *rb* than *Q. robur*, despite their similar leaf size.



Fig. 4. Variations in the boundary layer resistance to water vapour (rb) as a function of wind speed, calculated from the characteristic dimension (*d*, assuming either forced or free convection) or from leaf area (elliptical). For reference, a typical factory-calculated value for a 18 x 25 mm cuvette window is provided (rb=0.4 m² s mol⁻¹).

When applying energy-balance equations, and accounting for variations in *rb*, we found that the response of *gs* to ambient VPD under open-air conditions may be highly sensitive to other environmental factors, such as wind speed and incoming radiation, which in turn modify leaf-to-air VPD through changes in leaf temperature (Fig. 5). Under calm conditions (0.1-0.3 m s⁻¹) all species showed highly diverging values of leaf-to-air VPD, whereas under relatively strong wind (>6 m s⁻¹), *Q. pyrenaica* still shows high sensitivity, particularly in the upper range of ambient VPD (Fig. 5).



Fig. 5. Modelled variations in leaf-to-air VPD, as a function of ambient VPD in open-air conditions, under three ranges of wind speed (Calm: 0.1-0.3 m s⁻¹, 3 levels; Moderate: 0.5-4 m s⁻¹, 5 levels; Strong: 6.7-19 m s⁻¹, 3 levels). Each pane covers six levels of ambient temperature, seven levels of relative humidity, three to five wind speed levels, and three levels of photon flux density.

Due to the uncoupling between ambient VPD and leaf-to-air VPD, when plotting ambient VPD against modelled *gs* (Fig. 6), we obtained multiple response curves, i.e. highly sensitive to wind speed and radiation, particularly under calm to moderate wind speeds. $(0.1 \text{ to } 4 \text{ m s}^{-1})$.



Fig. 6. Modelled response of gs to ambient VPD in open-air conditions, for three ranges of wind speed. Each pane covers six levels of ambient temperature, seven levels of relative humidity, three to five wind speed levels, and three levels of photon flux density.

Conclusions

Our results indicate that to build plant response curves to VPD, it is crucial to fine-tune cuvette conditions. This can be better achieved under moderately low ambient temperatures, due to their lower saturated water vapour content, and using as high as possible mass flow rates inside the cuvette. This is particularly critical for water-spender species such as *Q. pyrenaica*. On the other hand, we demonstrate that under open-air conditions the association between ambient VPD and *gs* maybe masked by other environmental factors, such as radiation or wind speed.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Real-time trunk water potential combined with soil water content for precise irrigation in nectarine trees

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Abstract: Precision irrigation is needed to improve the efficiency use of water. This work aimed to integrate as the first-time, the novel plant water status indicator of trunk water potential (Ψ_{trunk}) into an irrigation protocol. During the summer 2023, two automated irrigation treatments were assessed in a nectarine orchard. The irrigation decision was based on (i) threshold values of soil water content (Θ_v) at 30% maximum allowable soil deficit (MAD), measured with capacitance probes (SOIL treatment), and (ii) on combined threshold values of Θ_v and $\Psi_{trunk} \leq$ -0.80 MPa measured with trunk microtensiometers (PLANT treatment). In both treatments, discrete measurements of midday stem water potential (Ψ_{stem}) and leaf gas exchange were also calculated. The threshold Ψ_{trunk} value was reached every day during the morning, promoting irrigation events whenever Θ_v fall below 30% MAD, then, irrigation gifts of 3h prevented water loss below effective plant roots. The Ψ_{trunk} showed the average minimum values of -1.3 and -2.2 MPa in the PLANT and SOIL treatments, respectively in the afternoon, whereas its maximum values (≤-0.04 MPa) were observed before dawn, in both treatments. Ψ_{stem} , average was -0.82 and -1.37 MPa in the PLANT and SOIL treatments, respectively. Leaf gas exchange also revealed better plant water status in the PLANT treatment. The proposed combination of real-time Ψ_{trunk} and Θ_v measurements for precise irrigation has been a successful rule for automated irrigation while keeping adequate water status in nectarine trees.

Keywords: automated irrigation, maximum allowable soil water depletion (MAD), stem water potential (Ψ_{stem}), trunk water potential (Ψ_{trunk}), water relations.

1. Introduction

Precision irrigation scheduling (PIS) uses methodologies that measure the water status in the soil-plant-atmosphere continuum, providing eco-friendly and circular economy solutions for fruit tree production. The traditional irrigation scheduling method is based on the standardized FAO-56 Penman-Monteith approach (Allen et al. 1998) which estimates crop evapotranspiration (ET_c) as the product of the reference crop evapotranspiration (ET_0) and the crop coefficient (K_c). However, it provides a degree of uncertainty due to the parameters that affect the K_c and the soil hydrodynamic properties (Ramírez-Cuesta et al., 2017).

Soil water content (Θ_V), along with a knowledge of soil characteristics and root exploration, is a state variable that has been proposed as a key input in PIS. In this sense, automated irrigation based on Maximum Allowed Deficit (MAD, Merriam, 1960) threshold values, and monitored by computed Θ_V real-time data, has been proved as a valid method for PIS in nectarine trees (Vera et al., 2019; Conesa et al., 2021).

Assessment of plant water status indicators leads to accurate information for irrigation management, as plants represent the interface between soil and atmosphere (Jones, 2007). Stem water potential at midday (Ψ_{stem}) and leaf gas exchange have been extensively identified as sensitive determinations to assess plant water status. Nevertheless, they are temporally discrete, destructive and non-automated measurements (Anderson and Brodbeck, 1988; McCutchan and Shackel, 1992).

Trunk microtensiometers (MTs) appear as an option for continuous monitoring of plant water status through measurements of trunk water potential (Ψ_{trunk}). Previous studies with MTs revealed that these sensors measure Ψ_{trunk} in the same units as Ψ_{stem} (with the pressure chamber), as proven in vineyard (Pagay et al., 2022), pear (Blanco and Kalcists, 2021), apple (Gonzalez-Nieto et al., 2023), and nectarine (Conesa et al., 2023) trees. However, there have been no studies that integrated this novel plant water status indicator, Ψ_{trunk} , into the irrigation decision-making process.

The goal of this work was to implement an automated irrigation protocol combining real-time Ψ_{trunk} and Θ_{V} threshold values to precise irrigate nectarine trees. Discrete plant water status (leaf gas exchange, Ψ_{stem}) were also measured to validate the approach.

2. Materials and Methods

2.1 Experimental conditions

The study was conducted in a 0.5 ha orchard of 13-year-old early-maturing (harvest early May) nectarine trees (*Prunus persica* (L.) Batsch, cv. Flariba, on GxN-15 rootstock), located at the CEBAS-CSIC experimental station (SE Spain). Trees were spaced 6.5 m x 3.5 m and trained to an open-center canopy. The soil was stony with a clay-loam texture. Θ_v at field capacity (FC) and at permanent wilting point (WP) was 0.29 and 0.14 m³ m⁻³, respectively. The irrigation system consisted of one drip-line per tree row, with 4 emitters (4 L h⁻¹) per tree located 0.5 and 1.3 m from the tree trunk.

2.2 Measurements

Environmental data was recorded by an automated agrometeorological station located close to the orchard. Vapor pressure deficit was calculated from temperature and relative humidity data.

 Θ_v was monitored with multidepth EnviroScan[®] capacitance probes (Sentek Sensor technologies, Australia), installed in PVC access tube at 0.1 m from the emitter in one tree per replicate (n=4). The probes had sensors at 0.1, 0.3, 0.5, and 0.7 m depth, and

were connected to a radio transmission unit that sent the data to a telemetry system (ADCON Telemetry, Austria) for data acquisition, processing and visualization.

Plant water status was assessed by monitoring Ψ_{trunk} with MTs (FloraPulse, USA), embedded into the trunk of four nectarine trees, and located at 0.4 m from soil surface. Data were obtained every 15 min, and transmitted using the same telemetry network. Also, Ψ_{stem} was weekly determined at midday (12:00 h solar time) using a pressure chamber in four shaded mature leaves (one per tree), covered with aluminum foil bags for at least 2 h prior to the measurements (McCutchan and Shackel, 1992).

Leaf gas exchange parameters: net photosynthesis (P_n), stomatal conductance (g_s) and transpiration (E) were weekly measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) in the early morning in four sunny leaves. From these data, the instantaneous water use efficiency (WUE_{inst}) was calculated as the ratio P_n/E .

2.3. Automated soil and plant-based irrigation treatments

During the post-harvest period (day of the year, DOY, 150-300), two automated irrigation treatments were imposed, which were randomly distributed in four replicates, each consisting of six nectarine trees (n= 24):

<u>- SOIL treatment</u>, was scheduled based on computed real-time θ_v threshold values. according to the management allowed deficit (MAD) concept (Merriam, 1960) as a percentage (α) of the available soil water content (AW) that can be depleted (MAD_{α} = 100 · FC – α · AW), where AW is the difference between field capacity (FC) and wilting point (WP). Irrigation was activated when mean θ_v values in the 0.1-0.5 m soil profile (active root zone) reached the MAD_{α} threshold value and stopped at FC. During the experiment $\alpha \ge 30\%$ (MAD₃₀) (corresponded to a light deficit).

<u>- PLANT treatment</u>, was scheduled based on a combination of computed real-time θ_v and Ψ_{trunk} . Threshold values were set to start irrigation when $\Psi_{trunk} \leq -0.8$ MPa (corresponded to well-irrigated trees, Conesa et al., 2023) and θ_v fall below MAD₃₀. A 3 h irrigation event was trigger whenever both variables fell below the threshold values. Recorded data were subjected to one-way analysis of variance (ANOVA) using the SPSSv.9.1 to discriminate between treatments ($p \leq 0.05$).

3. Results and Discussion

The experiment covered the post-harvest period of early-maturing nectarine trees (June to October), when climatic conditions were common for a semi-arid Mediterranean summer: high VPD (3.3 kPa) and low rainfall (112.6 mm) values (Fig. 1A).

 Θ_{v} was influenced by the irrigation protocols and the diurnal environmental changes (Fig. 1B). For SOIL treatment, the irrigation criteria of MAD₃₀ started on DOY 180, one month after the irrigation scheduling set up. From this, irrigation events occurred daily until DOY 240, when rainfall events and the initial leaf senescence, typical of deciduous fruit trees, occurred. In a long-term study in the same orchard, Conesa et al. (2021) found that an automated irrigation Θ_{v} -based on MAD, with $\alpha \geq 30\%$ during the

postharvest did not penalize nectarine yield, but increased the amount of total solid soluble compared to traditional FAO-56 irrigation schedule. For the PLANT treatment, the threshold Ψ_{trunk} -0.80 MPa was reached every day during the morning, promoting irrigation events whenever Θ_v fall below MAD₃₀ (Fig. 1C). Then, the episodes of irrigation with a duration of about three hours prevented water losses below effective plant roots. The addition of optimal Ψ_{trunk} value in the irrigation protocol clearly improved the soil water status compared with the SOIL treatment (Fig. 1C).

 $Ψ_{trunk}$ showed differences between both irrigation treatments (Fig. 1C). Minimum daily $Ψ_{trunk}$ values of -1.3 to -2.2 MPa was reached in the afternoon (17:00 GTM+2) when the climatic conditions were more demanding. However, daily maximum values (≤ -0.04 MPa), observed at sunrise, were similar for both treatments. Average $Ψ_{trunk}$ values were -0.82 and -1.37 MPa, while for $Ψ_{stem}$ were -1.0 and -1.45 MPa for PLANT and SOIL treatments, respectively. Both were indicative of non-limiting soil water conditions (Vera et al., 2019; Conesa et al., 2021). Interestingly, from DOY 240, both $Ψ_{stem}$ and $Ψ_{trunk}$ recovered their values to the initial experimental conditions (especially in the PLANT treatment) due to rainfall and mild meteorological conditions. A mean gradient $Ψ_{stem}$ - $Ψ_{trunk}$ of 0.2 and 0.1 MPa for PLANT and SOIL treatments was noted, which were in the same order as other authors reported (Blanco and Kalcits, 2021; Pagay et al., 2022; González-Nieto et al., 2023).

When comparing seasonal data of both plant water status indicators, a strong lineal relationship was obtained (R²=0.88, $p \le 0.001$). Similar dependence Ψ_{stem} vs. Ψ_{trunk} was found in vineyards (Pagay et al., 2022), and pear (Blanco and Kalcits, 2021) trees.

Mean values of P_n and g_s were 13 µmol m⁻² s ⁻¹ and 139 mmol m⁻² s ⁻¹ and 15 µmol m⁻² s ⁻¹ and 191 mmol m⁻² s ⁻¹ in SOIL and PLANT treatment, respectively. A high CO₂ fixation at the time of the maximum water demand (DOY 210-250, August) was observed but without limiting stomatal opening. As expected, P_n and g_s decreased as the season progressed (Anderson and Brodbeck, 1988), and this reduction was advanced in the SOIL treatment as a result of the slight soil water deficit (Conesa et al., 2023). WUE_{inst} was somewhat high in SOIL treatment, meaning that carbon fixation was higher than water losses through transpiration in this treatment (Vera et al., 2019).



Fig. 1 Seasonal values of: **(A)** Rainfall (bars) and mean daily vapor pressure deficit (VPD, line), **(B)** Soil water content (Θ_v) in the soil profile 0.1-0.5 m [blue and green lines indicate the field capacity (FC) and the allowable deficit of soil water (MAD₃₀), respectively], **(C)** Trunk water potential (Ψ_{trunk}) and Stem water potential at midday (Ψ_{stem}) in the SOIL (black) and PLANT (red) irrigation treatments, **(D)** Θ_v during 10 days, as in B. Asterisks indicate significant differences between treatments at $p \le 0.05$. DOY: day of the year.



Fig. 2 Seasonal values of: **(A)** Net photosynthesis (P_n), **(B)** Stomatal conductance (g_s), and **(C)** Transpiration water use efficiency (WUE_{inst}) in the SOIL (black) and PLANT (red) irrigation treatments. Asterisks indicate statistically significant differences between treatments at $p \le 0.05$. DOY: day of the year.

4. Conclusion

The plant water status indicator trunk water potential (Ψ_{trunk}), measured with novel trunk microtensiometers (MTs), was successfully real-time monitored in nectarine trees. This work is the first study that integrates real-time Ψ_{trunk} in an automated irrigation protocol. Our results showed that the PLANT treatment allowed better plant water status than the SOIL treatment, as data of stem water potential and leaf gas exchange

revealed. The combination of Ψ_{trunk} and soil water content threshold values resulted in a successful practice for precise automated irrigation in nectarine trees.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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A new empirical framework to quantify soil and atmospheric forcing on plant water status

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Abstract: Metrics to quantify regulation of plant water status at the daily as opposed to the seasonal scale do not presently exist. This gap is significant since plants are hypothesised to regulate their water potential not only with respect to slowly changing soil drought, but also with respect to faster changes in air vapour pressure deficit (VPD), a variable whose importance for plant physiology is expected to grow because of higher temperatures in the coming decades. We present a metric, the stringency of water potential regulation, that can be employed at the daily scale and quantifies the forcing exerted on plants by the separate and combined effect of soil and atmospheric drought. We test our theory using datasets from two experiments where air temperature and VPD were experimentally manipulated. In contrast to existing metrics based on soil drought that can only be applied at the seasonal scale, our metric successfully detects the impact of atmospheric warming on the regulation of plant water status. We show that the thermodynamic forcing of VPD on plant water status can be isolated and compared against that exerted by soil drought and the covariation between VPD and soil drought. Furthermore, in three of three cases, VPD accounted for more than 5 MPa of forcing on leaf water potential. We explore the significance of our findings in the context of potential future applications of this metric from plant to ecosystem scale.

Keywords: Soil drought, vapour pressure deficit, water status regulation, hydraulic forcing, isohydry, homoiohydry.

1. Introduction

Terrestrial plants live along a hydraulic continuum with their water potential bracketed between that of soil water (as high as 0 MPa) and that of atmospheric water vapour (as low as -100 MPa). The water status of terrestrial plants is closely connected to water potential, which is regulated via the control of water loss at the leaf surface and via water supplied by hydraulic transport through roots, stem and leaves given the constraints (forcing) from soil and atmospheric water availability (Tyree & Jarvis, 1982).

Several existing metrics aim to quantify the regulation of leaf water potential Ψ_{leaf} . The degree of isohydric/anisohydric control of water potential can be quantified by examining changes in the difference between pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials $\Delta \Psi = \Psi_{\text{pd}} - \Psi_{\text{md}}$, stomatal responses to Ψ_{md} (Klein, 2014), the slope σ of the relationship between Ψ_{pd} and Ψ_{md} (Martínez-Vilalta et al., 2014) and the hydroscape area (Meinzer et al., 2016). These metrics of isohydric were also found to relate with traits, i.e., leaf turgor loss point, vulnerability to cavitation and several other functional proxies (Flo et al., 2021).

Current metrics of isohydry have several limitations. Our aim here is to present a water potential stringency metric S^{ws} that a) quantifies the effects of both VPD and Ψ_{soil} and b) can be applied at daily and seasonal time scales. This paper shows that this is possible by c) quantifying the departure of observed water potential from the predictions of a model representing both thermodynamic forces (i.e., both soil and atmospheric drought) when no physiological regulation (no stomatal limitations for a given hydraulic supply) occurs. Using data collected in one field experiment as case study, we ask the following questions: 1) to what extent is S^{ws} sensitive to differences in Ψ_{leaf} regulation in response to experimental treatments including VPD manipulations? 2) How does its sensitivity compare against other common metrics, such as σ , $\Delta \Psi$ and the hydroscape area? 3) How does the regulation of Ψ_{leaf} under high VPD compare to the regulation under negative Ψ_{soil} ?

2. Materials and Methods

2.1 Regulation of leaf water potential

We derive a null model for the hydraulic forcing exerted on Ψ_{leaf} by VPD and Ψ_{soil} , such that the internal regulation of plant water potential can be examined. We start by assuming steady-state flow in plants, i.e., transpiration equals plant water transport. Here, we limit the analysis to the case of $\Psi_{\text{leaf}}=\Psi_{\text{md}}$. The original derivation for this steady-state water balance between supply and demand assumed well-coupled canopies (Whitehead & Jarvis, 1981):

$$\Psi_{\rm md} = \Psi_{\rm pd} - \frac{g_{\rm tot}}{k_{\rm L}} VPD \tag{Eqn.1}$$

In Eqn.1, g_{tot} (leaf vapour conductance, sum of stomatal and boundary layer conductances) and κ_L (whole-plant hydraulic conductance divided by leaf area, assuming $\Psi_{pd}=\Psi_{soil}$) are dependent on plant water potential. This formulation omits gravity-induced head-losses to the soil-to-leaf gradient since they cancel out in Eqn.5 below. In Eqn.1, VPD is vapour pressure deficit outside of the leaf boundary layer. The assumption can be relaxed in future studies if continuous observations of leaf canopy temperature are available.

The null model of hydraulic forcing presented here can be employed to assess the degree of regulation of water potential by comparing the measured Ψ_{leaf} against the corresponding Ψ_{hf} under the same forcing conditions (i.e., same VPD and Ψ_{pd}). We define the stringency of water potential regulation (MPa) as the departure from the forcing in Ψ_{pd} and VPD.

2.2 Empirical tests

We employ one experimental dataset to test whether S^{ws} provides a sensitive metric of the daily stringency of Ψ_{md} regulation. The dataset comes from the SUMO experiment conducted in New Mexico (Sevanto et al., 2018).

2.3 Statistics

To address question 1) (to test the sensitivity of the metric to experimental treatments), we fit linear mixed models to the two datasets to examine whether daily hydraulic forcing Ψ_{hf} and regulation stringency S^{ws} vary across treatments and species (SUMO) or treatment combinations (Australia). To address question 2) (to compare against existing seasonal metrics), we first fit the same mixed models to Ψ_{md} as a function of Ψ_{pd} and treatment to obtain σ , and to $\Delta\Psi$ as a function of treatment. Using the SUMO experiment as a test case, we also calculate the hydroscape and compare it against the equivalent stringency areas (i.e., A_s) across species and treatments. To address question 3) (to test the relative regulation against VPD and Ψ_{soil}), we quantify the relative importance of the forcing by VPD and Ψ_{pd} , as well as the regulation against the forcing, by plotting these variables against total forcing.

3. Results

Of all the water status metrics, only the stringency metric (e.g., S^{ws}) was able to discern treatment effects in these two experiments. A highly significant relationship between Ψ_{md} and Ψ_{pd} was found in the SUMO experiment for both *P. edulis* and *J. monosperma* (both P<00001). However, a treatment effect was not found in either species or for the two species combined (Table 1A). Similar results were obtained for $\Delta\Psi$, the water potential difference between pre-dawn and midday (Table 1B) and for hydroscape area A_{HS} (Table 1E). Ψ_{md} was highly significantly and linearly related to total hydraulic forcing Ψ_{hf} in both species (at least P<0.0001, Tables 1C). In contrast to the previous metrics, treatment effects for Ψ_{md} against Ψ_{hf} were found in *J. monosperma* and when the two species at SUMO were combined (Table 1C). Treatment effects on total regulation S^{ws} were also found in *J. monosperma* and for the two species combined at SUMO.

Consistent with these results, a significant relationship was found between the slopes of the Ψ_{hf} - Ψ_{md} relationships and the slopes of the Ψ_{pd} - Ψ_{md} relationships (not shown) but the confidence intervals were generally larger for the second. Across both species at SUMO and all treatments, the hydroscape area A_{HS} was inversely related to the equivalent area of stringency. However, stringency areas had often smaller standard errors compared to hydroscape area and showed significantly larger values in Heat and Heat+Drought relative to Ambient in the analysis combining both species (P<0.00001) and in *P. edulis* (P<0.00001), but not in *J. monosperma*. No such differences were apparent with hydroscape area (always P>>0.05, cf., Table 1D and 1E).

The 5% quantiles of forcing reached -8.4 and -6.0 MPa for juniper and piñon pine, respectively, and varied significantly with species and treatment (both P<0.0001). In both species, Heat and Heat+Drought were the treatments with the most negative values of forcing. The mean net forcing by VPD was -4.6 MPa (minimum, -7.3 MPa) in P. edulis and -3.8 MPa (minimum, -5.8 MPa) in J. monosperma (P<0.00001 for the difference across species). The absolute values of forcing by VPD were significantly more negative, as expected, for Heat and Heat+Drought relative to Ambient (21% and 27%, respectively).

Regulation was 40-48% more stringent in *P. edulis* (36-37% in *J. monosperma*) in Heat and 47-57% (42-43%) in Heat+Drought relative to Ambient (P< 0.00001 for both species), respectively. The maximum values of regulations also varied significantly as a function of species and treatment, again with higher values for Heat and Heat+Drought relative to Ambient (P<0.00001), reaching values of 4.0 MPa for piñon pine in those treatments.

4. Discussion

In our analysis, we quantify the magnitude of the changes in leaf water potential relative to the changes that would occur if no internal regulation of the liquid-phase and vapour-phase conductances took place. In essence, our approach provides a null model based on environmental forcing, against which internal regulation is assessed. Therefore, the *S*^{ws} metric quantifies the direction and magnitude of the internal regulation of water status under specific sets of external forcing conditions.

In response to question 1) (cf., Introduction), we show that our metrics behave more sensitively than earlier metrics, as predicted, across two case studies where VPD levels were manipulated. The interest here is that soil and atmospheric contributions to regulation stringency are examined on the same thermodynamic scale, by examining their forcing on plant water status (Grossiord et al., 2020). We also asked whether S^{ws} is sensitive to water status regulation in response to experimental VPD manipulations (question 2). We found that stringency against forcing by VPD was weaker in *J. monosperma*, reflecting the strong seasonal decline in Ψ_{md} with Ψ_{hf} or Ψ_{pd} in this species. Conversely, the regulation against VPD was stricter in *P. edulis*, as evidenced by the fact that Ψ_{md} showed a much more constrained response with Ψ_{hf} despite substantial increases in VPD at low Ψ_{hf} . Compared to traditional isohydry metrics (σ , $\Delta \Psi$ and the hydroscape area), the stringency metric S^{ws} detected responses to experimental manipulations of water availability and water demand in a much more sensitive manner (Tables 1).

We also asked how the regulation of water status against high VPD compares with the regulation against negative Ψ_{pd} (question 3). We showed that plant control against the forcing by VPD was at least as important as the control against Ψ_{pd} . Remarkably at SUMO, both forcing and regulation in the Heat treatments ranked higher than the equivalent properties under Drought, reinforcing the thermodynamic significance of VPD against soil drought forcing for both species at SUMO.

4. Conclusion

We presented a novel metric for the quantification of water status regulation stringency. We showed that this metric is more sensitive to manipulations of VPD compared to existing metrics.

Table 1. Results from the whole-tree chamber *Pinus edulis / Juniperus monosperma* experiment. Statistics of the linear models used to detect the effects of A) Ψ_{pd} and treatment on Ψ_{md} ($R_m^2 = 0.16/0.82/0.78$), B) treatment on $\Delta\Psi$ ($R_m^2 = 0.52/0.31/0.40$), C) Ψ_{hf} and treatment on Ψ_{md} ($R_m^2 = 0.07/0.80/0.76$), D) treatment on A_s ($R_m^2 = 0.75/0.03/0.50$) and E) treatment on A_{Hs} ($R_m^2 = 0/0.02/0.39$). Multiple R² values for each case refer to *P. edulis, J. monosperma* or the two species combined. Sample size n=1462/1512 for *P. edulis/J. monosperma*, for tests A) to C); Samples size n=25/27 for *P. edulis/J. monosperma* for tests D) and E); Appr. d.f., approximate number of degrees of freedom; *, P<0.05; **, P<0.01; ***, at least P<0.001. Results are from the ANOVA table of the mixed effect models using individual tree as the random factor. Interactions are included only when variance inflation factors < 5 and model AIC is lower.

A) Ψ_{md}									
		Pinus edul	lis	Juniperus monosperma			Combined		
Variable	F test	Appr. d.f.	P value	F test	Appr. d.f.	P value	F test	Appr. d.f.	P value
Ψ_{pd}	263.8	(1,1446)	<0.00001 ***	6507.8	(1,1504)	<0.00001 ***	2111	(1,2856)	<0.00001 ***
Treatment	0.19	(4,25)	0.94	1.39	(4,27)	0.26	0.89	(4,55)	0.48
Species							297	(1,71)	<0.00001 ***
Species * Ψ_{pd}							425	(1,2887)	<0.00001 ***
Β) ΔΨ									
	Pinus edulis			Juniperus monosperma			Combined		
Variable	F test	Approx. d.f.	P value	F test	Appr. d.f.	P value	F test	Appr. d.f.	P value
Treatment	0.19	(4,25)	0.94	1.4	(4,27)	0.26	0.89	(4,55)	0.48
Species							155	(1,784)	<0.00001 ***
Species * Ψ_{hf}							425	(1,2887)	<0.00001 ***
C) Ψ_{md}									
	Pinus edulis			Juniperus monosperma			Combined		
Variable	F test	Approx. d.f.	P value	F test	Appr. d.f.	P value	F test	Appr. d.f.	P value
Ψ_{hf}	101.4	(1,1419)	<0.00001 ***	6015	(1,1472)	<0.00001 ***	2401	(1,2900)	<0.00001 ***
Treatment	2.0	(4,25)	0.12	3.8	(4,27)	0.015 *	3.9	(4,56)	0.008 **
Species							478	(1,640)	<0.00001 ***
Species * Ψ_{hf}							1167	(1,2900)	<0.00001 ***
D) A _s									
		Pinus edul	lis	Juniperus monosperma			Combined		
Variable	F test	Approx. d.f.	P value	F test	Appr. d.f.	P value	F test	Appr. d.f.	P value
Treatment	22.6	(4,25)	<0.00001 ***	1.21	(4,27)	0.33	10.2	(4,52)	<0.00001 ***
Species							29.8	(1,52)	<0.00001***
Species * Treatment							1.7	(1,52)	0.16
E) A _{HS}									
	Pinus edulis			Juniperus monosperma			Combined		
Variable	F test	Approx. d.f.	P value	F test	Appr. d.f.	P value	F test	d.f.	P value
Treatment	0.10	(4,25)	0.98	1.2	(4,27)	0.34	0.30	(4,52)	0.30
Species							39.16	(1,52)	<0.00001 ***
Species * Treatment							0.79	(1,52)	0.54

We also showed that regulation against high VPD is a very large component of regulation of plant water status. Metrics for the quantification of water status regulation *in vivo* are complementary to metrics of water use regulation and to mechanistic hydraulic traits, since ultimately the behaviour of field metrics needs to be interpreted in the context of regulation relative to hydraulic damage traits.

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Relationship between trunk water potential and other plant-based indicators for continuous monitoring tree water status

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Abstract: Irrigation scheduling of deciduous fruit trees and vines based on tree water status can be more precise compared to traditional methods focused on weather and soil variables. Plant-based indicators that can be continuously monitored have potential for use in fruit production. Trunk water potential is a plant-based indicator for assessing tree water status and informing irrigation management that can be continuously measured using microtensiometers. However, this new indicator must be validated and key indicators need to be derived for using microtensiometers as a decision tool for irrigation management. Thus, trunk water potential was continuously recorded in a commercial 'Honeycrisp' apple orchard for a month through drought-irrigation cycles and compared with other continuous plant-based indicators such as diurnal trunk diameter variations, canopy temperature, and sap flow. As expected, daily patterns of trunk water potential corresponded well with trunk diameter fluctuations and were negatively related to canopy temperature and sap flow. Maximum trunk water potential occurred at dawn and minimum values occurred during the middle to late afternoon. Seasonal pattern of trunk water potential followed changes in soil water availability and environmental demand. Midday trunk water potential ranged between -0.7 and -2.2 MPa. In particular, values ranging between -0.7 and -1.8 MPa, which is the range from no stress to moderate-severe stress in apple trees, strongly corresponded to increases in trunk maximum daily shrinkage from 75 μ m to 250 μ m, and in a lesser degree with an increase of the tree water-use and an increase in the difference between canopy and air temperatures. Other indicators derived from the trunk water potential, such as the daily minimum and maximum values and the daily range, were also compared. These results suggest that the trunk water potential is a promising candidate indicator for continuous monitoring of tree water status for apples.

Keywords: Canopy temperature, irrigation scheduling, microtensiometer, plant-based sensor, trunk diameter fluctuations, sap flow.



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Fostering Low-cost Drought-phenotyping. Part I: Designing and mounting a custom-made multi-image platform

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Abstract: High-throughput plant phenotyping is one of the most pressing needs to speed up the plant breeding process, which is essential for dealing with climate change. A significant part of the need for plant phenotyping is and will progressively be felt in developing countries with limited financial resources, which has triggered the requirement to develop low-cost systems. Drought is already one of the main limitations to agricultural production and will tend to worsen in the future. Phenotyping plants for drought resistance is therefore crucial. At the Faculty of Science of the University of Lisbon, in a collaboration between the departments of Physics, Computer Science and Plant Biology, we designed and produced a cost-effective phenotyping platform based on image capture in different zones of the electromagnetic spectrum and produced the software to control the equipment and process the captured images. In a nutshell, this platform consists of a metallic backbone structure designed aiming the following main goals: A) To house plants of different dimensions to be sampled and imaged; B) To shield sampled plants from external light; C) To house different light sources emitting in the optical range; D) To hold different imaging devices, namely a thermal camera and 2 RGB cameras; E) To include spectral equipment for leaf analysis; F) To include electrical supply and control for installed devices and light sources.

Keywords: High-Throughput Plant Phenotyping, Low-Cost Phenotyping, RGB cameras, Thermal cameras, Drought-stress

1. Introduction

High-throughput plant phenotyping is one of the most pressing needs to speed up the plant breeding process, which is essential for dealing with climate change (Costa et al. 2019). A significant part of the need for plant phenotyping is and will progressively be felt in developing countries with limited financial resources, which has triggered the need to develop affordable systems (Reynolds et al. 2019; Cudjoe et al. 2023). Drought is already one of the main limitations to agricultural production and will tend to worsen in the future (Seleiman et al. 2021). Phenotyping plants for drought resistance is therefore crucial. In this communication, we will describe the platform in detail, identify the problems encountered in its design and production and indicate the costs associated with its construction.

2. Materials and Methods

At the Faculty of Science of the University of Lisbon, within a collaboration between the departments of Physics, Computer Science and Plant Biology, we designed and produced a cost-effective phenotyping platform based on image capture in different zones of the electromagnetic spectrum and produced the software to control the equipment and process the captured images. In a nutshell, this platform consists of a metallic backbone structure designed aiming the following main goals: a) To house plants of different dimensions to be sampled and imaged; b) To shield sampled plants from external light; c) To house different light sources emitting in the optical range; d) To hold different imaging devices, namely a thermal camera and two RGB cameras (on top and lateral, Fig. 1B); e) To include spectral equipment for leaf analysis; f) To include electrical supply and control for installed devices and light sources.

2.1 Backbone structure

Fig. 1A-C shows the different phases of the production of the prototype. A first preprotype was built with perishable materials (matt black color reinforced cardboard and metal rails for holding and sustaining it) (Fig. 1A).



Fig. 1 A) Set-up for spectra acquisition in the pre-prototype: (A) White LED; (B) Blue/Red LED; (C) White diffuse standard; (D) Fiber tip. B) Technical design of the prototype: a) Aluminum structure; b) RGB and thermographic cameras (a second RGB camera, lateral, is not represented); c) Diffuse illumination, emulating the solar spectrum, with UV range enhancement. C) Aspect of the built prototype and the controlling computer.

The purpose of building the pre-prototype was to evaluate different lighting sources and the best illumination conditions to be adopted in the final design. This preprototype enabled the acquisition of accurate reflectance spectroscopy data which was published in Barradas et al. (2021). The final backbone structure of the prototype was then designed (Fig. 1B) and built in black mate aluminum by an external provider (Fig. 1C).

2.2 Electrical setup

The electrical setup is composed by two main units: protection/power circuitry and switching controller. These units are mounted in a dedicated enclosure and located near the lateral service door (Fig. 2).





Fig. 2 Protection/power circuitry and switching controller; A) General view; B) Detail.

The functions of these two units are as follows:

- Protection/power circuitry: AC power input and breaker circuit. Includes emergency mushroom button for quick power down. Feeds all internal lighting and ventilation parts. A dimmer circuit allows power control of two LED sources.

- Switching controller: allows remote control of all internal active components. This is done using an Arduino microcontroller and a relay board for AC power switching. Arduino board is directly controlled from an external PC running the data acquisition software. The electrical schematic of power and switching circuitry is shown in Fig. 3.





2.3 Light sources

The prototype was equipped with different light sources to emulate as much as possible the wavelength range of the solar light spectrum (up to NIR wavelengths). To have a uniform lighting and to minimize shadow areas in the sample plant, the light sources were mounted on the top and on the sidebars of the cabin (Fig. 4). All light sources were remotely controlled by the dedicated software installed in a PC computer.





Fig. 4 A) Illumination sources: (1) UV lamp; (2) Vis + NIR tungsten lamp; (3) and (4) Visible LEDs; B) UV lamp spectrum; C) Square LED arrays spectrum; D) LED strips spectrum; E) Tungsten bulb lamp spectrum.

On the top of the cabin several light sources were fixed, namely a linear discharge UV lamp (a), a bulb tungsten lamp (Vis + NIR) (b) and two square arrays of visible light emitting diodes (c) with diffusing cover. Linear strip LED arrays (d) emitting in the visible spectrum were placed on two opposite sidebars of the cabin, for lateral illumination of the sample plant. The UV lamp (Eurolite Set 60 cm UV Light, 18W, see the spectrum on Fig. 4B) was chosen with the main aim of studying fluorescence emission from secondary metabolites. Square LED arrays of white light (reference spectrum on Fig. 2C), covered with a diffusing glass providing illumination to the plant from top in different angles were used to obtain visible images, minimizing shadow areas. A tungsten bulb lamp (Philips, Halogen classic, 2800K, 42 W) placed at the top of the cabin was chosen to enhance visible and near infrared images.

2.4 Data acquisition sensors

For data acquisition from the plants, image and spectral sensors were added to the cabin. The imaging sensors consisted of three cameras: Two compact RGB cameras FLIR Flea®3 USB 3.0, one of the cameras being fixed on the top of the cabin, and the other on the rear panel; and a small thermographic camera OPTRIS PI 400i with an optical resolution of 382 x 288 pixels and a 53° x 40° lens. In addition, a spectrometer SPARSEC SP-STD-UV/VIS/NIR with enhanced sensitivity in the UV, using an optical fibre OF-UV-

600-200, was used for light collection was also included in the top of the cabin; the collector tip of the fibre was manually positioned on the region to be analysed.

2.5 Dedicated software development for data acquisition and analysis

PHENO, an original software programmed in Python for data acquisition and analysis, was developed to provide a friendly user interface for adjustments on cameras configuration, selection of illumination, connection to the spectrometer and image analysis software.

2.5.1 Software modules

PHENO is formed by three autonomous modules: PHENO Light Control (PHENO_Lights) that directly controls the illumination system; PHENO Data Acquisition System (PHENO_DAS), designed to control the imaging system and to proceed to image acquisition, while also providing an alternative pathway to control the lightning; and PHENO Data Treatment System (PHENO_DTS), programmed to extract phenotypic traits from the images acquired. Current software version is V1.0.0.

2.5.2 Data acquisition

In the first screen of PHENO DAS it is possible to visualize the live image of the plant (top and side RGB camera and thermographic camera) while adjusting several settings to optimize the image: The exposition (automatic or manually adjusted from 1 to 19) and the gain (automatic or manually adjusted from 0 to 24), for the RGB cameras; the temperature scaling (automatic or manually adjusted from 0 to 100 °C, minimum and maximum temperature limits), for the thermographic camera. The exposition and the gain must be selected in an integrated way with the lighting, which can be chosen by switching on or off the 4 available light sources ("Stripes", "Pannels", "Incandescent" and "UV"). The illumination control panel also includes the possibility of switching the levelling laser on/off. When the acquisition conditions are fine-tuned, the data save directory must be indicated and an acquisition profile may be chosen. In the "Custom" profile, the data acquiring sensors ("RGB – Top", "RGB – Side" and "Thermal") must be chosen. At this point the user must also decide if preliminary analysis is to be performed ("Analyze Results?") and for which type ("Geometric Analysis", "Color" and "Number of Leaves"). In addition to "Custom", three pre-defined plant profiles (corresponding to plants with contrasting body plans) may be selected ("Arabidopsis", "Grapevine" and "Rice"), automatically selecting and locking the corresponding sensors and analysis parameters. After all parameters are set, image acquisition may start by pressing "Start Analysis" and the input of a filename is then requested ("Please input filename:"). The acquired images are thereafter presented on the left part of the screen and three options are offered: to repeat the acquisition ("Retake Snapshot"), to save the images and move for the next plant ("Save and Proceed with Acquisition") or to stop the process ("Finalize Batch Acquisition").

2.5.3 Data treatment

The data treatment proceeds through five stages, presented on the opening screen of PHENO_DTS: 1. Input Selection; 2. Clipping; 3. Segmentation; 4. Output Selection; and 5. Calibration (Fig. 5).



Once completed these five stages data analysis may start ("Launch"). In "1. Input Selection" the type of images to be treated (RGB or Thermal) must be selected and the directory with the images indicated. The number of images in the directory is showed and if the user decides to proceed to next stage those images will be imported. In "2. Clipping" the area of the acquired image to be analysed may be set. Three options are offered: "No Clipping", where the whole image will be processed; "Automatic", where the area to be analysed is automatically set by the software; and "Manual", where the area to be analysed is set by the user, by providing the pixel value of the top and bottom (0 to 1024) and the left and right (0 to 1280) limits of the image. In the third stage ("3. Segmentation") the segmentation method is chosen. If "Automatic" is chosen, the software will automatically select the segmentation method. If "Manual" is chosen, the user will be able to choose the preferred method and, when applicable, the method custom parameters. There are nine segmentation methods available: Three based on thresholding, two based on clustering and three based on other systems. In the next stage ("4. Output Selection") the output directory must be selected, and the type of output trait descriptors may be chosen. In the first level menu the user my select (or not) "Geometric Analysis", "Color Analysis" and "Leaf Characterization". If "Geometric Analysis" is selected, a second level menu allow to select different sets of geometric descriptors: "Basic", "Ellipse Fitting", "Image Moments", "Hull Characterization" and "Density". The number of plant divisions (for each one the descriptor will be presented) may be inputted. The user chooses to

perform (or not) "Enhanced Color Analysis" and also if to (or not) "Save Segmented Images". The final stage ("5. Calibration") is aimed to "Spatial Calibration". This may be automatic, semi-automatic or manual. Spatial calibration will convert pixel to cm². Thereafter, the program may be launch. Image processing may take several minutes. The resulting computed descriptors are presented as a spreadsheet (Fig. 6).

Top Area	Top Radius	Top Perimeter	Тор	Top Max Diameter	Top Hull Perimeter	Top Hull Area	Top Compactness	Тор	Top Skeleton	Top Density
(cm2)	(cm)	(cm)	Roundness	(cm)	(cm)	(cm2)		Circularity	Length (cm)	
45,501946	14,9488675	131,2597608	378,6458864	29,61477769	63,10922123	314,5635002	0,144651068	0,480796406	62,08524582	0,064813208
87,196089	21,0523677	193,6220261	429,9446156	38,6936152	87,68463104	494,5555604	0,176312018	0,45286496	100,7268441	0,062624639
42,533514	16,9174552	95,16477544	212,9221693	32,21155653	58,74385239	202,6675163	0,209868581	0,617285672	43,33032781	0,047305499
55,165228	23,3135150	124,6957019	281,8626612	46,42202953	89,57090153	158,0601569	0,349014131	0,71831587	58,36659829	0,032307253
72,943692	16,6186551	138,6369245	263,4936108	28,52613057	54,75573764	241,3729253	0,30220329	0,394957821	67,79795073	0,084070982

Fig. 6 Partial aspect of the computed results spreadsheet showing some of the most relevant geometric descriptors.

Depending on the selection at stage 4, up to 78 plant descriptors might be presented: 29 geometric descriptors, 45 color descriptors and 4 thermal descriptors.

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Fostering Low-cost Drought-phenotyping. Part II: Testing a custom-made system with *Arabidopsis thaliana* and *Vitis sp.*

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Abstract: The efficiency of the phenotyping platform described in this communication (Part I, Abreu et al. 2024) was tested using plants from two genus: Arabidopsis thaliana and Vitis sp. The model plant A. thaliana has a rosette growth habit, so the shape of its shoot, before flowering, is essentially expressed in the two horizontal axes, with little expression in the vertical axis, making 2D image processing seem appropriate. Vitis sp., on the other hand, are three-dimensionally shaped presenting a complex organization requiring 3D image analysis. Three Arabidopsis thaliana natural genotypes from different geographical origins were used. These genotypes present relative differences in resistance to water stress: Coimbra (Portugal, resistant), Columbia 0 (Germany, intermediate) and Rschew (Russia, sensitive). Two grapevine genotypes were used: one non-grafted Vitis vinifera cultivar ("Cabernet Sauvignon") and a hybrid of Vitis berlandieri and Vitis riparia ("Sélection Oppenheim 4", SO4). In two independent experiments, the plants were grown in a controlled environment and part of the group was subjected to water stress by completely suppressing irrigation. Throughout the stress period, images of the plants were taken on the phenotyping prototype and at the end of the experiment destructive methods were used to determine leaf area by planimetry. Correlation analysis was used to compare the data obtained through automatic image analysis and destructive methods. The correlations between the estimated and measured areas were satisfactory in Arabidopsis when a set of plants with quite different areas was processed, but in Vitis the method has not yet shown the necessary precision. Some causes of error and possible corrections are presented and discussed.

Keywords: Plant phenotyping, Custom-made system, *Vitis sp., Arabidopsis thaliana,* drought stress, computer-vision problems.

1. Introduction

The ability of the phenotyping platform described in this communication (Part I, Abreu et al. 2024) to accurately estimate total leaf area was tested using plants from two genus: *Arabidopsis thaliana* and *Vitis sp*. The model plant *A. thaliana* has a rosette growth habit, so the shape of its shoot, before flowering, is essentially expressed in the two horizontal axes, with little expression in the vertical axis, making 2D image processing seem appropriate. *Vitis sp.*, on the other hand, are three-dimensionally shaped presenting a complex organization requiring 3D image analysis. Several problems have been identified in image-based plant phenotyping. The extraction of quantitative phenotypic traits from images of plants poses a handful of demanding computer vision problems, e.g. analyzing densely packed and occluded plant organs and analyzing images with highly self-similar repeating patterns (Stavness et al. 2023).

2. Materials and Methods

2.1 Plant Material

Three *Arabidopsis thaliana* genotypes (natural accessions) from different geographical origins were used. These genotypes present relative differences in resistance to water stress: Coimbra (Portugal, resistant), Columbia-0 (Germany, intermediate) and Rschew (Russia, sensitive).

Two grapevine genotypes were used: one non-grafted *Vitis vinifera* cultivar ("Cabernet Sauvignon") and a hybrid of *Vitis berlandieri* and *Vitis riparia* ("Sélection Oppenheim 4", SO4).

2.2 Growth Conditions

Seeds of *A. thaliana* genotypes (Columbia (Col-0), Coimbra and Rschew) were surface sterilized and stratified for three days at 4 °C. The plants were grown in a controlled environment chamber (Fitoclima 5000 EH, Aralab, Rio de Mouro, Portugal) in 200 cm³ pots containing the same amount of a mixture of horticultural substrate (Compo Sana Universal, Compo Sana, Spain) and vermiculite (1:1). The substrate was sterilized at 190 °C for 4 hours. Potted plants of *Vitis* genotypes ("Cabernet Sauvignon" and "SO4") were acquired to a commercial supplier (Viveiros do Oeste, Portugal).

Pots were placed in trays, water was provided by sub-irrigation, and the trays were frequently rotated and moved in the chamber. Light, an average photosynthetic photon flux density (PPFD) of 180 μ mol m⁻² s⁻¹, was provided by fluorescent lamps (Osram Lumilux L 58W/840 cool white lamps) with a 14 h photoperiod, under controlled temperature (23/18 °C day/night for *Arabidopsis* and 25/18 °C day/night for *Vitis*) and 50% relative humidity.

2.3 Experimental Design

For each of the three *Arabidopsis* genotypes, 70 seeds were sown. Twenty-four days after sowing (DAS), 60 plants of similar size were selected (one per pot) and randomly assigned to the control group (with normal sub-irrigation along all the experiment, i.e. 100 per cent of field capacity) and the stress group (to be later subjected to water stress by withholding irrigation, see below).

For each of the two *Vitis* genotypes, 60 plants were used. Plants were randomly assigned to controls and water stress as described above.

In *Arabidopsis*, four measurements were taken on the three genotypes used, two before the application of stress (24 DAS and 34 DAS) and two after 7 (41 DAS) and 12 (47 DAS) days of stress. At 24 DAS, 10 plants were used to measure leaf area with non-destructive [PHENO_DTS] and destructive [ImageJ] methods (see below). At 34 DAS another 10 plants were used and the same analyses were carried out. After these analyses, the remaining plants were divided into two groups: 20 control plants under watering and 20 stressed plants with suspended watering. At 7 days of stress (41 DAS), 10 plants were carried out; At 12 days of stress (47 DAS), the last plants were collected for the final analyses.

In *Vitis*, four measurements were taken on the two genotypes used, two before the application of stress and two after 10 and 20 days of stress. Plants started acclimation to the growth chamber 18 days after potting (18 DAP). On the sixth day after acclimation (6 DAA = 24 DAP), 10 plants were used to measure leaf area by non-destructive [PHENO_DTS] and destructive [ImageJ] methods (see below). On the thirteenth day after acclimation (13 DAA), 10 more plants were used, and the same analyses were carried out. After the analyses, the remaining plants were divided into two groups: 20 control plants with sub-irrigation and 20 stressed plants with suspended watering. On the tenth day of stress (23 DAA), 10 plants were collected from each group (control and stress) and the destructive analyses were carried out. On the 20th day of stress (33 DAA), the last plants were collected for the final analyses.

2.3 Non-destructive and Destructive measurement of total leaf area

For non-destructive analyses (total leaf area) pots were placed on the center of the phenotyping prototype and the level of the portable lab jack and of the cabinet shelf, if needed, was adjusted to position the top of the plant canopy in line with the laser beam. The lightning was adjusted, and the images were captured making use of the dedicated PHENO Data Acquisition Software (PHENO_DAS) and analyzed with the PHENO Data Treatment System (PHENO_DTS) (see Abreu et al. 2024, these proceedings).

For destructive measurements of total leaf area all plant leaves were excised, flattened, and scanned in a commercial scanner. The area of scanned leaves was determined using ImageJ software (Schneider et al. 2012).

2.4 Statistical Analysis

Descriptive statistics (mean values and standard deviation) and correlatio analysis was done with Excel software.

3. Results and Discussion

Even though the PHENO_DAS output includes up to 78 plant descriptors (geometric, color and thermal) (Abreu et al. 2024, these proceedings), the aim of this work was to evaluate the ability of this software to accurately estimate plant leaf area and therefore only leaf area results, obtained with the top camera, will be addressed.

Fig. 1 shows the correlation between PHENO_DAS estimated and destructively determined plant leaf area for the three genotypes of *Arabidopsis thaliana* at 34 DAS.



Fig. 1 Correlation between estimated area (cm²) and measured area (cm²) for the *Arabidopsis* genotypes Col_0, Coimbra and Rschew, individually and pooled, 34 DAS.

When genotypes were considered individually, no significant correlations were found between the leaf area measured destructively and the area estimated using PHENO_DTS. This means that when the variations in area are very small (the variation between replicates of the same genotype and treatment would ideally tend towards zero) the measurement errors of both the destructive method and PHENO_DTS weaken the correlation between the two methods. However, when the results of the three genotypes are pooled and analyzed together, adding the inter-genotype variation, we obtained a stronger and statistically significant (p<0.001) correlation between the destructive leaf area measurements and their estimation using PHENO_DTS.

Fig. 2 shows the correlation between PHENO_DAS estimated and destructively determined plant leaf area for the *Vitis* genotype SO4, for each of the four periods after acclimation and for the two conditions (Control and Stress).



As in *Arabidopsis*, no significant correlation between measured and estimated area were found inside each experimental group (date of measurement and watering conditions), but the correlation increased when the results from different experimental groups were pooled (Fig. 2). However, the correlation coefficient in SO4 pooled results (r^2 =0.41), although statistically significant (p<0.01) was lower than the one obtained in *Arabidopsis* (r^2 =0.65; p<0.001). Furthermore, in *Vitis* the correlation seems to be strongly dependent on the results of a group of extremely stressed plants obtained at 28 DAA.

Area (cm², measured)

Both in *Arabidopsis* and *Vitis* the estimated areas are lower than the measured ones, but the difference is much deeper in *Vitis*. The overlapping of leaves can explain the underestimation of digital leaf areas. The overlapping and visual obscuring of different organs, including fruit, is a problem for image-based phenotyping. Recently Kierdorf et al. (2023) developed a method for estimating hidden organs in computer-vision processes. In their specific case, the aim was to estimate the number of grape berries,

bypassing the problem of occlusion by the leaves. Variants of this method may prove useful in overcoming the problem of juxtaposing leaves when estimating the plant's total leaf area.

The more marked underestimation of leaf area in *Vitis*, however, seems to have reasons other than the juxtaposition of leaves. The greater volume of the canopy makes it more common for the depth and field limits of the camera lenses to be exceeded. In addition, this volume tends to create zones with different lighting intensity, despite the care taken in the placement of light sources during the design of the prototype. Sheets that are identical but illuminated differently tend to be segmented differently, introducing errors in the quantification of their area.

The dependence of the accuracy of the results on the species under study is also illustrated by the fact that Pheno_DTS top and side digital areas have previously been successfully used to determine the digital biomass of *Artemisia annua* (García-García et al. 2023).

4. Conclusion

The correlations between the estimated and measured areas were satisfactory in *Arabidopsis*, but they didn't achieve the necessary precision in *Vitis*. Some causes of error have been identified and technological solutions are being sought to resolve them. The exploitation of plant image databases using new algorithms for their segmentation is also a promising avenue for advancing high-throughput image-based plant phenotyping.

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2. Oral Communications

Sesión V: "Aspectos relacionados con el movimiento de agua en plantas"





XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Effect of rootstock on xylem embolism vulnerability in grapevine: from leaf to stem

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Abstract: Severe water stress induces embolism processes in vines. When prolonged over time, these events can disable the hydraulic system of the plant by embolization of the xylem vessels causing its collapse. This work aimed to determine xylem embolism vulnerability in whole-plant through the percentage of embolized pixels (PLC) in different scion and rootstock tissues to understand the staged failure of xylem function under severe drought in Vitis vinifera L. One-year-old Vitis vinifera cv. Tempranillo clone 232 grafted onto two different rootstocks (110 Richter and SO4; n=5) were grown in 12L pots under well-watered outdoor conditions. We monitored water-stress induced xylem embolism in leaves and stems (above and below grafting junction) by using "Cavicams" system, determining the percentage of embolized pixels (at P12, P50 and P88). Pressurevolume curves were also obtained in fully expanded leaves. In both rootstocks, stem did not undergo embolism events until the leaf was fully embolized and the embolism process started from the top to the bottom of the vine, thus suggesting hydraulic segmentation. Moreover, regardless of the rootstock, the P₈₈ in leaves corresponded to less negative Ψ_{leaf} than at P₁₂ in stems (both in scion and rootstocks). Furthermore, significant differences were found in the Ψ at which P₅₀ and P₈₈ occur in scion and rootstock stem tissue. Neither significant rootstock effects were found on any of the parameters derived from pressure-volume curves in the scion leaf, nor on hydraulic segmentation.

Keywords: Vitis vinifera, embolism, water stress, hydraulic segmentation, rootstock.

1. Introduction

Viticulture has experienced significant impacts attributable to the progressive elevated temperatures and arid conditions derived from climate change (IPCC 2023). Water scarcity in Mediterranean regions is projected to intensify notably in the summer, particularly after dry winter and spring seasons (van Leeuwen et al. 2019). This conditions significantly raises the probability of encountering elevated water stress leading to xylem embolism. Xylem embolism involves changing xylem vessel from a water-filled to an air-filled state, which block the water flow within that specific vessel (Tyree and Sperry 1989). When this process occurs in many vessels, it can cause hydraulic collapse inside the plant (Choat et al. 2018). To address this problem in a sustainable and cost-effective way for vineyards, it is essential to select genotypes that exhibit increased tolerance to hydraulic failure caused by water scarcity.

It is well known that the scion genotypes exhibit a wide range of tolerance to soil water deficit (Mairata et al. 2022), and to suffer xylem embolism (Lamarque et al. 2023). Furthermore, selecting tolerant rootstocks to water deficit has been identified as a viable approach for improving vineyard sustainability (Buesa et al. 2023).

Most of phenotyping methods allow the screening of prominent genotypes, but with scarce information on the understanding of the plant hydraulics. However, Brodribb et al. (2016) developed a novel method called the Optical Vulnerability Method (OV) that allows the *in vivo* visualization of the plant's hydraulic system, providing a direct view of xylem cavitation events.

In this study, the hydraulic resistance to water deficit of 'Tempranillo' clone 232 vines grafted onto a more tolerant Richter 110 (R110) and more sensible Selection Oppenheim nº 4 (SO4) rootstocks. For this purpose, the hydraulic performance of leaves, the scion stem (above 5 cm from the graft junction), and the rootstock stem (below 5 cm from the graft junction) was monitored throughout a water stress period. The objectives of this study were: 1) to investigate whether different rootstocks affect water deficit resistance when grafted onto the same scion, and 2) to confirm if the optical vulnerability (OV) technique provides valuable insightful into vine hydraulic system behaviour under water deficit conditions.

2. Materials and Methods

2.1 Plant Material and experimental conditions

The study was carried out in 2023 at the University of Balearic Islands, Spain (39°38'14.8"N, 2°38'51.4"E). Two-year-old grafted plants were grown under field conditions and potted in 12L containers filled with a mix of organic substrate and perlite (4:1). The rootstocks were selected according to its contrasting response to water deficit (Buesa et al. 2023). Thus, Tempranillo clone 232 (as scion) was grafted onto two rootstocks: Richter 110 (R110) (tolerant) and Selection Oppenheim 4 (SO4) (sensible).

2.2 Pressure-volume curves

One leaf per plant was covered at 08:00 for one hour in darkness inside a zip-lock bag with a metallized high-density polyethylene reflective film (Sonoco RF, Sonoco Products Co., Hartsville, South Carolina, USA). The petiole was cut underwater, and then, the leaf (still enclosed) was promptly transferred to the laboratory. To generate the pressure-volume curves, leaf water potential (Ψ_{leaf}) was determined using a pressure chamber (Model 600, PMS Instruments, USA) and simultaneously, the weight of the leaf was recorded immediately before each leaf potential measurement with a precision scale (AS 60/220.R2, RADWAG Wagi Elektroniczne). Pressure-volume curves (n=5) were analyzed according to Dayer et al. (2020), and the following parameters were extracted: osmotic potential at full turgor (Po), leaf water potential at leaf turgor loss point (Ψ_{TLP}), relative water content at turgor loss point (RWC_{TLP}), modulus of elasticity at full turgor (ε), and relative capacitance at full turgor (C_{FT}) and at turgor loss point (C_{TLP}).

2.3 Embolism determination

At noon, a vine of each treatment was uprooted from the pot and the roots were gently cleaned (n=5). Afterwards, the roots were submerged in a container with water and covering the whole plant with a black plastic bag keeping under darkness during 12 hours. At midnight, the vine roots were taken out of the water and placed in the air and in darkness on the laboratory bench. The "Cavicams" were installed and fixed at two different positions on the stem (5 cm above and 5 cm below the graft junction). In addition, a fully expanded mature leaf per plant was installed and scanned in a high-resolution scanning process using an EPSON Expression 10000 XL scanner (Epson America, Inc., Long Beach, CA, USA). Throughout the desiccation process (7 days), images were systematically captured at intervals of approximately 5 minutes, and during first two days, the leaf water potential (Ψ_{leaf}) was measured (Brodribb et al. 2016) (Fig. 1). The slope of the resulting linear regression between the relation of Ψ_{leaf} and time when the leaf starts to embolize until it completes leaf embolism, allowed us to ascertain the potential rise 12%, 50%, and 88% of embolized pixels (P₁₂, P₅₀, P₈₈) across tissues (Brodribb et al. 2016).

2.4 Statistics

The normality distribution analysis using the Shapiro-Wilk test and accordingly, an ANOVA or Kruskal-Wallis tests were applied, and mean comparisons were conducted using the Tuckey's and Dunn's test, respectively. The statistical analysis was carried out using R Studio software (R Core Team, 2023).

3. Results

3.1 Water relations: pressure-volume curves

No significant differences were found between rootstock-scion combinations in the parameters derived from pressure-volume curves, mainly because the wide range of variation among replicates of the values for each parameter. For example, in Tempranillo 232-110R rootstock combination, P₀ values ranged from -1.16 to -0.83 MPa; or from - 1.48 to -1.14 MPa in Ψ_{TLP} (Table 1).

	Po (MPa)		RWC _{TLP} (%)	ε (MPa)	C _{FT} (MPa ⁻¹)	C _{TLP} (MPa ⁻¹)
T232-110R	-0.97 ± 0.03	-1,31 ± 0.03	92.7 ± 0.61	14.34 ± 1.46	0.06 ± 0.01	0.11 ± 0.01
T232-SO4	-1.01 ± 0.04	-1.27 ± 0.05	93.77 ± 0.56	16.43 ± 1.05	0.05 ± 0.00	0.13 ±0.02
Pr > F(Model)	0.872	0.457	0.26	0.306	0.285	0.365
Significant	No	No	No	No	No	No
Mean	-0.99 ± 0.04	-1.29 ± 0.04	93.24 ± 0.58	15.39 ± 1.26	0.05 ± 0.00	0.12 ± 0.01

Table 1. Mean values per combination of the parameters generated from pressure-volume curves.

3.2 Cavitation dynamics

Different levels of embolism (P_{12} , P_{50} and P_{88}) were achieved in the above and below section of the graft point (scion and rootstock respectively), as well as in the leaf in two combinations. There were differences in the leaf water potential values at P_{12} , P_{50} and P_{88} between each combination for the studied tissues. The lowest values were found below the grafting point and the highest values in the leaf tissue. These differences were significant at every moment, demonstrating the hydraulic segmentation of the vine (Table 2). No significant differences were found between combinations at any degree of embolism. The values of water potential from P_{12} to P_{88} leaf were similar in the two combinations, ranging from -1,28 to -1,90 MPa in T232-110R and from -1,53 to -1,87 MPa in T232-110R and T232-SO4 combination, respectively (table 2). Interestingly, embolism process (P_{12}) started at the same water potential above and below grafting point. However, only when the complete vessels were embolised in the upper part of the grafting point (-4,96 MPa), the embolised process at P50 started below grafting (rootstock) in both combinations (Table 2).

Table 2. Mean values of water potential at different levels of embolism (P_{12} , P_{50} , P_{88}) per organ and combination studied (n=5). Within the same genotype, capital letters mean differences between same level of embolism but different tissue, while lowercase letters represent differences between the levels of embolism from the same tissue.

	Leaf	Scion	Rootstock						
T232-110R									
P12	-1.28 ± 0.25A	-2.72 ± 0.31B a	-2.60 ± 0.29AB a						
P50	-1.42 ± 0.28A	-3.41 ± 0.42AB ab	-4.98 ± 0.73B ab						
P88	-1.90 ± 0.18A	-4.96 ± 0.53AB b	-6.85 ± 0.36B b						
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T232-SO4									
P12	-1.53 ± 0.16A	-2.60 ± 0.37A	-2.68 ± 0.52A a						
P50	-1.62 ± 0.16A	-3.65 ± 0.45AB	-4.58 ± 0.76B ab						
P88	-1.87 ± 0.21A	-4.96 ± 0.74B	-6.56 ± 1.50B b						
	Significance of effects between genotypes								
P12	ns	ns	ns						
P50	ns	ns	ns						
P88	ns	ns	ns						

4. Discussion

Our study shows that Tempranillo clone 232 exhibits similar values of P₁₂, P₅₀, and P₈₈ than other studies (Dayer et al. 2020; Lamarque et al. 2023). Moreover, our findings indicate that the stem had greater resistance to cavitation compared to leaves, as previously reported (Charrier et al. 2016; Lamarque et al. 2023). Furthermore, our results found a tendency for scion stem cavitation to begin earlier and at less negative water potentials than the rootstock stem.

5. Conclusion

The application of the optical vulnerability method made it possible to evaluate xylem embolism and hydraulic segmentation in grafted vines. Our study revealed the hydraulic segmentation from leaves to stem, regardless of the rootstock-scion combination analysed. These results, for the first time, reveal a potentially increasing gradient of cavitation tolerance through the graft-union, from leaves to roots. Nevertheless, the two contrasting rootstocks showed neither effects on its resistance to water deficit nor on leaf hydraulic parameters.

Further research should evaluate the effect of rootstocks on the vulnerability of grapevine xylem embolism in plants grown under water deficit conditions, so that rootstocks can better express their adaptive response to water deficit.

Acknowledgments

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Polyploid trees and water use efficiency: experiments with subtropical fruit crops

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Abstract: The coastal south of Spain is the main producer of subtropical fruit crops in continental Europe, but faces the negative effects of climate change, such as the extreme depletion of water for irrigation. Tree crops are affected by water scarcity, but their hydraulics is particularly understudied.

Given that the hydraulic function of trees depends largely on the structure and arrangement of the vascular tissues from source to sink organs, we performed detailed evaluations of the elements composing the phloem and the xylem tissues in diploid and polyploids of two important subtropical crops, mango and a hybrid of cherimoya named atemoya. We performed experiments in controlled conditions applying water scarcity treatments in the soil to understand their physiological performance.

Tetraploids of both tree species showed contrasting phenotypes depending on the soruce of polyploidization, more vigorous in autopolyploids and dwarf like in allopolyploids, but both displayed anatomical enlargement of the elements composing the xylem and the phloem along the transport pathway that runs from source (leaves) through the stems to sink (reproductive) organs. Xylem sap flow, measured through an implemented technique to track the conductivity of leaf veins and flower carpels in vivo revealed different speeds across individuals with different chromosome numbers.

Diploids showed a highest physiological performance under good irrigation, but consumed more water from the soil. Physiological variables were similar under good irrigation, but under water scarcity, diploids reduced plant conductance to a minimum compared with tetraploids. Further work is under evaluation, but our results point to polyploids as alternative fruit crops with higher resilience in times of water scarcity.

Keywords: polyploids, fruit tree crops, drought, phloem, xylem, conductivity.



Evaluation of Huber value role in carbon and water relationships across fruit tree species

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Abstract: Crop plants are particularly susceptible to water scarcity, likely due to historical breeding focused on productivity and fruit quality rather than drought resistance. In the context of climate change, the ability of plants to achieve productivity with minimal water usage is becoming increasingly relevant. To compare how different plant species respond to varying environmental conditions in terms of carbon and water utilization the trait-based approach of economic spectrum has been proved useful, especially in ecological studies. Among these traits, the Huber value (H_v), representing the ratio of sapwood area to leaf area, has been shown to play a pivotal role in understanding plant water transport and leaf function. The goal of this work was to evaluate the relevance of H_v in the water and carbon utilization patterns of various fruit tree species (almond, apricot, plum, peach, olive, nectarine, khaki, pomegranate, two varieties of vine, lemon, orange, grapefruit) under the same experimental conditions, with well-watered soil and high atmospheric demand. A significant positive correlation (p<0.05) between H_v and traits related to carbon use and allocation (e.g., photosynthesis, leaf mass area) was found. Conversely, there was a negative correlation with other anatomically derived stem hydraulic traits, such as vulnerability. This suggests that species with lower canopy areas relative to sapwood area tend to have high acquisitive leaves, i.e., larger radiation-intercepting leaf area for the required mass investment as well as high area-based photosynthesis. The lower leaf area compared to sapwood area would also act as a buffer against vulnerability. We found that Prunus species, particularly almonds, exhibited significantly higher H_v values compared to Citrus species, including orange, lemon and grapefruit. Our research offers new insights into the coordination of H_v with other ecophysiological traits which is not well understood.

Keywords: Hydraulic traits, huber value, crops, photosynthesis.



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Plant water relations in wild tomatoes and relatives are better explained by leaf venation traits than by hydraulics

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Abstract: The leaf hydraulics theory and the Leaf Economics Spectrum delineate a resource conservative-acquisitive axis of leaf trait variation and describe global tradeoffs between leaf morphology and function. Discrepancies arise within narrow plant groups due to phylogenetic constraints to trait variation, highlighting higher adaptive importance for particular leaf traits. The wild tomatoes and relatives constitute a group of closely related species inhabiting contrasting habitats across South America. We aimed to understand which are the most determining traits and trait relationships driving wild tomatoes' adaptation, and if global tradeoffs occur within this clade. All the species and some domesticated accessions were grown under the same conditions, outdoors in the Mediterranean summer. We studied water relations at leaf level, considering leaf hydraulics, venation and stomatal conductance, to determine which are the pivotal drivers of leaf gas exchange and leaf morphology. We framed it within a comparison with literature data. Results showed a coordination of leaf vein density with stomatal conductance and photosynthesis, and in turn with leaf size and leaf mass per area, which was not found among the vast range of species in literature. Contrarily, the correlation of hydraulic conductance with photosynthesis and gas exchange found in literature did not occur in the tomatoes, showing a decoupling of leaf hydraulics and venation traits in this group. Thus, limitations to gas exchange in the tomatoes are more related to CO₂ diffusion than hydraulics. Moreover, species from arid habitats followed a drought-escape strategy, favouring pulse-growth in suitable periods, resulting in leaf traits not predicted from global trends. In this framework, domesticated tomatoes had extreme values for most traits, reflecting modifications during domestication.

Keywords: Venation, hydraulics, gas-exchange, tomato species radiation.

1. Introduction

The Leaf Economics Spectrum (LES) delineates global tradeoffs in leaf morphology and structure (e.g., Wright et al. 2004, Díaz et al. 2016). Leaf hydraulics traits evolve in concert with leaf photosynthetic function (Scoffoni et al. 2016) and play an important role in plant evolution and adaptation to different ecological niches (e.g. Boyce et al. 2009, Rockwell and Holbrook 2017). Traits like leaf hydraulic conductance (K_{leaf}) and the bulk modulus of elasticity (ε_{max}) strongly affect stomatal control and closure (Brodribb and Holbrook 2003). Leaf venation properties that allow water supply more efficiently facilitate stomata to stay open, increasing CO₂ supply for photosynthesis. Furthermore, relationships between venation, hydraulics, and gas exchange are further modified by leaf morphology and climate (e.g. Sack and Holbrook 2006, Blonder et al. 2017).

The variable importance of hydraulic conductance and venation in defining photosynthetic function may depend on the phylogenetic group and the environment in which evolved (Sack et al. 2013, Blonder et al. 2016). Nevertheless, phylogenetic or habitat-related constraints may limit variation in specific parameters, pushing particular plant groups to increase variation in others (e.g. Jones et al. 2013 and references therein), which may be a major factor limiting the existence of the global tradeoffs.

In this study, we examine the leaf hydraulics theory and its relationship to the LES in wild tomatoes and relatives (*Solanum* sect. *Lycopersicon*, sect. *Lycopersicoides* and sect. *Juglandifolia*), a group of 15-17 closely related species with monophyletic origin, radiated ca. 6 Mya (Särkinen et al. 2013). Because of the extreme diversity in habitat conditions across the species (ca. 3500 km in latitude, and ca. 3500 m in altitude, reviewed in Peralta et al. 2008), this clade is well-suited to investigate the aforementioned relationships. Past studies in the tomatoes and relatives showed weak coordination of leaf morphology with gas exchange traits (Muir et al. 2017) and also with growth-related traits (Conesa et al. 2017). Because that, we hypothesize that leaf hydraulics and venation traits may drive the coordination between leaf structure and function in this group. We also compared wild species to cultivated tomato varieties to understand which traits and trait relationships have been selected upon during domestication.

2. Materials and Methods

2.1 Plant material, growth conditions, and climate classification

16 tomato accessions were grown outdoors at the University of the Balearic Islands. Wild accessions included the two *Solanum* sect. *Lycopersicoides* species (*S. lycopersicoides*, *S. sitiens*), the two sect. *Juglandifolia* species (*S. juglandifolium*, *S. ochranthum*), and 9 sect. *Lycopersicon* taxa (*S. pennellii*, *S. arcanum*, *S. peruvianum*, *S. neorickii*, *S. chmielewskii*, *S. pimpinellifolium*, *S. cheesmaniae*, *S. galapagense*, and *S. lycopersicum* var. *cerasiforme*). The domesticated accessions included 'Roma VF' and two 'Ramellet' accessions from the University of the Balearic Islands (UIB) seedbank. The wild species were classified into

different climatic groups: arid (AR; $M_{SP} < 12$), semi-arid (SA; $M_{SP} 12-24$) and humid (HU; $M_{SP} > 24$). HU was divided in shrub-like (HU-s) and the liana-like (HU-I). Domesticated as DO (see Conesa et al. 2017 for details).

2.2 Leaf morphology, venation and hydraulics

The leaf area per leaf (LA₁) was measured with Image J. Dried leaflets were weighed to calculate the leaf mass area (LMA). Venation was measured in leaflet pieces of 1 cm². Clearing was performed in customized PVC mini-trays following a protocol based on 1.25M NaOH, 2.5% NaClO, EtOH series of 50% and 100%, and stained with 1% safranin solution. Vein network was automated in LEAF GUI (Price et al. 2011). Average vein length (L) and width (W), total vein density based on vein length (VdL, mm mm⁻²), which was instead decomposed in the densities for major veins (MVdL; 1st to 3rd order in a leaflet) and for minor veins (mVdL; 4th and further order veins). Pressure-volume relationships were determined as in Corcuera et al. (2002) and Brodribb and Holbrook (2003). The water-relation parameters analyzed were: leaf water potential at the turgor loss point (Ψ_{TLP}), maximum bulk modulus of elasticity (ε_{max}), osmotic potential at full turgor (π_0) and the relative water content at the turgor loss point (RWC_{TLP}). The leaf hydraulic conductance (K_{leaf},) was measured following the methodology described by Brodribb and Holbrook (2003).

2.3 Leaf gas exchange measurements

The net CO₂ assimilation rate (A_N) and stomatal conductance to H₂O (g_s) were measured using an open-path infrared gas exchange analyzer (Li-6400, Li-Cor Inc., Lincoln, NE, USA) with a 2 cm² leaf chamber fluorometer. Point measurements were taken after steady-state conditions at leaf temperature of 25°C, 400 ppm of CO₂, saturating irradiance (1500 μ mol quanta m⁻² s⁻¹) with 10% of blue light.

3. Results & Discussion

The trend across tomato climatic groups for the total (VdL) and minor vein density (mVdL) was to decrease with humidity (Fig. 1). This depicts a positive relationship between hydraulic conductance (K_{leaf}) and VdL across tomato groups that agrees with global observations by Sack and Holbrook (2006). The correlation between K_{leaf} and VdL was non-significant for wild species (P>0.05). Thus, there is a lack of coordination between leaf hydraulics and venation in the tomatoes. The highest VdL in the AR (Fig. 1) agrees with studies showing that VdL correlates with aridity across diverse species (Sack and Scoffoni 2013). Thus, the maintenance of maximum VdL in AR despite of the low K_{leaf} could respond to vein over-investment allowing maximization of net photosynthesis (A_N) and stomatal conductance (g_s), being as high as in SA and HU-s (Fig. 2).

The lowest K_{leaf} values were found in HU-s and HU-I (Fig. 1). Within angiosperms, the hydraulics theory predicts higher K_{leaf} in species from moister habitats (Sack and

Holbrook 2006), the opposite to what we found in tomatoes. The unexpectedly high K_{leaf} in AR and SA (Fig. 1) implies a water-profligate strategy in arid climate species, which can be an advantage in dry and nutrient-poor habitats (Querejeta et al. 2018). In fact, AR species tended to exhibit a drought escape strategy with pulse-growth behavior in suitable periods, having cheap leaves with lowest LMA and fast turnover (Conesa et al. 2017). There were no differences in leaf water potential at turgor loss point (Ψ_{TLP}) and osmotic potential at full turgor (π_0) among tomato climatic groups (Fig. 1). Ψ_{TLP} is considered a key indicator of drought tolerance because leaves with lower Ψ_{TLP} are able to maintain function longer during soil dehydration (Bartlett et al. 2014). Consequently, hydraulics traits did not explain differences in aridity among tomato climatic groups. On the contrary, tomato species from humid habitat and with liana-like plant habit (HU-I) had lower Ψ_{TLP} and π_0 than any other group (Fig. 1).

There was no correlation of K_{leaf} with A_N and g_s in the tomatoes (Fig. 2), reflecting the lack of coordination of hydraulics also with leaf gas exchange parameters. On the contrary, the correlations were significant when considering diverse species (Fig. 2), indicating the role of K_{leaf} in determining maximum g_s and A_N at broader phylogenetic scales (Sack and Holbrook 2006, Blonder et al. 2017). In contrast to hydraulic traits, VdL correlated with gas exchange traits in the tomatoes (Fig. 2), reflecting the importance of venation rather than hydraulics in explaining variation in leaf function in this clade.



Fig. 1 Variation across climatic groups (arid, AR; semi-arid, SA; humid with shrub habit, HU-s, and with liana-like habit, HU-I; domesticated, DO), for (left column, top to bottom): total vein density (VdL), separating major (black) and minor (grey) vein densities, average vein length (L) and width (W), and vein areole eccentricity (Eccentricity), that ranges from 0 to 1 (completely round areoles to parallel veins,

respectively); and for (right column, top to bottom): hydraulic conductance on area basis (K_{leaf}), leaf water potential at turgor loss point (Ψ_{TLP}), osmotic potential at full turgor (π_0), and modulus of elasticity at full turgor (ϵ_{max}). Bars are averages including all replicates per species, and error bars are the S.E. Letters on the error bars indicate nested ANOVA-Tukey (species nested within climatic group) differences among groups at α =0.05.

Across climatic groups, the highest LMA corresponded to species from HU-s and HU-l habitats (data not shown). This agrees with past studies in wild tomatoes showing a trend to increase LMA with humidity (Easlon and Richards 2009, Conesa et al. 2017, Muir et al. 2017), which is opposite to predictions of the global leaf economics spectrum (LES), (Reich 1998, Wright et al. 2005). The low predictive power of LMA in tomatoes might relate to the narrow range that this parameter covers along the LES spectrum, there is the 2-fold variation in the tomatoes (Fig. 2) and the up to 100-fold variation in the global LES (Wright et al. 2004). Contrary to hydraulics traits, LMA correlated negatively with vein traits in the tomatoes (Fig. 2). The inverse relationship between both traits has also been described across diverse species sets (Sack et al. 2013). Therefore, these results suggest a central role of the leaf vein system in explaining environmental adaptation in the tomatoes, while other traits thought to have a central adaptive role, like LMA and K_{leaf} , cannot explain the variation existing among the tomatoes regarding climatic distribution, leaf morphology and gas exchange function.



Fig. 2 Correlation of hydraulic conductance (K_{leaf} ; A, C, E, G) and vein density (B, D, F, H) with leaf mass per area (LMA; A, B), and leaf area per leaf (LA₁; C, D). net photosynthesis (A_N; E, F), and stomatal conductance to water (g_s; G, H). Species codes and colors as in Fig.3. The S.E. is indicated in the tomatoes for all parameters (n=3-5). Grey dots represent data from higly diverse species from Brodribb et al. (2007), Flexas et al. (2013), Sack and Scoffoni (2013), Sack et al. (2013), Price and Weitz (2014), and Scoffoni and Sack (2017). Vein density corresponds to total veins (V_{dL}) for accessions in this study and for literature. Regression lines only indicated when *P*<0.05, always separately for grey dots (grey line) and accessions in this study (black line).

When compared to the wild species climatic groups, the domesticated accessions group (DO) had consistently higher K_{leaf} , Ψ_{TLP} , π_0 and RWC_{TLP} and among the highest A_N, and lower ε_{max} , VdL, MVdL, mVdL and W (Figs. 1,2). Furthermore, leaf morphology traits are the most obvious characterizing the DO, with ca. 3-fold larger LA₁ than those in the AR, SA and HU-s, and with the highest LMA among all groups. Leaf size increase related to the domestication process (Evans et al. 1993), as it seems also the high K_{leaf} values.

4. Conclusion

In the tomatoes, the weak coordination of gas exchange with leaf morphology, growth and hydraulics (Fig. 2) indicates that different trait combinations may be similarly adaptive. Results also indicate that hydraulic traits are not the main limitation to gas exchange in the tomatoes, which could better relate to CO₂ diffusion limitations and affected by the venation system (Fig. 2). Indeed, the pulse-growth behavior in arid habitat tomatoes may shape variation in leaf structure and function in this species group.

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3. Posters





XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Improving the soil-water management strategies in almond orchards under drought conditions

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Abstract: This work summarizes the main results obtained in terms of the agronomical and physiological responses of almond trees (*Prunus dulcis* Mill., *cv*. Guara) when are cultivated under organic (OS) and conventional (CS) agricultural systems, together with the introduction of regulated deficit irrigation (RDI) strategies and different soil management (SM) systems. In relation to the crop physiological responses, the highest differences were observed among the irrigation regimes for the stem-water potential (Ψ_{stem}), followed by the net photosynthesis rates (A_N). This physiological trait was also found in terms of almond yield, with kernel yield losses of 25% and 60% for RDI under CS and OS, respectively. According to these findings, it can be concluded that CS are congruent with RDI and cover crop implementations, without affecting the almond yield. In addition, when these strategies are developed under OS, although the soil quality parameters can be improved, the final production could be committed.

Keywords: Water stress, soil fertility, production systems, deficit-irrigation, water potential, net photosynthesis rates, irrigation water productivity.

1. Introduction

Almond tree (*Prunus dulcis* Mill.) represents the second woody crop most important in Andalusia (S Spain), and from 2017 to 2022 this surface has been increased by 22%, as result of implanting new almond orchards in irrigated areas (most of them with a structural water scarcity), significantly improving productivity and farmers' income in the long-term (Expósito and Berbel 2020). Consequently, for these scenarios it is urgent to enhance the water-use efficiency by implementing deficit-irrigation strategies (DI);

with other agronomical practices focused on reducing the evapotranspiration surface (García-Tejero and Durán 2022).

Additionally, in Andalusia, almost 25% of total almond area is developed under organic system (OS). Although the actual yields obtained under this strategy are below to those cultivated under conventional system (CS), the improvements in the nut quality can help to obtain higher prices (130% higher in almond obtained under organic system strategies in comparison to conventional practices). Moreover, taking into consideration the new European agriculture paradigm described in the Common Agricultural Policy 2023-2027, the performance of plant cover crops in agricultural soils would help to restore the soil quality and increasing the agroecological biodiversity (Cárceles et al. 2023). In this line, this strategy would suppose a key challenge under water scarcity scenarios, when DI must be applied.

We hypothesize that it is possible to combine the plant covers for improving soil health and quality with sustainable water saving strategies in almond plantation. Here we present the main results in productivity terms obtained from almond trees cultivated under CS and OS and subjected to different irrigation treatments combined with plant covers.

2. Materials and Methods

The trial was conducted in an experimental orchard of mature almond trees belonging to the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA) ($37^{\circ} 30' 38,55'' N; 05^{\circ} 57' 44,98'' W$). Trees are 6 x 7 m spaced, and with drip irrigation by using two pipelines. Two experimental plots, under CS and OS, were studied, defining two irrigation strategies as follows: i) a full irrigated treatment (FI), which received the 100% of the crop evapotranspiration (ET_c); ii) and a regulated-deficit irrigation (RDI) treatment, which was irrigated applying the 80% of ET_c during the vegetative stage and post-harvest stages, whereas, during the kernel-filling stage, trees were irrigated applying 40% of ET_c. According to this design, the ET_c during the irrigation period, rainfall and irrigation doses applied on FI and RDI were 820, 169, 625, and 362 mm; respectively.

In relation to soil management strategy; under OS, a commercial green crop (Arboplus Cover Nitro [®] by Limagrain Ibérica (Navarrra, Spain) was planted. At midday of April, and once the cover crop had reached a certain development, two different managements were imposed: i) its incorporation as green manure, managed by disk narrow incorporation (DNI) into the soil; ii) its conservation as plant-based mulching (PBM), based on leaving the plant cover residues on the soil once cleaned the cover crop. Regarding to the soil management in CS, two strategies were performed: i) keeping the surface without spontaneous plants applying mechanical control by DNI and chemical mowing, ii) a PBM strategy, sowing the same green crop used in OS and leaving the plant

cover residues on the soil once cleaned the cover crop at midday of April; with additional chemical mowing once done to avoid the spontaneous nascence.

Physiological measurements were fortnightly developed at midday, including the chlorophyll content index (CCI); stem water potential (Ψ_{Stem}), photosynthesis rate (A_n) and stomatal conductance (g_s).

At the end of the season, harvesting was done by using a mechanical vibrator and peeling to remove the hull. Almonds were air-dried and weighted once reached a humidity content below to 6%, estimating the kernel unit weight (with and without shell) and the ratio between them.

3. Results

Table 1 shows the average values of physiological parameters monitored during the kernel-filling (K-F) period. Non-significant differences were observed for the chlorophyll content index as response to water management (WM) and soil management (SM) strategies. By the contrast, WM promoted significant differences in the values of Ψ_{stem} , g_s, and A_n. These differences were more evident under CS in comparison to OS. Additionally, SM promoted differences on Ψ_{stem} under CS without observing a similar trend in the case of OS; and without being affected the remaining physiological parameters assessed during the K-F stage. Focusing the attention on the crossed effects (WM x SM), the main effects were determined as response to the WM, although it was remarkable the improvements on A_n under FI and the presence of mulching in comparison to FI and bare soil.

Yield values were in accordance with the physiological response for the different WM and SM strategies (Table 2). Thus, under conventional management, no significant differences were observed as result of the irrigation treatments imposed, whereas with respect to the soil management, significant improvements were found when the mulching strategy was implemented, taking into consideration the crossed effects, trees reduced its productivity in FI_DNI with respect to FI_PBM plots, and in RDI_PBM with respect to RDI_DNI.

Regarding to the kernel unit weight, higher values were reached under FI, whereas in the case of Ratio, the best results were denoted for RDI strategies. Finally, as it was expected, RDI promoted high improvements on IWP comparing to FI, without effects because of the presence/absence of cover crops.

In relation to the OS, significant yield reductions under RDI and without relevant impact by the implementation the cover crops were observed (Table 2), the best productivity being registered under FI_PBM, followed by FI_DNI and RDI_DNI, and the lowest yield under RDI_PBM. No effects on kernel unit weight and Ratio were observed, whereas, as it was observed for CS, the IWP was significant increased under RDI strategies. It was noticeable that, in the RDI strategy under OS, the PBM worsened the results by comparing to DNI, as the contrary to FI, where the PBM improved the results in comparison to DNI.

Management	W	M	SM		WM x SM				
System	FI	RDI	PBM	DNI	FI_PBM	FI_DNI	RDI_PBM	RDI_DNI	
	ССІ								
CS	21.1a	22.7a	22.3a	21.5a	21.8a	20.4a	22.8a	22.6a	
OS	19.4a	20.4a	19.8a	20a	19.9a	18.9a	19.7a	21.1a	
	$\Psi_{\sf stem}$ (MPa)								
CS	1.3a	-1.7b	-1.6b	-1.4a	-1.3a	-1.2a	-1.8b	-1.6b	
OS	-1.3a	-1.6b	-1.4a	-1.5a	-1.3a	-1.3a	-1.5b	-1.6b	
	g _s (mmol m ⁻² s ⁻¹)								
CS	0.2a	0.16b	0.19a	0.17a	0.21a	0.19a	0.16b	0.15b	
OS	0.16a	0.12b	0.15a	0.13a	0.16a	0.15a	0.13ab	0.10b	
	A _n (μmol m ⁻² s ⁻¹)								
CS	16.1a	11.5b	14.3a	13.4a	16.9a	15.3b	11.6c	11.4c	
OS	11.2a	10.4a	10.9a	10.7a	11.1a	11.2a	10.7a	10.1a	

Table 1. Average values of chlorophyll content index (CCI), stem water potential (Ψ_{stem}), stomatal conductance (g_s), and net photosynthesis (A_n).

CS, conventional system; OS, organic system; WM, water management; SM, soil management; FI, full irrigation; RDI, regulated-deficit irrigation; PBM, plant brushcutter/mulching; DNI, disk narrow incorporation; CCI, Chlorophyll content index; Ψ_{stem} , stem water potential; g_s, stomatal conductance; A_n, net photosynthesis rate. Different letters evidence significant differences (p <0.05) within each factor by the Tukey Test.

Management	WM		SM		WM x SM			
System	FI	RDI	PBM	DNI	FI_PBM	FI_DNI	RDI_PBM	RDI_DNI
	Yield (in-shell almond; kg ha ⁻¹)							
CS	1,405a	1,483a	1,578a	1,310b	1,562a	1,431ab	1,594a	1,372b
OS	1,097a	831b	984a	945a	1,253a	941b	714c	949b
	Kernel unit weight (g)							
CS	1.13a	1.00b	1.07a	1.06a	1.17a	1.09ab	0.96b	1.03b
OS	1.15a	1.14a	1.13a	1.16a	1.12a	1.17a	1.14a	1.14a
	Ratio							

Table 2. Average almond production, yield components, and irrigation water productivity

CS	0.33b	0.35a	0.34a	0.34a	0.33b	0.33b	0.35a	0.35a
OS	0.31a	0.31a	0.30a	0.31a	0.30a	0.31a	0.30a	0.31a
	IWP							
CS	0.24b	0.42a	0.35a	0.32a	0.25b	0.23b	0.44a	0.40a
OS	0.18b	0.23a	0.20a	0.21a	0.20b	0.15c	0.20b	0.26a

CS, conventional system; OS, organic system; WM, water management; SM, soil management; FI, full irrigation; RDI, regulated-deficit irrigation; PBM, plant brushcutter/mulching; DNI, disk narrow incorporation; IWP, irrigation water productivity. Different letters evidence significant differences (p <0.05) within each factor by the Tukey Test.

4. Discussion

Almond can be considered as a suitable option in agricultural lands with structural water limitations because of its positive response to DI strategies (García-Tejero and Durán, 2018), this being able to obtain acceptable yields when moderate RDI strategies are applied (~3,000 m³ ha⁻¹), as it has been recently discussed by Mirás-Avalos et al. (2023). Additionally, not only the water supply will determine the final yield, but the irrigation strategy imposed when the full crop water requirements cannot be applied, avoiding the maximum water stress levels during the most critical phenological periods. A large part of the recently peer-reviewed literature for almond response to water stress have been reported the absence of yield losses when the water restrictions are applied during the kernel-filling stage (Cornacchia et al. 2010; Egea et al. 2009). In our case, with water withholdings around 20% during vegetative and postharvest period and 60% during kernel-filling stage, yield reductions agreed to the yield functions defined by Mirás-Avalos et al. (2023), assuming that water savings around 50% would allow final yields similar to full-irrigated conditions or with moderate reductions ~20%.

Combining a proper management of soil and water resources, with an adequate plant cover control, avoiding competition with the main crop for the water and soil nutrients will be crucial in implementing cover crops under drought conditions. The findings from this work emphasize that plant covers can be used in CS without significant effects on final yield. Moreover, when this strategy is implemented under RDI strategies, the plant cover presence as mulching can improve the almond yield. Additionally, under OS the effects of cover crops under RDI strategies could reduce the yield, although all these issues must be studied during additional seasons to conclude the best strategy to reach an equilibrium between sustainable water savings and the implementation of cover crops.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Fiabilidad de la monitorización del contenido de agua del suelo para determinar el estado hídrico de la vid

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Resumen: La disponibilidad de agua es clave para el rendimiento de la viña y la calidad de la uva, por lo que monitorizar el estado hídrico de la vid es esencial para optimizar las prácticas vitícolas. Los sensores de capacitancia permiten determinar el contenido de agua en el suelo (θ_s); sin embargo, sus registros deben relacionarse con un indicador fiable del estado hídrico del cultivo. El objetivo de este trabajo fue evaluar la fiabilidad de las medidas obtenidas con sensores de capacitancia como indicadores del estado hídrico de la vid bajo dos estrategias de gestión del suelo (laboreo y vegetación residente). Durante la campaña 2023, en un viñedo (*Vitis vinifera* L. cv. 'Riesling') en Salas Bajas (Huesca) se monitorizó θ_s con sensores de capacitancia a tres profundidades: 10, 30 y 50 cm. El potencial hídrico de tallo a mediodía solar (Ψ_{tallo}) se usó como indicador de referencia del estado hídrico de la vid. Los datos se analizaron mediante regresión, observándose que θ_s a 10 y 30 cm se correlacionó significativamente con Ψ_{tallo} , con coeficientes de correlación (r) superiores a 0,92. A 50 cm, r fue inferior (0,71), pero significativo. En las condiciones de este estudio, los sensores de capacitancia pueden ser útiles para determinar el estado hídrico de la vid a tiempo real.

Palabras clave: potencial hídrico de tallo, Riesling, sensor capacitancia, Vitis vinifera L.

1. Introducción

La disponibilidad de agua es uno de los factores más limitantes para la productividad del viñedo, especialmente en climas áridos y semiáridos (Medrano et al. 2015). El déficit hídrico puede afectar negativamente al rendimiento de la vid y a la acumulación de azúcares en la uva. Por otra parte, en zonas más húmedas, el estado hídrico es un factor clave en la maduración de la uva y la calidad del vino resultante (van Leeuwen et al. 2009). El estado hídrico del viñedo se puede modular con el riego, pero también con

otras técnicas como la implantación de cubiertas vegetales, que ofrecen una amplia gama de beneficios medioambientales (Celette et al. 2008). No obstante, la cubierta vegetal compite por el agua con el viñedo (Medrano et al. 2015).

Existe una gran variedad de métodos que permiten estimar el estado hídrico de la vid (Rienth y Scholasch 2019). Entre ellos, el potencial hídrico de tallo medido al mediodía solar (Ψ_{tallo}) se considera el método de referencia debido a su estabilidad frente a variaciones ambientales en el momento de las medidas y su sensibilidad para detectar diferencias en el estado hídrico de plantas sometidas a diferentes tratamientos (Choné et al. 2001). Sin embargo, no permite obtener registros continuos de datos. Por el contrario, el contenido volumétrico de agua en el suelo (θ_s) puede monitorizarse utilizando sensores de capacitancia, lo que puede permitir una estimación en tiempo real del estado hídrico del viñedo. No obstante, es necesario validar los registros de estos sensores con un indicador de referencia. Algunos estudios han mostrado que θ_s y Ψ_{tallo} están estrechamente relacionados (Williams y Trout 2005), mientras que otros no detectaron relaciones significativas entre ambas variables (Asenjo y Yuste 2003).

El objetivo de este trabajo es evaluar la fiabilidad de los registros de θ_s obtenidos con sondas de capacitancia sin una calibración específica previa como indicadores del estado hídrico de cepas de vid (*Vitis vinifera* L. cv. 'Riesling') bajo dos estrategias de gestión del suelo (laboreo y vegetación residente).

2. Materiales y Métodos

2.1 Descripción del viñedo de estudio

El ensayo se llevó a cabo en el año 2023 en un viñedo particular de 1,8 ha localizado en Salas Bajas (Huesca), en la comarca del Somontano (42º 5' 33,5" N, 0º 5' 8,9" E, 440 m). Este viñedo se plantó en 2013 con *Vitis vinifera* L. cv. 'Riesling' injertada sobre 1103 Paulsen a un marco de 2,8 × 0,9 m (3968 cepas/ha). Las cepas se conducen en espaldera con un sistema de cordón simple y orientación este – oeste. El viñedo se maneja en condiciones de secano y, durante sus 5 primeros años de vida (2013-2017) se evitó el crecimiento de vegetación en las calles, mientras que durante los siguientes 5 años (2017-2022) se dejó crecer la vegetación residente, segándola al alcanzar una determinada altura. El 30 de mayo de 2023 se realizó un laboreo reducido (10-15 cm de profundidad) en 8 calles para determinar los efectos de esta práctica sobre el estado hídrico de la vid (Fig. 1). Esta operación se repitió a mediados de junio cuando el suelo estaba más húmedo debido a las lluvias registradas a finales de primavera.

El suelo es de textura franco-arenosa (62,9% arena, 23,2% limo y 13,9% arcilla), con un contenido medio en materia orgánica (1,97%) y pH básico (8,04). El θ_s a capacidad de campo es 22,3% en volumen, mientras que θ_s a una tensión de -1,5 MPa es 12,3% en volumen. La capacidad de almacenamiento de agua en este suelo se estima en 145 mm para los primeros 80 cm de profundidad.

Para el período 2005-2022, la temperatura media anual es 8,4 ºC, la precipitación y la evapotranspiración de referencia anuales ascienden a 462 y 1205 mm, respectivamente.



Fig. 1 Calles del viñedo de estudio: cubierta vegetal (izquierda) y laboreo (derecha).

2.2 Monitorización del contenido de agua en el suelo y del potencial hídrico en la planta Se instalaron dos conjuntos de sensores por tratamiento (laboreo y cubierta vegetal) para medir θ_s (TEROS10, Meter Group Inc. Pullman, Washington, USA) a 10, 30 cm y 50 cm de profundidad en las filas de cepas. Los sensores se colocaron entre dos cepas, a una distancia aproximada de 40 cm del tronco de cada una. Se emplearon dataloggers (ZL6 Pro, Meter Group Inc. Pullman, Washington, USA) para registrar los datos.

Entre junio y septiembre, se tomaron medidas de Ψ_{tallo} en las dos cepas que se encuentran al lado de los sensores con el fin de correlacionarlas con los registros de θ_s . Adicionalmente, se midió Ψ_{tallo} en otras 5 cepas por tratamiento para conocer el efecto de este sobre el estado hídrico de la vid. Las medidas se realizaron con una cámara de presión (Pump-Up, PMS Instruments Company, Albany, Oregón, USA) siguiendo las recomendaciones de Choné et al. (2001). Para ello, las hojas se envolvieron en bolsas zip cubiertas con papel de aluminio una hora antes de la medida. Se tomó una hoja sana y adulta del tercio medio del pámpano por cada cepa. La periodicidad de estas medidas fue mensual, realizándose en 4 fechas diferentes a lo largo del estudio.

2.3 Análisis de datos

En cada fecha de muestreo se verificó el efecto del manejo del suelo sobre el Ψ_{tallo} mediante un análisis de varianza. Las relaciones entre θ_s y Ψ_{tallo} se determinaron mediante regresión lineal y cuadrática utilizando R versión 4.3.0 (R Core Team 2023).

3. Resultados

Durante el período de estudio (1 de junio a 1 de septiembre de 2023), la temperatura media en el viñedo de ensayo fue 24,5 °C (con una mínima absoluta de 10,7 °C y una máxima absoluta de 40,4 °C), la precipitación acumulada ascendió a 150,8 mm (129,8 mm registrados en junio) y la evapotranspiración de referencia alcanzó 583,9 mm.

La evolución de Ψ_{tallo} a lo largo de la campaña fue similar bajo las dos estrategias de gestión del suelo (Fig. 2), detectándose diferencias significativas tan solo a principios de agosto, cuando Ψ_{tallo} fue más negativo en el tratamiento con cubierta vegetal (Fig. 2).



Fig. 2 Evolución temporal del potencial hídrico de tallo (Ψ_{tallo}) en cepas de la variedad 'Riesling' cultivadas bajo dos estrategias de gestión del suelo: laboreo y cubierta vegetal. El asterisco indica diferencias significativas entre tratamientos (p < 0,05).

Los registros de θ_s se correlacionaron significativamente con las medidas de Ψ_{tallo} , observándose el valor de r más elevado para la profundidad de 30 cm y el más bajo para la profundidad de 50 cm (Tabla 1). Las pendientes de las rectas de regresión apenas variaron en función de la estrategia de manejo del suelo (Fig. 3a). Cuando se combinan los datos de ambos tratamientos, una ecuación de segundo grado ofrece un coeficiente de determinación más elevado (Fig. 3b).

Tabla 1. Coeficientes de correlación lineal de Pearson obtenidos para los pares de datos de potencial hídrico de tallo a mediodía solar (Ψ_{tallo}) en vid y el contenido volumétrico de agua en el suelo (θ_s) a tres profundidades. Se indica el p-valor de cada relación.

	θ_s 10 cm	θ _s 30 cm	θ _s 50 cm	θ _s 10 - 50 cm
Ψ_{tallo}	0,923	0,961	0,708	0,947
p-valor	< 0,001	< 0,001	0,01	< 0,001



Fig. 3 Relaciones entre el contenido de agua en el suelo (θ_s) promedio entre 10 y 50 cm de profundidad y el potencial hídrico de tallo (Ψ_{tallo}) en cepas de la variedad 'Riesling' cultivadas bajo dos estrategias de

gestión del suelo: laboreo y cubierta vegetal. (a) Relaciones individualizadas por tratamiento de gestión del suelo y (b) relaciones generales con todos los datos disponibles.

4. Discusión

Bajo las condiciones de este estudio, la cubierta vegetal no provoca un estrés hídrico excesivo en la viña, ya que no se han apreciado diferencias significativas en los valores de Ψ_{tallo} entre las dos estrategias de gestión del suelo consideradas. Esto puede deberse a, por una parte, que se ha dejado libre de cubierta vegetal una zona debajo de la fila de cepas, donde se aloja la mayor parte del sistema radicular de la vid y, por otra parte, a que la viña capta el agua de capas más profundas del suelo a las que no alcanza el sistema radicular de la vegetación presente en la cubierta (Celette et al., 2008).

En este estudio, las medidas de Ψ_{tallo} se correlacionaron significativamente con θ_s , siendo los valores de r más elevados cuando los registros se corresponden con los obtenidos a 30 cm de profundidad. Esto parece indicar que la mayor parte del agua captada por las raíces de la viña en este estudio procede de esa capa de suelo. Las relaciones obtenidas son lineales, al contrario que otros autores que observaron relaciones curvilíneas (Williams y Trout, 2005). Sin embargo, no siempre se ha podido detectar una relación significativa entre θ_s y Ψ_{tallo} (Asenjo y Yuste, 2003), lo que parece estar debido a condiciones locales.

De acuerdo con la relación de la Fig. 3b, al 50% del volumen de agua disponible para la planta en este suelo (\approx 18%), Ψ_{tallo} sería -1,07 MPa, por lo que, según van Leeuwen et al. (2009), la vid se encontraría sufriendo unas condiciones de estrés hídrico moderado.

5. Conclusión

En las condiciones de este ensayo, las medidas de θ_s promediadas entre 10 y 50 cm de profundidad constituyen un indicador fiable de la disponibilidad de agua en el suelo y se encuentran estrechamente relacionadas con el estado hídrico del viñedo. Se ha observado que los registros a 30 cm de profundidad presentaron una correlación más estrecha con las medidas de Ψ_{tallo} , lo que sugiere que las raíces del viñedo extraen agua, fundamentalmente, de esta capa del suelo. Estos resultados ponen de manifiesto la enorme importancia de la elección de la profundidad a la que se instalan las sondas.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Unravelling the potential influence of grass cover on the physiological aspects of grapevine plants in both optimal and drought conditions

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Abstract: The effect of climate change on plant development is of great concern: the global climate models predict that Europe will experience a decrease in water precipitation in the coming decades, along with rising temperatures.

Photosynthesis is the central process of all primary production in the Biosphere. When analyzing the assimilation capacity of plants, it must be taken into account that CO2 assimilation is strongly conditioned by environmental conditions and crop management. One emerging trend in viticultural soil management is the use of vegetal covers, as they improve soil characteristics and biodiversity. Numerous works carried out at the leaf level and (to a lesser extent) at the entire plant level confirm that drought (followed by an increase in temperature and saline stress) induces strong decreases in photosynthesis (and plant growth) that can become greater than 80% (reference). These inhibitory effects are associated with stomatal closure to reduce water loss, oxidative stress damage, and decreased activity of genes involved in photosynthetic machinery

The current study examined the impact of water availability and the use of ray grass-based coverture approaches on responsiveness of photosynthetic apparatus of grapevine plants. To this end, gas exchange (photosynthesis and transpiration) analyses were carried out at the leaf and canopy level in young grapevine plants subjected to fully versus partial (50% of full capacity) irrigation conditions. Additionally, isotopic composition and stress markers were also analyses in order to better understand the strategies of plants to sustain water use efficiency (WUE). Along with these determinations, the impact of plant management on soil physico-chemical parameters is also studied in order to determine the role of water availability and crop management in soil health.

Keywords: Grapevine, crop cover, drought, gas exchange, physiology and water use efficiency

1. Introduction

Viticulture is highly susceptible to erosion and soil degradation. This is primarily due to several factors: firstly, the soil in vineyards often lacks sufficient organic carbon, leading to poor soil quality. Secondly, the steep slopes and shallow soils upon which some vineyards are established make them prone to runoff during heavy rainfall events. Besides, the process of soil tillage further exacerbates soil losses. Thirdly, the planting schemes that are frequently employed in viticulture result in a substantial portion of the soil surface being left uncultivated. The manner in which this particular section of the soil is managed has notable implications on various physiological aspects, including vegetative growth, yield, grape and wine quality. Moreover, it also has an impact on environmental factors, such as soil vineyard biodiversity, gas emissions and water use. The management of vineyard soils through the implementation of cover crops (CC) is a practice that is experiencing a growing trend on a global scale, even in regions where their use was previously limited due to a lack of rainfall. Therefore, is of utmost importance to carefully evaluate the advantages and disadvantages associated with this approach.

CC are extensively acknowledged for its many well-recognized ecosystem services, which effectively mitigates the loss of soil and water, reinstates organic matter, enhances biodiversity, and boosts fertility in deteriorated agricultural soils. However, the utilization of cover crops by farmers remains limited. Even scientific literature presents conflicting perspectives: certain researchers have documented elevated water stress in vineyards under cover crop management across distinct soil and climatic conditions (Montero and Lopes, 2007), while others have observed that cover crops do not have a detrimental effect on water stress when compared to conventional management (Celette et al., 2005).

Water plays a vital role in ensuring the sustainability of viticulture as the production, quality, and economic viability of the farm. However, in many viticultural regions, the total water consumption of vineyards, exceeds the annual average precipitation, posing a significant risk to the sustainability of vineyards (Medrano et al., 2015). Thus, it is of utmost importance to enhance the water use efficiency (WUE) in vineyards, particularly in semi-arid areas, in order to achieve a sustainable viticulture industry. On a physiological leaf scale, the relationship between net photosynthesis (An) and transpiration (Tr) or stomatal conductance (gs) is known as photosynthesis to water stress (Flexas et al. 2002) implies that all strategies involving deficit irrigation contribute to the enhancement of iWUE. the utilization of δ 13C as a reliable indicator for measuring the integrated WUE (WUEc) over the course of the time. Nevertheless, fully comprehensive reaction of the entire plant system seems to be the most ideal to calculate WUE (Douthe et al., 2018).

Despite decades of attempts on implementation, there is still a lack of a consistent State-of-the-Art that provides clarity regarding the impact of CC in wineyards. A deeper comprehension of the physiological implications that CC has on water resources would prove beneficial in determining the appropriate time and manner in which this practice can be implemented. Our working hypothesis is that CC competes for water with the vineyards, providing a narrow but enduring water limitation for the vineyard. In order to attain the objectives of conserving soil and water through agriculture, it is essential for agricultural lands to transition towards more sustainable practices.

2. Materials and Methods

2.1 Plant material.

Dormant cuttings of four clones of *Vitis vinifera* L. cv. Tempranillo were employed in this study. The cuttings were carefully sown in the middle of 7 L pots, which were filled with a mixture of agricultural soil and pure quartz sand in a 1:1 vol:vol of ratio. Subsequently, the pots were thoroughly watered until they reached their full capacity, ensuring that the cuttings received sufficient moisture for their initial establishment. As CC, a mixture of *Perennial ryegrass* and Trifollium repens in a 1:1 vol:vol of ratio was used.

To ensure a uniform and successful establishment of the CC several steps were taken to facilitate the implantation of the cover crop -the pots without cover undergo the same management-:

- 1. A layer of pure quartz sand, measuring approximately 3-5 millimeters in thickness, was carefully spread on top of the soil surface. This sand bed served as a suitable medium for the subsequent placement of the cover crop seeds.
- 2. A total of 5 grams of the mixture of cover crop seeds were gently and evenly distributed on the prepared sand bed. This ensured a uniform distribution of the seeds and minimized the chances of overcrowding or patchy growth.
- 3. The seeds were then covered with another layer of pure quartz sand, approximately 1 centimeter in thickness, acting as a safeguard against external influences and fostering an ideal setting for germination.
- 4. In order to maintain optimal moisture levels for seed germination, the cover crop seeds were hydrated once a day for a period of 3 weeks. This was achieved by using a pressure sprayer, which delivered a consistent amount of water (20 milliliters) daily.
- 5. After the 3 weeks, when the CC was implanted, the water restriction experiment started. Plants receive the contrasting watering for 9 weeks.

2.2 Plant growth and experimental design

The experiment was run in a fully-control greenhouse at the Institute of Agrobiotechnology IdAB (Mutilva Baja, Spain) during the months of June-August 2023. Only one branch was allowed to growth.

The experiment consists in two planting schemes: CC versus no CC. Moreover, plants within each group were subjected to two water regimes: well-watered (control) versus partial (50% of full capacity; drought) irrigation conditions.

2.3 Methods

2.3.1 Biomass parameters

After the 12-week, the plants were harvested. The leaves and stems of the plants were removed and fresh and dry weight was obtained.

2.3.2. Leaf level gas exchange and fluorescence

Measurements of leaf photosynthetic CO₂ assimilation (A) and gs were taken using a portable photosynthesis system Li-6400XT (LI-COR Bioscience, Lincoln, NE, USA) on the newest fully expanded leaf at the canopy top, generally corresponding to the third leaf from the bottom. Fluorescence parameters were also recorded. The measurements were done in the week 12 of growth, started 3 h after sunrise and extended over for about 4 h.

2.3.3. Canopy C and H₂O exchange

A custom chamber closed system was built for measuring whole pot total C gain and H2O loss. The chamber consisting of a base module, a cylindrical chamber, and a module to measure variation in CO₂, H₂O, and methane (CH₄) Li7810 (Li-COR). The chamber had a footprint of 0.25 m², a length of 40 cm. All connections sealed to prevent leaks. Air mixing was ensured with fans in both the middle and top modules. The fluxes were calculated using slope of the parameter after a stabilization (60-90 seconds). The measurement was completed within 180 seconds.

2.3.4. Carbon, Nitrogen and stable isotopes

Samples of leaf were used for analysis of the carbon and nitrogen isotope composition using an isotope ratio mass spectrometer and elemental analyzer.

3. Results

Under drought conditions, it was observed that the steam exhibited a lower dry biomass (Figure 1A). The findings indicate a significant difference when comparing plants without CC, while the difference was only marginally significant (*q*-value < 0.072) with CC (Figure 1B). However, the treatments did not have an impact on the dry biomass of the leaves.



Fig. 1 Biomass parameters in vineyard into contrasting crop cover management and edaphic water regime in A) steam dry weight (DW) and B) leaf DW. Individual observations, the average, the standard deviation and *q*-values are represented. Statistical power: Welch's ANOVA test and false discovery rate of one step-up procedure of Benjamini, Krieger and Yekutieli. (n= 9-12).

Under conditions of full irrigation, vineyard exhibited greater Amax as depicted in Figure 2A. Additionally, it was observed that when the vineyard was cultivated in bare ground, the Amax was higher than when growth with CC for both water regimes. The leaf nitrogen (N) content (data not shown) and electron transport rate (Figure 2C) were found to be unaffected by the water regime or CC; this suggests that the increased capacity of the plants to fix C is likely attributed to the higher gs observed (Figure 2B). In terms of the entire plant-soil system, a greater overall C gain was observed for CC under well water conditions, which is consistent with the larger coverage and photosynthetic area of the pot. The results indicate that despite variations in leaf C gain, the entire system as a whole does not exhibit differences under limited water

regime. Nevertheless, the entire system evapotranspiration was largely higher under well water conditions, and the highest, when vineyard and CC shared the environment.



Fig. 2 Photosynthetic capacity at 1000 mmol PAR of vineyard into contrasting crop cover management and edaphic water regime in A) maximun photosynthesis (Amax), B) stomatal conductance (gs), C) electron transport rate (ETR). Individual observations, the average, the standard deviation and *q*-values are represented. Black bars, dots and error bars represents Statistical power: Welch's ANOVA test and false discovery rate of one step-up procedure of Benjamini, Krieger and Yekutieli. (n= 9-12).



Fig. 3 Whole pot gas exchange and δ 13C. Data represents the slope of A) carbon gain and B) transpiration for 120 seconds selected into the linear phase. In C) data represents the δ 13C. Individual observations, the average, the standard deviation and *q*-values are represented. Black bars, dots and error bars represents Statistical power: Welch's ANOVA test and false discovery rate of one step-up procedure of Benjamini, Krieger and Yekutieli (A and B, *n*= 3; C *n*= 5).

The response of δ^{13} C to limited water conditions was observed both robust and consistent in our study. An intriguing finding of our study was the substantial influence of the CC treatment on the δ^{13} C, particularly when comparing it to the delta value of the bulk soil.

4. Discussion

CC have been recommended for the purpose of extracting excessive water and nutrients within the effective root zone of plants, thereby potentially inducing excessive vigor in grapevines. In areas where grapevines grow under water-limited conditions, ground covers can be managed in a manner that competes with the vines during the initial vegetative growth stage Consequently, this approach can lead to a reduction in the canopy leaf area and subsequently lessen transpiration losses at a later stage (Monteiro and Lopes 2007). In our work, we have not noticed limiting consequences for plant vigor under CC, neither under limiting water regime. Nevertheless, leaf-level photosynthetic characterization allows us to observe the limiting influence of CC on vineyard C fixation capacity: specifically, our results point to stomatal limitation as the main actor for this reduction. The results of δ 13C indicates that CC largely influence the C isotopic fingerprint along the growing time of the plants.

Conclusion

It is crucial to implement these strategies in a timely manner to prevent excessive water stress on the plants, which could potentially result in reduced fruit set or even premature defoliation. CC could be an alternative. The adoption of cover crops in semi-arid regions by farmers is still restricted because the disadvantages outweigh the advantages; our findings align with our hypothesis, showing that there is a persistent water limitation with CC. Further work regarding on the physiological and metabolic influences of this stress will be addressed.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Exploring the hydraulic functioning of *Viscum album* L. and its relationship with the host tree *Pinus sylvestris* L.

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Abstract: Excessive mistletoe infection tends to weak the host pine, which become more vulnerable to other threats such as intense droughts. Consequently, excessive mistletoe infection can cause anything from a slight stippling to partial or total death of the host tree. The objective of this study was to explore the hydraulic functioning of mistletoe in order to elucidate the possible hydraulic causes behind the host pine decline. For this reason, we have measured gas exchange, pre-dawn water potential and the stem water potential gradient at midday in branches of mistletoe and Scots pine during the summer of 2023, in two conditions: saturated soil field capacity and soil water deficit. We have also assessed the hydraulic conductivity and the loss of conductivity with increases in water potential in both species. Preliminary analysis revealed that infected branches of Scots pine at the beginning of summer (without soil water stress) showed a 30% less in stomatal conductance and carbon assimilation than non-infected branches. At the end of summer (with a predawn soil water potential of -1.40 MPa), both types of pine branches, infected and non-infected, showed a reduction in stomatal conductance and carbon assimilation of ca. 80%, while mistletoe barely reduced stomatal conductance by 20%, showing values ca. 360 mmol $H_2O m^{-2} s^{-1}$. The higher stomatal conductance of mistletoe together with its lower specific hydraulic conductivity can explain their more negative midday water potential values measured (ca. -2.90 MPa). By contrast, Scots pine showed a midday water potential around -1.90MPa. Moreover, when calculating the stomatal conductance of the whole branch ($g_{s,branch}$, mmol H₂O s⁻¹), the infected branches showed a much higher g_{s,branch} than the expected for a given xylem hydraulic conductivity measured in the pine prior the infection. This was specially noticed under soil water stress conditions. That is, it seems that in infected branches there is an imbalance between the whole-branch stomatal conductance and the xylem hydraulic conductivity, with in turn seems to harm the pine tissues after the mistletoe infection.

Keywords: Forest decline, gas exchange, mistletoe, Scots pine, summer aridity, water relations.

1. Introduction

According to the results of the Level I network of the Forest Damage Inventory, two of the main threats observed in the forests of the province of Teruel during the 2020 sampling continue to correspond to prolonged or intense droughts and the excessive spread of mistletoe (*Viscum album* subsp. *austriacum*). Regarding drought, it can cause water stress in the tree due to the absence of water in the soil. To cope with this situation, plants close stomata to avoid losing water through transpiration, which in turn prevents carbon uptake and, therefore, stops photosynthesis, slowing down their growth. If this stress situation continues excessively over time, the tree can exhaust the stored resources and begin to show symptoms of decay, from leaf yellowing, slight stippling, or even the death of the branch or the tree itself (Sancho-Knapik et al. 2017).

Regarding the mistletoe, also known as golden branch due to the color of its ripe fruits, it is a hemiparasitic plant species, considered by the Celts to be their most sacred plant, where only the druids were authorized to harvest it. Mistletoe, although not exclusive to conifers, is observed in forests of Scots pine (*Pinus sylvestris* L.), so its presence is common and necessary in ecosystems, since its fruits serve as food for different species of birds and its leaves for numerous insects (Hernández-Alonso et al., 2001). However, sometimes it can proliferate in excessive numbers and extension, being considered dangerous for the survival of the pine since the presence of mistletoe in a tree reduces its vigor and growth and can also exacerbate the water stress suffered by the host tree during periods of aridity (Sangüesa-Barreda et al., 2018; **Fig. 1**).



Fig. 1 Pine decline process due to mistletoe

Due to the harmful effect of the mistletoe-aridity binomial on pines, the objective of the study was to explore the hydraulic functioning of mistletoe in order to elucidate the

possible hydraulic causes behind the host pine decline. For this, we assessed the response to drought during summer 2023 of *Pinus sylvestris* branches with different levels of mistletoe infection in Orihuela del Tremedal (Teruel, Spain).

2. Materials and Methods

2.1 Plant material and experimental conditions

We selected a forest of *P. sylvestris* moderately infected with mistleto*e* located in Orihuela del Tremedal (40,54° N, 1.66° W, 1466 m a.s.l., Teruel, Spain). This site features

oro-Mediterraean climatic conditions, being characterized by cold winters and arid summers. The experimental plot selected within the forest was characterized by the existence of both infected and non-infected pines. In this plot, trees were 7-10 m in height, ca. 20-40 years old and trees were sparsely distributed. This low stand density allowed the existence of functional branches available for measurements close to the ground (**Fig. 2**).



Fig. 2 Pine experimental plot

2.2 Water potential and gas exchange measurements

In situ measurements of water potential and gas exchange were performed in the early summer (without soil water deficit conditions) and in the late summer (with soil water deficit). For each soil water condition, we first measured the predawn water potential (Ψ_{pd}) in 5 pine shoots of different infected trees with a Scholander pressure chamber. Then, around noon, we measured midday water potential (Ψ_{md}) and gas exchange parameters (stomatal conductance (g_s) and net assimilation rate (A_N)) on non-infected pine branches, infected pine branches and on mistletoe (**Fig. 3**). Gas exchange was measured with an open gas exchange system (CIRAS-3, PP-Systems, Amesbury, MA, USA) fitted with an automatic universal leaf cuvette (PLC6-U, PP-Systems, Amesbury, MA, USA).

On each pine branch, we measured Ψ_{md} on two shoots: the apical shoot (after mistletoe infection in case of infected branches) and a shoot located ca. 60 cm from the apical shoot (i.e. before the mistletoe infection in case of infected branches). This second shoot was previously covered with aluminum paper to avoid irradiance and therefore shoot transpiration, to obtain the stem water potential at that point of the branch (**Fig. 3**).



Fig. 3 Schematic representation of water potential and gas exchange measurements on Scots pine branches and Mistletoe. Ψ_{md} , midday water potential; g_s, stomatal conductance; A_N, net assimilation rate

2.3 Hydraulic conductivity

During summer 2023, plant material mas recollected from the nearest trees to the experimental plot for hydraulic laboratory measurements. We collected in the early morning infected branches and re-cut them under water, obtaining ca. 0.70 long branches. Then, branches were preserved in black plastic bags with the cut ends under water and were carried to the lab. The age of recollected mistletoe was ca. 8 years.

In the lab, the hydraulic conductivity (K_h) was determined in different years stem segments of *P. sylvestris* and *V. album*. Two to three stem segments per species were cut under water. The measurement pressure was set to 8 kPa. The flow rate was determined with a mass flow meter/controller (Liqui-flow, Bronkhorst, Holland). The conductivity measurements were carried out with distilled, filtered (0.22 μ m) and degassed water containing 0.005% (volume/volume) Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth. After the hydraulic measurement, segments were measured in length to obtain K_h and diameter without bark to compute the specific conductivity (K_s) as the hydraulic conductivity on a sapwood area basis. We have also measured the total leaf area each branch supported after the stem segment measured. This was used to calculate the stomatal conductance of the whole branch (g_{s,branch}, mmol H₂O s⁻¹) to be related with the K_h measured (as a proxy of water needed - g_{s,branch}- versus water supplied -K_h-).

3. Results and Discussion

Without soil water stress conditions (Ψ_{pd} close to 0 MPa), non-infected and infected pine branches showed similar mean values of Ψ_{md} : -1.22 and -1.18 MPa (measured in the apical shoot), respectively (**Fig. 4a**). The main difference in this condition was that infected branches of Scots pine showed a 30% reduction in stomatal conductance and carbon assimilation values than non-infected branches (**Fig. 4a**). At the end of summer with soil deficit conditions (Ψ_{pd} = -1.40 MPa) non-infected branches showed slightly less negative values of Ψ_{md} than infected branches: -1.77 and -1.86 MPa (measured in the apical shoot), respectively (**Fig. 4b**). This water stress condition caused in both types of pine branches, infected and non-infected, a reduction ca. 80% in stomatal conductance, going from 268 and 196 to 49 and 45 mmol H₂O m⁻² s⁻¹ i in non-infected and infected branches, respectively. This reduction in g_s entailed a high increase in pine WUE. By contrast, under water stress conditions mistletoe barely reduced stomatal conductance, going from 456 to 359 mmol H₂O m⁻² s⁻¹ (**Fig. 4b**), that entailed low rates of WUE in both, non-stress and stress water conditions.

The higher stomatal conductance of mistletoe together with its lower specific hydraulic conductivity (K_s, **Fig. 5**) can explain their more negative midday water potential values measured, ca. -2.56 and -2.88 MPa in non-stress and stress water conditions, respectively (**Fig. 4**).



Fig. 4 Gas exchange and water potential values in infected and non-infected branches with soil field capacity (a) and with soil water deficit (b)

When analyzing the relationship between K_h and $g_{s,branch}$, the non-infected branches showed a clear linear relationship ($R^2 = 0.95$) indicating that there is a consistency between the water needed and the water supplied. By contrast, we did not find any relationship in infected branches, showing a much higher $g_{s,branch}$ than the expected for a given xylem hydraulic conductivity. This was specially noticed under soil water stress conditions (**Fig. 6**).




Fig. 6 Hydraulic conductivity (Kh) and whole branch stomatal conductance (gs, branch) under soil water deficit conditions

4. Conclusion

Our exploratory analysis revealed that in infected branches there is an imbalance between the whole-branch stomatal conductance and the xylem hydraulic conductivity, with in turn seems to harm the pine tissues after the mistletoe infection.

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Local and systemic regulation of maize aquaporins by the arbuscular mycorrhizal symbiosis under drought stress

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Abstract: In recent years studies have shown that the arbuscular mycorrhizal (AM) symbiosis regulates the expression of a wide number of aquaporin genes in the host plant, concomitantly with an enhancement of the membrane water permeability of both intact root cortex cells and protoplasts from AM plants as compared to those of non AM plants. However, it is not currently known if the mycorrhizal effect on aquaporins and on cell water transport is local (at AM-colonized cells) or systemic (at the whole root system). The present study aims to elucidate if the regulation of plant aquaporins and root water flow occurs only in cells colonized by the AM fungus or if it is extended to the whole root system. Maize plants were cultivated in a splitroot system so that, half of the root system was inoculated with the AM fungus and the other half remained uninoculated. After plant cultivation and drought exposure, plant development, root hydraulic conductance and aquaporin gene expression was measured in each root fraction and in isolated cells (either containing arbuscules or non-colonized). Both root fractions had a similar development, except in AM plants cultivated under well-watered conditions, where the non-colonized root fractions grew more than the colonized root fraction. Total root hydraulic conductance (Lpr) was higher in AM plants than in the non-mycorrhizal plants, both under well-watered and under drought stress conditions. In any case, no differences in Lpr values were found between both root compartments. The expression of most maize aquaporin genes was different in the mycorrhizal root fractions than in the non-mycorrhizal root fractions of AM plants. At cellular level, differential aquaporin expression in AM-colonized cells and in uncolonized cells was also observed. Results indicate the existence of both, local and systemic regulation of plants aquaporins by the AM symbiosis.

Keywords: Aquaporin, arbuscular mycorrhizal symbiosis, drought stress,

1. Introduction

Aquaporins are membrane intrinsic proteins which locate in different cell membranes. In plants they constitute a highly diverse protein family, with over 30 isoforms in most higher plants. Aquaporins transport water and some of them can also transport other relevant molecules for the plant, such as, CO₂, metalloids, urea, ammonia, H₂O₂, oxygen or even ions (Fox et al. 2017; Zwiazek et al. 2017; Singh et al. 2020). Thus, it is clear that aquaporins isoforms contribute to several plant physiological functions (Chaumont and Tyerman 2014; Li et al. 2014; Singh et al. 2020).

The arbuscular mycorrhizal (AM) symbiosis involves a group of microscopic soil fungi and the roots of almost 80% of terrestrial plants, including many important agricultural species such as maize. After root colonization, the fungi develop specialized structures called arbuscules inside the root cells, where there is an exchange of nutrients and water between both symbionts (Genre et al. 2005; Ezawa and Saito 2018; Püschel et al. 2020). The AM symbiosis has been reported in literature as beneficial for improving the resilience of the majority of crops to water stress. Indeed, the AM symbiosis is essential for plants to acquire nutrients and water from the soil and increases their resistance to environmental stresses (Augé et al. 2015; Santander et al. 2017; Bahadur et al. 2019; Cheng et al. 2021).

Studies have also shown that the AM symbiosis has the capacity of altering root hydraulic conductivity (Lpr), enhancing it mostly under stress conditions (Aroca et al. 2007; Bárzana et al. 2012, 2014; Sánchez-Romera et al. 2016; Quiroga et al. 2017; 2018; 2019a,b; 2020). The involvement of plant aquaporins in these processes has been proved by a number of studies (reviewed by Ruiz-Lozano and Aroca 2017) and, recently, it has been shown that the presence of the AM fungus in the root increases the water permeability of root cells, related to induction of some aquaporin genes and increase of the phosphorylation status of PIP2s, which implies a higher activity of their water channels (Quiroga et al. 2019b). Results obtained showed for the first time an enhancement of the membrane water permeability of both intact root cortex cells and protoplasts from AM plants as compared to those of non AM plants (Quiroga et al. 2019b).

In any case, it must be taken into account that in the above mentioned study on cell hydraulic conductivity (Lpc) and Pf values were measured on isolated cortical cells. Some of these cells may be colonized by the AM fungus and others not colonized. We do not know yet if the mycorrhizal effect on cell water transport is local or systemic. Thus, the aim of the present study was to determine, at both tissue and cell levels, if the regulation of plant aquaporins by the AM symbiosis occurs only in cells colonized by the AM fungus or if it extends systemically to the whole root system.

2. Materials and Methods

2.1 Experimental design

The experiment consisted of a factorial design with two factors: (1) inoculation treatment, including plants inoculated with the AM fungus *Rhizophagus intraradices*, (Ri) and non-inoculated control plants (C); (2) water regime, so that one half of the plants were cultivated under well-watered conditions (WW) throughout the entire experiment and the other half of the plants were subjected to drought stress (DS) for two weeks just before harvest. Each treatment had 20 replicates giving a total of 80 pots.

In this study, maize plants were cultivated into a split-root system (Fig. 1) in containers prepared *ad hoc* for this split-root assay. These containers were constructed from two 1L plastic pots fastened together, side by side, with adhesive tape, as described by Neumann et al. (2009) and Bárzana et al. (2015). Thus, each maize plant had its root system divided into two fractions, the right one and the left one. In the AM treatments, the AM inoculum was applied only to a single root compartment (always to the left root compartment, see Fig. 1). Thus, we got control non-AM plants where none of the root fractions was colonized by the AM fungus and AM plants with only a root fraction colonized (the left portion), while the right root portion remained uncolonized, although it took part of an AM plant.



Fig. 1 Split root system used to cultivate maize inoculated or not (C) with the AM fungus *R. intraradices* (Ri). Plants were cultivated with the root system divided into two fractions (left and right). For AM plants, AM inoculum was applied only to the left root fraction, while the right fraction remained always uncolonized by the AM fungus, although belonging to a mycorrhizal root system.

2.2 Biological material and growth conditions

The growing substrate consisted of a mixture of soil and sand (v/v 1:1). Soil was collected at the grounds of IFAPA (Granada, Spain), sieved (2 mm), diluted with quartz-sand (<1 mm) and sterilized by steaming (100°C for 1 h on 3 consecutive days). Soil had a pH of 8.1 (water); 0.85% organic matter, nutrient concentrations (mg kg⁻¹): N, 1; P, 10 (NaHCO₃-extractable P); K, 110. The soil texture was made of 38.3% sand, 47.1% silt and 14.6% clay.

Seeds of *Zea mays* L. (cv PR34B39, Pioneer Hi-Bred, Spain) were pre-germinated on sand for ten days and then transferred to the containers prepared *ad hoc* for this split-root assay. Each root compartment was filled with 1300 g of the soil/sand mixture described above and harboured half root system from maize seedlings.

Mycorrhizal inoculum consisted of soil, spores and mycelia. It was a commercial inoculum provided by MycAgro Lab. (Bretenière, Dijon, France). The AM fungus was *Rhizophagus intraradices* (Schenck and Smith). Twenty grams of inoculum were added to appropriate compartments at sowing time. Non inoculated control plants received the same amount of autoclaved mycorrhizal inoculum together with a 10 ml aliquot of a filtrate (<20 μ m) of the AM inoculum in order to provide a general microbial population free of AM propagules.

Maize plants were grown under greenhouse conditions ($25/20^{\circ}C$, 16/8 light dark period, 50-60% RH and average photosynthetic photon flux density 800 µmol m⁻² s⁻¹) for a total of eight weeks. Four weeks after sowing, all plants started receiving 10 mL per pot and per week of Hoagland nutrient solution (Hoagland and Arnon, 1950) containing only 25% of P, in order to provide basic nutrients, but avoiding inhibition of AM symbiosis due to a high P application. Soil moisture was measured with the ML2 ThetaProbe (AT Delta-T Devices Ltd., Cambridge, UK) as described previously (Quiroga *et al.* 2017). Water was supplied daily to maintain soil in both root compartments at 100% of field capacity during the first 6 weeks after sowing. Then, half of the plants were allowed to dry (DS treatments) until soil water content reached 60% of field capacity (2 days needed), while the other half were maintained at field capacity (WW treatments). Plants were maintained under such conditions for 14 additional days. The same watering treatment was applied to both root compartments, so that plants were either well-watered of subjected to drought stress in both roots compartments.

2.3 Parameters measured

2.3.1 Biomass production and symbiotic development

At harvest (8 weeks after sowing) the shoot and root system were separated and weighed to determine fresh weights (FW). For each treatment, eight replicates were used to determine shoot and root dry weight (SDW and RDW) after drying in a forced hot-air oven at 70 °C for 2 days. In order to visualize and quantify AM fungal structures, roots (aliquots of both root compartments)

were stained with trypan blue according to Phillips & Hayman (1970). The percentage of mycorrhizal colonization was calculated by the gridline intersect method according to Giovannetti & Mosse (1980) in six replicates per treatment.

2.3.2 Hydrostatic root hydraulic conductivity (Lpr)

Lpr was determined at noon in eight plants per treatment with a Scholander pressure chamber as described by Bárzana et al. (2012). A gradual increase of pressure (0.3, 0.4 and 0.5 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 root hydraulic conductance (L) value. Lpr was determined by dividing L by root dry weight and expressed as mg H₂O g RDW⁻¹ MPa⁻¹ h⁻¹.

Lpr was measured in both root compartments on five replicates per treatment and per root compartment. Thus, to measure in the left root compartment, the root fraction contained in the right compartment was detached from the system. To measure Lpr in the right root compartment, the root fraction contained in the left compartment was detached from the system. Total Lpr for each treatment was also calculated as the sum of Lpr values in each root portion.

2.3.3 RNA extraction from maize roots

Three biological replicates of maize roots were used to extract total RNA as described in Quiroga et al. (2017). First-strand cDNA was synthesized using 1 μ g of purified RNA with the Maxima H Minus first strand cDNA synthesis kit (Thermo Scientific TM), following the manufacters' instructions.

2.3.4 Laser microdissection and RNA extraction from maize cells types

Maize roots cut into about 10 mm segments with a razor blade in fixative and embedded in paraffin as described by Balestrini et al. (2007) and Fochi et al. (2017). Neoclear was then gradually replaced with paraffin (Paraplast Plus) and samples were embedded in paraffin in Petri dishes as described by

Balestrini et al. (2007). Sections of 12 μ m thickness were cut using a rotatory microtome, placed and stretched out on Leica RNase-free PEN foil slides (Leica Microsystems) with ddH₂O (filtered with a 0.2 μ m filter). The sections were then dried on a 40 °C warming plate, stored at 4 °C and used within 2 days.

A Leica LMD 6500 Laser microdissection system (Leica Microsystems, Inc., Germany) was used to isolate the different cell types from the prepared tissue sections. Just before use, the slides with the sections were deparaffinized with Neoclear for 8–10 min, rinsed in 100% ethanol for one minute and then air-dried. The deparaffinised slides were placed face-down on the microscope and two different cell types were selected from maize roots, microdissected and collected separately: (i) cells containing visible fungal arbuscules from the mycorrhizal root fraction or left root and (ii) non-colonized cells from the non-mycorrhizal root fraction or right root. Approximately 1500 cells for each cell-type population were collected for each replicate, and the pools were brought to a final volume of 50 μ L with Pico Pure extraction buffer and processed for RNA extraction following manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). At least three independent biological replicates of each cell type were collected for downstream gene expression analyses.

2.3.5 Quantitative real-time RT-PCR at root tissue and at cell levels

The expression of seven previously selected maize aquaporins (ZmPIP1;1, ZmPIP1;3, ZmPIP2;2, ZmPIP2;4, ZmTIP1;1, ZmTIP2;3 and ZmTIP4;1; Quiroga et al. 2017) was measured by qRT-PCR using 1 μL of diluted cDNA (1:9) obtained from the different RNA samples (either from the root tissue or from the laser microdissected cells) with PowerUpTM SYBRTM Green Master Mix in a QuantStudioTM 3 system (Thermo Fisher Scientific). The reaction was repeated for 40 cycles at annealing temperature of 58ºC for all primers. Four reference genes were measured in all the treatments for normalization of gene expression values. These genes were poliubiquitin (gi:248338), tubulin (gi:450292), GAPDH (gi:22237) and elongation factor 1α (gi:2282583) (Bárzana et al. 2014). Standardization was carried out based on the expression of the best-performing reference gene under our specific conditions, "NormFinder" chosen by using algorithm (Andersen which were et al. 2004) (https://moma.dk/normfinder-software). Thus, expression levels were normalized according to ZmGAPDH gene. Fungal aquaporins (GintAQP1, GintAQF1 and GintAQF2) were analysed as previously described (Aroca et al. 2009; Li et al. 2013) using the fungal elongation factor 1α (Accession No. DQ282611) as reference gene for standardization. The relative abundance of transcripts was calculated using the 2^{-AAct} method (Livak & Schmittgen 2001). The threshold cycle (Ct) of each biological sample was determined in duplicate. Negative controls without cDNA were used in all PCR reactions.

3. Results

3.1. Plant biomass production

The shoot dry weight (SDW; Fig. 2) was enhanced by the AM symbiosis both under wellwatered conditions (by 30%) and even more under drought stress conditions (by 78%). Drought stress affected negatively the SDW, reducing it by 51% in non-AM plants and by 32% in AM plants. The root dry weight (RDW; Fig. 3) total was similar in AM and nonAM plants under wellwatered conditions. Under drought stress conditions AM plants maintained a RDW similar to the values under well-watered conditions, while nonAM plants decreased considerably their total RDW (by 54% as compared to the well-watered plants). Regarding the root development in each root fraction, it was similar in both fractions for all treatments except in AM plants under well-watered conditions, which had a higher root development (an increase of about 30%) in the non-mycorrhizal root fraction (the right one) than in the mycorrhizal root fraction (the left one).



Fig. 2 Shoot dry weight of maize plants inoculated or not (Control) with the AM fungus *R. intraradices* (AM). Plants were cultivated either under well-watered conditions or subjected to drought stress for 15 days before harvest. Data represents the means of 20 values \pm S.E. Different letter indicates significant differences between treatments (p<0.05) based on Duncan's test.



Fig. 3 Root dry weight in the different root fractions or in the whole root of maize plants inoculated or not (Control) with the AM fungus *R. intraradices* (AM). Plants were cultivated with the root system divided into two fractions (left and right). For AM plants, AM inoculum was applied only to the left root fraction, while the right fraction remained always uncolonized by the AM fungus, although belonging to a mycorrhizal root system. Plants were cultivated either under well-watered conditions or subjected to drought stress for 15 days before harvest. Data represents the means of 20 values \pm S.E. Different letter indicates significant differences between treatments (p<0.05) based on Duncan's test.

3.2. Hydrostatic root hydraulic conductivity (Lpr)

The total Lpr values at the whole root system were higher in the AM plants than in the nonAM plants (Fig. 4), both under well-watered conditions (by 130%) and under drought stress conditions (by 45%). Regarding the Lpr values in each root fraction, these values were similar in

the right root fraction and in the left root fraction for each treatment. However, under wellwatered conditions AM plants also exhibited a higher Lpr values in each root fraction as compared to the nonAM plants. Under drought stress conditions no differences were observed.



Fig. 4 Root hydraulic conductivity (Lpr) in the different root fractions or in the whole root of maize plants inoculated or not (Control) with the AM fungus *R. intraradices* (AM). Data represents the means of 10 values ± S.E. See legend for Fig. 3.

3.3. Expression of maize and AM fungal aquaporins at root tissue level

In control uninoculated plants, the expression of the different aquaporins was not significantly affected by the drought stress imposed, except in the case of ZmTIP1;1 and ZmTIP2;3 that increased their expression under drought stress (Fig 5E,F). Regarding AM plants, we measured the gene expression in the two root fractions (the one containing the AM fungus and the one uninoculated with the fungus). The expression of four out the seven maize genes showed a similar pattern. Thus, under well-watered conditions, ZmPIP1;1, ZmPIP1;3, ZmPIP2;4, and ZmTIP2;3 decreased their expression in the uninoculated root fraction as compared to the inoculated AM fraction. In contrast, under drought stress conditions, these genes increased their expression in the uninoculated root fraction as compared to the inoculated AM fraction (Fig 5A,B,D,F). ZmPIP2;2 decreased it expression in the uninoculated root fraction as compared to the inoculated AM fraction both, under well-watered and under drought stress conditions (Fig 5C). In contrast, ZmTIP4;1 increased it expression in the uninoculated root fraction as compared to the inoculated AM fraction both, under well-watered and under drought stress conditions (Fig 5G). Finally, as expected, the AM fungal aquaporin GintAQF2 was only detected in the inoculated AM fraction (Fig 5H) and decreased its expression by drought stress. The other two fungal aquaporins were not detected.



Fig. 5 Aquaporin gene expression in roots of uninoculated maize plants (Control, black bars) or in the two root fractions of maize plants inoculated with the AM fungus *R. intraradices* (AM). Plants were cultivated with the root system divided into two fractions (left and right). For AM plants, mycorrhizal inoculum was applied only to the left root fraction (AM, white bars), while the right fraction remained always uncolonized by the AM fungus (, dashed bars), although belonging to a mycorrhizal root. Plants were cultivated either under well-watered conditions or subjected to drought stress for 15 days before harvest. Data represents the means of 3 values \pm S.E. Different letter indicates significant differences between treatments (p<0.05) based on Duncan's test.

3.4. Expression of maize and AM fungal aquaporins at root cell level

In control uninoculated plants, the expression of *ZmPIP1;1* and *ZmTIP1;1* was upregulated by drought stress (Fig 6 A,E). The rest of aquaporin genes analysed did not show significant changes in these plants as a consequence of the water treatment. In AM plants, we measured the gene expression in cells colonized by the AM fungus (cells containing arbuscules), collected in the inoculated root fraction and in cells without the AM fungus collected in the uninoculated root fraction (See Fig. 1). The expression of most of the aquaporin analyzed showed a significant upregulation in the cells uncolonized by the AM fungus when plants were cultivated under well-watered conditions (Fig 6 A-F). In contrast, under drought stress, this upregulation was only found for *ZmPIP1;1*, but not for the rest of aquaporin genes (Fig 6A). *ZmTIP4;1* showed upregulation of gene expression both in arbusculated cells and in uncolonized cells, when cultivated under well-watered conditions only (Fig 6G). Finally, the AM fungal aquaporin *GintAQF2* was only detected in the inoculated AM fraction (Fig 6H) and enhanced its expression by drought stress. The other two fungal aquaporins were not detected.

4. Discussion

In this study, plant growth was improved by AM fungal inoculation, specially under drought stress conditions, where AM symbiosis alleviated the detrimental effects of the drought stress imposed. This has been widely observed in previous studies (Santander et al. 2017; Cheng et al. 2021) and confirmed here. A similar trend was observed for root hydraulic conductivity, that was also enhanced by AM symbiosis, specially under drought stress conditions, as previously observed (Aroca et al. 2007; Sánchez-Romera et al. 2016; Quiroga et al. 2019a,b). In any case, the main objective of this study was to determine, at both tissue and cell levels, if the regulation of plant aquaporins by the AM symbiosis occurs only in cells colonized by the AM fungus or if it extends systemically to the whole root system. For that, plants were cultivated in a split root system, so that we can have a mycorrhizal root fraction where the AM fungus is present and an uninoculated root fraction of the same root system without fungus (Barzana et al., 2015). This growing system did not affect plant growth, and the beneficial effects of AM symbiosis on this parameter were clearly visible here.

Using this system, we found that the different aquaporin genes analyzed were regulated differently in the root fraction containing the AM fungus and in the root fraction uninoculated with the fungus. Moreover, such regulation was also different at the cell level, when comparing the expression of arbusculated cells with that of uncolonized cells. At the root tissue level, the regulation varied depending on the watering conditions, thus, under well-watered conditions, *ZmPIP1;1, ZmPIP1;3, ZmPIP2;4,* and *ZmTIP2;3* decreased their expression in the uninoculated root fraction as compared to the inoculated AM fraction. In contrast, under drought stress conditions, these genes increased their expression in the uninoculated root fraction of the fungus in the root allows a high water uptake, probably mediated by the fungal hyphae (Ruth et al. 2011; Püschel et al. 2020; Cheng et al. 2021) and these aquaporins and the own fungal aquaporin GintAQF2 are involved in the mobilization of water. In contrast, the root fraction that remains uninoculated with the fungus decreases these aquaporins since there is no water uptake by the fungal hyphae and the amount of water mobilized is lower. In contrast, under drought stress the plant need to obtain



Fig. 6 Aquaporin gene expression in root cells isolated from uninoculated maize plants (Control, black bars) or from the two root fractions of maize plants inoculated with the AM fungus *R. intraradices* (AM). See the legend for Fig. 6.

water from the growing subtract and, as in the uninoculated root fraction there are no fungal hyphae to take up such water, the root expresses the plant aquaporins in order to mobilize water in such root fraction. Indeed, mycorrhization enhanced Lpr in all cases as compared to control plants and, curiously, the Lpr values in the uninoculated root fraction reached similar values than in the AM-colonized root fraction, probably by means of the overexpression of several aquaporins.

To study the regulation of these aquaporins also at the cellular level, we did lasermicrodisection of AM colonized cells (cells containing arbuscules) from the AM root fraction and uncolonized cells from the uninoculated root fraction (both from the same root system). Surprisingly, at the cell level the behaviour of aquaporin genes was the opposite than at root tissue level, with a higher expression in uncolonized cells than in arbusculated cells under well-watered conditions and no significant changes under drought stress conditions. This differential aquaporin expression at root tissue and at root cell levels remains to be explained and so far is not easy to be understood. We observed that drought stress enhanced the amount of root ABA accumulation and in AM plants such accumulation was higher in the uninoculated root fraction than in the inoculated root fraction (data now shown), indicating that the level of drought stress was higher in the uninoculated root fraction and supporting the previous idea that such root fraction suffered more the drought stress imposed and need to try to mobilize as much water as possible by expressing aquaporin genes. The own ABA may have affected the aquaporin gene expression (Lian et al. 2006; Beaudette et al. 2007; Ruiz-Lozano et al. 2009; 2022) and may explain why the expression of the aquaporin genes was different at the cell and root tissue levels. However, this remain to be elucidated.

5. Conclusion

Results indicate the existence of both, local and systemic regulation of plants aquaporins by the AM symbiosis. This was observed both at root tissue level and at cellular level. However, depending on the watering conditions, the regulation was different at root tissue and at cell levels and the reason for that remains to be elucidated.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Relaciones entre contenido de agua en el suelo y potencial hídrico de tallo al mediodía para optimizar la programación del riego en melocotoneros y perales

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Resumen: Monitorizar el estado hídrico del árbol es fundamental para ajustar las dosis de riego a los requerimientos del cultivo y obtener producciones rentables y de calidad. El uso de sondas de capacitancia se está expandiendo para programar el riego en cultivos leñosos a través de registros de contenido de agua en el suelo (θ_s) en tiempo real. Sin embargo, cuando no están calibradas pueden generar datos imprecisos que dificultan su uso para la gestión del riego. En este trabajo se relacionaron los registros de sondas de capacitancia sin una calibración previa con un indicador de referencia del estado hídrico en melocotoneros y perales, el potencial hídrico de tallo a mediodía (Ψ_{tallo}). Entre abril y agosto de 2023, en una plantación de melocotoneros y otra de perales, se monitorizó θ_s a tres profundidades: 15, 30 y 45 cm. Se experimentaron dos regímenes hídricos: i) riego según la práctica habitual del productor, y ii) riego gestionado de acuerdo con un modelo de balance hídrico, modulado según las medidas de θ_s . La segunda estrategia permitió ahorrar un 23% de agua en ambas plantaciones, sin penalizar rendimientos o calidad de fruta. En melocotonero, θ_s no se correlacionó con Ψ_{tallo} . No obstante, en peral, θ_s a 45 cm de profundidad se correlacionó significativamente con Ψ_{tallo} (r > 0,95).

Palabras clave: melocotonero, peral, potencial hídrico de tallo, sensor de capacitancia.

1. Introducción

Las limitaciones en el uso de agua para riego en áreas mediterráneas se verán agravadas por los efectos negativos del cambio climático (Masia et al. 2021). Por ello, la reducción de la cantidad de agua aplicada durante determinados períodos del año en los que los frutales son menos sensibles al estrés hídrico podría incrementar la eficiencia en el uso del agua, así como la conservación de este preciado recurso (Fereres y Soriano 2007). Una de las estrategias para alcanzar este objetivo sin reducciones en el rendimiento y la

calidad de la cosecha es el riego deficitario controlado (RDC) que consiste en aplicar una menor cantidad de agua que los requerimientos del cultivo durante las fases del ciclo en las que la producción y la calidad se ven escasamente afectadas, restaurando el riego total durante el resto del ciclo. Esta estrategia ha sido utilizada con éxito en frutales (Girona et al. 2003; Mirás Avalos et al. 2016). Sin embargo, para programar correctamente el RDC es necesario monitorizar el estado hídrico de los árboles de manera fiable. Para ello, el método de referencia es el potencial hídrico de tallo medido al mediodía solar (Ψ_{tallo}) que tiene como desventajas que sus medidas son puntuales y destructivas. Por ello, se está generalizando el uso de sondas que miden el contenido volumétrico de agua del suelo (θ_s) para gestionar el riego en las plantaciones de frutales. Sin embargo, el manejo de estas tecnologías requiere de valores umbral entre los que mantener el θ_s para que los árboles no sufran estrés hídrico. Por lo tanto, es necesario determinar estos valores umbral empleando un indicador robusto y fiable del estado hídrico de los árboles, lo que permitirá ajustar las dosis de riego a las necesidades de los frutales y, así, optimizar el manejo del agua. El objetivo de este trabajo es evaluar la fiabilidad de las relaciones entre los registros de $heta_s$ y las medidas de Ψ_{tallo} en melocotonero y peral con el fin de emplear θ_s para gestionar el RDC en estos frutales.

2. Materiales y Métodos

2.1 Descripción de las parcelas de ensayo

El ensayo se llevó a cabo en dos fincas particulares situadas en Binaced (Huesca) y dedicadas a la producción de fruta para la industria conservera. La zona se clasifica como Mediterránea cálida, de clima semiárido y subcontinental, con una temperatura media anual de 8 °C, precipitación y evapotranspiración de referencia anuales de 398 mm y 1130 mm, respectivamente.

La primera parcela (41º 49' 26,17" N, 0º 13' 23,72" E), de 2,14 ha, fue plantada entre 2018 y 2019 con melocotoneros de la variedad Catherine a un marco de plantación de 5 × 3 m (666,7 árboles/ha). La parcela posee un sistema de riego por goteo (un emisor de 2 L/h por cada 0,5 m lineales de manguera). El suelo de esta parcela es franco arcillo arenoso, con pH básico y un contenido en materia orgánica de 1,7% (Tabla 1).

La segunda parcela (41º 49' 59,8" N, 0º 12' 7,86" E), de 0,98 ha, fue plantada en 2005 y en 2014 con perales de la variedad Williams a un marco de plantación de 4 × 2 m (1250 árboles/ha). La parcela posee un sistema de riego por goteo (un emisor de 2 L/h por cada 0,5 m lineales de manguera). El suelo de esta parcela es franco arcilloso, con pH básico y un contenido en materia orgánica de 1,6% (Tabla 1).

de marchitamiento permanente); AU (Agua útil).								
Darcala	Arena	Limo	Arcilla	рН	MO	CC	PMP	AU
Faiceia		%					%	
Melocotonero	51,4	27,3	21,3	8,47	1,70	24,3	16,0	8,3
Peral	31,0	31,0	38,0	8,42	1,55	28,6	21,3	7,4

Tabla 1. Propiedades de los suelos (0-40 cm de profundidad) de las parcelas de ensayo de melocotonero y peral en Binaced (Huesca). Abreviaturas: MO (Materia orgánica), CC (Capacidad de campo), PMP (Punto de marchitamiento permanente); AU (Agua útil).

En cada parcela, se programó el riego: 1) siguiendo el criterio del agricultor y, 2) a partir de un balance hídrico del suelo siguiendo la metodología propuesta por Allen et al. (1998) y unos coeficientes de cultivo adaptados a la zona. Los datos meteorológicos se tomaron de la estación "Alfántega", situada a 13 km, aproximadamente, de las fincas experimentales. El coeficiente de uniformidad de la instalación de riego se fijó en 95% y se descontó la precipitación efectiva de la semana anterior a la recomendación. Las dosis semanales se dividieron entre 7 para obtener el volumen diario a aplicar. Dependiendo de los registros de las sondas, se espaciaba la frecuencia de eventos de riego.

2.2 Monitorización del agua en el suelo y el estado hídrico de los árboles

A mediados de abril de 2023, en uno de los sectores de cada finca se instaló un conjunto de sondas para el seguimiento en continuo del θ_s (TEROS10, Meter Group Inc. Pullman, Washington, USA) a tres profundidades (15 cm, 30 cm y 45 cm). El seguimiento del estado hídrico de los árboles se realizó con medidas periódicas (entre mayo y agosto, cada 3 semanas, aproximadamente) de Ψ_{tallo} empleando una cámara de presión (modelo Pump-Up, PMS Instruments, Albany, OR, USA). Estas determinaciones se llevaron a cabo sobre una hoja adulta de cada uno de los árboles seleccionados en cada parcela. Las hojas se cubrieron con una bolsa plástica forrada de papel de aluminio al menos dos horas antes de la medida (Mirás Avalos et al., 2016).

2.3 Análisis de datos

Las relaciones entre θ_s y Ψ_{tallo} se determinaron mediante análisis de regresión lineal y cuadrática usando R versión 4.3.0 (R Core Team 2023).

3. Resultados y Discusión

Durante el periodo de estudio (mayo a agosto 2023), la temperatura media fue 23 ºC, la evapotranspiración de referencia ascendió a 619 mm y la precipitación alcanzó 156 mm. En este periodo, el volumen de riego aplicado por el agricultor fue 1333 m³/ha y 1576 m³/ha para los melocotoneros y los perales, respectivamente. En el caso del tratamiento gestionado mediante un balance hídrico y las sondas de capacitancia, las dosis fueron de 1022 m³/ha y 1216 m³/ha para los melocotoneros y los perales, respectivamente. En el caso del tratamiento de 1022 m³/ha y 1216 m³/ha para los melocotoneros y los perales, respectivamente. Esto supone un ahorro del 23% en cada parcela.



Fig. 1 Evolución del contenido volumétrico de agua en el suelo (θ_s) a tres profundidades en una parcela de melocotoneros localizada en Binaced (Huesca).

Entre junio y agosto de 2023, los sensores instalados permitieron captar las variaciones de θ_s causadas por los eventos diarios de riego. En el caso de los melocotoneros (Fig. 1), se observó cierta irregularidad en los picos de θ_s durante la primera mitad de la campaña, así como un período de 15 días (entre el 16 de julio y el 1 de agosto) en los que no se regó. Cabe destacar también que θ_s decayó más rápidamente a 15 y 30 cm que a 45 cm de profundidad (Fig. 1). Los valores mínimo y máximo registrados durante este período fueron 5,1% y 15,6%, respectivamente.

En el caso de los perales, se observaron picos de θ_s relativamente constantes a lo largo de la campaña. Cabe destacar que θ_s decayó más rápidamente a 15 cm que a 45 cm de profundidad (datos no mostrados). De hecho, entre el 25 de junio y el 9 de agosto, el agua aportada a los 15 cm de profundidad del suelo se agota. Los valores mínimo y máximo registrados durante este período fueron 6,7% y 17,8%, respectivamente.



Fig. 2 Evolución temporal del potencial hídrico de tallo (Ψ_{tallo}) en melocotoneros y perales cultivados en Binaced (Huesca) durante el año 2023.

La Figura 2 muestra la evolución estacional del Ψ_{tallo} de melocotonero y peral. En el caso del melocotonero, en ninguna de las fechas de medida se superaron los umbrales de estrés considerados como moderado (-1,5 MPa) y severo (-1,8 MPa) en árboles de la variedad Catherine (Mirás Avalos et al. 2016). Por el contrario, los valores de Ψ_{tallo} para el peral se han situado muy próximos al umbral de estrés moderado (Marsal et al. 2002) y, a mediados de julio, han disminuido hasta alcanzar el umbral de estrés severo (Figura 2). Esto parece indicar que la dosis de agua aplicada es la óptima para esta parcela y que no existe margen de ahorro, o este es muy pequeño.

Tabla 2. Coeficientes de correlación lineal de Pearson obtenidos para los pares de datos de potencial hídrico de tallo a mediodía solar (Ψ_{tallo}) en melocotonero y peral y el contenido volumétrico de agua en el suelo (θ_s) a tres profundidades.

Melocotonero	θ_s 15 cm	θ _s 30 cm	θ_s 45 cm	θ_s promedio	
Ψ_{tallo}	0,430	0,176	0,009	0,213	
p-valor	0,717	0,888	0,994	0,863	
Peral					
Ψ_{tallo}	0,857	0,923	0,956	0,910	
p-valor	0,143	0,077	0,044	0,090	

En el caso del melocotonero, no se observó una relación significativa entre Ψ_{tallo} y el θ_s registrado por los sensores instalados en la finca de ensayo (Tabla 2). Por el contrario, en el caso del peral, se observó una relación significativa entre Ψ_{tallo} y el θ_s a 45 cm de profundidad (Tabla 2). Por tanto, a partir de estos registros se puede obtener una estimación del Ψ_{tallo} con la que poder gestionar el riego de manera más precisa, siendo interesante que Ψ_{tallo} en el peral no sea inferior a –1,5 MPa (Marsal et al. 2002) ya que estudios previos han demostrado la estrecha relación entre Ψ_{tallo} y producción y calidad de cosecha en esta especie (Shackel, 2007).

4. Conclusión

En este estudio, los melocotoneros no han sufrido estrés hídrico a lo largo de la campaña, mientras que los perales se han mantenido en condiciones de estrés hídrico moderado. Se han detectado relaciones significativas entre θ_s y Ψ_{tallo} para el peral, lo que puede contribuir a optimizar la gestión del riego en esta parcela. Sin embargo, no se han detectado relaciones significativas entre θ_s y Ψ_{tallo} para el melocotonero.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

Zaragoza 14-16 FEB 2024

Respuesta del crecimiento radial del tallo de *Cestrum nocturnum* en maceta al estrés hídrico y ambiental

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Resumen: En este estudio se evaluó la eficacia de los dendrómetros para detectar el estrés en arbustos ornamentales y su potencial aplicación en el manejo del riego. Para ello se cultivaron plantas de galán de noche (Cestrum nocturnum) en maceta y en invernadero. Primero se sometieron a estrés ambiental en verano y luego a sequía mediante la suspensión completa del riego. Para evaluar el impacto del estrés en el crecimiento del tallo, se analizaron cuatro índices dendrométricos (IDs): el crecimiento diametral diario del tallo (CDT), la máxima contracción diaria del diámetro del tallo (MCT), y los diámetros de tallo máximos y mínimos diarios (MXDT y MNDT). Bajo estrés ambiental, se observó una disminución en CDT a medida que el estrés se intensificaba. Por otro lado, la MCT mostró oscilaciones pronunciadas, marcadas por días con niveles elevados. El MNDT mostró una mayor sensibilidad en la detección del estrés en comparación con el MXDT, observándose una disminución progresiva en ambos a medida que se intensificaba el estrés. En condiciones de sequía, se registró un aumento de la MCT junto con la disminución en el CDT. Estos resultados indican que los índices dendrométricos pueden detectar el estrés en el galán de noche, ofreciendo así información valiosa sobre su estado fisiológico. Su utilidad para gestionar el riego es limitada, ya que los índices responden tanto al estrés ambiental como al hídrico, lo que significa que el estrés ambiental podría alterar los valores de los dendrómetros incluso cuando la planta no presentara falta de agua.

Palabras clave: dendrómetros, humedad ambiental, sustratos, floricultura

1. Introducción

La disponibilidad limitada de agua para el riego es un problema para el sector viverístico de plantas ornamentales, ya que hay que producir plantas de calidad a la vez que optimizar el uso del agua. Para abordar este desafío, la programación eficiente del riego

es una herramienta valiosa y, por lo tanto, es común el uso de sensores que miden el contenido volumétrico de agua del suelo (CVA). Sin embargo, en los cultivos en maceta, el uso eficiente de estos sensores puede estar condicionado por factores como las características físicas del sustrato, salinidad, temperatura y nivel de humedad (Bañón et al. 2021). En contraposición, la programación basada en el estado hídrico de la planta puede evitar dichas interferencias, y los indicadores obtenidos a partir de las variaciones diarias del diámetro del tallo (VDT) pueden reflejar el estado hídrico de las plantas, aunque las condiciones ambientales de cultivo pueden afectar a dichas variaciones (Miralles-Crespo et al. 2010). Los dendrómetros miden las VDT, las cuales son usadas para calcular los indicadores dendrométricos de tallo (IDs), útiles para conocer el estado hídrico y fisiológico de las plantas (Nortes et al. 2005). El objetivo de esta investigación fue determinar si los IDs diarios obtenidos en plantas de galán de noche cultivadas en sustrato pueden reflejar el estrés hídrico y ambiental de las plantas, y evaluar el potencial de estos para la programación del riego.

2. Materiales y Métodos

Se cultivaron plantas de *Cestrum nocturnum* (galán de noche) en macetas de plástico de 3 litros entre junio y septiembre de 2022. El cultivo fue en invernadero (La Palma, Cartagena) para intensificar el estrés ambiental. El experimento se dividió en tres fases: estrés ambiental ligero (brotación), estrés ambiental severo y estrés hídrico hasta la muerte. Las VDT se midieron con 4 dendrómetros DD-S1 (Ecomatik GmbH) y el CVA se estimó con 4 sensores GS3 (METER Group, Inc.), ambos conectados a un programadorregistrador CR1000 con registros horarios. Se utilizó el software Loggernet 3 para programar el riego, consistente en comprobar el CVA cada hora, para dar un riego de dos minutos cuando el valor estaba por debajo del umbral del 43%. El DPV se calculó con un registrador de datos de temperatura y HR LOG 32 TH (Dostmann electronic GmbH). Los valores de la contracción máxima del diámetro (MCT) y el crecimiento diametral del tallo (CDT) se calcularon a partir de los valores máximos y mínimos diarios de la VDT registrados por los dendrómetros (MXDT y MNDT). Los análisis de regresión y las figuras evolutivas se realizaron utilizando el software SigmaPlot 12.5.

3. Resultados y discusión

3.1. Crecimiento sin restricción de riego

En la Figura 1A se muestra la evolución de los valores de los IDs durante la fase de riego en los meses de junio, julio y agosto. Por otro lado, en la Figura 2 se presenta la evolución de los IDs durante la fase de sequía extrema en septiembre. Para facilitar la visualización de los IDs, se ajustó a cero el valor inicial del MXDT y CDT, y se registraron las pérdidas de diámetro como valores negativos.



Fig. 1 (A) Evolución diaria durante tres meses de cultivo del valor máximo del diámetro del tallo (MXDT), valor mínimo del diámetro del tallo (MNDT), máxima contracción del tallo (MCT), y crecimiento del diámetro del tallo (CDT). (**B**) Evolución diaria durante tres meses de cultivo del déficit de presión de vapor (DPV) y contenido volumétrico de agua diario en el sustrato (CVA). La barra vertical muestra el error estándar medio.

Durante el inicio del crecimiento de los brotes (1ª semana de junio) y hasta la 3ª semana de julio, la MCD y el CDT aumentan progresivamente con oscilaciones entre días, que son más pronunciadas para MCD conforme avanza el tiempo de cultivo (mayor estrés ambiental). Estas oscilaciones sugieren que la MCD es más sensible al estrés ambiental que el CDT.

Desde la 4ª semana de julio, el CDT disminuye progresivamente hasta finales de agosto (Fig. 1A), lo que indica que el tallo está disminuyendo su diámetro debido al estrés ambiental severo al que están sometidas las plantas, como resultado de un alto DPV en el invernadero (calor, alta luminosidad y ausencia de ventilación) (Fig. 1B). La evolución de la MCD fue muy oscilante desde la cuarta semana de julio hasta mitad de agosto, destacando un brusco aumento el 13 de agosto, consecuencia de la caída del MNDT en ese día. Esta caída se produjo por un fuerte aumento del DPV (>3 kPa, ver Fig. 1B) sobre plantas que ya estaban muy estresadas por las malas condiciones ambientales, aun teniendo un riego correcto.

En la figura 1A vemos las subidas en MCD se corresponden con bajadas en el MNDT, lo que sugiere que este índice es más sensible al estrés ambiental que MXDT. Los estudios de Griffin et al. (2021) y Ortuño et al. (2006) demostraron que el crecimiento radial de tallos en árboles urbanos responde a variaciones ambientales, incluso cuando están bien regados. Este hecho nos puede llevar, bajo condiciones ambientales severas, a malinterpretar los valores de IDs para la programación del riego. En la segunda mitad de agosto, las oscilaciones de la MCD se reducen (Fig. 1B).

El estudio de regresión (Tabla 1) muestra que no hay una relación lineal significativa entre el DPV y los diferentes IDs para los meses de junio y julio. Además, no hay una relación estadísticamente significativa entre el DPV y el MXDT ni el CDT en agosto, y aunque las relaciones DPV-MNDT y DPV-MCT fueron significativas, el r² indica una relación lineal muy pobre entre las variables. Todas las relaciones lineales de los IDs con el CVA durante el periodo de cultivo con riego no fueron significativas, probablemente porque las plantas estuvieron bien regadas.

Tabla 1. Análisis de regresión lineal entre y el CVA, y entre los índices dendrométricos y el DPV. Para las abreviaturas, ver leyenda de la Figura 1.

Relaciones	Brotación	Estrés ambiental	Estrés hídrico severo	
	(junio-julio)	(agosto)	(septiembre)	
CVA-MXDT	ns	ns	y=0,004x+27,322 r ² =0,62*	
CVA-MNDT	ns	ns	y=0,004x+28,227 r ² =0,65*	
CVA-MCT	ns	ns	ns	
CVA-CDT	ns	ns	ns	
DPV-MXDT	ns	ns	y=0,0001x+1,531 r ² =0,24*	
DPV-MNDT	ns	y=-0,001x+6,932 r ² =0,26*	y=0,0001x+1,581 r ² =0,20*	
DPV-MCT	ns	y=0,005x+0,985 r ²=0,35*	y=0,002x+1,095 r ² =0,29*	
DPV-CDT	ns	ns	ns	

El número de datos usados para cada regresión fueron n=59 (crecimiento), n=31 (estrés ambiental) y n=29 (sequía). *Diferencias estadísticas significativas con *P*<0,05, ns=sin significación estadística.

3.2. Estrés hídrico hasta la muerte de las plantas

A finales de agosto, antes del inicio del periodo de sequía, las plantas estaban visiblemente dañadas (defoliación y presencia de clorosis foliares) debido al estrés ambiental acumulado. En ese momento los valores de los IDs eran más o menos estables (Fig. 2).

Durante la primera semana de sequía, los valores de MNDT y MXDT bajan y comienzan rápidamente a distanciarse, induciendo un aumento significativo de la MCT y una disminución significativa del CDT (Fig. 2). Tras una semana de sequía, tanto MCT como CDT experimentaron fluctuaciones bruscas que se relacionan con el proceso de deshidratación de los tejidos vegetales del tallo (Remorini y Massai 2003). Estas fluctuaciones se mantienen posteriormente indicando daños severos en las plantas, que son confirmados porque los valores de MCT y CDT evolucionan hasta llegar a niveles estables y próximos a cero, que se corresponden con la muerte de las plantas (Fig. 2). Este comportamiento ha sido descrito anteriormente por Miralles-Crespo et al. (2010)

para la MCT en *Callistemon citrinus* sometido a sequía extrema. Después de la muerte, los valores de CDT y MCT no llegan a cero debido a la rehidratación nocturna (rocío) y a la deshidratación de los tejidos (radiación solar). El análisis estadístico (Tabla 1) muestra que solamente MXDT y MNDT tienen una relación lineal significativa con el CVA, mientras que para MCT y CDT no es significativa, debido quizás a su comportamiento errático durante el periodo de sequía extrema.



Fig. 2 Evolución diaria durante la fase de sequía hasta la muerte de las plantas del valor máximo del diámetro del tallo (MXDT), valor mínimo del diámetro del tallo (MNDT), máxima contracción del tallo (MCT), y el crecimiento del diámetro del tallo (CDT). Los datos del 15 y 16 de septiembre para MCT y CDT no están disponibles debido a un fallo en el datalogger. La barra vertical muestra el error estándar medio.

4. Conclusiones

El MNDT fue más sensible que el MXDT para detectar el estrés ambiental en plantas de galán de noche, respondiendo a esta situación con una bajada. Tanto el CDT como la MCT tiende a generar oscilaciones frente al estrés ambiental, pero de forma más pronunciada y precoz en MCT. Un estrés ambiental más severo conduce a la caída del CDT y a una MCT con oscilaciones pronunciadas. El aumento de la MCT junto con la reducción del CDT sería la primera respuesta a la sequía, indicando la necesidad de regar. Un estés hídrico más severo produciría variaciones bruscas de la MCT y/o el CDT, indicando la necesidad extrema de riego para la planta. La respuesta de los indicadores dendrométricos tanto al estrés ambiental como al déficit hídrico dificulta su uso en la programación del riego.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Protocolo de uso de la dendrometría como herramienta de programación del riego deficitario en olivar

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Resumen: El agua es uno de los factores de producción más limitantes en todos los cultivos. El olivar es una especie tradicional de secano, sin embargo, la intensificación ha llevado a incrementar de forma importante la superficie de riego. El riego deficitario controlado en olivar tiene como mayor limitación la definición de umbrales de estrés hídrico en las diferentes fases del cultivo. La fluctuación del diámetro del tronco son curvas diarias de expansión y contracción conocidas desde los años 60 del siglo pasado. A partir de los 90 comenzó su estudio en gran cantidad de frutales, con poca aplicación en los últimos años, incluso en trabajos científicos. Esta falta de uso está habitualmente relacionada con el efecto de la demanda evaporativa en las curvas obtenidas y la gran variabilidad entre días, incluso en árboles en condiciones de ausencia de estrés hídrico. El objetivo de este trabajo es presentar un protocolo de manejo de la fluctuación del diámetro del tronco aplicado al riego deficitario controlado en olivar. Se presentan dos experimentos en dos fincas diferentes, años distintos y varios tratamientos de riego. En la primera finca, la frecuencia de aparición en la tasa de crecimiento del tronco de valores entre -0.1 a 0.3 mm día⁻¹ ("buenos") fue máxima en momentos de ausencia de estrés, mientras que las más negativos de -0.3 mm día-1 ("severos") fue casi nula. En los momentos de incremento de estrés hídrico los valores "buenos" disminuyeron su frecuencia mientras se incrementó la de "severos". En la segunda finca se observó el mismo patrón, pero durante un periodo de ausencia de riego, con niveles de estrés severos, las frecuencias presentaron valores óptimos. Este error en este indicador fue identificado mediante la comparación de la máxima contracción diaria medida que, descendió muy por debajo de la esperada para condiciones óptimas.

Palabras claves: Diámetros máximos, fluctuación del diámetro del tronco, línea base, máxima contracción diaria, tasa de crecimiento del tronco.

1. Introducción

Las fluctuaciones del diámetro del tronco son ciclos diarios de expansión y contracción que tienen lugar en todas las especies (también en el tallo de herbáceas) y que fueron descritos a partir de los años 60-70 del siglo pasado (Klepper et al. 1971). Se han descrito diferentes indicadores derivados de estas curvas diarias que pueden describir el estado hídrico de los árboles (Ortuño et al., 2010). Sin embargo, a pesar del gran impulso que supuso la década de los 90 en cuanto a publicaciones científicas su uso actual en investigación o a nivel comercial son escasos.

El presente trabajo tiene como objetivo presentar un protocolo de manejo de los indicadores derivados de estos ciclos que permitan una programación en continuo del riego deficitario.

2. Materiales y Métodos

Este trabajo presenta datos de dos campañas diferentes de riego. La primera, en la finca comercial "El Morillo", año 2019. Se trata de una plantación superintensiva de olivar de 13 años. Se realizaron tres tratamientos de riego: Control, sin estrés hídrico a lo largo del año, riego deficitario controlado (RDC-1) y riego deficitario controlado (RDC-2). En ambos se restringió el riego durante el endurecimiento del hueso, pero a diferentes niveles de estrés hídrico, variando también su rehidratación. La segunda, en la finca comercial "El Valenciano", año 2021, en una plantación superintensiva de olivar de 7 años. En esta última finca solo se monitorizó a modo de validación el riego del agricultor. El riego fue deficitario y no se rego durante el mes de agosto. En ambas fincas se monitorizó la variación del diámetro del tronco con sensores perimetrales (D6, UMS, Alemania) y el potencial hídrico de tallo con una bomba tipo Scholander al mediodía en hoja cubierta. Los indicadores obtenidos fueron la tasa de crecimiento del tronco (la diferencia entre dos máximos consecutivos) y la máxima contracción diaria (la diferencia entre el máximo y el mínimo diarios).

3. Resultados

La tasa de crecimiento del tronco (TCT) presentó grandes variaciones entre días, incluso para los árboles con los tratamientos más regados, aunque aparentemente suponían una tasa constante en la curva de diámetros máximos (Fig. 1). A pesar de la gran variabilidad en los valores de la TCT se obtuvieron diferencias significativas entre tratamientos, especialmente durante el periodo de recorte del riego (endurecimiento del hueso) y la rehidratación (Fig. 1a). Las tendencias en la curva de diámetros máximos, incluso, permitiría establecer de forma más visual las diferencias entre los tratamientos de riego planteados (Fig. 1b).

Los mismos datos al convertirlos en frecuencias de aparición semanales incrementaron las diferencias y pudieron ser evaluados de una forma más clara (Fig. 2).



Fig. 1 Evolución de la tasa de crecimiento del tronco (a) y los diámetros máximos (b) del experimento de riego de olivar en el año 2019. Cada símbolo/línea es la media de 4 datos. Los asteriscos indican diferencias significativas en la TCT en esas fechas. (Fuente: Martín-Palomo et al 2021).

La caída de la frecuencia de los valores denominados "buenos" (entre -0.1 y 0.3 mm día⁻¹) fue significativamente menor en los tratamientos estresados en lugar del Control, especialmente durante el periodo de endurecimiento del hueso (Fig. 2a). Por el contrario, hubo un gran incremento de los valores "severos" (menores de -0.3 mm día⁻¹), en este periodo (Fig. 2b). Es importante señalar que, durante la rehidratación, la mejora fue sólo parcial, recuperándose los valores de las frecuencias a partir del día del año 250. La evolución estacional del potencial hídrico fue similar a la obtenida en la curva de diámetros máximos, coincidiendo con los periodos de estrés hídrico planteados por las frecuencias de la TCT (Fig. 2c). La recuperación del potencial fue rápida en los tratamientos estresados, aunque parcial en uno de ellos.

Los datos de potencial hídrico obtenidos en la Finca el Valenciano sugieren tres partes diferentes de la estación de riegos (Fig. 3).

La primera con un nivel de estrés hídrico moderado, incluso con una recuperación a finales de julio. La segunda, durante el mes de agosto con valores más negativos de potencial hasta finales del mes de agosto donde se recuperaron los árboles de forma parcial (Fig. 3a). Esta evolución fue similar a la sugerida por las frecuencias semanales de la TCT tanto en el mes de julio como en la rehidratación a finales de agosto (Fig. 3b). Sin embargo, no coincidió con los valores del mes de agosto, en los momentos de mayor

estrés hídrico. En este periodo, las frecuencias de la TCT indicaron un estado hídrico óptimo con máximos en los valores "buenos" y cercanos a cero en los valores "severos". Los datos obtenidos por la máxima contracción del tronco permitieron identificar estos valores anómalos encontrados durante el mes de agosto (Fig. 3c). Los valores de este indicador fueron muy similares a los esperados en condiciones óptimas al inicio de julio y a primeros de septiembre, pero no así desde la segunda mitad de julio.



Fig. 2 Evolución de la frecuencia de valores buenos (a), severos (b) y potencial hídrico a lo largo del ensayo. Las líneas verticales indican el periodo de endurecimiento del hueso. La línea sólida presenta el potencial hídrico estimado en condiciones óptimas de riego según la ecuación de Corell et al 2016). Los asteriscos indican diferencias significativas en la fecha en la que aparecen. (Fuente. Martín-Palomo et al., 2021)



Fig. 3 Evolución del potencial hídrico (a), frecuencia TCT (b) y MCD (c) a lo largo del ensayo en la finca El Valenciano. Los símbolos son la media de 5 datos. La línea sólida presenta el potencial hídrico (a) y la MCD (c) estimado en condiciones óptimas de riego según la ecuación de Corell et al (2016 y 2013, respectivamente).

4. Discusión

La frecuencia de aparición de diferentes rangos de valores TCT permitió evaluar el estado hídrico de los árboles. Estos valores tuvieron un rango de variación con el potencial hídrico similar al encontrado en otros dos años anteriores de estudio (Corell et al 2019, Martín-Palomo et al., 2021). Solo en condiciones de ausencia de riego y estrés severo las frecuencias dieron valores falsos de estados hídricos óptimos. Esto es debido a que el tronco adaptó su evolución a las condiciones más severas. Este tipo de respuesta estaría relacionada con una restricción severa del intercambio gaseoso, ya que la contracción diaria se ha relacionado tradicionalmente con la traspiración (Herzog et al., 1995). También, muy probablemente, con el nivel de embolismo debido al estrés hídrico, puesto que podría restringir el flujo de agua desde la corteza al xilema y dar lugar a cambios casi nulos de la TCT.

5. Conclusión

El uso de la dendrometría precisa de la combinación de los dos indicadores más habitualmente empleados, la TCT y la MCD. La frecuencia de aparición de la TCT permitiría una monitorización en continuo del estado hídrico en un amplio rango de estado hídricos. Los valores de MCD permitirían validad estos resultados para evitar la aparición de falsos positivos derivados de la respuesta al estrés hídrico de los árboles.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Strawberry fruit quality and production under drought stress conditions

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Abstract: The increase in temperature and the decrease in water resources predicted by climate change models will directly influence the productivity, ripening rate, and organoleptic quality of fruit crops. Strawberry is an important crop in Spain, Spain being the sixth largest strawberry producer in the world. This crop is particularly susceptible to drought stress; climate change will significantly challenge strawberry production in the short and medium term. This assay aimed to characterize the performance of different strawberry (Fragaria × ananassa, Duch.) varieties under water restriction conditions. For this purpose, potted plants of the cultivars Chandler, Camarosa, Amiga, and Rociera were grown in a greenhouse during the production months under sufficient (3.3 L·m⁻²·day⁻¹) or deficient (0.5 L·m⁻²·day⁻¹) irrigation conditions. Vegetative growth of plants under stress conditions decreased significantly in the same proportion in all lines tested. However, water use efficiency, estimated as photosynthetic rate/transpiration rate, increased in water-stressed plants of cv. Chandler. Under drought conditions, a significant decrease in fruit weight and size was observed in all genotypes and in the number of fruits produced per plant. The cv. Rociera showed the most significant decrease in fruit yield, 90%, despite this genotype being the most productive in control conditions. Regarding fruit quality, no differences were observed in firmness, except in the Rociera genotype, which produced firmer fruits under stress conditions. Likewise, water restriction did not affect the concentration of soluble solids except in cv. Amiga, in which this variable was significantly increased. As for color, there were no significant changes in the parameters measured. Experiments are underway to determine transcriptomic differences among the different genotypes subjected to drought conditions.

Keywords: drought stress, strawberry, fruit quality, Fragaria

1. Introduction

The most likely environmental scenarios predicted by climate change models indicate that agriculture in Mediterranean regions will face greater water constraints than currently. Providing enough food for a growing population will therefore be one of the most important challenges of the 21st century (Kang et al., 2009). Thus, a thorough understanding of plant water relations is of vital importance for successful crop adaptation (Iglesias & Garrote, 2015).

Strawberry (Fragaria × ananassa Duch.) is considered one of the most important fruit crops in the world, with a high socioeconomic value due to its organoleptic properties and its high content in vitamins, minerals and antioxidants (Cordoba-Novoa et al., 2022). Spain represents the sixth largest strawberry producer worldwide and the first at European level, with 97% of the crop residing in the province of Huelva (FAOSTAT 2021; Romero-Gámez & Suárez-Rey, 2020). In Huelva, there has been a transition from a practice in which almost the entire productive area was dedicated to the cultivation of a single variety, Camarosa preceded by Chandler, to diversifying production with different genotypes, more than 40 different varieties at this moment. In recent years, the predominant variety has been Florida Fortuna, accounting for 36,2% of the area, followed by Rociera, which accounts for 25,6% (Fischer et al., 2021).

Both the development and the production of strawberries are severely affected by water shortage. Strawberry plants are very vulnerable to water deficit, as they require a high water supply due to their superficial root system, their extensive leaf surface and the high water content of the fruit. Therefore, this shortage has a direct influence on productivity, ripening rate and organoleptic quality of the fruit (Cordoba-Novoa et al., 2022). The decrease in strawberry production is due to a reduction in the number of fruits produced per plant, to a lower fruit weight or to a combination of both factors, depending on the experimental conditions and the genotype tested (Grant et al., 2010; Martínez-Ferri et al., 2016). In any case, the few studies carried out in this respect show a significant genotypic variability, which suggests that there are real possibilities in the improvement of this crop to produce strawberries under limiting irrigation conditions.

The present study aimed to characterise the behaviour of different strawberry cultivars under water restriction conditions, as a first step to deepen the genetic and molecular basis of strawberry plant response to drought.

2. Materials and Methods

2.1 Plant material.

The strawberry cultivars (*Fragaria x ananassa*, Duch.) Chandler, Camarosa, Amiga and Rociera were used for the experimental essay. The plants were established from runners.

2.2 Essay conditions.

The plants were grown in 6-litre pots, 18 plants per variety, and were kept in a greenhouse at the IFAPA Centre in Malaga during the production months (April to July) with natural photoperiod. Two irrigation conditions were used for this essay: daily

irrigation 6 days a week (equivalent to 3,3 L·m⁻²·day⁻¹, control treatment), irrigation two days a week (equivalent to 0,5 L·m⁻²·day⁻¹, deficit irrigation treatment). Temperature ranged from a minimum of 18.2°C to a maximum of 31.3°C, while relative humidity was maintained between 50.5 and 80.7%.

2.3 Vegetative growth analysis.

Photographs of the zenith projection of 6 plants of each variety and treatment were taken and area values were obtained using ImageJ software. In addition, leaf area was determined by scanning 9 leaves of different plants of each variety and treatment. In addition, leaf water content was determined. Measurements of CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (gs), and intracellular CO_2 concentration (Ci) were made using the IRGA (LCpro T). Water use efficiency was estimated as the A/E ratio.

2.4 Production analysis and phenotyping of fruits.

Fruits were harvested at the mature red stage and the total weight and number of fruits per plant were obtained. For phenotyping, fruits were weighed and measured using a precision balance and a digital caliper. In addition, firmness, soluble solids content and colour were determined using a penetrometer, refractometer and colourimeter, respectively.

3. Results

3.1 Vegetative growth.

The vegetative growth of plants under stress conditions decreased significantly in all genotypes (*Fig. 1*). Similarly, a significant decrease in leaf area and leaf water content was observed, although this decrease was not significant in the case of Rociera.

Photosynthetic parameters were measured in the genotypes Chandler and Amiga. In both varieties, a significant decrease in photosynthetic rate and transpiration was observed under deficit irrigation conditions (**Table 1**). However, variations in stomatal conductance and internal CO₂ were not significant. Water use efficiency, estimated as photosynthetic rate/transpiration rate, was slightly increased in cv. Chandler, but decreased in Amiga.

3.2 Production and phenotyping of fruits.

Under deficit irrigation conditions, a significant decrease in fruit weight and size was observed in all genotypes and in the number of fruits produced per plant. The cv. Rociera showed the most significant decrease in fruit yield, 90%, although this genotype was the most productive under control conditions (*Fig. 2*).


Fig. 1 (A) Zenith projection, (B) leaf area and (C) leaf water content in plants grown under water limitation conditions. Representative photograph of the zenithal projection of the Rociera variety (D) and leaf area of the Amiga variety (E). Statistical significance with respect to the control was determined by Student's t-test. ****P-value < 0.0001, **P-value < 0.01, "ns" not significant.

Variety	Treatment	A (μmol·m⁻ ²·s⁻¹)	E (mmol·m⁻ ²·s⁻¹)	Gs (mmol·m⁻ ²·s⁻¹)	Ci (vpm)	WUE (A/E)
Chandl er	Control	11,78±1,52	2,17±0,22	0,23±0,06	298,7±8,51	5,42±0,2 8
	Water stress	8,88±0,21*	1,54±0,02**	0,17±0,01 ^{ns}	303,3±6,66 ^{ns}	5,78±0,0 9 ^{ns}
Amiga	Control	11,45±0,86	2,253±0,15	0,32±0,03	322±10	5,09±0,4 8
	Water stress	8,59±1,08*	1,87±0,10*	0,29±0,04 ^{ns}	340,3±12,22 ^{ns}	4,61±0,8 4 ^{ns}

 Table 1. Photosynthetic parameters measured on Chandler and Amiga varieties.

A (photosynthetic rate), E (transpiration rate), Gs (stomatal conductance), Ci (intracellular CO_2), WUE (water use efficiency). Statistical significance with respect to the control was determined by Student's t-test. **P value < 0.01, *P value < 0.05, "ns" not significant.

Regarding fruit quality, no differences in firmness were observed, except in the Rociera genotype, which produced 20% firmer fruit under stress conditions. Likewise, water restriction did not affect the concentration of soluble solids except in cv. Amiga, where this variable increased significantly. As for colour, there were no significant changes in the parameters measured, but an increase in lightness (L*) was observed in the fruit of the Chandler and Amiga varieties (*Fig. 3*).



Fig. 2 Production and fruit size. (A) Number of fruits produced per plant, (B) fruit weight, (C) fruit length and (D) fruit width. Data represent the mean \pm SD of between 30 and 120 fruits of each variety and treatment. ****P-value < 0.0001, ***P-value < 0.001, **P-value < 0.01, *P-value < 0.05.



Fig. 3 Firmness (A), soluble solids (B) and L* or lightness of the fruit (C). Data represent the mean ± SD of between 30 and 120 fruits of each variety and treatment. ****P-value < 0.0001, **P-value < 0.01, *P-value < 0.05, "ns" not significant.

4. Discussion

Strawberry plants are very vulnerable to water deficiency, as they require a large water supply due to their superficial root system, their extensive leaf surface and the high water content of the fruit. Therefore, several studies show that under water deficit conditions there is a reduction in vegetative growth of the whole plant, directly affecting productivity, ripening rate and organoleptic quality of the fruit (Cordoba-Novoa et al., 2022; Deaquiz et al., 2014).

In this study it has been shown that cultivation of strawberry plants under limiting irrigation affects the crop in multiple factors, causing a significant decrease in vegetative development, leaf area and water content per unit leaf weight. In addition, this water deficit also leads to changes in gas exchange processes. In cv. Chandler and Amiga, there was a significant decrease in photosynthesis and transpiration rate of 25% and 20-30%, respectively, under deficit irrigation conditions. The decrease in transpiration rate resulted in intracellular CO_2 accumulation, reaching 20% for Amiga. In addition, in the cv. Chandler there was an increase in water use efficiency, coinciding with the results

previously described by Martínez-Ferri et al. (2016). Despite these changes, no visual signs of leaf stress damage were observed.

As for the number of fruits produced per plant, we found that under water stress there was a significant reduction in their number, with the Rociera cv. producing the greatest reduction (80%), followed by Amiga (67%), Chandler (59%) and Camarosa (46%). On the other hand, fruit quality was also affected, with a reduction in weight, length and width of 40%, 21% and 15%, respectively.

Regarding fruit quality parameters, under deficit irrigation conditions, the cv. Rociera significantly increased fruit firmness by 20%. For their part, the cv. Amiga increased the soluble solids content (28%) and the lightness (7%) of the fruit. These results suggest that growth under deficit irrigation may cause osmotic adjustments and/or changes in the characteristics of the cell walls of the fruit parenchyma cells, affecting firmness and sugar content. However, these changes are genotype dependent. These modifications of fruit quality could enhance the attractiveness of the fruit for commercial purposes (Reigada et al., 2021).

In order to deepen the understanding of the behaviour of these varieties, gene expression experiments are being carried out to determine the transcriptomic changes produced in the different genotypes in response to water stress.

5. Conclusion

The results of this study, along with those of previous works on strawberry cultivation, can be a useful tool to improve the efficiency of irrigation water use and the sustainability of water resources in strawberry cultivation. The use of varieties with good yield but reduced leaf area would reduce total plant transpiration and could be considered as a good method to reduce irrigation needs. Furthermore, the adaptability of cultivars such as Rociera and Amiga under water scarcity conditions are attractive options, not only in terms of quality preservation, but also in the context of their commercial potential. This study underlines the importance of considering the resilience and adaptability of plant varieties in the face of environmental challenges, providing valuable information for the agricultural and commercial sector in future decision making.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Response of grape anthocyanin profile to elevated temperature and water deficit on four commercial grapevine varieties

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Abstract: The objective of the study was to assess the impact of elevated temperature and water deficit, either individually or in combination, on the concentration and profile of berry anthocyanins in four commercial grapevine varieties. Fruit-bearing cuttings of Tempranillo, Cabernet sauvignon, Merlot and Grenache were grown from fruit set to maturity in temperature gradient greenhouses under two temperature regimes: ambient temperature (T) or ambient temperature + 4° C (T+4). At veraison, the plants of each variety and temperature regime were divided into two groups subjected to: full irrigation (FI, 90% substrate field capacity) or water deficit (DI, 50% substrate field capacity). At maturity, berry skin anthocyanins were analyzed by HPLC-DAD. The varieties studied differed in the concentration and composition of anthocyanins. Overall, T+4 reduced total skin anthocyanins, especially under FI. T+4 and DI increased the abundance of methylated forms, in particular malvidins. DI increased the proportion of try-hydroxylated anthocyanins, especially when DI was combined with T+4, whereas grapes developed under elevated temperature had a higher abundance of acetylated and coumaroylated forms. Tempranillo and Cabernet Sauvignon were more affected by changes in temperature and water availability than Grenache and Merlot. The results suggest a significant impact of elevated temperature and drought on anthocyanin concentration and profile, this effect being dependent on the genotype.

Keywords: anthocyanins, drought, climate change, cultivar, warming, Vitis vinifera L.

1. Introduction

As a consequence of the emissions of greenhouse gases, the global mean temperature has increased since the pre-industrial era and this tendency will likely continue in the coming decades (IPCC 2021). At the same time, changes in the precipitation regimes are projected, affecting both the intensity and frequency of rainfall. In particular, for the

Mediterranean area, water deficit is expected to occur more often and in higher intensity.

Anthocyanins are phenolic compounds of the flavonoid group that are responsible for the color of red grapes. The negative impact of elevated temperature on grape anthocyanin concentration has been broadly reported (Carbonell-Bejerano et al. 2013; Torres et al. 2017; Rienth et al. 2021). The impact of water deficit on grape composition strongly depends on the duration and the intensity of the drought period, as well as on the grape developmental stage when it occurs (Scholasch & Rienth 2019). The modulation of anthocyanin concentration and diversity in response to either temperature or water availability seems to be highly genotype dependent (Fernandes de Oliveira et al. 2015; Rienth et al. 2021; Scholasch & Rienth 2019). However, climate change impacts on grapevine performance are a multi-stress issue and interactions need further investigation (Gray & Brady 2016).

The objectives of the study were: i) to assess the impact of elevated temperature and water deficit, acting either individually or in interaction, on the concentration and profile of grape anthocyanins, and ii) to evaluate whether the multi-stress response depends on the grapevine genotype.

2. Materials and Methods

2.1 Plant material and growth conditions

Dormant cuttings of Tempranillo, Cabernet sauvignon, Grenache and Merlot were induced for fruit-bearing cuttings according with Mullins (1966), with some modifications (Ollat et al. 1998). The number of bunches was limited to one per plant. After fruit set, plants were transplanted to 13 L plastic pot with peat:perlite (1:1, v/v) and placed in temperature gradient greenhouses (TGGs). Plants of each variety were subjected to two temperature regimes: ambient (T) vs. ambient + 4° C (T+4). At veraison, half of the plants of each variety and temperature regime was exposed to water deficit (50% of substrate field capacity, DI) and the other half remained as well-irrigated controls (90% substrate field capacity, FI). The experiment included 5 plants per variety and treatment. Plants were irrigated with water alternated with nutrient solution (Ollat et al. 1998) and grew under these conditions until maturity (total soluble solid content, TSS, of ca. 23°Brix).

2.2 Determinations

Pre-dawn leaf water potential (Ψ_{pd}) was measured 7, 14 days after veraison, and at maturity in young fully expanded leaves, using a pressure chamber (Scholander et al. 1965). At maturity, 10 berries per plant were sampled and manually peeled. The concentration of TSS was measured in the pulp using a refractometer. The skins were freeze-dried, ground and extracted in methanol:water: 7M HCl (70:29:1, v:v:v). The extracts were filtered and analyzed using reversed-phase high performance liquid

chromatography (HPLC) coupled to a diode array detector (DAD) according to Martinez-Lüscher et al. (2019). A commercial standard of malvidin-3-O-glucoside was used for the quantification of anthocyanins.

2.3 Statistical analysis

Statistical analysis was performed with XLstat-Pro (Addinsoft). Data were subjected to a three-way ANOVA to evaluate the main effects of the factors studied (variety, temperature and water availability). Differences among treatments were tested with a Least Significant Difference (LSD) post-hoc test.

3. Results and Discussion

Plants exposed to water deficit (DI) combined with elevated temperature (T+4) had significantly lower Ψ_{pd} values in the four varieties studied (Figure 1). Differences were more evident at day 7 and 14 after veraison, and disappeared at maturity in all the varieties except in Grenache (Fig. 1).



Fig. 1 Evolution of the predawn leaf water potential in four grapevine varieties subjected to ambient temperature (T) and ambient temperature + 4°C (T+4), combined with full irrigation (FI) and deficit irrigation (DI). Points represent means \pm SE (n=5). Means with letters in common within the same variety and stage are not statistically different (LSD test). *P*, probability values of the main factors, temperature (T) and irrigation (I) and their interactions. ***, *P*<0.001; **, *P*<0.05; ns, not significant.

The level of TSS at maturity was similar among varieties and treatments (P_v =0.287; P_T =0.099, P_I =0.395). Tempranillo was the variety with the highest concentration of total grape anthocyanins. Grenache showed the lowest levels (Fig. 2). T+4 significantly reduced anthocyanin levels, especially in Tempranillo and Cabernet sauvignon, probably due to a reduced biosynthesis and/or degradation (Rienth et al. 2021). DI, overall, tended to increase the concentration in all the four varieties (Fig. 2).



Fig. 2 Total skin anthocyanins in four grapevine varieties subjected to ambient temperature (T) and ambient temperature + 4°C (T+4), combined with full irrigation (FI) and deficit irrigation (DI). Means with letters in common are not statistically different. The rest as for Fig. 1.

Grenache showed the highest proportion of methylated forms, namely malvidins and peonidins, and the lowest abundance of acylated forms (Figs. 3 and 4). Both T+4 and DI increased the proportion of methylated derivatives, through the increase in malvidins at the expense of delphinidins (Fig. 3A and 4A). DI significantly increased the abundance of try-hydroxylated forms, especially in combination with T+4 (Fig 3B), whereas T+4 enhanced the acylation level of anthocyanins (Fig. 4B). These changes induced by T+4 and DI may contribute to higher intensity and stability of color in grapes and wines (Gambetta et al. 2020). Tempranillo and Cabernet sauvignon showed significant increases in malvidins, methylated and acylated forms in the treatment with combined T+4 and DI (Fig. 3 and 4). In contrast, the effect in Grenache was less marked, despite this variety showed the highest decrease in Ψ_{pd} under the DI treatments (both T DI and T+4 DI) (Figs. 3 and 4).

4. Conclusion

DI partially alleviated the decrease in total anthocyanins produced by T+4 in Tempranillo and Cabernet sauvignon. The results show additive effects of T+4 and DI on anthocyanin composition, favoring the accumulation acylated and methylated forms, which may contribute to higher color intensity and stability in grapes and wines. The response of anthocyanin composition to changes in temperature and water availability was modulated by the genotype, Tempranillo and Cabernet sauvignon being the most responsive cultivars. Despite its lower concentration of total anthocyanins, Grenache was revealed as a variety less sensitive to changes in temperature and water availability.





Fig. 3 Anthocyanin profile (A) and hydroxylation level (B) in four grapevine varieties subjected to ambient temperature (T) and ambient temperature + $4^{\circ}C$ (T+4), combined with full irrigation (FI) and deficit irrigation (DI). In (A), * indicate significant differences with T FI for each compound and variety. In (B), letters in common indicate no significant differences. The rest as for Fig. 1.



Fig. 4 Anthocyanin methylation (A) and acylation (B) level in four grapevine varieties subjected to ambient temperature (T) and ambient temperature + 4° C (T+4), combined with full irrigation (FI) and deficit irrigation (DI). The rest as for Fig. 3.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

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Cannabis sativa a new water-efficient alternative. New approaches for measuring water stress

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Abstract: Climate change has generated new agricultural challenges, which require the use of more demanding irrigation efficiency techniques, as well as alternative crops that are not affected by dry periods throughout the agricultural season. For this reason, the effect of water deprivation has been studied in four cultivars of *Cannabis sativa* L. during the different phases of growth. During the 2022 growing season, the trials were carried out under a plastic greenhouse located at Las Torres - IFAPA research center, Sevilla, southern Spain.

The water deprivation experiment was carried out in each of the three periods of the cycle (vegetative, flowering and pre-harvest) with one week of irrigation cut, which meant a decrease of 10% of the total water used. After the period of water deprivation, irrigation was restored until the end of the cycle. An exhaustive real-time monitoring of plant stress was carried out employing techniques such as thermography and spectrometry, whose results were in turn compared with other classical methods such as determination of leaf gas exchange, fluorescence, water potential, and the foliar contents of malondialdehyde and anthocyanins.

The results showed that although the magnitude of the effect of water deprivation is dependent on the genotype, in general terms the occasional water deprivation in any case had an impact on biomass or cannabinoid production. In addition, the methodology and sensors used have demonstrated a high potential to be used as a decision-making tool for more efficient irrigation.

Keywords: cannabis, water deprivation, water stress, physiological traits, thermography, spectrometry, dendrometry

1. Introduction

In Europe, and other parts of the world, unprecedented periods of drought are being experienced, and throughout climate change, it is anticipated that these drought events will occur more frequently (Thevs y Nowotny 2023). The cannabis plant is showing high potential as a drought-resistant industrial crop and offering intriguing possibilities as a sustainable crop in climate change scenarios (Gill et al. 2023; García-Tejero et al. 2014).

Similarly to various studies on the effect of water deficit on industrial crops (p.e García-Tejero et al. 2014; 2019; Park et al. 2022; Duong et al. 2023), there are few studies related to medicinal cannabis. In fact, Payment y Cvetkovska (2023) indicate that all the studies they have examined regarding the drought resistance of *Cannabis sativa* have been conducted on hemp varieties with low or moderate cannabidiolic acid (CBDA) accumulation. In studies involving cannabis for medicinal purposes Caplan, Dixon y Zheng (2019) found that controlled exposure could even increase the production of Δ -9-tetrahydrocannabinol (THC) and cannabidiol (CBD). This same study noted a reduction in the photosynthetic rate and the water potential compared to the control.

Duong et al. (2023) found that the response to water stress depends on the variety. The two industrial hemp cultivars in their trials responded very differently to water stress in terms of growth and water relations, but less so in cannabinoid concentration. One variety exhibited a more anisohydric behavior, with high transpiration rates and management of water potential and stomatal conductance as regulators of water loss, while the other responded to water stress with rapid defoliation, balancing productive and evapotranspirative factors. Although in no case did stress significantly affect THC and CBD concentrations, the strategy of the anisohydric-responsive cultivar reduced flower and CBD production, while the yield of the other cultivar was similar to that of well-irrigated plants.

Non-destructive leaf assessments using optical methods such as leaf reflectance and absorbance in the visible and infrared spectra, fluorimetry, or thermography have been used, among others, to estimate leaf nutrient concentration (Oliveira y Santana 2020), for early disease detection(Owomugisha et al. 2020), to determine the effect of herbicides (Yao et al. 2012), for decision-making in irrigation (Parihar, Saha y Giri 2021), cannabinoids concentration (Birenboim et al. 2022) or for assessing photosynthetic conditions and the effects of different actions and stresses on plants (Shin et al. 2021).

This study aimed to verify the response of cannabis varieties for medical use to punctual water stress at different phenological stages of the crop. Similarly, it aimed to explore the possibilities of new non-destructive methods for stress determination performed *in situ*, intended for real-time decision-making and recommendations purposes.

2. Materials and Methods

2.1 Vegetal material

Trials were carried out under greenhouse conditions at Las Torres center (IFAPA, Sevilla), located in the Guadalquivir river basin, SW Spain (37° 301 47" N; 05° 581 0211 W), in 2022. Irrigation and fertilization were based on the cannabis crop demand and corresponded to the crop management standards recommended by Phytoplant Research. The plant material was rooted cuttings obtained from Phytoplant facilities. Transplanting into soil was carried out on June 06th, 2022, at a density of 1 plant m-2. Fertilization started (7 days after transplanting), applying exclusively Terra Vega[™] (CANNA España SL) fertilizer till the second week of august. After this, Terra Flores[™] (CANNA España SL) was applied until last week before harvest. Both Terra Vega and Terra Canna are liquid fertilizers for fertigation with a NPK composition of 3-1-3 and 2-2-4, respectively. Both compositions contain other relevant micronutrients as Mg, B, S, Na, Cu, Fe, Mn, Mo and Zn in different composition and chelates as EDDHA, EDDTA, and DTPA to ensure the nutrient absorption by roots. At the end of the season, plants had received 101, 43, 115 kg· ha⁻¹ of NPK.

2.2 Experimental Design

Four varieties belonging to Phytoplant Research were used: Raquel, Beatriz, Goya, and Magda. The trial design was a Split-plot, with three repetitions. Within each block, all varieties were randomly arranged, and within each variety, irrigation treatments were randomly distributed in elementary plots. This design was chosen to prevent interference due to different development rates among the varieties. Each elementary plot consisted of 11 plants, totaling 132 plants per variety.

The treatments involved approximately one-week irrigation cuts (depending on climatic conditions) at different phenological stages: vegetative (July 18th to July 25th), flowering (August 4th to August 12th), and pre-harvest (September 8th to September 19th). In all cases, these were compared with a Control treatment that remained irrigated by demand throughout the entire period.

2.2 Measurements

Throughout the irrigation deprivation period, measurements of various physiological parameters such as Gas exchange, leaf water potential, thermography, leaf fluorescence, and spectrometry were taken at three time points (start day, intermediate day, and final day). These measurements were taken from both the deprivation treatment and the control. Sampling or measurements were conducted between 12:00 pm and 2:00 pm.

All measurements or samples for subsequent analysis were taken from 3 plants per elementary plot, totaling 9 measurements per variety and treatment.

The measurements on leaves were taken on the central leaflet of one of the youngest fully expanded leaves from the apical part of the plant. These determinations included gas exchange, fluorescence, and spectrometry.

The photosynthetic rate (An) and Stomatic conductance (gs) was recorded under specific greenhouse conditions using an infrared gas analyzer (IRGA) in an open circuit (ADC BioScientific Ltd., LCPro, Herts, England). To compare was fixed the air CO₂ at 400ppm CO2 and 1000 μ mol(photon) m⁻² s⁻¹ of PAR. The temperature and humidity were taken from surrounding air in greenhouse.

Fluorescence data were measured using a Handy PEA+ (Hansatech Instruments Ltd., Norfolk, UK). Leaves were kept in the dark for at least 30 min and after this time, Chl a Fluorescence Induction Curves were measured using PEA with red irradiance of 3,000 μ mol(photon) m⁻² s⁻¹. From these measurements, the maximum quantum yield of PSII (Fv/Fm) was taken.

Foliar reflectance and transmittance from the upper side of the leaves between 400 and 1000 nm was recorded. The CI-710 Miniature Leaf Spectrometer (CID Bio-Science, Inc., Camas, Washington, USA) was used to record the spectra. For each genotype in each replicate, we calculated spectral vegetation indices. We used 4 indices previously associated with chlorophyll and carotenoid concentration, health and stress status:

- CCI: Chlorophyll Content Index. In situ measurement of leaf chlorophyll concentration (Parry, Blonquist y Bugbee 2014).

$$CCI = \frac{T_{931}}{T_{653}}$$

- Ctr2: Carter index 2. Index that has been associated with plant stress (Carter 1994).

$$Ctr2 = \frac{R_{695}}{R_{760}}$$

- RENDVI: Red Edge Normalized Difference Vegetation Index. This index has been linked to the plant's nitrogen content (Oliveira et al. 2017)

$$RENDVI = \frac{R_{750} - R_{705}}{R_{750} + R_{705}}$$

 PRI: Photochemical Reflectance Index. It's a vegetation index associated with changes in the carotenoid/chlorophyll ratio and linked to the interconversion of xanthophylls (Filella et al. 2009)

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$$

Leaf water potential (Ψ_{leaf} o WPot) was concurrently measured on a excised (measured immediately), fully exposed mature leaf per plant at apical level with a Scholander-type pressure chamber (Soil Moisture Inc, Santa Barbara, CA, USA), where plants were measured immediately after excision.

The thermal images were taken using a ThermaCam (Flir SC660, Flir System, USA, 7–13 μ m, 640 × 480 pixels) and an emissivity of 0.96. Readings were developed at the sunny side of the canopy, placing the camera at a 1 m distance from the monitored plant, approximately. Afterwards, images were analyzed using the Flir Research Pro Software (Flir System, USA), which allows to select different zones of the images (in our case; 3 different sunny areas per image were selected); each pixel corresponding to an effective temperature value.

For the determination of malondialdehyde (MDA) and anthocyanins (ATC), leaves from the same zone (apical) were taken. The leaves were immediately frozen until analysis. Anthocyanin extraction was carried out as described in Rabino y Mancinelli (1986), with slight modifications. MDA extraction was performed according to Hodges et al. (1999), subsequently modified by Landi (2017).

After harvesting, gravimetric measurements of yield were taken. At harvest, plant height and fresh weight were measured. Following the plant's air-drying period in a controlled environment, the dry weight of the entire plant and the weight of branches, flowers, and leaves from each plant were measured. From the latter two, crushed samples were taken for cannabinoids analysis.

Inflorescences and leaves samples were analyzed by near infrared spectroscopy (NIRS) for the determination of the content of cannabinoids and moisture content. An AURORA NIR (GraiNit S.r.l., Italy), equipped with a InGaAs detector, was used for reflectance measurement of the samples from 950 to 1650 nm, every 0.5 nm. High performance liquid chromatography coupled to a diode array detector (HPLC-DAD) and Karl Fischer titrator were employed as reference techniques to assure the correct performance of NIRS measurements for the determination of cannabinoids and moisture content, respectively. An Agilent 1260 Infinity series (Agilent Technologies, Inc, Santa Clara, USA) equipped with a G1329B autosampler and a G1316A DAD was employed (Garrido et al. 2022). Moisture content determination was performed using a Karl Fischer titrator (Metrohm 917 Coulometer, Switzerland). This measurement was intended for correcting the cannabinoid content on a dry weight basis. From these analyses, the total content of THC (% dry weight basis) and CBD (% dry weight basis) in the samples were obtained. The statistical analysis was conducted using R software, performing ANOVA, followed by Tukey's test (α =0.05). Pearson's regression analysis was performed using the GGally library (Schloerke B, et al, 2023)

3. Results and Discussion

At the end of the period, 377 mm was supplied to the Control treatment, 339 mm to the water deprivation treatment during the Vegetative stage, 317 mm during the flowering phase, and 355 mm during the pre-harvest phase. This represents a reduction in water supply of 10.1%, 15.9%, and 5.8%, respectively.

The results obtained on harvest parameters are shown in Table 1. Overall, the effect of the different irrigation cuts performed is dependent on the variety. These results align with those found by Duong et al. (2023). Similarly, several studies have demonstrated that drought significantly affects hemp's final yields (Babaei y Ajdanian 2020; García-Tejero et al. 2019; Herppich et al. 2020). However, these studies often speculate on the effect of drought at different growth stages.

The final plant height was not affected by the treatments, including the vegetative stage that occurs during linear growth (Herppich et al. 2020). The same can be applied to the production of dry weight per unit area. Observing by varieties, "Goya" showed no significant differences in any of the parameters studied. "Raquel" reduced the final branch production affected by the cut in the vegetative stage, but it did not affect any economically significant element. In the case of "Magda", the THC concentration decreased in flowers when subjected to water deprivation in the pre-harvest phase. This led to a non-significantly reduction in THC's final production ,. The most significantly affected variety was Beatriz, which had a decrease in Fresh Plant Weight in all water deprivation treatments, especially during the flowering phase. Similarly, water deprivation affected the production of dry weight in flowers and leaves, particularly during the flowering phase. One possible explanation is that "Magda" showed a greater reduction in stomatal conductance in the water deprivation treatments compared to the control treatment (data not shown). Although water deprivation did not significantly affect THC and CBD concentrations, the reduction in dry weight production decreased the final production of both CBD and THC (g/m^2) .

The results obtained are consistent with Duong et al. (2023) who conducted water deprivation treatment during the flowering stage. Similar to our results, water stress applied during this phase did not affect the concentration of THC or CBD in any of the varieties.

VC: Not calculated.							
	Parameter	Treatment	RAQUEL	BEATRIZ	GOYA	MAGDA	
	Height (cm)	Control	211.6+4.3a	206.3+4.6a	142.6+3.3a	149.3+5.1a	
		Vegetative	204.7+5.6a	201.2+7.7a	137.8+3.0a	140.3+2.7a	
		Flowering	204.8+5.2a	187.7+4.9a	137.2+3.3a	147.0+6.5a	

193.9+10.9a

3186+267a

2776+153ab

139.1+3.4a

2393+140a

2423+180a

148.2+7.6a

2270+187a

2246+116a

222.9+5.5a

2590+262a

2563+244a

Pre-Harvest

Control

Vegetative

Plant FW (g/m²)

Table 1. Harvest results. FW: Fresh Weight, DW: Dry Weight. RAQUEL, BEATRIZ, GOYA and MAGDA, are the varieties tested. ND: Not detected, the concentration falls below the measurement range of the method. NC: Not calculated.

	Flowering	2401+259a	2279+183b	2309+123a	2493+193a
	Pre-Harvest	2880+184a	2509+175ab	2396+141a	2210+222a
	Control	797.9+81.1a	977.5+73.3a	733.3+82.9a	726.7+50.2a
Plant DW	Vegetative	768.3+122.5a	867.1+59.7a	648.3+47.5a	648.4+36.9a
(g/m²)	Flowering	710.0+84.2a	731.7+63.4a	617.5+36.3a	742.3+60.1a
	Pre-Harvest	882.9+54.9a	865.4+82.2a	635.4+37.9a	701.8+71.8a
	Control	122.5+13.9a	145.7+13.4a	83.0+7.7a	139.0+9.5a
Leaves DW	Vegetative	126.9+17.7a	141.2+6.2ab	86.8+7.0a	128.3+8.7a
(g/m²)	Flowering	116.8+14.9a	104.2+9.6b	81.3+9.1a	134.7+10.5a
	Pre-Harvest	142.7+8.9a	116.6+10.2ab	86.9+6.6a	130.8+14.0a
	Control	272.5+34.8a	396.3+35.1a	286.5+18.6a	326.4+26.7a
Flowers DW	Vegetative	284.0+27.8a	332.4+15.9ab	304.9+24.7a	300.0+17.6a
(g/m²)	Flowering	281.9+30.6a	289.6+25.9b	285.1+14.8a	327.3+25.5a
	Pre-Harvest	265.0+19.1a	291.5+19.8b	265.9+15.8a	284.5+29.8a
	Control	402.9+35.2ab	435.5+34.5a	363.8+63.2a	261.3+16.8a
Stems DW	Vegetative	268.1+76.4b	393.5+54.0a	256.6+17.0a	220.1+16.5a
(g/m²)	Flowering	311.3+56.8ab	337.9+31.9a	251.0+18.9a	256.9+35.8a
	Pre-Harvest	475.2+32.7a	457.2+71.3a	282.5+19.6a	262.6+37.8a
	Control	ND	8.17+0.11a	15.28+0.24a	0.09+0.04a
Flowers CBD	Vegetative	ND	8.15+0.15a	15.96+0.24a	0.08+0.04a
(% DW)	Flowering	ND	8.00+0.14a	15.35+0.25a	0.02+0.06a
	Pre-Harvest	ND	7.75+0.11a	15.51+0.31a	0.16+0.04a
	Control	10.1+0.3a	7.3+0.2a	1.0+0.1a	14.5+0.3a
Flowers THC	Vegetative	10.8+0.3a	7.0+0.1a	1.1+0.1a	14.4+0.2a
(% DW)	Flowering	10.3+0.3a	7.0+0.1a	0.9+0.1a	14.4+0.1a
	Pre-Harvest	10.3+0.4a	7.0+0.1a	1.1+0.1a	13.4+0.3b
	Control	ND	1.16+0.10a	4.19+0.23a	ND
Leaves CBD	Vegetative	ND	1.37+0.12a	4.13+0.17a	ND
(% DW)	Flowering	ND	1.52+0.10a	4.14+0.25a	ND
	Pre-Harvest	ND	1.30+0.11a	4.08+0.18a	ND
	Control	2.5+0.2a	1.8+0.1a	ND	2.9+0.2a
Leaves THC	Vegetative	2.4+0.1a	1.7+0.1a	ND	3.1+0.3a
(% DW)	Flowering	2.7+0.2a	1.4+0.1a	ND	2.9+0.2a
	Pre-Harvest	2.5+0.1a	1.7+0.2a	ND	2.4+0.1a
	Control	NC	33.90+2.87a	46.91+2.68a	NC
Plant CBD	Vegetative	NC	28.92+1.33ab	53.08+4.93a	NC
(g/m²)	Flowering	NC	24.83+2.34b	47.24+2.91a	NC
	Pre-Harvest	NC	24.18+1.79b	44.67+2.66a	NC
	Control	30.68+3.85a	31.07+2.43a	2.27+0.24a	51.46+4.48a
Plant THC	Vegetative	33.88+3.80a	25.64+1.16ab	2.81+0.34a	47.29+3.03a
(g/m²)	Flowering	32.22+3.81a	21.75+2.07b	2.13+0.24a	50.88+3.91a
	Pre-Harvest	30.59+2.22a	22.43+1.69b	2.18+0.23a	41.77+4.49a

From all measurements taken during the three water deprivation periods—(vegetative, flowering, and pre-harvest)—the correlation between different variables was analyzed using the Pearson correlation coefficient. The result is shown in Fig. 1. A significant

correlation is observed among the different vegetation indices obtained by reflectance or transmittance, both among themselves and with thermographic determinations. All analyzed vegetation indices show good significant correlations with physiological parameters such as photosynthesis, stomatal conductance, and maximum quantum yield of PSII, especially in the second cutting period conducted in August (flowering).

Among the analyzed indices, CCI seems to exhibit a stronger correspondence with the rest of the physiological parameters. Although thermography only uses data from the second irrigation cut, it shows a significant correlation with the other parameters, except for water potential and fluorescence. Regarding Fv/Fm, it only shows lower than 0.8 means in the first irrigation cut, where the DPV was higher (data not shown).

Biochemical stress indicators such as MDA and ATC show a strong correlation with each other, with plant temperature taken through thermography, and with the photosynthetic rate. Leaf water potential shows correlations with phase-dependent indicators. It seems to have a stronger correspondence with physiological parameters (An, Fv/Fm) during the flowering period and with biochemical parameters (MDA and ATC) in the first period.

Vegetation indices like CCI and SPAD, often linked to leaf chlorophyll concentration (Rodriguez-Yzquierdo, Patiño-Moscoso y Betancourt-Vásquez 2021; Anderson et al. 2021; Parry, Blonquist y Bugbee 2014) have shown a strong relationship between them and a good association with factors such as gas exchange or fluorescence. While usually associated with fertilization (Anderson et al. 2021; Sandhu et al. 2022), this study has found that these indices are related, especially the CCI index, to the effect of water stress. An explanation for this is presented by Parry, Blonquist y Bugbee (2014) who show that these indices depend on leaf morphology, leaf thickness, and chloroplast distribution. The latter are actually responsible for differential absorption of red light versus far-red light. Water stress can alter leaf morphology and chloroplast distribution (Sun et al. 2015).

Thermography has been used as an indicator of the plant's water status and as a potential basis for irrigation scheduling (Parihar, Saha y Giri 2021; Brown et al. 2021). The obtained results and the significant correlation with most of the analyzed parameters suggest that this methodology could be a valuable tool for crop management.



Fig. 1 Correlation matrix of the different parameters measured throughout the periods of the various phenological moments of irrigation cuts. In the case of thermography, only the data obtained in the flowering phase are used. 1:, 2:, and 3: correspond to the water deprivation periods of the Vegetative, Flowering, and Pre-harvest phases. In scatter plots, the line represents the linear fit and shadows depict the confidence interval. Acronyms in MyM..

4. Conclusion

The effect of water deprivation over a significant period (7-10 days) has varied effects depending on the variety and the phenological stage where this deficiency occurs. Among the studied varieties, three did not show significant effects on the economic production parameters: Raquel, Goya, and Magda, while one variety, Beatriz, exhibited a reduction in biomass production, consequently impacting the final production of cannabinoids (THC and CBD).

The use of non-destructive, immediate, and *in situ* methodologies emerges here as potential tools for immediate determination of the plant's water status.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Relación entre la conductancia estomática y el potencial de tallo en tres variedades de pistacho

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Resumen: La superficie de cultivo de pistachero en España, y especialmente en Castilla y León, ha crecido notablemente en los últimos años debido a sus elevados rendimientos económicos; sin embargo, es esencial la selección del material vegetal más adecuado a las condiciones locales para garantizar la viabilidad de las explotaciones. Para ello, el objetivo de este trabajo fue evaluar las diferencias en el comportamiento estomático y la eficiencia intrínseca en el uso del agua (WUE_i) en tres variedades de pistacho (Pistacia vera L. cvs. Golden, Lost y Kerman) bajo la misma estrategia de riego. Periódicamente, se midió el potencial hídrico de tallo (Ψ_s), la conductancia estomática (g_s) y la tasa de fotosíntesis neta (P_n) al mediodía en 12 árboles por variedad. Los valores más bajos de P_n y g_s se encontraron en Kerman, siendo este efecto más pronunciado en agosto, cuando los árboles alcanzaron los valores más negativos de $\Psi_{\rm s}$ (-1,3 MPa). En general, Kerman también mostró WUE_i más altos que Golden y Lost durante todo el período experimental. Las tres variedades mostraron correlaciones significativas entre g_s y Ψ_s . Las pendientes de estas relaciones fueron más elevadas para Kerman y Golden que para Lost, lo que refleja la diferente capacidad de respuesta estomática de las tres variedades y que Kerman y Golden tienen un control estomático más estricto que Lost.

Palabras clave: Pistacia vera L., estado hídrico, intercambio gaseoso, eficiencia en el uso del agua

1. Introducción

En los últimos años, la superficie de cultivo de pistachero en España, y especialmente en Castilla y León, ha crecido notablemente debido a sus elevados rendimientos económicos; sin embargo, es esencial la selección del material vegetal más adecuado a las condiciones locales para garantizar la viabilidad de las explotaciones (Memmi et al. 2016; Álvarez et al. 2020). La regulación estomática es un mecanismo clave para modular y optimizar la fotosíntesis en relación con la pérdida de agua. Cuando el agua disponible es limitada o en condiciones de alta demanda evaporativa, el cierre estomático actúa como una respuesta efectiva, amortiguando la caída del potencial hídrico, lo que minimiza el riesgo de cavitación y fallo hidráulico (Tombesi et al. 2015), que podría llevar a la muerte de tejidos e, incluso, del propio árbol. En este contexto, el objetivo del presente trabajo fue evaluar, en condiciones de campo, la respuesta estomática y la eficiencia intrínseca en el uso del agua de tres variedades de pistacho de interés agronómico y sometidas a la misma estrategia de riego.

2. Material y Métodos

El estudio se llevó a cabo en condiciones de campo durante 2023. La parcela experimental está situada en Carpio, Valladolid (41°12′ N 5°5′ O, 759 m). El material vegetal utilizado fue arboles de pistacho (*Pistacia vera* L.) plantados en 2012 de las variedades 'Golden', 'Lost' y 'Kerman' con un marco de plantación de 5x6 m. Los árboles están injertados sobre patrón UCB1 y recibieron el mismo volumen de riego (130 mm), tanto en dosis como en frecuencia de eventos. Por tanto, se establecieron las tres variedades como tratamientos a comparar: Golden, Lost, y Kerman. Las variables meteorológicas (temperatura, precipitación y evapotranspiración) fueron registradas empleando una estación automática situada en Torrecilla de la Orden, a 9 km de la parcela experimental. De marzo a octubre de 2023, la temperatura media fue de 17,1 °C, con mínima de -6,0 °C y máxima de 39,6 °C. La precipitación total acumulada fue de 254 mm y la evapotranspiración potencial fue de 1073 mm.

De mayo a octubre, cada dos semanas, se realizó el seguimiento del estado hídrico de los árboles con medidas del potencial hídrico de tallo (Ψ_s) y parámetros de intercambio gaseoso. El Ψ_s se midió con una cámara de presión tipo Scholander (Mod. 3000, Soil Moisture Equipment Co., Santa Barbara, CA, USA) en hojas que habían estado previamente tapadas 2 h antes de la medición. Se utilizaron hojas sombreadas en el interior del dosel, cerca del tronco o los brazos principales. Para la medida de la conductancia estomática (g_s) y la tasa de fotosíntesis neta (P_n) se utilizó un aparato portátil LI-COR 6800 (LI-COR Inc., Lincoln, NE, USA). Todas estas medidas se realizaron al mediodía solar en una hoja por árbol y en 12 árboles por tratamiento. La ratio P_n/g_s se usó como una estimación de la eficiencia intrínseca del uso del agua (WUE_i). La significación de los efectos de la variedad de pistacho fue evaluada mediante un análisis de varianza, usando Statgraphics Plus y las medias se separaron con la Prueba de Rango Múltiple de Duncan ($P \le 0.05$).

3. Resultados y Discusión

El Ψ_s decreció progresivamente durante la campaña, mostrando una tendencia similar entre variedades y alcanzando los valores más negativos a finales de agosto (-1.3 MPa) (Fig. 1). A partir de ese momento, los valores de Ψ_s se recuperan parcialmente debido a varios eventos de lluvia registrados en septiembre. Al final del ensayo, Kerman mostró valores de potencial más negativos que Golden y Lost.



Fig. 1 Evolución del potencial hídrico de tallo (Ψ_s) en tres variedades de pistacho y de la precipitación durante 2023. Asteriscos indican diferencias significativas entre variedades. G = Golden, L = Lost, K = Kerman.

Los valores de g_s mostraron una tendencia similar entre variedades durante el ensayo (Fig. 2a). La disminución del contenido de agua en el suelo durante los meses de julio y agosto (datos no mostrados) produjo una reducción de los valores de g_s en las tres variedades, especialmente en Kerman. El descenso de conductancia observado en nuestro ensayo durante el verano sugiere un control eficiente de la transpiración en esta especie, limitando así la pérdida de agua (Moriana et al. 2018). En general, Kerman presentó valores de g_s menores que Golden y Lost durante todo el ensayo. A diferencia del Ψ_s , las diferencias entre variedades fueron significativas desde las primeras semanas del ensayo. Esta reducción fue más acusada en Kerman y se mantuvo hasta el final del ensayo. Las reducciones de Kerman con respecto a Golden y Lost fueron más marcadas en g_s que en Ψ_s . Como resultado de este descenso de g_s, P_n se vio inevitablemente reducida en Kerman respecto a Golden y Lost (Fig. 2b), aunque este efecto es menos evidente en P_n que en g_s.

En cuanto a la evolución de WUE_i, Kerman mostró mayores ratios P_n/g_s que Golden y Lost durante el periodo experimental (Fig. 2c). Los árboles de pistacho presentan una mayor eficiencia cuando los valores de g_s se sitúan en torno a 75-100 mmol m⁻² s⁻¹ y según aumenta el grado de apertura estomática, WUE_i disminuye progresivamente (Fig. 3a). Golden y Lost presentaron valores superiores a estos umbrales, por encima de 200 mmol m⁻¹ s⁻¹. Kerman osciló entre valores de g_s que sugieren mayor eficiencia intrínseca en el uso del agua (P_n/g_s). Este comportamiento ha sido observado en almendro por Vivaldi et al. (2020).



Fig. 2 Evolución de la conductancia estomática (g_s ; a), tasa de fotosíntesis neta (P_n ; b) y eficiencia intrínseca en el uso del agua (WUE_i; c) en tres variedades de pistacho durante 2023. Asteriscos indican diferencias significativas entre tratamientos. G = Golden, L = Lost, K = Kerman.



Figura 3. Relación entre la conductancia estomática (g_s) y la eficiencia intrínseca en el uso del agua (WUEi) (a) y relación entre el potencial hídrico de tallo (Ψ_s) y la g_s (b) en tres variedades de pistacho: G - Golden, L - Lost y K - Kerman.

La Fig. 3b muestra la respuesta de g_s en las tres variedades al descenso de Ψ_s . En general, g_s se mantuvo relativamente alta (>200 mmol m⁻² s⁻¹) hasta que Ψ_s alcanzó valores en torno a -0.8 MPa. En general, g_s decreció gradualmente en todas las variedades, aunque con diferencias. En Kerman, cuando Ψ_s estaba por debajo de -0.8 MPa, g_s fue menor de 200 mm m⁻² s⁻¹. Mientras que, en Golden, este umbral fue cercano a -1.3 MPa. Lost prácticamente no presentó valores de g_s por debajo de este umbral.

Tabla 1. Componentes (pendiente e intercepto) de las rectas de regresión entre la conductancia estomática (g_s) y el potencial hídrico de tallo (Ψ_s) para tres variedades de pistacho durante 2023. Se indica el número de datos para cada variedad y los coeficientes de regresión y correlación con su significancia estadística.

0						
Variedad		Componentes recta regresión		Coeficientes de regresión y correlación		
	n	Pendiente	Intercepto	R ² ajustado	r	p-valor
	-	mmol m ⁻² s ⁻¹	mmol m ⁻² s ⁻¹	K ajustauo	I	
Golden	100	140.05	380.95	0.3206	0.572	< 0.0001
Kerman	82	129.63	291.44	0.2770	0.535	< 0.0001
Lost	88	60.33	317.45	0.0473	0.241	0.024
Todos	270	118.53	339.19	0.1776	0.425	< 0.0001

Las pendientes de la relación entre g_s y Ψ_s reflejan la diferente capacidad de respuesta de g_s al estado hídrico de los árboles (Tabla 1). Las tres variedades mostraron correlaciones significativas entre estos dos parámetros. Las relaciones para Kerman y Golden presentaron una pendiente más marcada que para Lost, lo que refleja que las variedades de pistacho Kerman y Golden tienen un control estomático más estricto que Lost, algo previamente observado en variedades de vid (Bota el al. 2016).

4. Conclusión

Bajo las condiciones de este estudio, la variedad Kerman presentó valores más bajos de g_s que Golden y Lost para valores similares de Ψ_s . Además, la eficiencia intrínseca en el uso del agua fue superior en Kerman que en las otras dos variedades. Según los resultados obtenidos, Kerman y Golden poseen un control estomático más estricto que Lost. Teniendo en cuenta que los datos proceden de una sola campaña, se espera confirmar estos hallazgos en el futuro.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Cambios en las relaciones hídricas y calidad ornamental de plantas de *Euonymus* en respuesta al efecto individual y combinado de la salinidad y el riego deficitario

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Resumen: En algunas regiones mediterráneas, la práctica de utilizar agua salina a la vez que se aplican estrategias de riego deficitario se está generalizando en los últimos años, por la escasez de los recursos hídricos convencionales. La respuesta de las plantas a estos factores (salinidad y riego deficitario) no es extrapolable cuando se aplican por separado o conjuntamente. En este trabajo se estudia individualmente el efecto del déficit hídrico, la salinidad, y la combinación simultánea de ambos factores sobre algunos parámetros fisiológicos, morfológicos y ornamentales en Euonymus japonica. Las plantas de Euonymus se cultivaron en macetas al aire libre y se sometieron a tres niveles de riego y dos niveles de salinidad durante 12 meses: control (0,8 dS m⁻¹, 100% de capacidad de retención de agua; C), déficit hídrico moderado (0,8 dS m⁻¹, 60% del agua aplicada en control; MW), déficit hídrico severo (0,8 dS m⁻¹, 40% de agua aplicada en control; SW), salino (4 dS m⁻¹, misma cantidad de agua aplicada como control; S) y déficit de agua salina (4 dS m⁻¹, 60% del agua aplicada en el control; W+S). El déficit hídrico y la salinidad disminuyeron el crecimiento de la arte aérea y raíz, especialmente en las plantas sometidas a salinidad. Los efectos de los dos estreses combinados fueron aditivos en la biomasa acumulada, mostrando los valores más bajos de peso seco total de las plantas. El potencial hídrico de tallo fue más negativo en plantas SW, aunque las plantas en condiciones de salinidad (S y W+S) exhibieron una ligera deshidratación debido a la dificultad en la absorción del agua del sustrato. La aplicación de agua de baja calidad (salina) con el riego deficitario no es una práctica recomendada ya que puede tener un efecto adverso en la calidad de las plantas y provocar una reducción muy significativa del crecimiento. como consecuencia de daños en el aparato fotosintético.

Palabras clave: biomasa, clorofila, cociente raíz parte aérea, compacidad, conductancia estomática, pérdida de electrolitos.

1. Introducción

Euonymus es una especie muy utilizada en jardinería principalmente por su colorido follaje, con atractivas hojas de color amarillo y verde (Gómez-Bellot et al. 2013). Para producir cultivos más eficiente, reducir la cantidad del agua aportada o la calidad de la misma, sin incidir en el valor ornamental de la planta resulta trascendental en el manejo del riego. Para ello debemos conocer el nivel de estrés hídrico y salino tolerable y más adecuado para cada especie. En este sentido, la aplicación del riego deficitario con agua salina es una práctica común en algunas zonas Mediterráneas. Las respuestas de las plantas a la combinación de riego deficitario y salinidad no se pueden extrapolar de la respuesta cuando se aplican ambos factores por separado (Sucre y Suarez, 2011). Por ello el objetivo principal fue conocer el efecto del déficit hídrico, la salinidad y de ambos factores aplicados conjuntamente sobre algunos parámetros fisiológicos, morfológicos y ornamentales en plantas de *Euonymus japonica*.

2. Material y Métodos

En este ensayo se emplearon 150 plantas de *Euonymus japonica* de 1-2 años de edad, que fueron trasplantadas a macetas de 5 l de volumen. El sustrato fue una mezcla de fibra de coco, turba negra + turba rubia y perlita (8:7:1 en volumen) y enriquecido con 2 g l⁻¹ de un fertilizante de liberación lenta (Osmocote Plus: 14:13:13 N,P,K más microelementos). Estas macetas fueron trasladadas a una parcela al aire libre en la finca experimental del CEBAS, ubicada en Santomera (Murcia), y sometidas durante doce meses a cinco tratamientos de riego: un control (C), regado hasta cubrir sus necesidades hídricas (100%, CE; 1 dS m⁻¹), un tratamiento de riego deficitario moderado (MW) y un tratamiento de riego deficitario severo (SW), que corresponden a un 60 y 40% de la cantidad de agua aplicada a las plantas control (60 y 40%, CE; 1 dS m⁻¹), un tratamiento salino (S) regado hasta cubrir sus necesidades hídricas con agua salina (100%, CE; 4 dS m⁻¹) y un tratamiento de riego deficitario salino (W+S), regado al 60 % de las plantas control con agua salina (60%, CE; 4 dS m⁻¹).

Para la evaluación del crecimiento se realizó un muestreo destructivo en el que se determinó el peso seco (PS) en 8 plantas por tratamiento al término del ensayo. En estas plantas también se midió el número de hojas, la altura de la planta y la compacidad y se determinaron los parámetros relacionados con el color usando un colorímetro Minolta CR-10. Periódicamente, se midió el contenido relativo de clorofila (CRC) usando un Minolta SPAD-502 y los valores de florescencia se tomaron con un fluorímetro OptiScience (Modelo OS 30) en 7 plantas por tratamiento. Para la pérdida de electrolitos, parámetro que informa sobre la alteración de la permeabilidad de las membranas celulares, se utilizó la técnica descrita por Lafuente et al (1991) y se estimó al final del ensayo en 9 plantas por tratamiento. Periódicamente se mido la conductancia estomática (g_s) y la fotosíntesis neta (P_n) al mediodía solar con un LI-COR 6400, así como el potencial hídrico de tallo (Ψ_s), usando una cámara de presión (Mod. 3000). La humedad volumétrica del suelo fue medida usando un TDR (Tektronic,

mod1502B) en 6 plantas por tratamiento. La significación de los tratamientos de riego fue obtenida mediante un análisis de varianza simple, usando Statgraphics Plus y las medias se separaron con la Prueba de Rango Múltiple de Duncan (P≤0.05).

3. Resultados y discusión

Al finalizar el ensayo, el peso seco aéreo y radical se redujo con el riego deficitario y, especialmente en las plantas de los tratamientos salinos (S y W+S). Estas reducciones fueron más marcadas en las plantas del tratamiento de riego deficitario combinado con el salino, en el que la biomasa total se redujo más del 75% en comparación a las plantas control (Tabla 1). Las plantas regadas con agua salina tuvieron un mayor cociente PS raíz/PS aéreo comparado con las plantas control, especialmente en las plantas sometidas a W+S, debido a la mayor pérdida de hojas como consecuencia de la acumulación de sales en las mismas.

	С	MW	SW	S	W+S	Р
PS aereo (g pl⁻¹)	70.6±4.8 c	37.2±4.0 b	38.3±5.6 b	20.6±2.4 a	12.6±1.9 a	* * *
PS raíz (g pl ⁻¹)	22.2±1.3 d	15.5±0.6 c	14.7±1.2 bc	12.1±1.0 ab	9.8±0.6 a	***
PS total (g pl⁻¹)	92.9±5.3 c	52.7±4.5 b	53.0±6.4 b	32.7±3.3 a	22.4±2.5 a	***
Número de hojas	462±30.3 c	283±39.5 b	271±20.4 b	103±23.2 a	31±11.1 a	***
PS raíz/PS aéreo	0.32+0.02 a	0.45+0.04 a	0.44+0.07 a	0.61+0.05 b	0.84+0.07 c	**
Altura de la planta(cm)	41.3±1.8 c	37.3±1.5bc	34.0±1.2 ab	37.6±1.4 bc	31.1±1.4 a	**
Compacidad	3.8+0.3 d	2.3+0.3 c	2.4+0.4 c	1.2+0.3 b	0.3+0.1 a	***
Pérdida electrolitos (%)	28.8±2.0 a	30.7±2.3 ab	33.4±3.2 ab	38.5±1.4 b	66.0±1.8 c	***
Luminosidad	38.0±1.2 a	38.9±1.4 a	38.8±.6 a	13.9±0.9 b	48.5±1.2 c	***
Croma	21.8±0.7 a	22.7±1.1 a	22.9±1.3 a	29.0±1.1 b	33.5±1.1 c	***
Ángulo hue	119.3±1.9 c	119.1±1.7 c	118.0±0.8 bc	114.3±0.9 b	106.0±1.7 a	***

Tabla 1. Biomasa y parámetros relacionados con el crecimiento y el color en plantas de *Euonymus* sometidas a distintos tratamientos de riego al final del ensayo.

La salinidad afectó en mayor medida a la altura, siendo las reducciones más evidentes (25%) en el tratamiento combinado (W+S). La compacidad disminuyó con el riego deficitario, independientemente del nivel de déficit hídrico aplicado. El efecto de la salinidad resultó más consistente, especialmente en las plantas del tratamiento de riego W+S. No se observaron diferencias en los parámetros de color de las hojas en ambos niveles de riego deficitario en comparación con el control. Estos niveles de riego deficitario no modificaron la calidad ornamental en las plantas de *Euonymus*, aspecto importante en este tipo de plantas. Los valores más altos de luminosidad y croma y el menor valor de hue y contenido relativo de clorofila registrados en las hojas de las plantas sometidas a estrés hídrico y salinidad confirmaron el color más amarillo y

menos verde oscuro del follaje en estas plantas en comparación con las plantas control (Tabla 1, Fig. 1A).



Fig. 1 Evolución del contenido relativo de clorofila (CRC; A) y de la fluorescencia de la clorofila (F_v/F_m ; B) en plantas de evónimo sometidas a diferentes tratamientos de riego.



Fig. 2 Evolución del contenido volumétrico de agua en el suelo en plantas de evónimo sometidas a distintos tratamientos de riego.

El daño a la membrana, evaluado por el porcentaje de pérdida de electrolitos, no se vio afectado por el contenido de agua del suelo, mientras que las plantas regadas con agua salina, y especialmente las sometidas a los efectos combinados del estrés hídrico y la salinidad tuvieron un aumento significativo de daño, en comparación con las del control (Tabla 1). El contenido volumétrico de agua del sustrato fue claramente diferente desde el inicio del experimento, siendo mayor en las plantas salinas y disminuyendo proporcionalmente con el nivel de déficit hídrico impuesto (Fig. 2). En cuanto al estado hídrico, los valores más negativos del potencial hídrico de tallo se observaron en las plantas con déficit hídrico severo al final del experimento. Las plantas bajo salinidad también tuvieron valores más bajos que el control, debido a la menor disponibilidad de agua en el suelo (Fig. 3). En cuanto a la fluorescencia (F_v/F_m), el efecto más pronunciado se observó para el tratamiento combinado de déficit hídrico y estrés salino, con valores más bajos que las plantas control (Fig. 1B). En general, la disminución del contenido de agua en el suelo en las plantas de los tratamientos de riego deficitario (Fig. 2) produjo una reducción de los valores de g_s y P_n, especialmente en las plantas del tratamiento más deficitario (Fig. 4) (Mugnai et al. 2009 y Álvarez et

al. 2012). Las reducciones en la tasa de fotosíntesis de los tratamientos regados deficitariamente pudieron estar más relacionadas con descensos en la conductancia estomática, que, con daños en el aparato fotosintético, ya que no hubo diferencias en los valores de fluorescencia (F_v/F_m) y pérdida de electrolitos. Al final del experimento, las tasas de fotosíntesis más bajas se encontraron en plantas regadas con agua salina, especialmente en plantas sometidas a los efectos combinados del estrés hídrico y la salinidad. En estas plantas factores no estomáticos pudieron estar implicados en el descenso de la fotosíntesis como consecuencia de los daños provocados por las sales.



Fig. 3 Evolución del potencial hídrico de tallo (Ψ_s) en plantas de evónimo sometidas a diferentes tratamientos de riego.



Fig. 4 Evolución de la conductancia estomática (g_s; A) y la tasa de fotosíntesis neta (P_n; B) en plantas de evónimo sometidas a diferentes tratamientos de riego.

4. Conclusión

De acuerdo con nuestros resultados, las plantas de *Euonymus* resultan más afectadas por la salinidad que por el déficit hídrico en cuanto a la morfología y características ornamentales. Los niveles de riego deficitario aplicado produjeron una deshidratación severa de las hojas, pero no tuvieron un impacto negativo en la calidad ornamental de las plantas. La aplicación conjunta de agua de baja calidad (salina) con el riego deficitario provocó alteraciones importantes en el crecimiento y valor ornamental, así como daños en el aparato fotosintético. Los diferentes estreses aplicados de forma separada o combinada indujeron diferentes respuestas de *Euonymus*, lo que indica que

el tipo de estrés y su combinación debe ser considerado un aspecto importante cuando se use agua salina y/o déficit hídrico como estrategia de riego.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Exploring the use of water sensors to monitor the effect water availability on tomato plants exposed to different soil and irrigation levels: HYDROSTICK

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Abstract: As a consequence of the high level of clays and the use of heavy machinery in the fields, a reduction of up to 30% in production has been observed. The current study aimed at optimizing the management of tomato by providing different combinations soil (clay, sand and peat) under different irrigation conditions that were continuously monitorized with soil humidity sensor. For this purpose, tomato together with plant characterization (biomass, photosynthesis, stomatal conductance, N, chlorophyll, flavonoids and anthocianins) soil hydraulic properties were monitored. The results showed that the study found that tomato plants grown in soils with high clay content had the lowest biomass values, regardless of water irrigation. Gas exchange analysis revealed that such diminishment in biomass was associated with the low photosynthetic activity of those plants linked with stomatal closure phenomena detected in those plants. In summary, the abundant clay and poor oxygenation in the soil have led to the formation of dense and compacted soils, were available water content for plants remained limited with the consequent effect in crop yield.

Keywords: Crop, fertilization, precision agriculture, sensors, water content

1. Introduction

The climate change causes extreme weather fluctuation, which manifests in longer periods of drought on one hand and extreme overflow of watercourses on the other hand. In particular, the consequences for Europe is that, while Northern Europe is getting significantly wetter and winter floods could become common, southern and central Europe are seeing more frequent heat waves, forest fires and droughts, and the Mediterranean area is becoming drier, making it even more vulnerable to drought and wildfires. In addition, the consequences of the climate change on the rainfall patterns are aggravated by the population growth, which means that there is a greater demand for water resources.

Together, with water availability, soil characteristics are another target factor conditioning plant performance. The soil is a system composed of three phases. Optimal soils for plant growth would be composed (by volume) of 50% solid matter (texture particles and organic matter) and 50% pore space. The latter is equally divided between 25% liquid (circulating solution) and 25% gas (soil atmosphere) and an increase in liquid is associated with a decrease in gas and vice versa. The adequate presence of oxygen is crucial for the growth and development of roots (and therefore the plant), which guarantees access to water and nutrients. In this sense, the high presence of clay in the soils and their low oxygenation has contributed to the development of heavy and compacted soils that have a negative effect, among others, on the crop yield.

The climate change manifests in longer periods of drought on one hand and extreme overflow of watercourses on the other hand. This will significantly disrupt the agriculture sector, which strongly relies on certain temperatures and precipitation levels. Further another target factor such as high presence clay on the soil can also condition crop yield due tot the excess moisture in the soil. The current study aimed to analyze the effect of different levels of water irrigation on growth and physiology of tomato plants grown with different substrates combining clay, sand and peat.

2. Material and Methods

Seedlings of tomato (cv. Marglobe) were transplanted into 10 L pots (5 plants per pot) filled with a mixture of 100% soil, 75% clay soil-25% sand, 25% clay soil-75% sand and 100 % and 50% peat-50% clay. In each pot a sensor determining soil water content and oil water tension was placed. The plants were cultivated at the greenhouse located at the Agrobiotechnology Institute (IdAB-CSIC). The plants were fertilized with the Osmocote Exact Standard 8-9M (ICL) an encapsulated fertilizer composed of granules of micronutrients, nitrogen, phosphorus and potassium (NPK) wrapped in a resin of plant origin and biodegradable.

Half plants were irrigated at pot capacity (Control) whereas the other half was watered at 50 % of pot capacity (Drought). The plants were randomly distributed among four treatments (three pots per treatment). To mitigate chamber effects, pots were rotated weekly between greenhouse. Throughout the experiment, soil volumetric water content levels and oil water tension values were registered. After one month of growth at the corresponding growth condition, the following parameters were determined:

Plant biomass.
After one month of growth at the corresponding soil and water treatment condition, shoot biomass was harvested and dry mass was determined.

Gas exchange and chlorophyll fluorescence.

Fully expanded apical leaves were analysed with a portable Licor 6400 XT gas exchange analyser. Net photosynthesis (A_n), leaf conductance (g_s) and transpiration (E) were calculated as described by von Caemmerer and Farquhar (1981).

Nitrogen, chlorophyll, flavonoids and anthocyanin content.

Determination were conducted with a Dualex leafclip meter in the same leaves where the gas exchange and chlorophyll determinations were carried out.

3. Results and Discussion

The study showed that, regardless of water irrigation, the lowest biomass values were detected in tomato plants grown in soils with high (100 and 75%) presence of clay. The highest values were detected in soils composed of 50% peat and 50 % clay. Drought had no effect in plant biomass in soils with 100 % and 75% of clay.





Soil water content values showed (Figure 2) that the highest values were registered in tomato plants with the lowest clay content (25%). Lower water irrigation only decreased soil water content in soils with 50% and 25 % of clay. Soil water tension analyses (data not shown) confirmed that in pots with high presence of clay water was less available.

In the other hand, gas exchange analyses (Figure 3) revealed that, soil combination and irrigation treatments had a significant effect on gas exchange parameters. Under both soil water conditions, plants grown in 100% clay soil, showed the lowest photosynthetic activity A_n. Further, available data also showed that although soil composition significantly affected stomata conductance of tomato plants', irrigation treatment did not show a clear trend (Figure 3). Further, available data also showed that also showed that soil composition's effect on stomatal conductance was independent of water stress. Leaf transpiration values showed a similar pattern.



Fig. 2 Soil water content of tomato plants grown under different combinations of soil and water availability.



Fig. 3 The soil combination and water availability effect on net photosynthesis (A_n), leaf conductance (g_s) and transpiration (E) values of tomato plants. Each value represents the mean of 4 values.

Finally, as it is show in Figure 4, the current study did not show remarkable differences on leaf nitrogen, chlorophyll, flavonoids and anthocyanin content associated with soil and irrigation treatments.



Fig. 4 The soil combination and water availability effect on nitrogen, chlorophyll, flavonoids and anthocyanin content. Each value represents the mean of 4 values.

4. Conclusions

The current study showed that the tomato plants cultivated in soils containing high levels of clay exhibited the smallest values of biomass, irrespective of water irrigation. The examination of gas exchange unveiled that this reduction in biomass was linked to the diminished photosynthetic activity of said plants, which was connected to the decreased values of stomatal opening. In summary, the copious presence of clay and insufficient oxygenation within the soil have resulted in the development of dense and compacted soils, wherein the availability of water for the plants remained limited, thereby impacting crop yield.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Scheduling irrigation in a hedgerow olive orchard with an improved version of the crop coefficient approach

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Abstract: We compared two methods to schedule irrigation in hedgerow olive orchards with high plant densities, aimed to apply full irrigation (FI). One of the methods (FI-FAO) was based on the FAO crop coefficient approach (Allen et al. 1998), while the other (FI-A&P) was based on the approach described by Allen and Pereira (2009). While FI-FAO relies on adjusted crop coefficients for the orchard conditions, the FI-A&P approach considers the dynamics of leaf area, ground cover, tree height, and stomatal conductance, among other factors. We applied the two treatments along the 2023 irrigation season in a super-high-density olive orchard close to Seville, southwest Spain. Measurements of soil water content, leaf area, leaf water potential, stomatal conductance and net photosynthesis were made along the irrigation season. Fruit yield was also recorded. We found no differences among treatments, for any of the considered variables. Results showed that the FI-A&P approach estimated greater irrigation doses (25% more) than the FI-FAO approach. Using actual values of wind speed, minimum relative humidity, tree height and ground cover recorded at La Hampa olive orchard led to estimate even greater irrigation doses with the FI-A&P approach. For further improvements, we propose the use of models to evaluate both evaporation from the soil and leaf resistance.

Keywords: evapotranspiration, irrigation requirements, water consumption.

1. Introduction

Constant efforts by both the scientific community and private companies are being made to optimize irrigation management (Kannan and Anandhi 2020). Some of them aim to a more effective irrigation scheduling, i.e. to the better assessment of the timing and amount of irrigation. Irrigation can be scheduled from soil water measurements, meteorological records or water stress related variables measured in the plant, both at the field level or by remote imagery (Fernández et al. 2018). The FAO crop coefficient (K_c-ET_o) approach, based on meteorological measurements, is one of the most popular approaches to schedule irrigation (Allen et al. 1998). Still, the performance of this approach is curtailed by the empirical character of the crop coefficient, K_{c} . Also, most users do not consider the seasonal dynamics of leaf area and ground cover by the crop, among other variables highly conditioning crop water consumption. Advances on the K_c- ET_{o} approach, recently gathered by Pereira et al. (2021), shows that those limitations are being palliated by new versions of the K_c -ET_o approach. Among them, Allen and Pereira (2009) proposed a more sophisticated method to estimate the crop coefficients, based on considering actual values of ground cover, crop height, leaf area and stomatal conductance in the orchard, among other factors. The method was tested for a superhigh-density olive orchard by Paço et al. (2019), but doubts remain on the performance of the method when applied to other orchards. The aim of this work is to compare this method vs. the K_c -ET_o method to estimate the water needs in a 'Arbequina' olive orchard with 1667 trees ha⁻¹ near Seville, representative of hedgerow olive orchards in the area.

2. Materials and Methods

The experiment was performed during the irrigation season of 2023, at La Hampa experimental farm (23 km west of Seville, 37,28303 -6,06877), in a 'Arbequina' olive orchard planted in 2018 with 1-year-old trees at 4 m × 1.5 m (1667 trees ha⁻¹). We had two treatments, FI-FAO (full irrigation estimated with the FAO crop coefficient approach) and FI-A&P (Full irrigation estimated with the approach described by Allen and Pereira 2009). We had three replicates per treatment distributed in a randomized plot design. Each replicate consisted of a plot containing 5 tree rows with 24 trees in each row. The irrigation system consisted of one lateral per tree row, close to the trunk, with one 2 L h⁻¹ dripper every 0.5 m. We used an Agronic 2000 irrigation controller (Sistemes Electrònics PROGRÉS) to apply the calculated irrigation doses.

Key meteorological variables were measured along the season, with the Campbell weather station of La Hampa. Measurements of the volumetric soil water content (Θ_v) at 0.1, 0.2, 0.3, 0.4, 0.6 and 1 m depth were made every 7-10 days with a previously calibrated PR2 Profile Probe (Delta-T Devices) and two access tubes per tree, in one tree per plot. One access tube was in between two drippers and the other at 0.3 m from the dripper. The Θ_v readings were used to derive the water stored in the soil. Monthly measurements of leaf area (LA) (LAI-2000 Plant Canopy Analyzer, Li-cor), crop height and

width, and ground cover, were made along the irrigation season. On the same days we measured midday leaf water potential (Ψ_l) with a Scholander-type chamber (PMS Instrument company), in two leaves per tree sampled from one tree per plot. The same leaf sampling protocol was used to measure maximum daily stomatal conductance ($g_{s,max}$) and maximum daily net photosynthesis ($A_{N,max}$), with a Licor-6400 portable photosynthesis system (Li-cor). Fruit production was recorded at harvesting, on October 23rd. We used linear mixed models for repeated measures to test the differences between treatments, by using the *ImerTest* (Kuznetsova et al. 2017) and *emmeans* (Lenth 2023) packages for R software (R version 4.3.2, 2023-04-05).

3. Results

The time course of the grass reference evapotranspiration (ET_o) derived from the FAO 56 Penman-Monteith equation is shown in Fig. 1. Also shown are the time courses of the crop evapotranspiration derived from the FAO crop coefficient approach (ET_c -FAO) and that derived from the Allen and Pereira approach (ET_c -A&P). The latter was usually greater than ET_c -FAO, except at the end of the season, when



Figure 2. (A) Time courses of the irrigation needs (IN) estimated with the FAO crop coefficient approach (FI-FAO), and with the approach described by Allen and Pereira 2009 (FI-A&P). FI-A&P₁ was estimated with the actual values of tree height and ground cover. FI-A&P₂ was estimated with the actual values of those two variables plus those of minimum relative humidity and wind speed. Also shown are the total IN estimated for each treatment, in m³ ha⁻¹. (B) Time courses of the applied irrigation (AI) for the two treatments. Also shown are the total Al in m³ ha⁻¹, and the precipitation (P) recorded in the orchard. DOY = day of year (DOY 100 = April 10).



Figure 1. Time courses of the grass reference evapotranspiration derived from the FAO 56 Penman-Monteith equation (ET_o), of the crop evapotranspiration derived from the FAO crop coefficient approach (ET_c FI-FAO), and of that derived from the Allen and Pereira approach (ET_c FI-A&P). FI accounts for full irrigation, since both treatments were aimed to replace the crop water demand. DOY = day of year (DOY 100 = April 10).

the atmospheric demand was low.

In Fig. 2A we can see the time courses of the irrigation needs (IN) calculated for both treatments. As expected, they show the same trends than those of ET_c. The total IN, also shown in the Fig. 2A was 25% greater for FI-A&P than for FI-FAO. The figure shows that using actual values of tree height, ground cover, minimum relative humidity and wind speed had a low impact on the estimated IN (0,7% for FI-A&P₁ and 2,9% for FI-A&P₂). Fig. 2B is similar than Fig. 2A but for the applied irrigation amounts (IA). Fig. 2B also shows the precipitation recorded at La Hampa. The time courses of water stored in the soil (WS) are shown in Fig. 3. No significant differences were found in between treatments. For both of them, the WS trends were about horizontal along the season, which indicates that the IAs supplied in each treatment were similar to the crop water consumption. The lowest WS values were recorded in the FI-FAO treatment in July and August, the months with



Figure 3. Time courses of the water stored in the soil (WS) for the two treatments (avg \pm SE). See Fig. 1 for details on the treatments. DOY = day of year (DOY 100 = April 10).

greater atmospheric demand (Fig. 1). The values of the recorded physiological variables



are shown in Fig. 4. No differences between treatments were found except on DOYs with * (p < 0.05). Values of Ψ_1 were lower than -1.4 MPa in July and August, a threshold value for water stress in olive. This agree with the decreasing $g_{s,max}$ and $A_{N,max}$ values in that period. No significant differences between treatments were found either in leaf area (Fig. 5), although p = 0.07 for the last measurement of the season.



Figure 4. Time courses of (A) the midday leaf water potential (Ψ) , (B) the daily maximum stomatal conductance $(g_{s,max})$ and (C) the daily maximum net photosynthesis $(A_{N,max})$ recorded in trees of the two treatments (avg±SE). See Fig. 1 for details on the treatments. DOY = day of year (DOY 100 = April 10).

Figure 5. Time courses of the leaf area (LA) recorded in trees of the two treatments (avg \pm SE). See Fig. 1 for details on the treatments. DOY = day of year (DOY 100 = April 10).

Yield results were 27320.7 \pm 3583.9 kg ha⁻¹ for the FI-FAO treatment and 20420.7 \pm 2349.3 kg ha⁻¹ for the FI-A&P treatment. The difference was not significant.

4. Discussion

The IN value of 4859,8 m³ ha⁻¹ estimated with the FAO crop coefficient approach (FI-FAO treatment) (Fig. 1) agrees with values found in the area for this type of orchards. Thus,

IN values of ca. 5000 m³ ha⁻¹ are reported for super-high-density (SHD) olive orchards with 1500-2000 trees ha⁻¹ (Grattan et al. 2006, Padilla-Diaz et al. 2016). We actually supplied 2.5 % less (4738,4 m⁻³ ha-¹) than the estimated IN, and our findings show that that amount was enough for the recorded variables to show similar values than those recorded in the FI-A&P treatment, which received 5435,9 m³ ha⁻¹ (Fig. 2). This amount was nearly 11% lower than the estimated IN for this treatment (Fig. 2), due to occasional problems with the irrigation system. This suggests that the IN = 6095,2 m³ ha⁻¹ estimated with the Allen and Pereira (2009) approach was over the orchard requirements. Fig. 2 also shows that using actual values of tree height and ground cover (FI-A&P₁), plus those of minimum relative humidity and wind speed (FI-A&P₂), instead of the tabulated values (Pereira et al. 2021, 2023), did not improve the estimated IN. Although differences were not significative, both the daily LA time courses recorded in both treatments (Fig. 5) and the p value (p = probability of the null hypothesis being true) for the final seasonal measurement (0.07) suggest greater crop growth in the FI-A&P treatment. But records of shoot length and number of internodes (data not shown) were similar for both treatments.

We are not surprised on the good performance of the FAO crop coefficient approach observed in this work. This is because we have a long experience on using this approach to schedule irrigation in SHD olive orchards in the area (Fernández et al. 2018), so our K_c values are well adjusted. And, for the Allen and Pereira (2009) approach, we believe it has a great potential and it is worthy to improve the method. We suggest three potential improvements: (1) to use the model of Orgaz et al. (2006) or that by Allen et al. (1998) to estimate the time course of soil water evaporation, (2) to use the model of Buckley (2019) to estimate leaf resistance values from the time course of main meteorological variables and leaf parameters adjusted for olive, and (3) to use a high-performance weather forecast system, such as those relying on artificial intelligence and fed with a database from the area, to estimate oncoming ET_o values.

5. Conclusion

The Allen and Pereira (2009) approach overestimated the crop water requirements, but its capacity to overcome the empirical character of the FAO crop coefficient approach makes worthy to improve it as suggested in the Discussion section.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES

Zaragoza 14-16 FEB 2024

Influence of Altitude on the water requirements and water status of Cherry Trees in the Jerte Valley

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Abstract: Cherry tree cultivation represents the main productive orientation in the northern mountain area of Extremadura, Spain. Currently, these mountain irrigated lands present a structural deficit in water storage capacity that compromises the availability of water for cherry cultivation. Local irrigation communities distribute water equally, regardless of tree size, phenological stage and altitude. Water management in mountain agriculture is a critical challenge today, given the growing problem of water scarcity because of climate change.

In response to this problem, CICYTEX has initiated a project to evaluate the differences in water requirements depending on the altitude of the plots.

Three cherry tree plots were selected at altitudes of 470, 600 and 1030 meter above sea level. Two irrigation treatments were applied in every plot: sustained deficit irrigation according to the farmer's normal practice (C), and regulated deficit irrigation (RDI) at 100 % of seasonal crop evapotranspiration (ETc) in preharvest and at 25% ETc during post-harvest. Potential evapotranspiration for each plot was obtained from data from meteorological stations located in each plot. Crop response to altitude and irrigation treatments was analyzed in relation to stem water potential (Ψ_{stem}).

Preliminary results indicate the influence of altitude on the Ψ_{stem} of cherry trees. An increase in altitude correlates with a reduction in Ψ_{stem} , indicating the need for an irrigation strategy adapted to altitude. In cherry trees located at higher altitudes, no differences between irrigation treatments were observed. However, at lower altitudes, significant differences in Ψ_{stem} were observed between irrigation treatments, which underlines the importance of applying an appropriate irrigation strategy to maximize water availability in these areas.

These results justify the implementation of an irrigation management strategy better adjusted to the specific demands of the different altitudes. This approach will contribute to optimizing the use of water resources in cherry cultivation in the mountainous areas of northern Extremadura.

Keywords: Stem water potential, Irrigation strategy, mountain, regulated deficit irrigation.

1. Introduction

Cherry tree cultivation represents the main productive orientation in the northern mountain area of Extremadura, Spain. Currently, these mountain irrigated lands present a structural deficit in water storage capacity that compromises the availability of water for cherry cultivation. Local irrigation communities distribute water equally, regardless of tree size, phenological stage and altitude. Water management in mountain agriculture is a critical challenge today, given the growing problem of water scarcity because of climate change.

In response to this problem, CICYTEX has initiated a project to evaluate the differences in water requirements depending on the altitude of the plots.

2. Materials and Methods

2.1 Experimental sites, plant material, and treatments

The study on the influence of altitude on water requirements and water status was conducted in the Jerte Valey in the northern mountain area of Extremadura, Spain in 2023. Three plots were selected according to the altitude at 470, 600, and 1030 m above sea level (m.a.s.l.), and twenty trees were selected per plot (Table 1). The soil texture is sandy load and sandy loam, with a high percentage of coarse elements, which gives it average water retention properties. All plant material consisted of sweet cherry trees (*Prunus avium* L.) 'Lapins' grafted on Avium rootstock and cultivated in terraces.

Plot	Latitude (N)	Longitude (E)	Altitude (masl)	Pending (%)
1ET	40º 7' 10,48''	5º 57' 4,68''	480	15-35
3NA	40º 10' 31,52''	5º 50' 45,23''	600	47
5ET	40º 9' 9,27''	5º 56' 4,67''	1030	17-25

			C a i		
Table 1.	Geographical	locations	of Cherry tr	ees in Jert	e Valey.

The trial featured two irrigation treatments applied in each plot: sustained deficit irrigation according to the farmer's normal practice (Control), and regulated deficit irrigation (RDI) at 100 % of seasonal crop evapotranspiration (ET_c) in preharvest and during flower differentiation (20 days after harvest) and at 25% ET_c during post-harvest (Nieto et al, 2017). Ten trees per plot and treatment were monitored. Potential

evapotranspiration for each plot was obtained from data from meteorological stations located in each plot.

Crop water requirements under drip irrigation (ETc) were calculated using the following equation: $ET_c = ET_o \times K_c \times K_r$, where ET_o es reference evapotranspiration, K_c is a crop-specific coefficient for sweet cherry reported by Marsal (2012) and K_r is a localization factor (Fereres and Castel 1981) related to the percentage of ground covered by the crop.

Trees were drip-irrigated using a single drip line for each tree row, with two and four pressure-compensated emitters per tree for Control and RDI treatment, respectively, each with a discharge rate of 4 L h^{-1} .

2.2 Water status measurements.

The plant water status was measured every fifteen-twenty days by measuring midday stem water potential (Ψ_{stem}) at noon under field conditions according to the methodology proposed by McCutchan an Shackel (1992) with a Scholander pressure chamber (Model 3000, Soil Moisture Equipment, Santa Barbara, CA, USA) on twenty shaded, healthy and mature leaves located near the trunk, per irrigation treatment, two leaves per tree in each plot.

2.3 Statistical Analysis

A Linear Mixed Model (MLM) was fitted to estimate the effect of irrigation treatment, altitude and date of measurement on midday stem water potential. Irrigation treatment, altitude, and date were included in the interaction as independent factors in the model. Tree ID was included in the model as a random factor. Normality and homoscedasticity of all data were estimated with Shapiro-Wilks and Bartlett's tests, respectively. Tukey's test (HSD) at a significance level P<0.05 was used to compare means and establish differences between the different levels of each factor.

3. Results and Discussion

Flum bloom was in early April, and no differences were observed with respect to altitude. Fruit was harvested at 48, 56, and 90 days after full bloom (DAFB) in the plots at 470, 600, and 1030 masl, respectively. For every 100 m increase in elevation, fruit ripening is delayed by 7,5 days.

The reference crop evaporation (ET_o) accumulated during the growing season (April-October) in 2023 was between 841 and 967 mm; and the rainfall ranged between 669 and 975 mm, of which more than 50 % occurred in October and between 23 % (600 masl) and 33 % (470 masl) during fruit harvesting which cracked a high percentage. The mean amounts of water applied were 176, 105 and 125 mm in the RDC treatment and 53, 55 and 79 mm in the control treatment (Control), for 470, 600 and 1030 masl, respectively (Table 2). The control treatment has provided information on the use of irrigation water by farmers in the area.

	Precipitation (mm) (ETo	ETo		Irrigation water applied (mm)						
			(mm)		RDI		Control					
Altitude	470	600	1030	470	600	1030	470	600	1030	470	600	1030
April	12	13	15	121	135	117	7	0	0	6	1	0
May	134	94	135	124	137	113	36	20	20	9	19	18
June	118	58	112	147	149	131	19	28	32	8	1	20
July	0	0	0	188	201	173	40	20	43	7	14	18
August	0	0	0	177	188	163	42	21	24	13	12	15
September	124	106	146	93	96	86	19	10	4	6	7	5
October	380	397	567	63	62	58	13	6	1	4	0	3
Total	768	669	975	913	967	841	176	105	125	53	55	79

Table 2. Mean monthly and seasonal values (Abril-October) of precipitación, reference crop evapotranspiration (ETo), irrigation water applied for deficit irrigation treatments (RDC) and control treatment (C).

The fitting linear mixed models for midday stem water potential reflected a significant effect for all three factors analyzed (Fig 1). The effect of irrigation treatment was highly significant ($F_{1,733}$ =79,61, p < 0.0001), altitude exhibited a significant effect ($F_{2,733}$ =272.72, p < 0.0001), and weekly progression also had a significant effect ($F_{6,733}$ =434.52, P<0.0001) indicating its strong influence on Ψ_{stem} .

When considering the effect of altitude on the Ψ_{stem} throughout the crop cycle, an identical pattern was described at all altitudes, with maximum values at the beginning and end, and minimum values during the summer, coinciding with the period of maximum evaporation demand. During July and August (weeks 28, 30, 32, and 34), there was a significant difference in Ψ_{stem} between the different altitudes.

In the plot located at 1030 masl, the Control treatment resulted in a water saving of 37 % compared to the RDI. At this altitude, the highest Ψ_{stem} values were obtained in both irrigation treatments and did not exceed the threshold values established by Marsal et al. (2010) (Ψ_{stem} =-1.5 MPa) and by Blanco et al. (2018) (Ψ_{stem} =-1.3 MPa). This indicates the absence of water stress during the entire crop cycle at higher altitudes. The minimum Ψ_{stem} values were reached by trees at lower altitudes in treatment Control from week 28 to week 37, where the severe deficit threshold value (Ψ_{stem} =-1.8 MPa) established by Nieto (2020) was continuously exceeded, indicating that the trees are under severe stress conditions, and that prolonged overshoot could negatively affect the next season's crop. During the summer period, Ψ_{stem} reflected large differences between treatments at lower altitudes, as shown in Fig. 1. These findings suggest that altitude plays a crucial role in determining Ψ_{stem} , particularly during the summer months.



Fig.1 Midday stem water potential (Ψstem) for the irrigation treatments control (C) and regutated déficit irrigation (RDI) according altitude. Each value is the mean of the 20 measures.

4. Conclusion

Preliminary results indicate the strong influence of altitude on the midday stem water potential of cherry trees. In cherry trees located at higher altitudes, no differences between irrigation treatments were observed. However, at lower altitudes, significant differences in Ψ_{stem} were observed between irrigation treatments. This underlines the importance of considering altitude in irrigation strategies to optimize water distribution in mountain areas, improving resource productivity and plantation yields. A second and third year of trials are needed to know whether the irrigation strategy has effects on yield and fruit size.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Respuesta de la higuera frente a diferentes estrategias de riego

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Resumen: La higuera es una especie bien adaptada a condiciones semiáridas de clima mediterráneo y en el caso de producción de higo seco es habitual el cultivo en secano. Existe poca información sobre la respuesta de este árbol frente a una disponibilidad creciente de agua de riego, aspecto que adquiere relevancia para el diseño de estrategias de riego que persiguen un uso eficiente de los recursos hídricos, junto con el incremento de las producciones. En este trabajo se presentan los resultados de dos campañas (2022 y 2023) de un experimento de campo en una plantación con la variedad Calabacita para higo seco con tres tratamientos de riego: Control, regado para cubrir las necesidades hídricas del árbol; RD1, regado como el Control hasta el 1 de julio que se redujo al 50% del riego del control y RDS un riego deficitario sostenido aplicando el 60% del agua del Control. La campaña de riego tuvo una duración total de 216 y 191 días en los años 2022 y 2023 respectivamente. Los tratamientos deficitarios mostraron diferencias en estado hídrico en relación con el control, más acusadas en 2022 que fue un año más seco. Las diferencias fueron mayores en conductancia estomática foliar que en potencial hídrico de tallo, llegando a estar más estresado RD1 que RDS. RDS tuvo menor desarrollo de la copa que los otros dos tratamientos como reflejan los datos de porcentaje de suelo sombreado y madera de poda. En los tratamientos deficitarios hubo una reducción en el volumen de agua de riego entorno a 19 y un 38 % para 2022 y 2023 respectivamente con menor cosecha tan solo en 2022 en un 18%. Las estrategias de riego deficitario han demostrado ser efectivas para reducir el riego afectando mínimamente a la cosecha en producción y calidad de los higos.

Palabras clave: potencial hídrico de tronco, conductancia estomática foliar, productividad, déficit hídrico.

1. Introducción

Extremadura es la región española con mayor superficie dedicada al cultivo de la higuera siendo el destino principal la producción de higo seco, que se cultiva casi en su mayoría

en condiciones de secano. Sin embargo, en los últimos años se está incrementando la superficie de nuevas plantaciones con riego. Frente al escenario de escasez hídrica al que se enfrenta la Cuenca Mediterránea resulta fundamental disponer de información para realizar programaciones de riego dirigidas a un uso eficiente del agua. Existen muy pocos trabajos publicados sobre las necesidades hídricas de la higuera y estos se refieren a riegos de apoyo en plantaciones de secano (Honar et al. 2021) o a riegos sostenidos con diferentes criterios (Goldhammer y Salinas 1999) y, cuando se trata de producción de higo seco en riego, es prácticamente inexistente.

El objetivo de este trabajo es proporcionar información sobre la respuesta de la higuera para producción de higo seco, frente a tres estrategias de riego diferentes de cara al diseño de programaciones de riego adaptadas a este cultivo.

2. Materiales y Métodos

2.1 Descripción de la parcela experimental

Se realizó un experimento de campo en las Vegas del Guadiana (38º51'N, 6º40'W, altitud 184 m Guadajira, España) en los años 2022 y 2023 en una parcela de higuera cv. Calabacita (Ficus carica L.) para producción de higo seco, plantada en la primavera de 2018 con un marco de plantación 5 x 4 m. El clima es mediterráneo, con veranos secos y calurosos. La temperatura media anual (2000-2022) fue de 16,28 ºC, las precipitaciones de 447,08 mm y la evapotranspiración de referencia (ETo) de 1.296 mm. El suelo es franco arcilloso. El sistema de riego consta de 2 líneas portagoteros por hilera de árboles con goteros de 3,5 l/h espaciados 1 m. El suelo bajo la línea de árboles dispone de malla anti-hierba. El diseño experimental consistió en tres tratamientos en un diseño de bloques completos al azar con 4 repeticiones: Control, regado para cubrir las necesidades del cultivo con la información proporcionada por un lisímetro de pesaje con una higuera, RD1 regado como el Control hasta el 1 de julio con reducción posterior a un 50% del mismo hasta finalizar la campaña de riego y RDS al 60% del Control durante toda la temporada. La parcela experimental consistió en 4 hileras contiguas de 5 árboles cada una, con los 6 árboles centrales como árboles medida. En la Tabla 1 se presentan las fechas de inicio y fin de la campaña de riego, los volúmenes de agua aplicados en cada tratamiento y año, junto con la Evapotranspiración de referencia (ETo) y la Pluviometría efectiva (Pe).

2.2 Medidas en plantas y frutos

El potencial hídrico del tallo (Ψ s), se midió semanalmente al mediodía utilizando una cámara de Scholander (modelo 3005; Soil Moisture, Santa Barbara, CA, EE.UU.) en 8 árboles por tratamiento (dos por parcela individual). La conductancia estomática (GI) se midió también semanalmente con un porómetro LI-600 (LI-COR Corporate, Nebraska, EE.UU.) en los mismos árboles, de 10:00 a 11:30 en días soleados y sin nubes, con 3 hojas

por árbol. El Porcentaje de Suelo Sombreado (%SS) se midió sobre una imagen visible tomada con una cámara desde un dron y los programas de procesado de imagen Pix4D y QGIS.

Tabla 1. Volumen de agua de riego, inicio y fin de las campañas de riego, Evapotranspiración de referencia (ETo) y Pluviometría efectiva (Pe) en los años 2022 y 2023 desde brotación a caída de hojas.

۸	Volumen de riego (mm)			Campaña	de riego		Ре
Ano	Control	RD1	RDS	Inicio	fin	EIO (mm)	(mm)
2022	434	269	272	11-mar	19-oct	1187	69
2023	393	319	307	04-abr	12-oct	1191	235

Los higos de cada árbol se recogieron tras su caída natural en una malla sobre el suelo, con 6 recolecciones en 2022 y 4 en 2023. Los frutos se pesaron y contaron al recogerlos y se pesaron de nuevo tras un periodo de secado al aire bajo cubierta. La producción total se expresa como la suma del peso seco de todas las recolecciones y de la misma manera el número de frutos. En cada una de las recolecciones se tomaron 5 frutos por cada 6 árboles en buen estado, y se trituraron para medir el ^oBrix (refractómetro digital) y el pH. En la última recolección de 2022 no se pudieron hacer estas medidas por el mal estado de los frutos.

2.3 Análisis estadístico

El análisis de los datos de producción se realizó mediante una ANOVA y el Test de Tukey para comparación de las medias utilizando el programa Statixtics.

3. Resultados

2022 fue el año más seco con pluviometría inferior a 2023 (Tabla 1) de forma que en este primer año se establecieron mayores diferencias en el estado hídrico de los árboles de los diferentes tratamientos, siendo más evidentes en Gl (Fig. 1) que en Ψ s (Fig. 2). En el año 2022 RD1 alcanzó los valores más bajos de Ψ s y Gl, mientras que en 2023 los tratamientos deficitarios se mantuvieron en todo momento igualados e inferiores al Control.



Fig. 1 Evolución estacional de la conductancia estomática foliar (GI) en los años a) 2022 y b) 2023. Las barras representan la desviación estándar de la media.



Fig. 2 Evolución estacional del Potencial de Tallo (Ψ s) en los años a) 2022 y b) 2023. Las barras representan la desviación estándar de la media.

Como se puede ver en la Fig. 3 los tres tratamientos parten de valores similares de porcentaje de suelo sombreado (%SS) tras la brotación hasta los 160-170 días del año (dda). Durante el resto del ciclo hasta caída de hojas el Control se mantiene por encima y RDS empieza a quedar por debajo de los otros tratamientos en el día 180 en 2022 y el día 160 en 2023. En el caso de RD1 el retraso en el recorte del riego provoca una caída en el día 210 en 2022 y mantiene valores similares a RDS en 2023.



Fig. 3 Evolución estacional de Porcentaje de Suelo Sombreado (%SS) en los años a) 2022 y b) 2023. Las barras representan la desviación estándar de la media.

El peso de higos seco fue menor en los tratamientos deficitarios en 2022 igualándose en 2023 y en ningún caso se encontraron diferencias en el número de frutos por árbol (Tabla 2). El contenido de azúcares del fruto (ºBrix), fue similar en todos los tratamientos y recolecciones en los dos años, salvo en uno de los días en que fue más alta la del Control. En pH solo hubo diferencias entre tratamientos en el último día de recolección del año 2023, con RDS con el valor más bajo. En ambos años se observa en todos los tratamientos un descenso en el pH el último día de recolección (datos no presentados).

Tabla 2. Producción de higo seco y número de frutos por árbol en la recolección de los años 2022 y 2023. Peso seco de la madera de poda de la campaña 2022. Letras diferentes indican diferencias significativas para $p \le 0,05$.

	Producciór	n(kg/árbol)	Nº fruto	os/árbol	Madera de poda 2022	
	2022	2023	2022	2023	(kg/árbol)	
Control	8,721 a	8,729	770	1057	1,966	
RD1	7,266 b	7,789	740	1074	1,919	
RDS	7,324 b	7,844	788	964	1,493	

4. Discusión

El volumen de agua aplicado en los tres tratamientos ha sido reducido si se compara con las dotaciones habituales de las plantaciones frutales de la zona. Los tratamientos deficitarios han supuesto un ahorro considerable de agua (entre el 19 y 38 %), afectando poco a la producción del primer y segundo año, posiblemente por ser un nivel de estrés ligero, que se produce en una etapa avanzada del cultivo, cuando los brotes del año han alcanzado casi su tamaño final. En estas ramas es donde se sitúan los frutos y los últimos frutos en la zona apical no suelen completar la maduración, quedando en el árbol. La producción del ensayo ha estado entre 3.633 y 4.364 kg/ha, inferior a los 5.859 kg/ha de media de la región en regadío, pero es de suponer que la estadística incluya tanto plantaciones de higo fresco, como seco. En ambos años la evolución de ºBrix y pH parecen señalar la última recolección recomendable a principio de septiembre. La alta variabilidad entre los árboles hace difícil hacer afirmaciones apoyadas en diferencias estadísticas, aunque la tendencia observada en %SS y el peso de la madera de poda indica que RDS inicia el periodo de estrés antes que RD1 y que este ajuste del volumen de copa pudo ser la causa de que soporte niveles de estrés menos severos que RD1, manteniendo mayor intercambio gaseoso en la copa y es de esperar que mayor actividad fotosintética.

El rango de valores de Ψ s de los tratamientos se mantuvo entre -0,6 y -1,8 MPa, mientras que en el trabajo de Abdolahipour et al. (2019) en condiciones de secano árido no llegaron a bajar los potenciales de hoja por debajo de -1,9 MPa, lo que confirma en esta especie el cierre estomático frente a incrementos de Déficit de Presión de Vapor (DPV) que se acentúa con el estrés hídrico (Ammar et al. 2022) de forma que evita un descenso acusado del potencial hídrico. En estas condiciones Gl resultó mejor indicador del estado hídrico de los tres tratamientos.

5. Conclusiones

Las tres estrategias de riego incluidas en este trabajo, aunque con diferencias considerables en el volumen de agua de riego, no tuvieron apenas diferencias en producción, lo que supone un incremento en la eficiencia en el uso del agua cuyas consecuencias sobre la productividad de la plantación deberían evaluarse a medio y largo plazo.

Los niveles de estrés impuestos con el recorte del 50% (RD1) y el riego sostenido con el 60% de la ETc fueron ligeros, incluso en el año con menor pluviometría afectando al estado hídrico de los árboles, en un periodo aproximado de un mes antes del inicio de la caída de los frutos, de forma que el efecto sobre la cosecha fue reducido. Los dos tratamientos deficitarios supusieron ahorros similares de agua (aunque con diferencias notables entre años) y producción.

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XVI SPANISH-PORTUGUESE SYMPOSIUM ON PLANT WATER RELATIONS NEW SOLUTIONS FOR ANCIENT CHALLENGES Zaragoza 14-16 FEB 2024

Citrus water requirement in water-scarce conditions: A comprehensive approach to efficient water management

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Abstract: Precise measurement and management of crop water requirements is crucial for water saving and improving water use efficiency in irrigated agriculture, particularly in water-scarce regions. Therefore, obtaining a local and accurate estimation of crop water needs is essential. This study implements a new approach that separately calculates crop transpiration using basal crop coefficient and soil evaporation using the Ritchie model. This methodology was applied for drip-irrigated mature citrus trees (Citrus sinensis) during the 2015 irrigated season for the semi-arid climate of eastern Spain, Valencia, one of country's leading producer zones. Crop coefficients, transpiration rates, evaporation rates, and crop evapotranspiration were compared with other values from alternative approaches and outcomes of other studies. A high concordance was observed, underscoring the robustness of the approach developed in this study. A key finding was the differentiation between beneficial water usage (transpiration, 83.7%) and non-beneficial water loss (evaporation, 16.3%). This distinction is vital in arid and semi-arid regions where water scarcity poses a significant challenge to sustainable agriculture. The outcomes of this research demonstrate that the applied approach is valuable and practical for enhancing water use efficiency, mainly due to its simplicity and minimal data requirements for calculating local values.

Keywords:

Water use efficiency, Irrigation management, Water stress, Crop coefficient, Crop evapotranspiration, Citrus.

1. Introduction

Efficient water use has become a significant challenge facing irrigated agriculture, especially in arid and semi-arid regions such as a large part of the Iberian Peninsula (L. S. Pereira et al., 2009; Poveda-Bautista et al., 2021). This region has a Mediterranean

climate with limited water availability, and it is known for its irrigated agriculture, which primarily grows perennial crops like citrus (*Citrus sinensis*)(Daccache et al., 2014; Martínez-Ferri et al., 2013). Citrus orchards, already water-intensive, are under additional pressure from changing rainfall patterns and rising temperatures, leading to higher evapotranspiration rates and increased irrigation demands (Maestre-Valero et al., 2017).

Spain, a leading contributor in the citrus industry, significantly contributes to the European Union's citrus production, with over 56% of the total, surpassing countries like Greece and Italy (MAPA, 2023). In Spain, citrus orchards cover an area of approximately 307.000 hectares, yielding around 7 million tons annually (MAPA, 2022). Notably, Spain's most productive citrus-growing regions are located in the country's southeast, including Andalusia, Murcia, and Valencia, and feature a semi-arid climate with average annual precipitation not exceeding 500 mm, coupled with water scarcity and drought periods (Jiménez-Bello et al., 2015; Poveda-Bautista et al., 2021).

Therefore, optimizing water use efficiency in irrigating citrus crops is crucial. Ensuring efficient water use necessitates accurately quantifying the exact water requirement of the crop which represents the crop evapotranspiration (ETc).

Accurate estimation of ETc is of key importance for efficient water management and sustainable agricultural practices in citrus orchards, balancing irrigation needs and conserving water resources (Jiménez-Bello et al., 2015; Rana & Katerji, 2000). It enables precision irrigation scheduling, minimizing water losses, improving water productivity, and preserving water resources, particularly in water-scarce regions such as a large part of the Iberian Peninsula.

Various methods and approaches can be used to determine water requirements in citrus irrigation, including energy balance, eddy covariance, remote sensing, and crop coefficient (Rana & Katerji, 2000). However, these techniques are often time-consuming, tedious, and costly (Taylor et al., 2015). For example, energy balance and eddy covariance measurements require specialized equipment and expertise, making them time-consuming and tedious to implement (Peddinti & Kambhammettu, 2019). In addition, remote sensing can be expensive to implement due to the high equipment cost and the need for specialized expertise (Jiménez-Bello et al., 2015). The FAO56 method (Allen et al., 1998), which is based on the crop coefficient approach, is prevalent for quantifying crop evapotranspiration, which represents the net water needs of the crop, by multiplying the crop coefficient Kc (which assumes a single value for both crop transpiration and soil evaporation processes) with reference evapotranspiration (ETO), determined using the FAO Penman-Monteith equation (A. R. Pereira et al., 2006). However, single crop coefficient Kc can lead to inaccuracies in ETc estimation (L. S. Pereira et al., 2020). Furthermore, it neglects the soil evaporation rates, which can be significant in arid and semi-arid regions where soil evaporation is a significant component of ETc.

To address this limitation, the dual crop coefficient approach (Kc= Kcb + Ke) has been developed (Allen et al., 1998; L. S. Pereira et al., 2020). This approach separates the kc

value into two components: the basal crop coefficient (Kcb) and the soil evaporation coefficient (Ke). This distinction allows for a more accurate estimation of ETc by considering both evaporation and transpiration separately (Darouich et al., 2022; Ramos et al., 2023). Nevertheless, the basal crop coefficient can be site-specific, varying significantly between orchards and locations due to factors such as crop characteristics and environmental conditions (Jiménez-Bello et al., 2015). This variation limits its transferability across different sites. To overcome this limitation, Allen and Pereira (2009) developed an approach to estimate the basal crop coefficient values, considering parameters such as crop height, stomatal control during transpiration, and canopy cover fraction. This approach provides a more site-specific and accurate Kcb estimation that can improve ETc estimation accuracy in citrus orchards.

In this study, we have developed a comprehensive methodology to estimate the water requirements of citrus cultivation. This approach involves separately calculating soil evaporation and crop transpiration. Soil evaporation is determined using the Ritchie method (Ritchie, 1972), which predicts evaporation from row crops with incomplete cover and accounts for the two stages of evaporation. On the other hand, crop transpiration is calculated using the method developed by Allen and Pereira (2009). This method estimates the basal crop coefficient by considering key parameters such as crop height, the degree of stomatal control during transpiration, and the canopy cover fraction.

2. Materials and Methods

2.1 Case study site description

This study was conducted in experimental citrus plots in Picassent, Valencia, Spain (Figure 1) using data of the 2015 irrigation season. A semi-arid Mediterranean climate characterizes the region. The plots were planted with two varieties, Navelina (Citrus sinensis navel) and Navel Lane Late (Citrus sinensis (L) Osbeck), with a planting spacing of 5 m x 5 m. The plots were irrigated using a drip irrigation system with a double line with 10 emitters of 4 L h-1 per tree. The soil had a sandy loam texture with a stone content of 18% by weight. This specific environment offers a pertinent and representative setting for exploring crop water requirements and irrigation efficiency in citrus orchards under Mediterranean conditions.

2.2 Crop evapotranspiration calculation approach

To accurately calculate the citrus evapotranspiration, the method used in this study combines the equations proposed by Allen and Pereira, (2009) for crop transpiration estimation and the model introduced by Ritchie, (1972) for soil evaporation determination. The potential transpiration of citrus crops (Tr, mm.d⁻¹) is determined by multiplying the basal crop coefficient (K_{cb}) by the reference evapotranspiration (ET_o, mm.d⁻¹), as expressed in the following equation:

$$T_r = K_{cb} * ET_o \tag{1}$$



Fig. 1 Location of the case study plots: Picassent, Valencia (Spain)

 K_{cb} is computed by multiplying the density coefficient (K_d) by a maximum basal coefficient representing full cover conditions ($K_{cb_{full}}$), following the formula proposed by Allen and Pereira (2009):

$$K_{cb} = K_{c\,min} + K_d * (K_{cb\,full} - K_{c\,min})$$
(2)

Where, $K_{c_{min}}$ is the minimum crop coefficient for bare soil, approximately equal to 0.15 under typical agricultural conditions, as suggested by Allen and Pereira, (2009). The $K_{cb_{full}}$ is the maximum crop transpiration coefficient during peak plant growth with nearly full ground cover. This coefficient is initially calculated based on the crop height, accounting for climatic variations, and subsequently adjusted for stomatal control in trees using a reduction factor (Fr) derived from mean leaf stomatal resistance (Allen & Pereira, 2009). Allen and Pereira's (2009) study provides extensive insights, for a detailed understanding of these equations.

Soil evaporation (*Es*, expressed in mm.d-1) is calculated using the model proposed by Ritchie (1972). This model divides the process of evaporation into two stages. In the first stage, the soil evaporation is limited by energy availability and is equal to the potential evaporation. The first stage finishes once a specified amount of water (*U*, mm) has been evaporated. Upon reaching this threshold, soil evaporation switches to stage two (RAES et al., 2012). In this stage, the evaporation rate is lower than the potential evaporation rate and is proportional to the square root of time.

Mathematically:

$$E_{s} = \begin{cases} E_{s,pot} & \text{if} & \sum E_{s} \leq U \\ \alpha(\sqrt{t} - \sqrt{t-1}) & \text{if} & \sum E_{s} > U \end{cases}$$
(3)

Where, $\sum E_s$ is the cumulative soil evaporation, $E_{s,pot}$ (mm.d-1) is the potential soil evaporation amount, t (days) is the time since the start of stage two, and α (mm.d-0.5) is the Ritchie coefficient and it depends on soil hydraulic characteristics.

The model accounts for irrigation and precipitation events that re-wet the soil surface, potentially causing a transition back to stage one from stage two. The potential evaporation in stage one is calculated as follows:

$$E_{s,pot} = \left[\frac{\Delta}{\left(2.45 * (\Delta + \gamma)\right)}\right] * R_{ns}$$
(4)

Where, Δ (kPa ${}^{\circ}C^{-1}$) is the saturated water vapor pressure slope, R_{ns} (MJ.m⁻².d⁻¹) is the net radiation at the soil surface, and γ (kPa ${}^{\circ}C^{-1}$) is the psychrometer constant.

3. Results and Discussion

The citrus basal crop coefficient was calculated daily using the methodology proposed and developed in this study. However, monthly averages were computed to facilitate comparison with values derived from alternative approaches. **Figure 2** illustrates the comparison of the monthly values calculated in this study (Kcb_sim) with values proposed by FAO for a citrus crop under similar conditions as the crop in this study, with a 50% canopy cover, a crop height of 3 meters, and no active soil cover. Under these conditions, FAO published a Kcb value of 0.60 at initial, 0.55 at mid-season, and 0.60 at the end of the season. The FAO values reflect standard values and are not local values.



Fig. 2 Monthly Kcb values from different approaches

Furthermore, **Figure 2** includes Kcb values derived from the Kc values provided by the Valencian Institute of Agricultural Research (IVIA) (Kcb_IVIA). Although the study plots are in the same edaphoclimatic conditions as the IVIA plots, the Kcb_IVIA is overestimated compared to the Kcb_sim, especially during the late spring and summer when the temperature is higher. Two reasons can explain this: Firstly, Kcb_IVIA is considered for a citrus crop with a canopy cover equal to or more than 70%, whereas citrus in this study has a canopy cover of 50%. Secondly, this difference may be due to the fact that the approach used in this study for calculating the citrus basal crop coefficient accounts for the plant's stomatal control by applying an adjustment factor (Fr) relative to crop stomatal control. In addition, various studies have demonstrated that the citrus crop has a regulatory mechanism for stomata in conditions of high humidity, wind, and high temperatures. This is an advantage that reasonably justifies the application of this method to calculate citrus water requirements.

The crop transpiration calculated in this study using the basal crop coefficient (Tr_sim) was compared with the transpiration calculated using the equation developed by Villalobos et al. (2013) (Tr_Rs). This equation is developed for the calculation of the transpiration of fruit trees and combines climatic and crop factors. It is expressed as a function of total daily solar radiation as follows:

$$Tr_{Rs} = 37.08 * 10^{-3} * \frac{Q * R_s}{a + b * VPD} * \frac{VPD}{P_{at}}$$
(5)

Where, Q (dimensionless) is the fraction of PAR intercepted by the canopy, R_S (MJ.m⁻².d⁻¹) is daily solar radiation, VPD (kPa) is the vapour pressure deficit, P_{at} (kPa) is the atmospheric pressure, and a (μ E.mol⁻¹) and b (μ E.mol⁻¹.kPa⁻¹) are crop factors.

The results depicted in **Figure 3** show a good correlation between Tr_Rs and Tr_sim with a determination coefficient (R^2) of 0.72. This agreement underscores the robustness of the the method used to calculate citrus water requirement.



Fig. 2 Daily values of Tr_sim and Tr_Rs

In addition, crop evapotranspiration (ETc) was evaluated. *Figure 4* compares the calculated ETc in this study (ETc_sim) with ETc values published by the Valencian Institute of Agricultural Research (IVIA) from a study conducted under similar conditions to those of the experimental plots in this study. The results indicate a good agreement between ETc_sim and ETc_IVIA, with an R² of 0.86.



Fig. 3 Daily values of ETc_sim and ETc_IVIA

It is important to highlight that during the 2015 irrigation season, the evaporation fraction calculated in this study was 16.3%, which is a high value, while transpiration was 83.7% of the total crop evapotranspiration. Evaporation flow represents a non-beneficial loss for the crop. Therefore, it is crucial to quantify this component of evapotranspiration in order to develop measures aimed at minimizing this loss and improving water use efficiency.

4. Conclusion

Precise measurement and management of crop transpiration and soil evaporation components can significantly enhance water use efficiency in agricultural practices, particularly in arid and semi-arid regions which are often water-scarce and depend on irrigation to meet crop water requirements.

The approach developed in this study for calculating crop water requirements provides a more accurate understanding of the water needs of citrus crops, thereby enabling more efficient and targeted irrigation strategies. This approach is implemented with other functions, considering essential aspects such as root distribution and soil heterogeneity within the agronomic model titled AquaCitrus. This model calculates the soil water balance, crop water use, water productivity, etc. The model is currently under development and will be published soon.

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Grape berry cuticle plasticity under heat waves

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Abstract: Global warming is challenging grapevine cultivation, particularly in Mediterranean regions. Enhanced heat waves frequency and extended drought are projected to continue rising over the century, with penalties in grapevine yield and quality. Berry cuticle plays a central role in berry water loss control and serve as a protective barrier against desiccation. Maintaining these functions requires an intact cuticular membrane along ripening. Nonetheless little is known about berry cuticle in grapevine, particularly in what concerns its plasticity among varieties under heat waves, its role in shriveling disorder and in the berry water relations and quality. In this study, the incidence of simulated heatwave events, on grapevine water relations and berry cuticle was assessed. The study was conducted in Gandesa (Tarragona) in 2023, comparing Grenache and Syrah. Simulated heatwaves were performed using an open heating system (OHS) structure, either at veraison or at harvest. Leaf water potential (Ψ_{leaf}) , stomatal conductance (g_s), and canopy temperature were measured at three phenological stages: pea size, veraison and harvest. Soil water content was monitored hourly at 20 and 40 cm depths. Bunch microclimate was continuously monitored using thermistors installed at both sides of the canopy. At harvest, cuticles from healthy and shriveled berries were isolated and analyzed in terms of epicuticular waxes content. Results showed that OHS structure increased the berry temperature in both varieties, with Syrah more prone to highest increases. OHS do not impact on Ψ_{leaf} , although slight difference was observed in the g_s. Wax content was function of variety, being highest in Syrah. A tendency toward high waxes was found in OHS berries. High water control was observed in Grenache, under control and OHS. Wax analysis revealed varietal differences in the total amount of waxes.. Further studies are needed to unveil the role of cuticle in berry adaptation to global warming.

Keywords: Berry, cuticular transpiration, desiccation, shrivel, Vi is vinifera

1. Introduction

The importance of the cuticle for protection against water loss is widely recognized in plant kingdom (Kerstiens, 1996; Schreiber and Riederer 1996). In grape berries, cuticle plays a central role in the ripening process by providing the mechanical framework to support the integrity of the fruit and has a protective role barrier against desiccation (Zarrouk et al. 2018). In addition, cuticle characteristics affects the interaction of the grape berry surface to different microorganisms (Herzog et al. 2015), with critical consequences in berry and wine qualities.

Under abiotic stress conditions, namely water stress, several reports showed the increase of cuticular wax content both in leaves (Cameron et al. 2006; Kosma et al. 2009) and in fruits (Romero and Rose 2019; Zhang et al. 2020; Zarrouk et al. 2018; Dimopoulos et al. 2020), confirming its essential role in plant adaptation to environmental cues.

Global warming is challenging grapevine cultivation, particularly in Mediterranean regions. Enhanced heat waves frequency (and extended drought) is projected to continue rising over the century, with penalties in grapevine yield and quality. During the last years, sunburn, shriveling and altered sugar-flavour-aroma balance are becoming more prevalent in grapevine grown in the Mediterranean region as result of climate change incidence. Berry shrivel is economically disastrous for wine-growers due to significant loss of yield. The importance of intact berry cuticle is this scenario is crucial due to its protection role against solar radiation (Benitez et al. 2021), and high temperatures (Belge et al. 2019; Trivedi et al. 2019). Nonetheless little is known about berry cuticle in grapevine, particularly in what concerns its plasticity among varieties under heat waves, its role in shriveling disorder and in the berry water relations and quality.

In this study, the implication of grape berry cuticle in heat stress adaptation and in berry water relations was assessed in two different varieties, using field simulated heatwave conditions.

2. Materials and Methods

2.1 Experimental design and methodology

The study was carried out at IRTA experimental research station in Gandesa (Spain) during the 2023 growing season. The plot is included within "Terra Alta" *terroir*, characterized by a dry continental Mediterranean type, with cold winters and hot and dry summers. Twenty-year-old grapevines from Grenache and Syrah varieties grafted on 110R rootstocks were used. Grapevines, east/west oriented, were short pruned in spur

and conducted in Royat on a VSP system, at a density of 3200 vines ha⁻¹. Simulated heatwaves were performed using an open heating system (OHS) structure (Sadras et al. 2017), applied either at veraison or at harvest for one week.

The measurements of leaf water potential (Ψ_{leaf}) (Pressure chamber SAPS II, Soilmoisture), stomatal conductance (g_s) (SC1-Leaf porometer, Decagon), and canopy temperature (bunch and leaf (ThermoSpot Plus, Laserliner)) were all performed at solar noon and at three phenological stages: pea size, veraison and harvest.

2.2 Berry epicuticular wax analysis

At harvest, cuticles from healthy and shriveled berries were isolated and analyzed in terms of epicuticular waxes content. Five replicates of five frozen fresh berries (n≥5) were selected. Berry skins were separated from the pulp and one disc (1 cm diameter) per berry was selected for cuticle isolation. Cuticle were enzymatically isolated using pectinase/cellulase cocktail mixture (4% and 2%) following protocol by Chandra (2015). 1 ml of chloroform was added to the isolated cuticles and left gently shaking overnight. Then, the wax extract was completely dried with liquid nitrogen. Cuticular wax wight was gravimetrically determined at University of Girona.

2.3 Statistical analysis

All statistical analyses were carried out with "stats" package (R Core Team, 2012) functions in R Statistical Software version 4.2.2 (R Core Team, 2022).

3. Results and Discussion

Results showed that OHS structure was efficient in increasing the berry temperature in both varieties (Fig. 1). Interestingly, Syrah showed highest increases compared with Grenache both at veraison and harvest.



Fig. 1 Berry bunch temperature (°C of Grenache (a and Syrah (b under control (CTRL and heat treatments (OHS-V and OHS-H, from Veraison to Harvest. Grey and pink columns refer to treatments' periods. Bars are means ± SE (n=3).

OHS treatments do not impact significantly on Ψ_{leaf} (Fig. 2). In contrast, g_s decreased in OHS-V treatment in both varieties (Fig. 3). However, OHS-H treatment only decrease g_s in Syrah, while no effect of heat stress in stomatal opening was observed in Grenache.



Fig. 2 Leaf water potential, Ψ_{leaf} (MPa) of Grenache (a) and Syrah (b) under Control, and heat treatments (OHS-V and OHS-H) at three phenological stages (Peasize, Veraison and Harvest). Bars are means ± SE (n≥5).



Fig. 3 Stomatal conductance gS (% to control) of Grenache (a) and Syrah (b) under heat treatments (OHS-V and OHS-H) at three phenological stages (Peasize, Veraison and Harvest). Bars are means \pm SE (n \geq 10).

Wax content was function of variety, being highest in Syrah (Fig. 4). In both varieties, berry wax content decreased under OHS-V. Nonetheless, the level of decrease was higher in Grenache, while in Syrah only a slight decrease was observed in healthy berries (Fig. 4). On the other hand, shrivel tend to increase wax load in grape berries at veraison, namely in Grenache. No differences in wax content were observed between healthy and shriveled berries in Syrah. Preliminary analysis in 2022, revealed varietal differences in wax compositions (*data not shown*).



Fig. 4 Berry cuticular wax content per surface area (% to control) of Grenache (a) and Syrah (b) under heat treatments (OHS-V and OHS-H) of healthy and shriveled berries at harvest. Bars are means \pm SE (n≥15).

4. Discussion

Simulated heat wave stress does not induce changes in plant water status. This is not surprising since no differences in soil water content were observed among treatments (*data not shown*). In contrast, heat stress applied at veraison drove a significant stomatal closure in both varieties. OHS structure increased the canopy temperature with likely effect on the leaf-to-air VPD which may reduce g_s. In previous report the high sensitivity of stomata to high VPD has been demonstrated (Bunce, 1996; Prieto et al. 2010; Rogiers et al. 2012).

The fact that only Syrah showed a decrease in g_s at harvest under heat stress, while no changes were observed in Grenache, suggests a differential sensitivity of grapevine genotypes to heat stress occurring at later phenological stages. Syrah variety is reported to modulate its leaf hydraulic conductance even at very late stages (post-harvest/predormancy), with consequences in stomatal conductance (Damasio et al. 2023). The seasonal plasticity of stomatal regulation (Herrera et al. 2022) could thus explain the observed varietal response to heat stress in this study. In fact, differences in developmental rates or osmolyte accumulation rates between cultivars could be part of the source for the variability in stomatal regulation (Schultz, 2003). In addition, leaf temperature assessment at harvest showed increase in leaf temperature in Syrah compared to the control, while no differences were observed in Grenache. This could suggest a better leaf evaporative cooling capacity in Grenache than in Syrah. Corroborating this hypothesis, a higher leaf desiccation rate was observed in Syrah than in Grenache vines under OHS (data not shown).

Increased epicuticular wax was showed in cranberry fruits as strategy to mitigate heat and desiccation stresses by controlling the fruit surface temperature (Erdwein et al. 2023). Surprisingly, in our study, heat stress triggers a decrease in berry wax content, particularly in Grenache. In addition, fruit surface temperature was higher in Syrah (showing higher waxes per unit area) than Grenache. Trivedi et al. (2022) reported that only northern clones of bilberries showed wax load increased with temperature likely due to a higher sensitivity of these clones to heat stress. More recently, Cabodevilla et al. (2024) showed no differences in berry wax load between Tempranillo controls and heat treatment (+4°C). Overall, these results showed different heat stress response strategies among species and grapevine genotypes. The absence of differences between healthy and shriveled berries in terms of wax content, particularly in the high shrivel susceptible variety Syrah, corroborate previous data by Rogier et al. (2004). These authors showed that the berry weight loss during late ripening experienced by this variety is not only result of cuticle disruption and/or high transpiration rates but is related to a decreased vascular flow of water into the berry combined with continued transpiration.

5. Conclusions

In this study, we showed the phenotypic plasticity of berry cuticle among grapevine varieties under heat stress. Contrasting with previous reports, berry cuticular waxes were reduced under heat stress, and do not relate with neither reduction in berry temperature nor berry transpiration. Further studies are needed to unveil the role of cuticle in berry adaptation to global warming.

Although heat stress does not impact on vine water status, it reduces its stomatal conductance. This suggests that longer heat stress periods could have penalties on vine water relations and photosynthesis. Further studies are necessary to reveal the vine's adaptation strategy to heat waves.

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Soil water status modulates the temperature dependence of stem CO₂ efflux in *Quercus ilex* and *Quercus faginea* trees

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Abstract: The contribution of stem respiration to the overall forest carbon balance is currently uncertain due to the difficulty to directly measure it. Recent research has demonstrated that not all the CO₂ produced by respiration in woody tissues escapes immediately to the atmosphere. Instead, it can be partially stored inside the tree or dissolved and transported within the xylem. Furthermore, the CO₂ respired within the soil by heterotrophs can even move upwards and contribute to the measured CO₂ efflux at a given height of the tree stem. To disentangle this complex process, we have developed an automated system to quantify and trace the metabolic origin of stem CO₂ efflux. This system is composed by dynamic steady-state stem chambers where the CO₂ efflux and its stable isotope composition (δ^{13} C and δ^{18} O) are measured in real time together with plant transpiration, photosynthetic active radiation, vapor pressure deficit, and soil, stem, and air temperature. In this work, we present a mesocosm experiment where we monitored stem respiratory fluxes of two Quercus species with contrasting traits that are extensively distributed across the Iberian Peninsula, Quercus ilex subsp. rotundifolia and Quercus faginea. Given that the CO_2 transport within and outside the stem depends on xylem sap velocity and morphology of woody tissues, we hypothesize that both the magnitude and isotope signal of stem CO₂ efflux will differ between species as well as its relationship with ambient variables. Preliminary results show that Q. ilex has a greater stem CO₂ efflux compared with *Q. faginea*, especially during nighttime. Additionally, moderate soil drought (in terms of soil water potential) reduced the temperature dependence of stem CO₂ efflux for both species. Under moderate drought, the efflux increased in Q. *ilex* but decreased in Q. *faginea*. Similarly, δ^{13} C and δ^{18} O of CO₂ efflux also varied depending on the stem temperature and soil water status.

Keywords: respiration, isotopic tracing, woody tissue, water potential, dynamic chamber, Isotope Ratio Infrared Spectrometry (IRIS)

1. Introduction

The contribution of stem respiration to the overall forest carbon balance is currently uncertain. Some studies point out that it can represent between 5 and 35% of total ecosystem respiration (Campioli et al. 2016; Salomón et al. 2017). This high uncertainty stems from the different challenges associated to its direct measurement. For instance, recent research has demonstrated that not all the CO_2 produced by respiration in woody tissues escapes immediately to the atmosphere. Instead, it can be partially stored inside the tree or dissolved and transported axially within the xylem. Furthermore, the CO_2 respired within the soil by heterotrophs can even move upwards and contribute to the measured CO_2 efflux at a given height of the tree stem. Additionally, the little research that have been developed is mostly focused on temperate tree species. The main aim of our study is to quantify stem CO_2 respiration and its isotopic composition in two Mediterranean tree species, *Quercus ilex* and *Quercus faginea*, in order to understand mechanistically how this CO_2 efflux respond to the different influencing factors.

2. Materials and Methods

The mesocosm experiment was performed in the Agri-Food Research and Technology Centre of Aragon (CITA; 41.723 °N, 0.809 °W, Zaragoza, Spain) from the 5th of October to the 5th of November 2022 under Mediterranean climatic conditions (mean annual temperature 15.4 °C, total annual precipitation 298 mm). During this period, ambient conditions, stem CO₂ efflux and its isotopic composition were continuously measured by using an ad-hoc system specifically designed to continuously monitor two tree individuals of each species Quercus ilex subsp. rotundifolia and Quercus faginea, at the same time. Over the whole study period, three replicates of each species were monitored. These trees were planted in pots 13 years ago and irrigated during summer season to guarantee their survival. Ambient variables measured during the experiment corresponded to photosynthetically active radiation (PAR), relative humidity (RH), air temperature (Tair), vapor pressure deficit (VPD), and precipitation. For each plant, we also monitored soil, stem and air temperature inside the chamber (Tsoil, Tair, and Tchamber, respectively), and soil water potential (SWP). We used the difference between Tchamber and Tair as a proxy of incident PAR in each tree because raw PAR measurements at a single point did not reflect the shading pattern of the surrounding buildings and trees. Stem CO₂ efflux and its isotopic composition were measured every 4 minutes for each pair of trees by using a home-made dynamic chamber systems connected to a controlled air circuit to sample the incoming and outcoming air by using an infrared gas analyzer (CIRAS3-DC, PP-Systems) and an isotope ratio infrared spectrometer (Delta-Ray, Thermo Fisher Scientific). Details on the design of the automated system can be found in Figure 1.



Fig. 1 Automated system designed to measure real-time stem CO₂ efflux and its isotopic composition in two tree individuals. FCO, FC1 and FC2 represent flow controllers used to limit air flow along the circuit.



3. Results

Fig. 2 Relationships between stem CO₂ efflux and stem (**a**, **c**) and soil (**b**, **d**) temperature under daytime (**a**, **b**) and nighttime (**c**, **d**) conditions for *Quercus ilex* (green) and *Quercus faginea* (brown) trees over the study period.



Fig.3 Relationships between stem CO₂ efflux and stem temperature under daytime **(a, b)** and nighttime **(c, d)** conditions for *Quercus ilex* (left panels) and *Quercus faginea* (right panels) trees. Red and grey dots represent effluxes measured under moderate drought and normal conditions based on soil water potential values (SWP), respectively.



Fig.4 Relationships between isotopic composition of stem CO₂ efflux (δ^{13} C, δ^{18} O) and stem temperature under daytime (**a**, **b**, **e**, **f**) and nighttime (**c**, **d**, **g**, **h**) conditions for *Quercus ilex* (left panels) and *Quercus faginea* (right panels) trees. Red and grey dots represent effluxes measured under moderate drought and normal conditions based on soil water potential values (SWP), respectively.



Fig. 5 Relationship between isotopic composition of stem CO₂ efflux of *Quercus ilex* (left panels) and *Quercus faginea* (right panels) trees and the difference between the air inside and outside the stem chamber as a proxy of incident photosynthetic active radiation. Colors represent stem temperature.



Fig. 6 Change in time of the isotopic composition of water (δ^{13} C, δ^{2} H) in branches (**a**, **b**) and soil (**c**, **d**) samples of the *Quercus ilex* (green) and *Quercus faginea* (brown) trees. Line types represent different monitored trees.

4. Discussion and Conclusions

Our automated system designed to measure stem CO_2 effluxes together with their isotopic composition in real time (Fig. 1) worked well. The stem CO_2 efflux differed among species. During nighttime, stem CO_2 efflux of *Quercus ilex* trees was higher compared to *Quercus faginea* trees (Fig. 2). A similar pattern was observed under moderate drought conditions (Fig. 3). For both species, we found positive and negative

relationships between stem temperature and δ^{13} C and δ^{18} O signals, respectively, with more negative values corresponding to moderate drought conditions (Fig. 4). Higher δ^{13} C values in the stem CO₂ effluxes under high radiation conditions (i.e., higher difference between the air temperature inside and outside the chamber) suggest that CO₂ assimilation by photosynthetic woody tissue was relevant for *Quercus ilex* trees (Fig. 5a). The water isotopic composition in branches and soil changed over the study period, which must be considered in further statistical analysis (Fig. 6). Further research will comprise the analysis of stem tissue carbohydrates as well as statistical analyses that include potential temporal autocorrelation among observations.

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Physiology, gene expression, and epiphenotype of two *Dianthus broteri* polyploid cytotypes under temperature stress

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Abstract: Increasing evidence supports a major role of abiotic stress response in the success of plant polyploids, which usually thrive in harsh environments. However, understanding the ecophysiology of polyploids is challenging due to interactions between genome doubling and natural selection. Here, we investigated physiological responses, gene expression, and the epiphenotype of two related Dianthus broteri cytotypes -with different genome duplications (4× and 12×) and evolutionary trajectories- to short extreme temperature events (42/28 °C and 9/5 °C). 12× D. broteri showed higher expression of stress-responsive genes (SWEET1, PP2C16, AI5L3 and ATHB7) and enhanced gas exchange compared to 4×. Under heat stress, both ploidies had largely impaired physiological performance and altered gene expression, with reduced cytosine methylation. However, the 12× cytotype exhibited remarkable physiological tolerance (maintaining gas exchange and water status via greater photochemical integrity and probably enhanced water storage) while downregulating *PP2C16* expression. Conversely, $4 \times D$. broteri was susceptible to thermal stress despite prioritising water conservation, showing signs of non-stomatal photosynthetic limitations and irreversible photochemical damage. This cytotype also presented genespecific expression patterns under heat, upregulating ATHB7. These findings provide insights into divergent stress response strategies and physiological resistance resulting from polyploidy, highlighting its widespread influence on plant function.

Keywords: Cytosine methylation, gene expression, photosynthesis, polyploidy, temperature stress, water relations.



Evaluation of yield and quality of six cultivars under rainfed conditions

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Abstract: Future scenarios predict an increase of drought events in Mediterranean areas that affects seriously the crops production. The alfalfa (Medicago sa iva L.) has been reported as a potential crop to withstand water stress, with high persistence and nutritive value for livestock feeding. The main objective of this study is to evaluate the productivity, leaf water content (LWC), water use efficiency (WUE), fibres and protein content of six alfalfa cultivars under irrigated and rainfed conditions. The experiment was carried out in the field trials located in the Public University of Navarre (Pamplona, Spain) during 2021. The following cultivars were evaluated: San Isidro and Aragon commonly cultivated in Spain, Magna in Argentina and Secret of Desert, SW-10 and WH-9598 in Saudi Arabia. The plants subjected to irrigated conditions were supplied with the sufficient water to satisfy the needs of the plants per year (7.231 m^3 ha⁻¹) and the plants under rainfed conditions received the water provided by precipitations (2.246 m³ ha⁻¹). Under rainfed conditions, the highest biomass yield was obtained in Magna cultivar whereas the lowest in San Isidro. Likewise, the lowest loss in biomass under rainfed condition was observed in Magna and WH-9598 with a percentage of reduction of 37.52 and 28.19%, respectively. In this sense, Magna and WH-9598 cultivars were able to maintain the LWC higher than San Isidro and Aragon and, the highest WUE was observed in Magna. Moreover, a significative decrease in fibres content was observed for all cultivars analysed under rainfed conditions and, a significative increase in protein content was observed specially in Magna cultivar, as well as an accumulation of the asparagine amino acid as a possiblemechanism to face the water stress. These results highlight that the cultivars on of Magna and cultivars in Mediterranean zones could be used as strategy to deal with future droughts events.

Keywords: Alfalfa, drought, water use efficiency, quality



Ameliorating effect of foliar applied proline on the nutritional status of drought stressed UCB1 rootstock

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Abstract: Water deficit is one of the most important stresses that significantly impacts crop and fruit production worldwide. Proline is an essential amino acid involved in osmotic regulation and plays a crucial role in mitigating cell damage and enhancing drought tolerance. This study aimed to investigate the effects of foliar proline application on leaf nutrient content of drought-stressed UCB1 pistachio plants. The experiment was conducted in a greenhouse environment for 60 days following a factorial completely randomized design. The first factor encompassed three water levels (100% as the control, 70% and 40 % of field capacity) representing varying degrees of drought stress and the second consisted of proline applied in three levels (0 as the control, 75 and 150 mg L⁻¹). Under drought stress conditions, UCB1 pistachio plants exhibited a notable decrease in mineral leaf content (P, K, Ca, Mg, Fe, and Zn). However, droughted-plants which were sprayed with proline accumulated minerals in the leaves. Specifically, the application of 150 mg L⁻¹ proline notably improved the nutritional status of UCB1 pistachio plants under drought stress conditions, leading to increased leaf mineral content (P, K, Ca, Mg, Fe, and Zn), as well as enhanced relative water content. In summary, foliar proline application has the potential to enhance both foliar mineral nutrient levels and relative water content in plants subjected to water stress, offering a promising strategy in horticulture for improving drought tolerance in fruit crops.

Keywords: Pistachio, minerals, osmolyte, rootstock, water deficit



Hormonal changes involved in the endocarp hardening period of *Olea europaea* as the soil dries

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Abstract: Changes in the hormonal responses were investigated in hedgerow olive orchard (cv Manzanilla de Sevilla), in La Hampa experimental station in Coria del Río (near Seville, Spain, 37°17′ N, 6°3′ W, 30 m altitude), under water scarcity. Two irrigation treatments were applied during a growing season, described as control treatment, irrigated at 100 % of crop evapotranspiration and rainfed treatment, non-irrigated. The phytohormones abscisic acid (ABA) and jasmonic acid (JA) were determined in leaves. The results showed a clear difference in their response as the soil moisture decreased during endocarp hardening (from 15th of June to 28th of July). ABA concentration increased by 3-fold at the beginning of the endocarp hardening period in comparison to control plants, while its decreased progressively during the experiment. In contrast, JA increased progressively, reaching the maximum values (3-fold) at mid of endocarp hardening period (between 30th of June and 7th of July), while its concentration decreased (not significant difference) as the endocarp hardening period ends. Taken together biochemical mechanisms induced by water withheld period on rainfed olive orchard may lead to understand the hormonal changes produced by during endocarp hardening period suggested as the most drought-resistant phenological period.

Keywords: Abscisic acid, drought, hormones, jasmonic acid, pit hardening.



Comparative Analysis of Physiological Responses of Two Chestnut Varieties to Climate Change - Marsol vs CA-90

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Abstract: The cultivation of chestnut trees (*Castanea sativa* Miller), one of the main forest crops in the Iberia Peninsula, has been relying on grafting techniques with selected genotypes resistant to pests and diseases, such as the ink disease. However, the current climatic instability urges the selection of genotypes also capable of enduring prolonged periods of drought and heat. Therefore, this study aimed to compare the response of young chestnut plants (3 months old) from two varieties (Marsol and CA-90, used as disease-resistant rootstocks), to single and combined drought (null irrigation) and heat (4 hours/day at 42 °C) for 21 days.

After 21 days of exposure, stress conditions impaired Marsol's leaf area, but the relative water content (RWC) was only decreased by drought, alone or in combination, while the production of new leaves was not affected. Contrastingly, CA-90 plants were affected to a higher extent, showing greater inhibition rates for all biometric parameters. The photosynthetic performance followed the same pattern, with the Marsol variety

showing less inhibitory responses concerning leaf chlorophyll fluorescence analysis and leaf gas-exchange parameters, when compared to CA-90. Oxidative stress markers showed that, while in CA-90 stresses led to increased hydrogen peroxide levels, no variations of this ROS were detected in Marsol, which also presented lower superoxide anion (O_2^{-}) values and unchanged lipid peroxidation (LP) degree in all conditions. Contrariwise, CA-90 chestnuts had upsurged O_2^{-} levels upon heat, alone and combined, and a higher LP degree in response to drought, pointing to higher oxidative damage. Finally, a boost of proline levels was found in both Marsol and CA-90 plants, especially upon the combined stress. Altogether, results suggest that the combination of heat and drought severely impacts the physiology of chestnut plants, with CA-90 variety showing a higher susceptibility, evidenced by their growth performance, photosynthetic yield, and redox metabolism.

Keywords: Chestnut plants; Drought; Global warning; Heat; Oxidative stress; Photosynthesis



Efecto del injerto de cv Chiquitita en las relaciones hídricas del cv Manzanilla de Sevilla

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Abstract: El proceso de intensificación del cultivo del olivar en los últimos años ha estado muy relacionado con el aumento de la densidad debido al uso de explotaciones superintensivas. Este sistema de cultivo ha estado restringido a la producción de aceite debido a que los cultivares mejor adaptados para su manejo son aquellos con menor vigor y crecimiento. En el caso de la aceituna de mesa una de las posibles estrategias para poder incrementar la densidad sería el uso de patrones que transmitiesen una reducción en el vigor. El objetivo del ensayo fue estudiar el efecto de un cultivar de porte compacto y bajo vigor (cv Chiquitita) como portainjerto de un cultivar tradicional de mesa (cv Manzanilla de Sevilla). El ensayo se llevó a cabo durante varios años en la finca experimental "La Hampa" del IRNAS en Coria del Rio (Sevilla). El diseño experimental fue en bloques al azar, con 7 repeticiones. Cada parcela tenía de 6 árboles, 3 injertados y 3 sin injertar, que fueron plantados en el año 2013 a una distancia de 4*1.5 m. A lo largo de varios años se realizaron medidas de estado hídrico, desarrollo vegetativo y evaluación de la cosecha. Los resultados sugieren que no hubo ningún efecto significativo del portainjerto sobre el vigor de la variedad. Sin embargo, durante los periodos de estrés los árboles injertados tuvieron significativamente valores más negativos de potencial hídrico en algunas fechas. El diámetro de los vasos de xilema no estuvo significativamente afectado en la variedad. Los efectos en calidad y cantidad de cosecha no fueron significativos.

Keywords: Water relations, olive trees, stem water potential, xylem vessels, hedgerow olive



Algal wrack - a sustainable fertilizer solution for barley plants under drought-induced stress

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Abstract: Drought is a major problem faced by agriculture as it severely impacts crop productivity worldwide. Aligned with the Farm to the fork strategy, sustainable and efficient tools are needed to ensure food security. Thus, this study aimed to assess the biostimulant potential of algal wrack, an organic waste of marine origin, when applied as a soil amendment to Hordeum vulgare L. (barley plant) under different water regimes. For that, batches of uncontaminated agricultural soil were mixed, or not, with wrack (1%, m/m) followed by a 15-day stabilization period. Afterwards, for both groups (non-amended and amended), barley seedlings were grown for 14 days under the following treatments: control – plants continuously irrigated; drought – plants only irrigated during the first 7 days of growth. Results showed that wrack application promoted leaf's biomass production and relative water content even in conditions of drought. Still, wrack did not alleviate the drought-induced impacts on the photosystem II efficiency and electron transport rate of barley plants. Regarding the production of reactive oxygen species, the content of superoxide anion remained unchanged among treatments, but high levels of hydrogen peroxide were found under drought-induced stress, being lowered by wrack co-application. Moreover, drought promoted a higher membrane damage, regardless of wrack amendment. Concerning plants' antioxidant defenses, drought increased the accumulation of proline, glutathione, sugars and phenols, which were subsequently reduced upon the co-application of wrack. Interestingly, a tradeoff effect was observed, as plants exposed to both drought and wrack activated enzymaticdefenses, including catalase and glutathione reductase. Overall, the data showed that wrack efficiently acted as a fertilizer, rather than just a stress mitigator, for barley plants grown under drought conditions, making it a promising alternative to chemical substances. However, further studies are needed to understand the mode of action behind this fertilizing activity.

Keywords: marine organic residues; biostimulants; water scarcity; redox homeostasis; chlorophyll fluorescence; circular economy



The protective role of the leaf cuticle against water loss and mechanical damage during leaf development in *Quercus ilex*

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Abstract: The cuticle is the outermost layer of leaves and is the first to interact with the environment. It is a key structural element at a functional and ecological level, mainly composed of cutin and cuticular waxes. The leaf cuticle acts as a barrier to water loss and provides mechanical resistance to biotic and abiotic damage. This layer is of utmost importance during leaf development, especially in stressful conditions such as high herbivore pressure or water scarcity common in Mediterranean regions. However, there is a lack of knowledge about the protective role of the cuticle in the expanding leaves of species living in Mediterranean environments.

To understand the protective role of cuticle in developing leaves we studied physiological, morphological, and anatomical changes in expanding leaves of holm oak (*Quercus ilex* L.), a Mediterranean evergreen with a high sclerophylly degree. We focused on the changes in leaf minimum conductance (g_{min}) and leaf hardness, which are key parameters for water loss and mechanical resistance throughout the vegetative period. We also measured other parameters such as leaf dry mass per area (LMA), leaf surface and thickness, cuticle and epidermis thickness, and other anatomical parameters. We hypothesized that expanding leaves would differ from fully expanded leaves in these parameters, which would help to understand possible changes in g_{min} and leaf hardness and their relationship with the cuticle.

Keywords: Minimum leaf conductance (g_{min}), Leaf cuticle, Sclerophylly, Leaf development, Holm oak (*Quercus ilex* L.), Mediterranean climate.



Short-term water stress and heat on water relations, growth, and nutrient partitioning in pear

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Abstract: Arid and semiarid regions are experiencing severe droughts combined with short term exposure to extreme temperatures. The combination of both stressors, water limitations and heat stress, often occurs simultaneously, and interactions might be expected. However, parsing out the individual contributions of these two factors is important. Two-year-old potted pear trees ('D'Anjou' grafted onto 'OHxF.87') were grown in the greenhouse and four treatments were applied: (i) A control treatment (CTL) irrigated to satisfy the tree's water needs in an unheated environment. (ii) A water stress treatment (WS), in which trees were submitted to two irrigation withholding cycles in an unheated environment. (iii) A heat stress treatment (HS), irrigated as CTL but the air temperature was elevated to 10 °C above CTL. (iv) A heat and water stress treatment (WS+HS), irrigated as WS trees, and the air temperature was heated as HS trees. Tree water relations, growth, and nutrient partitioning were measured over the two drought cycles. At the end of each cycle, WS trees had midday stem water potentials close to -2.0 MPa, while WS+HS had values closer to -3.0 MPa. However, in the recovery period after 6 days of full irrigation, both WS and WS+HS trees had values similar to CTL trees (-0.5 MPa). Nitrogen uptake and distribution were more affected by water deficit than by heat. Leaf senescence occurred in both WS and WS+HS trees, reducing their leaf area at the end of the experiment by 66 and 96 % compared to the CTL trees. Although leaf senescence did not occur for the HS treatment, leaf area was also lower than CTL trees. The results show that water stress had a greater impact on the physiological response and nutrient uptake of pear trees than heat stress. However, heat stress magnified the effects of water limitations.

Keywords: Drought, gas exchange, nitrogen, stem water potential, temperature.



Análisis de Ciclo de vida de la producción de cerezas en el Valle del Jerte (Prunus avium L. cv Burlat) producidas con diferentes estrategias de riego.

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Abstract: Ante la escasez de disponibilidad de agua en el sector agrícola ocasionada por el cambio climático y el incremento de la competencia entre sectores por este recurso, las estrategias de riego deficitario desarrolladas en cerezo en el Valle del Jerte (Extremadura) se han mostrado viables para gestionar de manera más eficiente el agua de riego disponible, optimizando la producción final. En este estudio se analizan 7 categorías de impacto generadas en la producción de cereza en esta comarca y se comparan entre sí escenarios con diferentes aportaciones de riego. A lo largo de tres años se aplicaron en una misma parcela 4 estrategias de riego diferentes y en todas ellas se realizó un Análisis de Ciclo de Vida de la cereza, desde su producción hasta el punto de almacenaje para su posterior comercialización. Las estrategias de riego empleadas fueron: T100, consistente en cubrir las necesidades hídricas del cultivo, T50 y T25 consistentes en reducir un 50 y un 25% respectivamente las aportaciones de riego en postcosecha y TAGR, que siguió la estrategia planteada por la comunidad de regantes de la zona. La metodología de análisis empleada fue "Environmental Footprint 3.0" con su posterior normalización de las categorías de impacto. En todos los escenarios estudiados, la instalación de riego supone la mayor aportación en todas las categorías de impacto, seguido por las operaciones culturales y la aplicación de pesticidas. La comparación anual de las estrategias de riego muestra que la contribución anual de cada estrategia se mantiene estable en el tiempo, variando aquellas categorías de impacto relacionadas con el volumen de agua aplicado, como el uso del agua o la eutrofización y ecotoxicidad de agua dulce.

Keywords: Inventario, Impacto ambiental, Uso agua, Cambio Climático, Horticultura.



Caracterización del estado hídrico de la higuera mediante mediciones manuales y automáticas del potencial hídrico

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Abstract: El potencial hídrico de la hoja y el tallo a mediodía se consideran indicadores fiables del estado hídrico de los cultivos. Es una medida rápida y fácil de realizar pero tediosa por ser manual y requerir ser tomada al mediodía solar en un corto periodo de tiempo. Aun así, es el indicador más utilizado en los estudios de las relaciones hídricas de los cultivos y las estrategias de riego. En los últimos años han aparecido dispositivos automáticos que registran de forma continua el estado hídrico de los cultivos leñosos. El objetivo de este trabajo es evaluar la capacidad de microtensiómetros en continuo instalados en higueras para caracterizar la evolución de su estado hídrico sometido a diferentes regímenes hídricos. Durante el año 2022 se realizó un experimento de campo en un huerto joven de higueras en regadío, cv. Calabacita, situado en la Finca La Orden (Guadajira, España). Los tratamientos de riego fueron: Control, regado para cubrir las necesidades del cultivo con la información proporcionada por un lisímetro de pesada con una higuera y 2 tratamientos deficitarios: RDS al 60 % del Control durante toda la campaña y RD3, que sólo recibió riego hasta junio. Los microtensiómetros reflejaron el estado hídrico de los árboles durante el ciclo de cultivo, con potenciales más negativos en los tratamientos que recibieron menos agua. Mostraron una buena relación con el déficit de presión de vapor (VPD), alcanzando en el tratamiento RDS un R2 = 0,76 para el periodo de crecimiento activo de los brotes, y mostraron más sensibilidad a los cambios en el VPD que el potencial hídrico del tallo medido periódicamente, pero con una buena correlación entre ambas medidas. De acuerdo con los resultados obtenidos, las medidas obtenidas con microtensiómetros parecen prometedoras como alternativa o complemento a las medidas puntuales del estado hídrico.

Keywords: Ficus carica, potencial hídrico del tallo, microtensiómetros, DPV.



Reflections on why angiosperms acquired a hydroactive stomatal regulation mechanism mediated by ABA

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Abstract: Higher plants have two mechanisms for regulating stomatal opening: one is hydropassive, and the other is hydroactive. In truth, considering the efficiency of the hydropassive mechanism in ferns and to a large extent in gymnosperms in response to hydraulic disturbance, the need for angiosperms to include a hydroactive mechanism that complements this is not evident. However, whether out of necessity or to acquire new capabilities, the hydroactive mechanism played a key role in the dominance of angiosperms over other plant groups during evolution. The need to incorporate a hydroactive mechanism may have been related to the race to increase their photosynthetic capacity and, consequently, their growth potential. This process, associated with the miniaturization of cell size, had a side effect: the mechanical advantage of epidermal cells over guard cells. The inability to close stomata upon turgor loss, e.g., when the air's water vapor pressure deficit increased, necessitated the incorporation of a hydroactive mechanism that could independently modify the turgor of the guard cells. Another challenge that may have driven angiosperms to develop a hydroactive mechanism is the coordination of CO₂ and H₂O fluxes in the leaf. In hydropassive species, photosynthesis has little influence on stomatal regulation. This prevents their stomata from responding to abrupt changes in light or CO₂ and, as a result, from achieving high water use efficiency. In this case, the driving force for the incorporation of the hydroactive mechanism in angiosperms was the colonization of nutrient-rich, arid environments that basal plant groups had not been able to reach.

Keywords: Plant evolution, stomatal conductance, hydroactive mechanism, stomatal regulation.



Pine provenances differ in physiological and biochemical mechanisms to cope with drought

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Abstract: Maritime pine (*Pinus pinaster* Ait.) is a well-adapted Mediterranean tree species, occupying large areas in a wide variety of edaphoclimatic conditions in SW Europe. In Portugal, Maritime pine areas have decreased in the last decades, reflecting poor management practices and the effects of multiple biotic and abiotic factors, resulting in a significant reduction in timber production. Under the framework of project rePLANT, germplasm from *Pinus* breeding programs is being tested to improve the success of new afforestation actions. However, a broader knowledge of the physiological responses of these materials to abiotic and biotic stresses is needed.

We tested two provenances of *Pinus pinaster* (Portuguese, French) and *P. radiata* D. Don (Galician, French) for physiological and biochemical responses under imposed drought. Two-year-old potted plants (1.7 L pots) were maintained in a greenhouse under differing watering regimes: periodical watering to field capacity (control, C); and complete water suppression (water stress, S) for 92 days. From day 92 to 120 all plants were watered to field capacity. Monitoring was done throughout the assay through indirect assessment of stomatal conductance (thermal imaging), water status evaluation (RWC) and changes in biochemical traits, namely, total phenolics, antioxidant activity, soluble sugars, chlorophylls, and carotenoid content.

In both species, French provenances exhibited lower antioxidant activity and total phenolic in C and S plants, suggesting efficient cell protection mechanisms, probably resulting from genetic adaptation to the region of origin. Imposed drought significantly reduced needle chlorophyll and carotenoid content in the French provenance of *P. pinaster*, with negative consequences for photosynthetic activity and carbon gain. Stomatal conductance was significantly reduced by water stress in all provenances, but only the French provenance of *P. pinaster* recovered upon rewatering. The results suggest that different metabolic responses in *Pinus* provenances play a significant role in coping with drought.

Keywords: *Pinus pinaster* Ait., *Pinus radiata* D. Don, improved tree germplasm, water stress, physiological and biochemical traits



Omeprazole as a tool to improve water and nitrogen use efficiency in tomato

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Abstract: There is a growing need to improve resource use efficiency in modern agriculture given the exponentially rising population, resource scarcity and climate change that pose serious challenges towards sustainability. Water and nitrogen are among the most important factors affecting tomato growth and productivity. Omeprazole (OMP) has shown promising results in promoting plant growth and tolerance under abiotic stresses. The objective of this research was to explore OMP effect on tomato plants under water (W), nitrogen (N) and combined (W + N) deficit. To that end, plants were grown in a climate chamber and were subject to four growth conditions: control plants; 50% single W deficit; 50% single N deficit; and combined W and N deficit. It was found that OMP promoted tomato tolerance against the studied deficits, translated by higher dry weights in all treatments (up to 31%). Moreover, W use efficiency and N use efficiency have increased in tomato plants exposed to OMP. Other aspects of plant metabolism were also positively significantly affected by OMP including hormones' concentration, lipid peroxidation and total antioxidant activity which suggests its role in mitigating the effects caused by the imposed deficits. Therefore, it can be concluded that, in tomato plants, OMP has a dual role as a plant growth enhancer and stress alleviator, even under combined W and N deficit conditions. We acknowledge that using OMP on a large scale may be limited due to its high production costs. However, it is important to notice that OMP is used in micromolar concentrations and that these results can be an encouragement to the exploration of less expensive alternatives, such as cheaper analogs, for widespread use.

Keywords: abiotic stress, elicitor, hormone regulation, proton pump inhibitor, *Solanum lycopersicum* L, sustainability

4. Best doctoral thesis in water relations



Estudio del efecto a corto y medio plazo de la técnica del "forzado de yemas" sobre la productividad y la calidad de la vendimia 'Tempranillo' en Extremadura

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RESUMEN

Una de las limitaciones del viñedo en las zonas cálidas es la pérdida de calidad de las uvas para vinificación debido a la incidencia de las altas temperaturas durante el periodo de maduración de las mismas. La consecuencia final es un aumento del grado alcohólico y una disminución de la acidez y del color del vino. Además, esta situación podría verse agravada en el futuro debido a que, según las predicciones de los modelos climáticos, se producirá un aumento de las temperaturas durante los próximos años, que ya empieza a ser evidente en las principales zonas de producción vitícola, incluida la extremeña.

Retrasar el proceso de maduración de la uva hacia periodos con temperaturas más suaves, podría ser una solución para promover una producción rentable y sostenible de uvas de calidad adaptadas al contexto del cambio climático. Mediante el "forzado de yemas" o "crop-forcing", se produce el rebrote de las yemas francas y se reinicia el ciclo de desarrollo de la vid "forzada" desplazando su fenología en el tiempo y, por tanto, la maduración de la uva hacia fechas donde las temperaturas son más bajas.

El objetivo de este trabajo es evaluar el efecto del "crop-forcing" en un viñedo de 'Tempranillo' para las condiciones semiáridas de Extremadura sobre aspectos relativos a la productividad del viñedo, la composición de la uva y los vinos elaborados y el posible desgaste del nivel de reservas de la cepa. El estudio se realizó durante cuatro años (2017 – 2020), aplicando el "crop-forcing" tres años consecutivos sobre las mismas cepas (2017 – 2019) en dos fechas diferentes y junto con dos estrategias de riego y dejando el año 2020 como año de recuperación sin forzar. Se establecieron tres tratamientos de poda: dos fechas diferentes de aplicación del "crop-forcing", entre los estados fenológicos de floración y cuajado de la uva (F1), entre cuajado y tamaño guisante (F2) y un control con poda convencional en invierno (NF). Cada tratamiento de poda fue sometido a dos regímenes hídricos: riego para cubrir las necesidades hídricas de las plantas (C) y riego deficitario con estrés hídrico moderado durante pre-envero (RI).

Capítulo 1:

El "crop-forcing" retrasó la vendimia una media de 32 y 56 días para F1 y F2 respectivamente en relación a NF, pero disminuyó el rendimiento del viñedo. La aplicación de RI también provocó un descenso en la producción respecto a C. El "crop-forcing" incrementó la acidez titulable y los contenidos en ácido málico, polifenoles y antocianos totales de las uvas en comparación con las NF. La combinación del "crop-forcing" y riego RI, supuso una mejora de la composición de las uvas en comparación con la aplicación del "crop-forcing" con riego C, sin embargo, supuso una pérdida mayor de rendimiento.

Capítulo 2:

El "crop-forcing" demostró ser una técnica eficaz para incrementar el contenido fenólico total y el de en antocianos en particular, y la acidez total de las bayas, para un mismo contenido en sólidos solubles totales. Por ello, puede ser una técnica útil para lograr un mayor acoplamiento entre la madurez fenólica y tecnológica. La aplicación conjunta del "crop-forcing" y el déficil hídrico mantuvo este aumento en los parámetros de calidad a la vez que supuso una mejora en la productividad del agua de riego.

Capítulo 3:

La aplicación del "crop-forcing" después de floración (F1) no supuso un desgaste en el nivel de reservas, sin embargo, cuando se aplicó después de cuajado (F2) se observó una leve disminución en el contenido de carbohidratos en sarmientos y hojas en algunos momentos del ciclo vegetativo durante los años de aplicación del forzado, pero con una rápida recuperación en la campaña posterior (año 2020) al restablecer una única poda invernal. El "crop-forcing" modificó de la distribución de biomasa con respecto a NF, incluso al dejar de aplicar el forzado (2020). La aplicación conjunta de "crop-forcing" con RI no modificó los resultados en relación con los correspondientes tratamientos con C.

Capítulo 4:

El "crop-forcing" aplicado después cuajado (F2) tuvo mayor efecto que aplicado después de floración (F1) sobre la composición fenólica de las bayas. En F2 aumentó el contenido de antocianos y catequinas en las bayas. En todos los años de aplicación (2017-2019), e independientemente del tratamiento de riego aplicado, esta técnica modificó su perfil antociánico: aumentó el contenido de las formas monoglucósidas, y el de los derivados de delfinifina, cianidina, petunidina y peonidina. El efecto del déficit hídrico fue menos significativo y tuvo mayor variabilidad interanual. No se encontró interacción entre el efecto del "crop-forcing" y el tratamiento de riego.

Capítulo 5:

En los vinos de F2 se observaron mayores concentraciones de ácido málico, acidez total, antocianos, catequinas, taninos y polifenoles totales y un aumento en los contenidos de derivados de delfinidina, cianidina, petunidina y peonidina y antocianos copigmentados en relación con NF. Ello repercutió en las características cromáticas, siendo los vinos de F2 los de mayor intensidad de color. Sin embargo, el efecto del "crop-forcing" sobre el pH fue escaso. El efecto de las técnicas de riego sobre estos parámetros dependió del año considerado, y no se observó interacción significativa entre los dos efectos.

5. Group Picture



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