XII Portuguese-Spanish Symposium on Plant Water Relations

Water to Feed the World

BOOK OF ABSTRACTS

Évora, 30th of September – 3rd of October

Portugal

Organising committee

President: João Santos Pereira (ISA-UL, Lisboa, Portugal)

Secretary: Margarida M. Vaz (Universidade de Évora, Portugal)

Vocals: Renato Coelho (Universidade de Évora, Portugal)

Luís Leopoldo da Silva (Universidade de Évora, Portugal)

Ana Elisa Rato (Universidade de Évora, Portugal)

Arturo Torrecillas (CEBAS - CSIC, Murcia, España CEBAS-CSIC)

Scientific committee

João Santos Pereira	ISA-UL, Lisboa, Portugal
M. Manuela Chaves	ISA & ITQB, Lisboa, Portugal
Margarida M. Vaz	Universidade de Évora, Portugal
João Mota Barroso	Universidade de Évora, Portugal
Teresa Soares David	INIAV, Lisboa, Portugal
Jorge Marques da Silva	FCUL-UL, Lisboa, Portugal
M. Manuela David	Universidade do Algarve, Portugal
M. Margarida Oliveira	ITQB, Lisboa, Portugal
Arturo Torrecillas	CEBAS - CSIC, Murcia, España
Aurelio Gómez Cadenas	Universitat Jaume I de Castelló, Barcelona, España
Hipólito Medrano	Universitat de les Illes Balears, España
Robert Savé	IRTA, Barcelona, España
José Enrique Fernández	IRNAS-CSIC, Sevilla, España
Ismael Aranda	INIA-CIFOR Madrid, España
Jaume Flexas	Universitat de les Illes Balears, España
Félix Moreno	IRNAS-CSIC, España

Cover
Ricardo Mata

Graphic editor
Fabiana Vieira

Editors
Margarida M. Vaz
Renato R. P. Coelho

Organising entities

UNIVERSIDADE DE ÉVORA

Instituto de Ciências Agrárias e Ambientais Mediterrânicas

sociedadespañola FISIOLOGIA VEGETAL

Sociedade Portuguesa de Fisiologia Vegetal
http://www.spfv.pt

Sponsors

DELTA CAFÉS

solfranc
Tecnología para la Agricultura y el Medio Ambiente

DIVINUS Gourmet

LabFerrer

Contents

PLENARY CONFERENCES AND THEMATIC CONFERENCES

Molecular Mechanisms of Plant Adaptation to Drought

Oliveira MM

Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Genomics of Plant Stress, Av. da República, 2780-157 Oeiras, Portugal and iBET, Apartado 12, 2781-901 Oeiras, Portugal
* e-mail: mmolive@itqb.unl.pt

Water is one of the limiting factors of plant growth and productivity. The increasing pressure on fresh water resources, due to the increasing population and to climate changes, constitutes a major threat for food security and for the sustainability of ecosystems. To cope with water scarcity, some plants have evolved complex mechanisms that allow them to adjust to the adverse environment, by altering the molecular and developmental programs. The diversity of strategies that plants use to survive water scarcity is diverse and breeding for increased tolerance is complicated. However, the tools nowadays available to study those strategies were never these many. Because of that same reason, designing the best experiment in order to retrieve the most useful information is not trivial, and raises a number of questions not always addressed. This is obvious for instance when one wants to compare experiments and results of different groups. The level of stress imposed varies with the level of water limitation (intensity, duration) and putative recovery period, and with the different genotypes, developmental stages, growth conditions or tissues, among many other variables. In spite of such diversity, still common molecular pathways of plant response to water deficit could be identified. In crop production, high water use efficiency is especially important and the ability to grow with limited amount of water is an increasingly important trait. Water deficit affects photosynthesis efficiency and the expression of associated genes in a dose-dependent manner. Although growth arrest is a way to reduce the water usage, for many crops it is important that the water limitations do not completely prevent grain/seed production. More holistic approaches may better contribute to develop comprehensive models of plant behavior under drought. In this lecture we will cover some examples that illustrate how plants cope with water deficit, and focus in more depth in the underneath molecular mechanisms and on their impact in morphophysiological adaptations.

Water Relations in the Irrigation Scheduling of Olive Orchards

Moriana A[a,b]*, Corell M[a,b], Girón IF [b,c], Martín-Palomo MJ[a,b], Moreno F [b,c]

[a] Dept. Ciencias Agroforestales, EUITA, University of Seville, Carretera de Utrera Km 1, 41013 Sevilla, Spain;
[b] Unidad Asociada al CSIC de Uso sostenible del suelo y el agua en la agricultura (US-IRNAS). Crta de Utrera Km 1, 41013, Sevilla, Spain.
[c] Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS (CSIC), P.O.Box 1052, 41080 Sevilla, Spain
* e-mail: amoriana@us.es

Olive trees (*Olea europea* L) are traditional Mediterranean specie. The agricultural management of olive orchards has been changed from 90's of the last century. The most important change is the great increase of the irrigation surface. This new water demand has been produced in water scarcity areas. Such conditions and the traditional rainfed management of the orchards have produced very restrictive water used. The traditional irrigation scheduling based on water budget is a useful tool in conditions of full irrigation, but most of the olive orchards are deficit irrigated. In the last decades, plant water status measurements have been suggested in different fruits trees in order to improve the management of deficit irrigation. In this work, results of several experiments in different olive orchards using midday stem water potential and trunk daily diameter are presented. The water stress sensitivity and the real commercial utility are discussed.

Physiological Limits for Plant-Based Water Stress Indicators

Fernández JE

Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Avenida Reina Mercedes n.º 10. 41012-Sevilla
* e-mail: jefer@irnase.csic.es

The need for a rational use of water in agriculture has impelled the development of new methods tomonitor water stress in farms and orchards. Most of them are based on plant measurements, such that the outputs reflect the soil and atmospheric water status, as well as the response of the plant to the surrounding conditions. Additional benefits are derived from the new methods being nondestructive, suitable for automatic and continuous measurements, and easy to implement with data transmission systems for the user to have nearly real time access to the collected data from a remote computer, smart phone or similar. These advantages confer the new methods a great potential as water stress indicators, as compared to conventional plant-based methods relying on the monitoring of plant water status or gas exchange, i.e. on the use of Scholander-type pressure chambers or gas analysers. The new, automatic methods, however, are quite demanding in terms of installation and maintenance, and training is required for a proper analysis and interpretation of the collected data.

This work shows an assessment of the capacity of three automatic methods, based respectively on measurements related to sap flow, trunk diameter variation and leaf turgor pressure, to monitor water stress and schedule irrigation in fruit trees orchards. We evaluated installation, maintenance and data processing requirements when the three methods were applied to olive, our species of interest. Special attention was paid to errors on the interpretation of data derived from the impact of main physiological traits on the recorded variables. In our assessment we included different approaches to overcome the spotted drawbacks. We concluded that the three analysed methods can be suitable tools both to monitor water stress and schedule irrigation, provided the user is aware of the limitations of each method and uses a suitable approach to minimize the effect of limitations inherent to each method.

Water Use in *Montado* Ecosystems

Teresa S. David

Instituto Nacional de Investigação Agrária e Veterinária IP (Quinta do Marquês, Oeiras, 2780-159 Oeiras, Portugal); Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa (Tapada da Ajuda, 1349-017 Lisboa, Portugal)Members of the team relevant for the presented results: C.Pinto, J.S. David, J.S.Pereira, N.Nadezhdina, M.O.Henriques, T.Paço, C.Besson, M.C.Caldeira, T.Quilhó, H.Cochard, M.M.Chaves, M.Vaz, F.Valente, L.Gazzarini, I. Ferreira, J.Cérmak
* e-mail:teresa.david@iniav.pt

Montado ecosystems are characterized by a two-layered structure, with a sparse tree cover dominated by Mediterranean evergreen oaks - *Quercus suber* and *Quercus ilex* - and an understory of shrubs and herbs. Over the last two decades our team has been undergoing research on the water-use strategies of *montado* vegetation (mainly the tree component) to cope with seasonal drought stress. We analyzed tree behavior under different climatic and edaphic conditions. The two main strategies rely on maximizing water uptake and controlling excessive transpiration losses through a tight stomatal regulation. These processes smartly operate on the two exchange surfaces: roots for water uptake and leaves for water release. However, the xylem structure, that transports water from roots to leaves, also plays an important role in the functioning and survival of these oaks, determining the hydraulic conductance, embolism resistance and the degree of embolism spread. The dimorphic root system has a dense network of superficial roots linked to sinker roots and a taproot diverting into tangles of fine roots filaments. Depending on soil traits and lithology, these deep roots may access the groundwater table. Under these conditions, trees use surface water during the wet season (autumn, winter and spring) and groundwater in summer when the shallow soil dries out. The roots also perform hydraulic lift in summer, when surface soil is dry. A small amount of groundwater is released at night into the topsoil by superficial roots, providing water for the next day tree and understory transpiration and facilitating nutrient absorption.When root access to groundwater is unrestricted, trees do not show signs of summer water stress (high predawn leaf water potentials), contrary to what occurs when the access is somehow limited.Even in well-watered sites stomatal control plays an important role. During the summer, under high vapor pressure deficit (VPD) the atmospheric demand exceeds tree maximum water uptake capacity (hydraulically limited). Stomata closure, by reducing transpiration, prevents leaf water potential from falling below the critical xylem embolism thresholds. Under fully watered conditions, the relationship between transpiration (T) and VPD shows a unique maximum plateau, irrespective of the season, and the relationship between canopy conductance (gc) and VPD is seasonally invariable. Under limited or nonexistent access to groundwater, the maximum plateau of the relationship between T and VPD and the gc values for the same VPD decrease as the summer drought progresses due to stronger stomatal closure.The xylem is tolerant to drought-induced embolism and has a high degree of hydraulic modularily (sectoriality). It is highly sectored in the above parts of trees (branches and stem) and in the taproot, confining damages to localized sectors and reducing the risk of embolism spread. It is highly integrated (equally connected to all aboveground plant parts) in other roots, which is advantageous in water and nutrient patchy environments, but facilitates the spread of root diseases. In the studied sites, root water uptake and stomatal control were efficient in maintaining comfortable safety margins above the xylem water potential causing 50% loss in hydraulic conductivity ($\Psi_{xyl,50PLC}$), both in *Q. ilex* and *Q. suber*. However, in poor site conditions and under severe drought the hydraulic safety limits might be surpassed predisposing these species to decline.

Hydrological, Engineering and Physiological Approaches to Water Conservation

Luciano Mateos
Instituto de Agricultura Sostenible, CSIC. Alameda del Obispo, 14004 Córdoba, Spain.
* e-mail:ag1mainl@uco.es

In water-limited environments, water conservation is a means to increase agricultural production. It is also related to the protection of soil against erosion and the preservation of water quality. Water conservation can be approached at different scales. In closed basins (basins with no discharge of usable water even in the wet season), water is conserved only by reducing consumptive use. There is a wide range of hydrological solutions to conserve water at basin scale. Water productivity (the production to water use or consumption ratio) is increased by reducing non-beneficial use and by regulating irrigation applications as to reduce transpiration in smaller proportion than yield. Irrigation efficiency (the beneficially-used irrigation water to irrigation supply ratio) can be enhanced through various engineering solutions such as subsurface drip irrigation, site-specific variable rate irrigation, etc. These solutions reduce the use of irrigation water and energy although they do not necessarily reduce consumptive use.

Therefore, engineering solutions for water conservation at farm level do not imply basin-scale water conservation always. However, physiological solutions such as regulated deficit irrigation or drought tolerant crops are directly linked to basin water conservation because they goal is to reduce transpiration and the ratio yield : transpiration. In some situations, this goal is plausible; however, in others it conflicts with food production and food security, thus it should not be generalized.

From Leaf to Whole Plant Water Use Efficiency: Solving the Gaps

Medrano H*, Tomás M, Martorell S, Flexas J, Bota J, Hernández E, Gago X, Escalona JM

Research Group on Plant Biology under Mediterranean Conditions
Department of Biology , IMEDEA (CSIC -Universitat de les IllesBalears
Carretera de Valldemossa Km 7.5 , 07122 Palma de Mallorca , Spain
* e-mail: hipolito.medrano@uib.es

Current climatic conditions as climatic change predictions are showing clear increases in temperature and drought in semi-arid regions, thus to improve crop water use efficiency is an unavoidable mandatory objective for global food production. The plant water use efficiency (WUE) is therefore becoming a key issue in these areas as crop production largely relays on the use of important water volumes. Therefore, improving water use efficiency is a challenge to secure environmental sustainability of food production.

The WUE is commonly measured at the leaf level because portable equipment facilitates the simultaneous measurement of photosynthesis and transpiration. However comparing those measurements with the daily integrals or whole plant estimates of the WUE the relationship results with and without agreement depending on the particular experiment. Scaling up from single leaf to whole plant WUE was tested in grapevines comparing daily integrals of A_N/E with midday ones to evaluate the importance of the spatial and time variations of carbon and water balances at the leaf and plant level. The leaf position (i.e average light interception) inside the canopy showed a marked effect on the instantaneous and daily integrals of leaf WUE. The night transpiration and mainly, respiration rates were also evaluated, as well as the respiration contributions to the total carbon balances.

From these data, two main components were identified to solve the gap between leaf and whole plant WUE, the important effect of leaf position in the daily carbon gain and water loss and the large flux of carbon losses by dark respiration. These results show that WUE evaluation among genotypes or treatments would need to be revised.

Efficient Use of Water Under Mediterranean Conditions: Agronomic Tools

Mário Carvalho

University of Évora, Institute for Agriculture and Environmental Sciences (ICAAM), Portugal
* e-mail: mjc@uevora.pt

The efficient use of water is crucial under Mediterranean conditions, due to the development of water stress during late spring and summer. However, an astonishing intra-annual variability of rainfall makes any solution very complex. Production techniques need to be adapted to this variability, thereby increasing the availability of and the efficiency with which crops utilize water.

Tillage plays a major role in both aspects. No-till reduces runoff and therefore increases the amount of water stored in the soil, particularly in dry years. If crop residues are maintained on the soil surface, No-Till reduces the water lost by direct evaporation, although the benefits depend on the frequency of rain or irrigation events. The volume of soil porosity available for water storage is also affected by tillage, although information in the literature is somewhat contradictory. However, if organic matter increases under conservation agriculture the water storage capacity of the soil tends to increase. Crop rooting depth also plays an important role on the water available to the crop and for depths greater than tillage the presence of continuous bi-pores might be crucial for the rapid elongation of the root system into deeper soil layers. This aspect might be particularly important in Luvisols where the growth of the roots in the B horizon can be enhanced by long term No-Till. Under these circumstances No-Till improves saturated hydraulic conductivity of the soil that, together with greater soil cohesion, improves the soil bearing capacity, contributing for timelier field operations, such as seeding, fertilizer and herbicide applications, that can improve the water use efficiency by the crop.

The effect of the seeding time on crop yield depends on the crop and the precipitation of the year. The best seeding time for winter crops is from mid-November to mid-December, for seasons when rainfall at least equals the annual average value but October sowing is better in drier than average years. For rainfed spring crops the best seeding time is the beginning of February, irrespectively of the rainfall. The amount and timing of nitrogen application to winter crops depends on the amount of rainfall. In wet winters a top-dressing in January is crucial, but timing is depended on the trafficability of the soil. This aspect is also important for early application post-emergence herbicides. Both applications can be decisive for good cereal yields in wet years and therefore for water use efficiency.

Crop rotation also plays a role in the water available in the soil. Rainfed, long-season spring crops like sunflower depletes the soil water by the end of summer and impair the yield of the next crop in a dry year.

New avenues in crop breeding for drought tolerance

José Luis Araus[1]*, Maria Dolors Serret[1], Jordi Bort[1]; Mainassara Zaman-Allah[2], Jill E. Cairns[2]

1Unit of Plant Physiology, Department of Plant Biology, University of Barcelona, 08028 Barcelona, Spain,
2Global Maize Program, CIMMYT Southern Africa Regional Office, Harare, Zimbabwe;
*e-mail: jaraus@ub.edu

Genetic advances on yield potential and resistance to stresses such as drought have been reduced in the past decades in spite of the increased adoption of molecular approaches (e.g. marker assisted selection, transformation, etc). Increased evidence exists that phenotyping, particularly at the field level, is actually limiting the efficiency of conventional breeding as well as preventing the delivery of molecular breeding at its full potential. Constraints in field phenotyping limit our ability to dissect the genetics of quantitative traits, particularly those related to abiotic stress tolerance. The development of effective field-based high-throughput phenotyping platforms (HTPPs) remains a bottleneck for future breeding advances. However, progress in sensors, aeronautics, and high-performance computing are paving the way. Examples of the performance of different high throughput (including low-cost) phenotypical approaches assessing grain yield in different crops are provided. In addition utilizing technological advances with regard to phenotyping instrumentation must also go hand-in-hand with methods to characterize and control field site variation (for improving repeatability), adopting appropriate experimental designs, selection of the right traits, and finally, proper integration of heterogeneous datasets, analysis, and application, including prediction models.

ORAL PRESENTATIONS

Looking into the Sumoylation Machinery in Rice Tolerant Vs Sensitive Genotypes during Drought Stress: Contrasting Results

Rosa MG[1], Almeida DM[1], Saibo N[1,2], Oliveira MM[1,2], Abreu IA[1,2]

1 Genomics of Plant Stress Unit, ITQB-UNL, Av. da República (EAN), 2780-157 Oeiras, Portugal
2 IBET, Av. da República (EAN), 2780-157 Oeiras, Portugal
* e-mail: amgrosa@itqb.unl.pt

Rice is one of the most important food crops in the world, but it is particularly sensitive to several abiotic stress conditions, including drought, salinity, submersion and cold. Therefore, it is crucial to understand the molecular mechanisms underlying abiotic stress tolerance and relate them to the physiological data collected. Sumoylation is a post-translational modification which regulates numerous cellular processes, is essential for plant development and has been associated with stress response in Arabidopsis and also recently in rice. To understand how sumoylation responds to abiotic stress in rice we have performed a drought stress assay using PEG6000. We have used two different rice genotypes Nipponbare (drought sensitive) and LC-93-4 (drought tolerant) and submitted them to 48h of 30% PEG6000, followed by a recovery period of 6 days. Shoots and roots were collected separately at different time points and tested for biomass parameters. Gene expression was performed with RT-qPCR of genes involved in sumoylation and the respective alternative splicing forms which, so far, have not been studied in stress response. These genes include SUMO-activating enzymes (OsSAEs), SUMO-conjugating enzymes (OsSCE1s), SUMO ligases (OsSIZs) and SUMO proteases. The results showed that after 6 days of recovery period, the Nipponbare sensitive genotype did not survive, while the LC-93-4 drought tolerant presented a 90% survival rate. This difference could not be explained by the rate of water loss, which was not substantially higher in the sensitive genotype. However, when looking at the sumoylation machinery expression levels, there was a clear difference not only between the genotypes, but also when comparing roots versus shoots. Antagonic responses in roots were found between the genotypes, where the drought tolerant showed a generalized downregulation, contrary to Nipponbare where there was an upregulation. However, in shoots the scenario was the opposite, and the expression levels of most of the sumoylation machinery genes were in general higher in the drought tolerant genotype. These results indicate an important role for sumoylation in abiotic stress response and how the response is organ and genotype dependent.

Work funded by FCT PhD Fellowships SFRH/BD/84219/2012 and SFRH/BPD/78314/2011.

Three Common Drought-Stress Induction Methods Compared in Arabidopsis: a Proteomic Approach

Rodrigues MAA[1], Oliveira MM[1], Abreu IA[1,2]

1 Instituto de Tecnologia Química e Biológica, Oeiras, Portugal
2 Instituto de Biologia Experimental Tecnológica, Oeiras, Portugal
* e-mail: maar@itqb.unl.pt

Drought is a major environmental stress factor that severely affects plant growth and development. As a direct effect of an erratic climate, unsustainable crop yield losses are expected and will have a tremendous impact on world economy. To deal with this problem, plant/crop-response to drought stress is currently an important focus of the scientific community. Due to practical reasons, “Water-Withdraw”, “Dehydration”, and “PEG/Mannitol-induced” stresses have been recurrently used as assays to investigate drought stress responses in plants, regardless of the specific mechanisms that are being triggered by each of them. Consequently, conclusions about the physiological response of plants to drought using these stress-assays are made, despite the assays themselves not being truly-physiological. This issue has been a subject of controversy in the past years, but so far no study was conducted to determine how (de)similar is plant-response to each one of these distinct assays.

Drought stress can induce three main types of molecular modifications in plants: (a) alteration in gene expression (transcriptome-level); (b) dynamics in protein production, modification and degradation (proteome-level) and (c) alterations in the metabolic pool (metabolome-level). Being proteins the true effectors of cell-response, we aim to identify the main players involved in the response mechanisms of “Water-Withdraw”; “Dehydration”; and “PEG-induced” stress responses, by a proteomic strategy. At the same time, we also aim to conduct a fair comparison of these three distinct and widely used drought-stress induction methods. Using a 2-DE methodology, improved by the use of a highly sensitive fluorescent dye (RuBPS), we were able to qualitatively compare the Arabidopsis proteome response to each stress-assay and pinpoint some common effectors between these stress responses, using MALDI-TOF/TOF analysis.

By using proteomics to address different drought-mimicking induction methods, we are opening the doors to a new understanding of plant drought-stress responses and, moreover, to enable a more conscientious use of the current stress induction methods by the plant science community.

Work funded by FCT Fellowships SFRH/BD/87420/2012; SFRH/BPD/78314/2011. Pest-OE/EBQ/LA0004/2011. MS performed at UniMS (http://www.itqb.unl.pt/facilities/UniMS-Mass-Spec-Services).

Aquaporin Isoforms in Cv. Touriga Nacional Grapevine Under Water Stress and Recovery - its Role in Leaves and Roots Hydraulic Dynamics

Zarrouk O[1]*, García-Tejero I[2], Pinto C[3], Genebra T[1], Sabir F[4,5], Prista C[4], Soares David T[3], Loureiro Dias C[4], Chaves MM[1]

1 Instituto de Tecnologia Quimica e Biologica, Oeiras, Universidade Nova de Lisboa, Portugal
2 Inst. Andal. Investig. Form. Agrar. Pesq. Prod. Ecol. (IFAPA), Cent. "Las Torres-Tomejil", Ctra. Sevilla-Cazalla, Km. 12,2, Alcalá del Río (Sevilla), Spain
3 Instituto Nacional de Recursos Biológicos, Quinta do Marquês, Av. da República, 2784-505 Oeiras, Portugal
4 CBAA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
5 Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal.
* e-mail: zolfa@itqb.unl.pt

Aquaporins are trans-membrane proteins that facilitate rapid and passive water transport across cell membranes in all living organisms and show a large diversity of isoforms divided into seven subfamilies. The different function and regulation of aquaporins are highly variable among these distinct isoforms and across different species. Several aquaporins were cloned in cv. Touriga Nacional grapevine (Vitis vinifera L.), and five of them showed their ability to water transport in a yeast system (VvPIP2;1 - VvTIP1;1 - VvTIP2;1- VvTIP2;2 and VvSIP1).

In this study, ungrafted grapevines cv. Touriga Nacional were subjected to two different irrigation treatments: i) a well watered treatment (WW), in which vines were daily irrigated, being maintained at field capacity; ii) a water stress treatment (WS), in which vines were not irrigated along seven days, with a later recovery (Rec), these vines being irrigated at field capacityMeasurements started at 48 h after the beginning of the trial (all plants were at field capacity), and from this point on, every 48 h until the maximum water stress point 144 h.

Finally, 48 h after the recovery, measurements were done again in both treatments. We studied the expression pattern of the different aquaporins in roots and leaves of WW vines at 48 h and 144 h, of WS and 48 h after Rec in both treatments. In addition and with the same periodicity, leaf and root hydraulic conductances (Ks-leaf; Ks-root), abscisic acid (ABA) concentration, xylem sieve pH and leaf temperature measurements were assessed. Both VvPIP2;1 and VvTIP2;2 were down regulated under water stress treatments in roots, which may explain the decrease in hydraulic conductivity in roots (Ks root) measured in these conditions. In leaves, non-significant effect on the leaf hydraulic conductivity (Ks leaf) was observed under water stress, but thermal-imaging showed a significant increase in leaf temperature related to stomatal closure. On the other hand, VvPIP2;1 and VvTIP1;1 were up-regulated under water stress in leaves, whereas VvTIP1;2 was down-regulated and VvSIP1 unchanged. Abscisic acid (ABA) analysis showed that ABA increased both in roots and leaves of water stressed vines since 48h of WS treatment, in line with the results of vine water status and pH of xylem sieves. In general, the results demonstrated differential responses of different aquaporins isoforms in mediating water transport of grapevine, and the regulatory mechanisms differed according to stress duration and specific organs.

Acknowledgement: European Community's Seventh Framework Program (FP7/2007-2013) under the grant agreement nº FP7-311775, Project Innovine

Genetic Control of Functional Traits Related to Photosynthesis and Water Use Efficiency in Pinus Pinaster Ait. Drought Response: Integration of Genome Annotation, Allele Association and QTL Detection for Candidate Gene Identification.

de Miguel M.[1,2], Cabezas J-A.[1,2], de María N.[1,2], Sánchez-Gómez D[1], Guevara M.A.[1,2], Vélez M.D.[1,2], Sáez-Laguna E.[1,2], Díaz L.M.[1,2], Mancha J.A.[1,2], Barbero M.C.[1,2], Collada C.[2,3], Díaz-Sala C.[4], Aranda I[1], Cervera M.T.[1,2]

1Departamento de Ecología y Genética Forestal. INIA-CIFOR. Ctra. de La Coruña Km 7.5, 28040 Madrid, Spain, demiguel.marina@gmail.com
2Unidad Mixta de Genómica y Ecofisiología Forestal, INIA/UPM, Madrid, Spain.
3ETSIM, Departamento de Biotecnología, Ciudad Universitaria s/n 28040 Madrid, Spain.
4Departamento de Ciencias de la Vida. Universidad de Alcalá. Ctra. de Barcelona Km 33.6, 28871 Alcalá de Henares, Madrid, Spain.
* e-mail: demiguel.marina@gmail.com

Understanding molecular mechanisms that control photosynthesis and water use efficiency in response to drought is crucial for plant species from dry areas. Complex functional trait dissection can be achieved through the analysis of Quantitative Trait Loci (QTL) mapping. QTL mapping allows to locate the genomic regions underpinning the expression of quantitative traits and estimate their effect and interactions. This study aimed to identify QTL for photosynthesis and water use efficiency in a Mediterranean conifer (Pinus pinaster Ait.) at three water irrigation regimes to test their stability under drought conditions.

High density genetic maps were used in the detection of QTL for the analyzed traits. A total of 28 significant and 27 suggestive QTL were found. QTL detected for photochemical traits accounted for the higher percentage of phenotypic variance. Functional annotation of mapped genes was performed through the analysis of sequence homology with genes from other species with known function. Functional annotation of genes within the QTL suggested 58 candidate genes for the analyzed traits. Besides, allele association analysis in selected candidate genes showed three SNPs located in a MYB transcription factor that were significantly associated with efficiency of energy capture by open PSII reaction centers and specific leaf area.

The integration of QTL mapping of functional traits, genome annotation and allele association yielded several candidate genes involved with molecular control of photosynthesis and water use efficiency in response to drought in a conifer species. The results obtained highlight the importance of maintaining the integrity of the photochemical machinery in P. pinaster drought response.

Effect of Deficit Irrigation and Elaboration Process of Spanish-Style Green Table Olives on Phytoprostanes Content in Manzanilla de Sevilla Olive Flesh

Collado-González J[1], Moriana A[2], Girón IF[3], Corell M[2], Medina S[1], Durand T[4], Guy A[4], Galano JM[4], Galindo A[5], Ferreres F[1], Moreno F[3], Torrecillas A[e], Gil-Izquierdo A[1]*

1 Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology. Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, Espinardo, E-30100 Murcia, Spain
2 Departamento de Ciencias Agroforestales, EUITA. University of Seville, Carretera de Utrera Km 1, 41013 Sevilla, Spain
3 Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC), P.O.Box 1052, 41080 Sevilla, Spain
4 Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 - CNRS - University Montpellier I and II - ENSCM, Faculty of Pharmacy, Montpellier, France.
5 Departamento de Riego. Centro de Edafología y Biología Aplicada del segura (CSIC), P.O. Box 164, Espinardo, E-30100 Murcia, Spain
*e-mail: angelgil@cebas.csic.es

No previous information exists on the effect of different deficit irrigation treatments during pit hardening and the elaboration process of Manzanilla de Sevilla Spanish style green table olives on the phytoprostanes (PhytoPs) contents in the fruit flesh. In this paper, the influence of three different irrigation treatments on the PhytoPs content of just harvested and processed olive flesh was studied. During two seasons, control (T0) trees were overirrigated (125 % ETc) and T1 and T2 trees were submitted to regulated deficit irrigation (RDI) according to trunk diameter fluctuations data with a pronounced irrigation water restriction during pit hardening. Seasonal total water amounts received by each treatment, without considering precipitation, were 412, 130 and 111 mm in 2012 and 369, 207 and 106 mm in 2013 for T0, T1 and T2 treatments, respectively. Deficit irrigation during olive pit hardening did not affect neither olive table yield nor fruit size, maximizing farmer in comes. Nevertheless, this phenological period was clearly critical for olive table composition because water deficit enhanced the PhytoPs content in the flesh of just harvested and Spanish style processed olives. Stress integral (SI) was better indicator than minimum midday stem water potential (Ψ_{stem}) for predicting the PhytoPs content in the olive flesh, pointing to the idea that the increase in PhytoPs depends in a greater extension of the length of water stress instead of maximum stress achieved. Some PhytoPs (16-B1-PhytoP + *Ent*-16-B1-PhytoP and 9-L1-PhytyoP + *Ent*-9-L1-PhytyoP) measured in the just harvested olive flesh were not detected after the olive processing, while others PhytoPs (9-F1t-PhytoP and 9-*epi*-9-F1t-PhytoP) increased their content around 10 folds. These results suggested that the olive fruit processing enhance the accumulation of total PhytoPs, but decrease the number of these chemical compounds. Consequently, table olive tree culture under deficit irrigation conditions during pit hardening and the processing of its fruits to obtain Spanish-style olives can be considered as complementary actions to enhancing the PhytoP content and hence their potential beneficial effects on human health.

Jujube Fruit Water Relations during Fruit Maturation Stage Under Different Irrigation Conditions

Cruz ZN[1], Rodríguez P[1], Galindo A[2], Pérez-López D[3], Collado-González J[2], Ondoño S[2], Moreno F[4], Moriana A[5], Torrecillas A[2]*

1 Instituto Nacional de Ciencias Agrícolas (INCA), Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba
2 Centro de Edafología y Biología Aplicada del segura (CSIC), P.O. Box 164, Espinardo, E-30100 Murcia, Spain
3 Escuela Universitaria de Ingeniería Técnica Agrícola. Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain
4 Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC), P.O.Box 1052, 41080 Sevilla, Spain
5 Escuela Universitaria de Ingeniería Técnica Agrícola. Universidad de Sevilla, Carretera de Utrera Km 1, 41013 Sevilla,Spain
*e-mail: atorreci@cebas.csic.es

The fruit maturation stage is considered the optimal phenological stage for implementing water deficit in jujube (Zizyphus jujuba Mill.), since a low, moderate or severe water deficit at this time has no effect on yield, fruit volume or eating quality. However, no information exists at fruit water relations level on the mechanisms developed by Z. jujuba to confront drought. The purpose of the present study was to analyse the fruit water relations in jujube in order to clarify the mechanisms developed in response to water deficit during the fruit maturation stage. For this, adult jujube trees (cv. Grande de Albatera) were subjected to different irrigation treatments. Control (T0) plants were drip irrigated in order to guarantee non-limiting soil water conditions. T1 plants were subjected to deficit irrigation throughout the season, according to the criteria frequently used by the growers in the area. T2 treatment was irrigated as T0 except during fruit maturation, in which irrigation was withheld.

The results indicated that the jujube fruit maturation period was clearly sensitive to water deficit. During most of this stage water could enter the fruits via the phloem rather than via the xylem. From the beginning of water withholding to when maximum water stress levels were achieved, fruit and leaf turgor were maintained in T1 and T2 plants. However, in contrast with the axiom that expansive cell growth requires the presence of cell turgor, jujube fruit size in T2 plants was reduced despite the fact that fruit turgor was maintained, perhaps due to an enhancement of the cell elasticity mechanism (elastic adjustment) in order to maintain fruit turgor reducing fruit cell size. The enhancement of flesh sweetness during the final stages of fruit maturation could be mediated by a degradation of some of the polymers previously constructed using osmolites and by a diminution of fruit tissue water.

Differences on the Decline of Leaf Hydraulic Conductance with Dehydration in Olive and Almond: Effects on Stomatal Conductance Regulation and Methodological Considerations.

Hernandez-Santana V*, Rodriguez-Dominguez CM, Díaz-Espejo A

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

A major resistance component of the plant hydraulic system has been demonstrated to be the leaf and, consequently, leaf hydraulic conductance (Kleaf) may play an important role in the regulation of stomata conductance (gs) but their relationship is still poorly understood. Reliable and fast Kleaf measurements would be really helpful for studies relating Kleaf and gs. Although there are new approaches providing Kleaf measurements within minutes some uncertainties are still unresolved such as the potential embolism caused by cutting the xylem under tension to make Kleaf measurements. Moreover, the extent of the Kleaf decline with water stress and its recovery varies from species to species, even within a particular habitat and the knowledge of its relationships to leaf structure and ecological strategy remains incomplete.

We conducted an experiment to quantify the dehydration response of Kleaf in two agricultural Mediterranean species with different ecology (olive-Olea europea and almond-Prunus dulcis) in well irrigated young individuals. Data obtained with two methodologies were compared and the effect of air entry into xylem conduits upon cutting the petioles under water was assessed. Vein density (VD) was also determined in both species to explain the potential differences between them.

The results showed that leaf water potential (Ψleaf) – Kleaf relationships determined by the two methods were statistically indistinguishable (P=0.87 and 0.12 for olive and almond, respectively). However, a clear effect was observed due to of the experimental artifact of air entry into xylem conduits upon cutting the petioles. Kleaf was always higher in almond than in olive for decreasing Ψleaf which is in accordance with the fact that almond is able to keep higher gs than olive for lower Ψleaf. Higher Kleaf for high Ψleaf in almond than in olive is in accordance with higher VD in almond (22.28±1.05 mm mm-2) than in olive (15.51±0.75 mm mm-2). Potential methodological implications and gs regulation strategies are discussed.

Jatropha Curcas Maintains a High Water Status under Drought Stress Due to a Strict Stomatal Control and Reduced Transpiration Area

Sapeta H[1]*, Lourenço T[1], Lorenz S[3], Grumaz C[3], Costa JM[2], Sohn K[3], Oliveira MM[1]

1 Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Genomics of Plant Stress, Av. da República, 2780-157 Oeiras, Portugal and iBET, Apartado 12, 2781-901 Oeiras, Portugal
2 Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Plant Molecular Ecophysiology Lab, Av. da República, 2780-157 Oeiras, Portugal and iBET, Apartado 12, 2781-901 Oeiras, Portugal
3 Functional Genomics Lab, Fraunhofer IGB, Nobelstr. 12, 70569, Stuttgart, Germany
* e-mail: hsapeta@itqb.unl.pt

Jatropha curcas is a multipurpose plant emerging as an excellent source of biodiesel, due to its quality and high oil content. Moreover, *J. curcas* is amenable for cultivation in areas with limited water availability. Since water scarcity is an increasing problem, our study aimed to contribute to understand this ability at the transcriptomic and morpho-physiological levels. We used two accessions from different climate origins (wet tropical and semi-arid). One month-old potted plants were subjected to continuous well-watered conditions, or water withholding for 49 days followed by re-watering (another 7 days). Soil and plant water status, growth and biomass partitioning, leaf gas exchange and chlorophyll (Chl) *a* fluorescence were assessed. Simultaneously, leaf and root samples were collected along drought and re-watering for RNA extraction and Next Generation Sequencing (RNA-Seq). We found no marked differences between accessions in the response to water stress at the morpho-physiology and transcriptome level (R=0.913-0.996). In fact, both *J. curcas* accessions presented a similar dehydration-avoidance strategy, maintaining a high water status, strict stomatal control and reduced transpiration area. Maximum stress (day 49) resulted in impaired growth and photosynthesis, as well as extreme transcriptomic changes, with over 4000 genes (2000 in roots and 2000 in leaves) differentially regulated. Moreover, Chl *a* to *b* ratio was found to be reduced under stress, due to an increase in Chl *b* content. RNA-Seq supported these observations. Further validation (RT-qPCR and HPLC) confirmed expression of genes putatively involved in Chl biosynthesis/breakdown, and the expression was correlated with Chl metabolites. Upon re-watering, despite the strong reduction of photosynthetic parameters observed during stress, the plants were able to fully recover within 3 days, suggesting a high photo-oxidative protection during severe water stress. This recovery was also observed at the transcriptomic level. The results presented suggest that *Jatropha curcas* rapidly responds to drought, withstanding severe water stress and rapidly recovering after rehydration, independently of its provenance. This reinforces this species plasticity to unfavorable conditions.

Acknowledgments: Funding by FCT through Project PTDC/AGRGPL/101435/2008, and through the fellowship grants SFRH/BD/89781/2012 (HS), SFRH/BPD/34429/2006 (JMC) and SFRH/BPD/34943/2007 (TL).

Diurnal and Seasonal Performance of Two Grapevine Varieties (Touriga Nacional and Aragonez (Syn. Tempranillo) Under Different Irrigation Regimes

Costa JM[1,2],García-Tejero I[1,3],Tomás M[4], Egipto R[2], Vaz M[5], Lima RSN[5], Lopes C[2], Chaves MM

1 LEM-ITQB, UNL, Apartado 127, 2780-901 Oeiras, Portugal;
2 LEAF- ISA, Tapada da Ajuda, 1349-017 Lisboa, Portugal;
3 Centro IFAPA, LasTorres–Tomejil Ctra. Sevilla-Cazalla Km12, 2 41200. Alcalá del Río, Sevilla, Spain
4 Grup de Recerca en Biologia de les Plantes en Condicions Mediterrànies, Dep. Biologia Univ. de les Illes Balears, 07122 Palma de Mallorca, Spain
5 ICAAM, Univ. Évora, 7002-554 Évora, Portugal
* e-mail: miguelc@itqb.unl.pt

Water is the most limiting natural resource for irrigated agriculture, especially in the dry regions of the Mediterranean. Deficit irrigation can help to optimise water use in irrigated viticulture and to improve wine quality parameters without major negative effects on yield. This study examines the daily and seasonal variation of physiological and biophysical parameters in two Vitis vinifera genotypes, Aragonez (syn. Tempranillo) and Touriga Nacional in response to irrigation. Vines were subjected to two irrigation regimes; ¡) a sustained deficit irrigation (SDI, ~40% ETc); and ii) a regulated deficit irrigation (RDI, ~20% ETc). Diurnal curves of leaf water potential (ψleaf), canopy temperature (Tc) and leaf gas exchange were done along the season of 2013: i) mid-June (171 DOY, green berry stage), ii) mid-July (198 DOY, veraison), iii) early August (220 DOY, early ripening) and iv) before harvest (233 DOY, late ripening). Additionally, leaf parameters such as specific leaf area, total Chl content and δ 13C were quantified. Mesophyll conductance and the photosynthetic response to leaf temperature were measured for SDI vines. Reflectance indices (NDVI and Water Index) were determined to assess eventual optical differences between genotypes. ARA showed more negative values of ψleaf than TOU, especially under the RDI regime and unfavourable atmospheric conditions (very high VPD and Tair). Punctual differences were found between genotypes for leaf gas exchange but the RDI regime resulted in lower stomatal conductance and net photosynthesis and higher Tc than the SDI, particularly when VPD and Tair were very high (e.g. 198 and 233 DOYs). In both genotypes the daily Tc raised 5-10ºC above the typical optimal leaf temperature range for photosynthesis during large part of the photoperiod, and mainly at the most stressful conditions of the trial, but no leaf senescence was observed. The leaf Chl content was not influenced by irrigation regimes but it decreased in ARA along the season. This may explain the lower NDVI values observed in ARA. The lack of marked differences between genotypes may be related to the high soil water content available along the summer due to the atypical rainy spring of 2013. Genotype behaviour will be discussed considering scenarios of more severe water deficits.

Key-words: genotype, deficit irrigation, leaf gas exchange, leaf temperature, leaf reflectance, senescence

More Fruit per Drop: Optimizing Irrigation in "Rocha" Pear Orchards in Central Portugal

Silvestre S[1]*, Leão de Sousa M[2], Ribeiro D[3], Pires S[3], Rodrigues B[3], Arrabaça JD[1], Matos AR[1], Bernardes da Silva A[1], Marques da Silva J[1]

1 Universidade de Lisboa, Faculdade de Ciências, Center of Biodiversity, Functional and Integrative Genomics (BioFIG), Campo Grande, 1749-016 Lisboa, Portugal.
2 Campotec, S.A., EN 9, Zona Industrial de Casalinhos de Alfaiata, 2560-393 Silveira, Torres Vedras, Portugal.
3 Hidrosoph, Lda., Rua Alcárcova de Baixo, 54 Sala C, 7000-841 Évora, Portugal.
* e-mail: smsilvestre@fc.ul.pt

Water availability is the main limiting factor for agricultural production worldwide. Water consumption in irrigated fruit orchards is an important determinant of economic viability. This work aims to optimize water use in irrigated orchards of "Rocha" pear in Central Portugal. Four homogeneous microclimatic and edaphic areas were selected (Alcobaça, Cela, Alfeizerão and Lourinhã) and, in each, one highly productive orchard was chosen for in depth studies, guaranteeing that half of them were high density orchards to get additional data. These orchards were provided with moisture soil probes that monitor irrigation by an irrigation management system (Smart Irrigation Management - Hidrovolution™, Hidrosoph, Évora, Portugal) connected to an integrated data management system (Irristrat™, Hidrosoph, Évora, Portugal). The water economy of the orchards is expected to be fully characterized along an entire production cycle. Furthermore physiological parameters related with the primary productivity are being periodically measured in representative trees of each orchard. The photosynthetic performance, assessed by light response curves using infrared gas analysis, is currently being monitored in parallel with photosynthetic pigments and soluble sugars content spectrophotometrically determined. Photochemical efficiency, measured by OJIP fast fluorescence induction transient, and cell membrane integrity (electrolyte leakage) are also under study. Preliminary results show photosynthetic and photochemical differences between orchards, closely related to the pigment and soluble sugar content, probably reflecting different agronomic procedures.

Different Barley Cultivars Responses to Several Climate Change Stress Factors

Pérez-López U[1]*, Barco-Antoñanzas M[2], Miranda-Apodaca J[1], Lacuesta M[2], Muñoz-Rueda A[1], Mena-Petite A[1]

Departamento de Biología Vegetal y Ecología, Facultad de Ciencia y Tecnología[1] y Facultad de Farmacia[2], Universidad del País Vasco (UPV/EHU).
* e-mail: usue.perez@ehu.es

Climate is a major influence affecting agricultural production, and any change on it can reduce crop productivity. Therefore, the maintenance and the increase of production under future environmental conditions is a major challenge. Predictions of climate change pose a continued increase in CO2 in the atmosphere associated with higher temperatures, an increase in the duration and intensity of drought. This implies that the productivity of crops will be influenced by complex interactions between the effects of increased atmospheric CO2 and other climate-related changes such as drought and increased temperature.

Barley (*Hordeum vulgare*) is the fourth largest cereal in the world in terms of production, which is used for both human and animal nutrition (feed cultivars) and in industrial processes such as brewing (malting cultivars). Barley is relatively resistant to drought which makes it a good candidate as a crop adapted to future climate conditions, helping to alleviate malnutrition and increasing food security. For this it is essential to analyze the interactive effects of the factors linked to climate change on the morphological and physiological characteristics of barley, focusing on cultivars that differ in their sensitivity to stress and ensure adequate production and quality under changing environmental conditions.

Therefore, the objective of this work was to analyze the water relations in two malting and two feed barley cultivars, commercially available. Water, osmotic and turgor potential, dehydration, osmotic potential, cumulative transpiration and aboveground biomass were used to characterize the responses to drought stress, temperature stress, and the combination of both stress.

We detected that each barley type responded differently. Generally, the feed barleys were more drought tolerant than the malting ones. Besides, within each type of barley differences between cultivars were observed. This variability will permit to identify the most interesting morphological and functional indicators of stress response, highlighting cultivars with a greater productivity under a future climate change scenario.

Acknowledgements: MICINN-BFU2010-16349/BFI cofounded by ERDF, and GRUPO Gobierno Vasco-IT577-13.

Short-Term Responses of Water and Carbon Fluxes to Cork Stripping

Correia AC[1], Costa e Silva F.[1], Pinto C[2], David TS [2], Hernandez-Santana V[3], Pereira JS[1]

1Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal, alexandrac@isa.utl.pt
2Instituto Nacional de Investigação Agrária e Veterinária, Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal
3Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), Av de la Reina Mercedes, 10, 41012 Sevilla, Espanha
* e-mail: alexandrac@isa.utl.pt

There is still a knowledge gap regarding cork stripping effects in tree water and carbon balance. Reliable and appropriate methods are mandatory to evaluate tree water losses and its partition in tree-compartments. The experimental site is a 50-yr-old evergreen cork oak open woodland (*Quercus suber*) located in herdade da Machoqueira do Grou in Coruche region (39°08_20.9_ N, 9°19_57.7_ W, 165 m a.s.l.). The average annual precipitation is 608 mm with a mean annual temperature of 15.9 °C. This site is integrated in the research infrastructure *ICOS - Carbon Observation System*, and is measuring continuously since 2009 the water and carbon fluxes at the ecosystem level using the *eddy covariance* method.

In the early summer of 2014 we have selected two similar pairwise sets of stripped and unstripped control trees (12 trees in total) and monitored water and carbon fluxes throughout the summer. Daily and seasonal measurements comprise: 1) Integrated branch carbon uptake, respiration and transpiration (Licor 840); 2) Stem respiration and transpiration (LCPro+, Bioscience); 3) Continuously sap flow monitoring (sap flow sensors, Granier methodology) 4) Tree water status (predawn and midday xylem leaf water potential).

This integrated study intends to assess the short term effects of cork stripping in the tree water and carbon fluxes and its partition between compartments. It will provide invaluable information regarding the timing for tree carbon and water balance recovery after cork stripping, therefore providing experimental knowledge to improve management practices towards montados sustainability.

Effects of an Extreme Dry Winter on Cork Oak Woodland: Net Ecosystem Exchange and Phenology Adjustments

Costa e Silva F*, Correia AC, Piayda A, Dubbert M, Werner C, David JS, Pereira JS

CEF, Instituto Superior de Agronomia. Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa (Portugal).
* e-mail: filipecs@isa.utl.pt

In seasonally dry climates, such as the Mediterranean, lack of rainfall in the normally wet winter season may originate severe droughts and a great variability in annual precipitation. Droughts, in turn, are a main source of inter-annual variation in carbon sequestration when winter rainfall diminishes. This may alter the seasonal pattern of photosynthetic uptake, which is determined by leaf phenology and gas exchange limitations.

The current study is based on the monitoring of an extremely dry winter in an evergreen cork oak woodland under the Mediterranean climate of central Portugal. Results are centred on net ecosystem exchange (*NEE*), phenology and tree growth measurements during two contrasting years: 2011, a wet year with a standard summer drought pattern and 2012, with an extreme dry winter (only 10 mm of total rainfall) that exacerbated the following summer drought effects. The main aims of this study were to assess: 1) effects of winter drought in annual and seasonal *NEE*; 2) interactions between cork oak phenological events and *NEE*. The dry year 2012 was marked by a 45% decrease in carbon sequestration (-388 vs. - 214 gC m-2 year-1), a 63% reduction in annual tree diameter growth but only a 9% reduction in leaf area index compared to the wet year 2011. A significant reduction of 15% in yearly carbon sequestration was associated with leaf phenological events of canopy renewal. On the contrary to male flower production fruit setting was severely depressed by water stress showing a reduction of 54% during the dry year.

Our results suggest that leaf growth and leaf area maintenance are ecophysiological traits preserved under drought winter and are a sink priority for photoassimilates contrarily to tree diameter growth. Thus, carbon sequestration reductions under low water availabilities in cork oak woodland should be ascribed to stomatal regulation or photosynthetic limitations and in a much less extent to leaf area reductions.

Bryophyte Morphology, Desiccation Tolerance and Ecosystem Water Availability

Cruz de Carvalho R[1,2]*, Marques da Silva J[1,3], Branquinho C[1,2]

1 Faculdade de Ciências (FC), Universidade de Lisboa (UL)
2 Centre for Environmental Biology (CBA)
3 Centre for Biodiversity, Functional and Integrative Genomics (BioFIG) Campo Grande, Edifício C2, 1749-016 Lisbon, Portugal
* e-mail: rfcruz@fc.ul.pt

Bryophytes were amongst the first organisms to explore the terrestrial environment, expanding to different habitats from deserts to full aquatic environments, with different life forms. Through evolution, bryophytes acquired the ability to tolerate extremely dry environments, undergoing desiccation, a process in which tissues virtually lose almost all water and returning to normal function upon rehydration. In dry habitats, bryophytes are organized in dense arrangements, like cushions, which naturally retain more water by capillarity and dehydrate more slowly than arrangements characteristic of species from damp locations, when submitted to the same drying conditions. Morphology, life form and colony structure may be determinant factors in the adaptation of bryophytes to each habitat.

We tested this hypothesis bryophytes from two contrasting habitats (semi-arid and aquatic) and discuss the morphological adaptations as the main evolutionary driver for ecological niche fitness.

La Invasión de Oenothera Drummondii de las Dunas Costeras del Litoral Onubense, Previsiones de un Suceso a Medio Plazo

Zunzunegui M, Ruiz E, Sert M, Díaz-Barradas MC, Fernández JB

Departamento de Biología Vegetal y Ecología, Universidad de Sevilla.
* e-mail: zunzu@us.es

La introducción de especies invasoras puede conducir a un nuevo estado de equilíbrio en la comunidad através de procesos de competencia o facilitación. Las especies exóticas interactúan con las autóctonas, pudiendo cambiar la dinâmica del ecosistema, ocasionando el incremento de unas y la desaparición de otras. No todos los taxones alóctonos suponen el mismo peligro,ni todos los ecossistemas sufren losmismos daños por esta invasión. Por ello es importante conocer la capacidade invasora de las especies.

En este estúdio mediante medidas ecofisiológicas analizaremos el grado de aclimatacion y la capacidad invasora de Oenothera drummondii, espécie nativa de las dunas costeras del Golfo de México que actualmente está invadiendo dunas del Golfo de Cádiz.

Durante un ciclo anual completo, hemos realizados medidas ecofisiológicas con el objeto de analizar la respuesta a las condiciones ambientales de la especie invasora frente a la autóctona Otanthus broteri, ambas de similares características estructurales y de habito de vida. Se seleccionaron 15 individuos de cada especie que crecian próximos unos a otros.

Durante todo el año Oenothera presentó menor eficiencia fotoquímica que la especie autóctona Otanthus. Pero pese a esta menor eficiencia, sus tasas fotosintéticas, excepto en invierno, fueron sempre superiores a las dela especie autóctona. La especie invasora también mostró mejor estado hídrico a lo largo del año, probablemente debido a una mayor eficiência en el uso del agua y mayor desarrollo radical.

Los resultados obtenidos demostraron claramente un alto grado de adecuación de la especie invasora a los ecosistemas dunares mediterráneos.

Relationship between Measurements with Scholander Pressure Chamber and ZIM Probes in Olive

Padilla-Díaz CM*, Rodriguez-Dominguez CM, Díaz-Espejo A, Fernández JE

Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Avenida Reina Mercedes n.º 10, 41012-Sevilla
* e-mail: cmpadilla@irnas.csic.es

Precise irrigation implies accurate monitoring of plant water stress. This can be achieved through the use of Scholander-type pressure chambers. The method, however, is time and labour consuming, hardly suitable for large orchards with high variability. This explains the development of new methods for automatic and continuous measurements of variables related to plant water stress. This is the case of the ZIM probe, a non-invasive tool that operates automatically and continuously under field conditions. The outputs of the probe are inversely related to leaf turgor pressure. The user can see the collected records through any computer connected to the Internet. These advantages confer the ZIM probe a high potential for monitoring water stress in commercial orchards. Previous comparative studies between the Scholander chamber and the ZIM probe showed robust relationships between the outputs of both tools, but with limitations derived from methodological constraints, osmotic adjustment and other aspects. Our aim was to evaluate the ZIM probe versus the Scholander chamber to monitor water stress in a commercial olive orchard, and determine whether the latter can be successfully substituted for the ZIM probe. We are comparing records from measurements with a scholander chamber and ZIM probes in an hedgerow olive orchard with 'Arbequina' trees at 4 m × 1.5 m, all along four irrigation seasons (May to October, 2010-2013). Trees were under three irrigation treatments, a full irrigation (FI) with daily supplies to replace 100% of the crop evapotranspiration (ETc) and two regulated deficit irrigation treatments that received 60% (60RDI) and 30% (30RDI) of ETc. The comparison is currently in progress, so we cannot provide results at this stage. We expect to identify at what extent the relationship between both outputs holds all along the irrigation season, as well as possible limitations derived from both the physiological behaviour of the plant and methodological aspects.

Combining a Process-Based Model of Stomatal Conductance with Leaf Turgor Pressure Related Probe Measurements to Study the Regulation of Plant Water Status and Stomatal Conductance under Drought

Rodriguez-Dominguez CM[1,2], Buckley TN[3], de Cires A[2], Fernández JE[1], Perez-Martin A[1,] Díaz-Espejo A[1]

1 Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain. crdominguez@us.es
2 Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla, Spain
3 IA Watson Grains Research Centre, Faculty of Agriculture and Environment, The University of Sydney, Narrabri, NSW 2390 Australia
* e-mail: crdominguez@us.es

The automatic monitoring of any physiological variable in a changing environment is of high interest in ecophysiological studies. The recently developed plant sensor of relative changes in leaf turgor pressure (leaf patch clamp pressure probe or LPCP probe) was compared with the turgor pressure output simulated with a process-based stomatal model (BMF model). In this study we aimed to gain a better understanding of regulation of plant water use by stomatal conductance and the actual physiological meaning of LPCP probe outputs.

Measurements were carried out in sunny and shaded leaves of adult olive trees in a hedgerow orchard under two irrigation treatments. Our results confirm the good agreement between the simulated turgor pressures and those derived from LPCP readings. But overall, the combined use of the BMF model and LPCP probes raised new insights into the regulation of hydraulic conductivity and osmotic pressure. Both variables emerged as highly dynamic at the seasonal and diurnal scales suggesting an important role in the regulation of stomatal conductance, and hence transpiration and water status, of leaves under drought conditions.

Impact of Estimated Versus Measured Meteorological Data on the Value of Reference Evapotranspiration in Southern Spain

Romero R*, Fernández JE

Instituto de Recursos Naturales y Agrobiología (IRNAS-CSIC), Aptdo. 1052, 41080-Sevilla, Spain.
* e-mail: rafaelromerovicente@gmail.com

Accurate estimations of crop evapotranspiration are crucial for an efficient use of water in agriculture. The most common procedure for computing this variable is the crop coefficient method, which needs the calculation of the reference evapotranspiration (ETo), that can be derived from meteorological data. In this work we evaluated possible errors in the estimation of ETo when one or more of the four main meteorological variables (temperature, solar radiation, relative humidity and wind speed) involved in the calculations are estimated instead of measured. With this aim we analyzed data from 87 automatic weather stations all over Andalusia (from 2011 to 2013). We completed our analysis with a new approach on which we have found no information in the literature. This consisted on an economical evaluation on the impact of using estimated, instead of measured, values of meteorological variables in the calculation of the irrigation needs in southern Spain. Our findings could be used to reduce the number of sensors in meteorological stations used for irrigation scheduling.

Biological Soil Crusts: a Tool for Soil Protection and Water Conservation

Adessi A[1], Cruz de Carvalho R[2,3]*, Rossi F[1], Marques da Silva J[2,4], Branquinho C[2,3]

1 Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 24 - 50144, Florence, Italy.
2 Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Vegetal
3 Centre for Environmental Biology (CBA)
4 Centre for Biodiversity, Functional and Integrative Genomics (BioFIG) Campo Grande, Edifício C2, 1749-016 Lisbon, Portugal
* e-mail: rfcruz@fc.ul.pt

It has been estimated that under cropland conditions, it takes between 200 and 1000 years for 2.5 cm of topsoil to form. Therefore, soil erosion and degradation is a major threat for global food security. Biological Soil Crusts (BSCs) are widely recognized as beneficial to soil fertility due to their contribution to the stabilization of soils and to the increase in their carbon, nitrogen and moisture content, as well as mitigating the effects of desertification. BSCs can play a relevant role in modifying the microenvironment for the growth of small plants, with the eventual effect of stabilizing the soil patches colonized by crusts. Amongst the wide diversity of desiccation tolerance organisms that compose BSCs, cyanobacteria are the first to appear after disturbance events, contribute to the C and N input in the soil and are major extracellular polymeric substances (EPSs) producers. Besides representing a huge carbon source, EPSs give an important contribution to the hydrological behaviour of the crusts. Therefore, using non-invasive techniques (IRGA and chlorophyll fluorescence) to analyse photosynthetic performance, we studied the ecophysiology of the autophototrophic community of BSCs and its response to partial removal of the polymeric matrix. Preliminary results suggest increased susceptibility to photoinhibition and decreased water retention after EPS extraction. Evaluating the role of the EPSs matrix (particularly the polysaccharide fraction) in retaining water and its overall effect in the community is essential to further understand the equilibrium of those systems and how they can prove beneficial for agriculture.

The Authors would like to acknowledge the COST Action “ES1104-Arid Lands Restoration and Combat of Desertification: Setting Up a Drylands and Desert Restoration Hub” for creating the opportunity for cooperation between the University of Lisbon and the University of Florence.

Monitoring Soil Moisture Dynamics in Root Zone System of Argania Spinosa Using Electrical Resistivity Imaging

Ain-Lhout F, Boutaleb S, Diaz-Barradas MC*, Zunzunegui M, Ouchbani A

Dpt. Plant Biology and Ecology. University of Seville
* e-mail: diaz@us.es

Argania spinosa is an endemic tree of the Southwest of Morocco, occupying 828,000 Ha, distributed mainly between Mediterranean vegetation and the desert. This tree can grow in extremely arid regions in Morocco, where annual rainfall ranges between 100-300 mm where no other tree species can live. It has been designated as an UNESCO Biosphere Reserve since 1998. Argania tree represents an important resource in human economy and animal feeding of rural population as well as for oil production, it is considered as a multi-usage tree. Admine forest located in the suburbs of Agadir city, 5 km inland, was selected to conduct this work.

The aim of the study was to investigate the temporal changes in root-zone moisture dynamic in response to variation in climatic conditions and vegetation water uptake, using a geophysical technique called Electrical resistivity imaging (ERI). This technique discriminates resistive woody roots, dry and moisture soil. Time-dependent measurements (from April till July) of resistivity sections were performed along the surface transect (94m Length) at 2 m fixed electrode spacing. Transect included eight Argan trees.

The interactions between the tree and soil moisture were estimated by following the tree water status variations accompanying the soil moisture deficit. For that purpose we measured midday leaf water potential and relative water content during each sampling day, and for the eight trees.

The first results showed that ERI can be used to accurately quantify the spatiotemporal distribution of root-zone moisture content and woody root. The section obtained shows three different layers: middle conductive one (moistured); a moderately resistive layer corresponding to relatively dry soil (calcareous formation with intercalation of marly strata) on top, this layer is interspersed by very resistant layer corresponding to woody roots. Below the conductive layer, we find the moderately resistive layer. We note that throughout the experiment, there was a continuous decrease in soil moisture at the different layers. With the ERI, we can clearly estimate the depth of the woody roots, which does not exceed 4 meters. In previous work on the same species, analyzingthe δ18O in water of xylem and in the range of possible water sources, we argued that rain is the main water source in winter and spring, but not in summer. Trees are notexploiting deep water from the aquifer as the popular assessment; instead of this they are using soil water at few meters depth. The results of the present work confirm the idea that the Argania spinosa is not a deep root species.

POSTERS

RNASeq Analysis of the Quercus Suber Root Response to Drought

Azevedo H[†,2], Magalhães A[†,1], Verde N[1], Martins I[1], Castro PH[1], Lino-Neto T[1], Tavares RM[1]

1 BioFIG, Center for Biodiversity, Functional & Integrative Genomics, Plant Functional Biology Center, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal;
2 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
† - co-authorship
* e-mail: tavares@bio.uminho.pt; hazevedo@cibio.up.pt

Cork oak (Quercus suber L.) is a typical species from the Portuguese Mediterranean forest, usually disseminated in dry environments. Mainly due to its high economic value, cork oak has been considered a protected plant species and a national species of interest. Climate alterations, in particular, increased temperature, high light intensity, drought and air pollution are factors that directly affect the development of plants and reduce their productivity. In the present work, the effect of soil water content was studied in Q. suber seedlings, as two-month-old plants were subject to different water stress regimes to impose drought stress. Photosynthetic activity measured by PAM fluorometry and photosynthetic pigment content were used as physiological indicators of stress. Subsequently, the transcriptome of Q. suber roots in response to moderate and severe drought stress was analyzed by Next Generation Sequencing using 454 (Roche) technology, with a total of over 1.8 million reads. Data was assembled into 21012 unigenes, and reads were subsequently mapped to the assembly, in order to identify differential expression. This allowed us to recognize 546 differentially expressed genes (353 up-regulated and 193 down-regulated). These genes were subjected to subsequent in silico analysis, starting with their functional annotation and assembly into functional categories. Differentially expressed genes include a significant number of effector proteins traditionally associated to drought responses, such as dehydrins and LEA proteins, several transcription factors and ubiquitin-associated genes are also present, suggesting a tight control of drought responses at both the transcriptional and protein turnover levels.

This work is funded by FEDER through the Operational Competitiveness Program - COMPETE - and by national funds through the Foundation for Science and Technology - FCT - in the scope of projects SOBREIRO/0033/2009 “Cork oak ESTs Consortium – Abiotic stress: drought, salt and oxidative stresses” and PTDC/AGR-GPL/118505/2010 “An integrated approach to identify stress-related regulatory genes in cork oak (SuberStress)”

SUMO Proteases Act as Modulators of *Arabidopsis Thaliana* Development and Drought/ABA Signaling

Castro PH[1,3], Couto D[1], Freitas S[1], Verde N[1], Ruiz-Albert J[3], Bejarano ER[3], Tavares RM[1], Azevedo H[2]

1 BioFIG, Center for Biodiversity, Functional & Integrative Genomics, Plant Functional Biology Center, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal;
2 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
3 Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga-Consejo Superior de Investigaciones Científicas, Departamento de Biología Celular y Genética, Universidad de Málaga, Campus de Teatinos, E-29071 Málaga, Spain
* e-mail: pedro1berto@bio.uminho.pt; hazevedo@cibio.up.pt

Water availability controls plant growth and, consequently, crop production. Unravelling mechanisms of plant tolerance to drought is one of the main research subjects of current plant biology. An appropriate response to environmental fluctuations needs to re-programme plant metabolism, physiology and morphology. Molecularly, many of these strategies rely on the quick and reversible post-translational modification (PTM) of key proteins. Sumoylation is a PTM performed by a small peptide called SUMO. Sumoylation is not only important for plant development but also the response to stress, including drought conditions. SUMO can control target protein activity by altering its conformation, creating or blocking interacting interfaces. SUMO peptides are first processed by SUMO proteases and then conjugated to a target protein via an E1 activation, E2 conjugation and E3 ligation cascade. The attached peptide can then be removed by action of SUMO proteases. In Arabidopsis, the main SUMO proteases belong to the ULP family and are essential regulators of the SUMO cycle.

Important advances in plant abiotic stress research have been possible due to forward and reverse genetic strategies using loss- and gain-of-function mutants of the model plant *Arabidopsis thaliana*. In the present study we show that, in Arabidopsis, a pair of ULP proteases functioned redundantly to regulate several developmental aspects. Determining their spatial expression pattern using GUS report assays proved their ubiquitous expression during plant development. A microarray analysis of differentially expressed genes in the double T-DNA mutant was carried out, suggesting a deregulation of drought and ABA-related genes. Taking this into consideration, a characterization of related phenotypes was performed in the mutant lines, leading us to conclude that ULPs control stomatal aperture and are modulators of the plant drought response.

This work is funded by FEDER through the Operational Competitiveness Program - COMPETE - and by national funds through the Foundation for Science and Technology - FCT - within the scope of project “SUMOdulator” (Refs. FCOMP-01-0124-FEDER-028459 and PTDC/BIA-PLA/3850/2012).

SUMO Proteases Control Development and Stress Responses in *Arabidopsis Thaliana*

Castro PH[1,3], Santos MA[1], Freitas S[1], Ruiz-Albert J[3], Tavares RM[1], Bejarano ER[3], Azevedo H[2]

1 BioFIG, Center for Biodiversity, Functional & Integrative Genomics, Plant Functional Biology Center, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal;
2 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.
3 Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga-Consejo Superior de Investigaciones Científicas, Departamento de Biología Celular y Genética, Universidad de Málaga, Campus de Teatinos, E-29071 Málaga, Spain
* e-mail: pedro1berto@bio.uminho.pt; hazevedo@cibio.up.pt

Plants are subjected to repeatedly changing environments that compromise survival. Several strategies were adopted to tolerate stress conditions including post-translational modifications (PTMs), which act as fast and reversible regulators of key proteins. Relevant PTMs includes small peptides such as ubiquitin and ubiquitin-like modifiers, namely the Small Ubiquitin-like Modifier (SUMO). SUMO may exert different effects depending on the target protein, either controlling its conformation, or even creating or blocking interacting interfaces that ultimately modulate its activity. SUMO attachment (sumoylation) requires SUMO peptides to first be processed by SUMO proteases (ULPs), and then conjugated to a target's lysine via SUMO E1 activase and SUMO E2 conjugase, aided by SUMO E3 ligases. Deconjugation of the SUMO peptide can subsequently be carried out by the SUMO proteases. A great number of SUMO targets are involved in nuclear-related functions, and are part of essential cellular processes. This is particularly evident in plants since loss-of-function mutants of most SUMO components display embryonic lethality or pleiotropic defects. SUMO-conjugation levels vary in respect to growth conditions, tissues and developmental stages. It is therefore expected that highly dynamic SUMO conjugation and deconjugation machinery is at play. Unlike ubiquitin, SUMO conjugation machinery components are less abundant in the plant genome. SUMO proteases (ULPs) constitute a fairly large gene family and are the potential sources of specificity within the pathway by displaying different enzymatic activity, subcellular localization and expression levels. We have characterized the developmental and environmental stress responses of previously uncharacterized Arabidopsis T-DNA insertion mutants disrupting two functionally redundant ULPs, resulting in diverse developmental defects and constitutively increased SUMO-conjugate levels. Since SUMO is important for plant stress responses, we tested whether the ULP mutants displayed new phenotypes in response to several abiotic stresses, including decreased water availability, as well several hormones associated to environmental stimuli.

This work is funded by FEDER through the Operational Competitiveness Program - COMPETE - and by national funds through the Foundation for Science and Technology - FCT - within the scope of project "SUMOdulator" (Refs. FCOMP-01-0124-FEDER-028459 and PTDC/BIA-PLA/3850/2012).

Metabolites Modulate Acclimation in Radiata Pine

Diego N[1], Saiz-Fernández[2], Pérez-López U[3], Muñoz-Rueda A[3], Moncalean P[4], Lacuesta M[2]*.

1 Centre of the Region Hana´ for Biotechnological and Agricultural Research, Palacký University, Czech Republic.
2, 3 Department of Plant Biology and Ecology, 2Faculty of Pharmacy, 3Faculty of Science and Technology. University of Basque Country UPV/EHU Spain.
4 Department of Plant Production and Protection, NEIKER-Tecnalia. Spain.
* e-mail: maite.lacuesta@ehu.es

Metabolic and physiological bases of plant tolerance and hardening to drought are essential to improve forest seedling survival, especially in productive species such as Pinus radiata. It is known that the exposure to different cycles and intensities of stress before transplanting plants to the forest improves their conditioning. For this reason, six different breeds with varied water stress tolerance were analysed during two consecutive drought cycles and their response was compared. Tolerance and drought hardening were the result of a combined action of metabolite changes and phytohormone signalling. Hexoses such as D-glucose and D-fructose played a main role in improving osmotic response and, together with □-aminobutyric acid (GABA), contributed to alleviate the effect of the reduction in carbon assimilation (photosynthesis inhibition). An increase of some amino acids could also improve the survival under drought, highlighting L-proline and GABA as a protector molecules that mitigate the negative stress consequences, and L-glutamic acid as their precursor. Regarding polyamines, high initial levels of spermidine and its conversion to spermine benefit the maintenance of plant development and tolerance under stress.

Acknowledges: This research was supported by MEC-INIA and Fondo Europeo de Desarollo Regional (FEDER) (grant RTA2010-00041-CO2-02), Grupo GV-IT326-10, Departamento de Agricultura y Pesca-Gobierno Vasco (VEC2007012, 72.0093.0), MSMT (CZ.1.07/2.3.00/30.0004). Iñigo Saiz Fernández has a fellowship from the UPV/EHU.

Phytohormone Profile of Selected Provenances of *Pinus Pinaster* Aiton.

Delatorre C*, Granda V, Dantas AK, Meijón M, Majada JP, Feito I, Rodríguez A.

Departamento de Biología de Organismos y Sistemas, Facultad de Biología, Universidad de Oviedo, C/ Catedrático Rodrigo Uría s/n, 33071, Oviedo, Asturias, España.
* e-mail: delatorre85@gmail.com

Pinus pinaster is a forestry species with great economic importance and one of the main species found in Spain, mainly due to its facility to adapt to different ecological conditions. But its adaptation ability is considered to be genotype dependent. Characterization and analysis of the various patterns of adaptation in the early growth stages of different genotypes is essential to determine which individuals are the most suitable for a specific environment.

In previous studies, it has been observed that certain traits, such as growth parameters, metabolomic profile or phenological and morphological traits, enable the arid or non-arid origin of the provenances to be discerned. In three selected provenances of *Pinus pinaster* from contrast climate conditions ecophysiological parameters have been analyzed and it was found that the differences among them were lower than expected.

Phytohormones play a crucial role in plant development and control biotic and abiotic plant responses. While they are structurally diverse compounds, each one of them having characteristic biological effects, their action mechanisms is interconnected through cross-talk, such that they can mediate synergic or antagonistic responses. Thus, monitoring the balance of several phytohormones in different plant developmental stages, as well as in different plant materials, is clearly of great importance.

The objective of this work was to study the phytohormone profile during two years of growth in five clones of three contrast provenances of *P pinaster* in order to get insight into the reasons behind phenotypic diversity. It was thus necessary to develop an analytical method for quantifying the greater number of plant growth regulators with the least plant material as possible. The method of analysis obtained was fast, sensitive and accurate enough to extract different principal classes of phytohormones from plant needles and quantify them. A total number of 19 compounds from several groups of phytohormones (comprising three stress related hormones, ABA, SA, JA; one auxin, AIA; seven cytokinins, DHZ, DHRZ, tZ, tRZ, iP, iPR, BA; five gibberellins, GA1, GA3, GA4, GA7, GA9; and three brassinosteroids, HBl, 24HB, BK) was quantified.

Although some differences in global phytohormone content between provenances were found, the results obtained showed that differences between provenances are not so large, at least during the juvenile developmental stages. The major differences in content levels of some of the compounds analyzed, for example gibberellins content, were found between seasons when all materials also exhibited the greatest changes in relation to water potential and gas exchange parameters, which furthermore coincides with the greatest differences in the ecophysiological parameters assessed.

Acknowledgments: Project funding by RTA-2010-00120-C02-00.

How Grape Berry Dehydrins Mrna and Protein Expression Profiles Relate with Deficit Irrigation?

Genebra T[1], Pinheiro C[1,2]*, Chaves MM[1], Zarrouk O[1]

1 Instituto de Tecnologia Química e Biológica (ITQB); Plant Molecular Ecophysiology Lab. Av. República, Estação Agronómica Nacional. 2780-901 Oeiras-Portugal
2 Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
* e-mail: pinheirotqb.unl.pt

Dehydrins (DHNs) are belonging to the late-embryogenesis abundant (LEA) family and they were proposed as protecting of plant cells from environmental stresses. It has been assumed that DHNs act as stabilizers for other proteins and/or membranes due to hydrophobic associations but their functions are not fully elucidated. Although the presence of DHNs in woody plants is infrequent, in grapevine (*Vitis vinifera* L.), DHNs have been reported to be major mesocarp proteins of mature berries. While their expression coincide with changes in the osmotic stress in mesocarp tissue resulted from hexose accumulation, their mechanism remains only elusive. More recently, four dehydrin proteins and four genes were identified in grapevine and their expression under abiotic stress was studied in leaves. To investigate DHNs involvement in the berry ripening and their response to water availability, we studied tissue specific protein accumulation and gene expression of the different DHNs in three different tissues of berries (pulp, skin and seeds) along berry development and under three different irrigation systems. Results showed that the four *VvDHNs* were expressed in the different berry tissues and analysis by qRT-PCR showed that the expression pattern of the different *VvDHNs* was modulated by ripening process and water availability. Immuno-blots, SDS and 2D PAGE profiles were used to qualitatively and quantitatively assess dehydrin expression. At the proteome level, DHNs were not detected in skin suggesting a post-transcriptional regulation in this tissue. At seed and pulp levels, DHN proteins respond both to the phenological stage and the irrigation treatment. The clear differences of DHNs expression profile among the different studied tissues suggesting a functional diversification within the grapevine DHN family.

Acknowledgement: European Community's Seventh Framework Program (FP7/2007-2013) under the grant agreement nº FP7-311775, Project Innovine

Growth Responses and Water Status of Transgenic Tobacco Harboring the Dehydrin Gene of *Vitis Vinifera* L. Under Water Deficit

Jamoussi RJ[1], Zarrouk O[2], Salem AB[1], Mejri S[1], Khiari B[1], Ghorbel A[1], Chaves MM[2], Mliki A[1], Pinheiro C[2,3*]

1 Laboratory of Plant Molecular Physiology, Biotechnology Center of Borj Cedia, BP901, 2050 Hammam-Lif, Tunisia
2 Instituto de Tecnologia Química e Biológica (ITQB); Plant Molecular Ecophysiology Lab. Av. República, Estação Agronómica Nacional. 2780-901 Oeiras-Portugal
3 Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
* e-mail: pinheirotqb.unl.pt

Drought is one of the most serious environmental constraints that limit grapevine productivity. In the framework of a drought genetic improvement program in grapevine, the dehydrin gene (VvDHN1) (accession N° AY634281) was identified as relevant in stress adaptation. The open reading frame (410 bp length), encodes for a YSK2 dehydrin, with 124 amino acid residues (predicted molecular weight of 13230.4 Da and isoelectric point (pI) of 9.04). To investigate the role of the VvDHN1 in drought tolerance enhancement, the candidate gene was transformed into tobacco under the 35S promoter control. Three transgenic tobacco lines were then assessed under controlled greenhouse conditions for their tolerance to drought by withholding water during 6, 12 and 18 days, followed by recovery (until one week). The effect of the insertion of the VvDHN in transgenic tobacco performance was monitored by comparing leaf, shoot and root biomass. The water relations were also examined through relative water content, osmotic potential and photosynthetic parameters (photosynthetic rate and stomatal conductance). The results revealed that transgenic tobacco plants exhibit a better performance under drought (water status, photosynthetic capacity and biomass production). The physiological evaluation of the VvDHN transgenic expression effect coping with drought would offer an effective candidate gene that may have potential usa in the genetic improvement of drought tolerance in grapevine.

Acknowledgements: Portuguese-Tunisian Joint Research Programme (2013)

Identification of an ABA-Dependent Signaling Cascade in *Quercus Suber* Roots in Response to Drought

Magalhães A[†,1], Verde N[†,1], Martins I[1], Lino-Neto T[1], Tavares RM[1], Castro PH[1], Azevedo H[2]

1 BioFIG, Center for Biodiversity, Functional & Integrative Genomics, Plant Functional Biology Center, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal;
2 CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.† - co-authorship
* e-mail: pedro1berto@bio.uminho.pt; hazevedo@cibio.up.pt

The Mediterranean forest is a genuine heritage of southern European countries. Cork oak (Quercus suber L.) is a typical species from Portuguese Mediterranean forests, usually growing in dry environments. Mainly due to its high economic value, cork oak has been considered a national species-of-interest. Climate changes pose serious threats to cork oak distribution, particularly with regards to heat and water availability issues. To address this matter we performed differential transcriptomic analysis in roots of young Quercus suber plantlets subjected to moderate and extensive drought stress conditions, as well as control (well-irrigated) plants. Analysis was carried out by 454 Next Generation Sequencing and resulted in the identification of 353 up-regulated and 193 down-regulated genes. In the present work we report subsequent in silico analysis of the differential root transcriptome of Quercus suber. Genes were annotated against the complete nucleotide dataset at NCBI, but also against the genome of the model plant Arabidopsis thaliana, allowing for the identification of orthologs in this species. This information was used to establish networks of GO functional assignment, gene co-expression, and known protein-protein interactions. We also analyzed orthologs for cis-element enrichment in their promoters. We thus established functional relationships between differentially expressed genes, ultimately identifying the presence, in Quercus suber roots, of an ABA-dependent signaling cascade. Elements of this signaling cascade range from ABA-sensing components to transcription factors and then to effector genes involved in the drought response.

This work is funded by FEDER through the Operational Competitiveness Program - COMPETE - and by national funds through the Foundation for Science and Technology - FCT - in the scope of projects SOBREIRO/0033/2009 “Cork oak ESTs Consortium – Abiotic stress: drought, salt and oxidative stresses” and PTDC/AGR-GPL/118505/2010 “An integrated approach to identify stress-related regulatory genes in cork oak (SuberStress)”.

Drought Induced Changes in Membrane Fatty Acids and Physiological Responses of Arabidopsis Plants Altered in the Expression of the Phospholipase A Gene *pplaiiα*

Marques da Silva J, Bernardes da Silva A, Ribeiro AS, Silvestre S, Arrabaça JD, Matos AR*

University of Lisbon, Faculty of Science, Department of Plant Biology and Center for Biodiversity, Functional and Integrative Genomics, Campo Grande, 1749-016 Lisbon, Portugal
* e-mail: armatos@fc.ul.pt

Drought is an abiotic stress with great impact on plant growth and productivity. Cell membranes are one of the targets of drought, and early changes in their lipid composition can contribute to tolerance, whereas in latter stages mainly reflect damage. Several lipid metabolizing enzymes have been identified in plants, however little is known concerning those involved in drought-induced membrane changes occurring in response to drought. We have identified a drought-induced patatin-like gene in Arabidopsis, *pPLAIIα*, encoding an enzyme that releases fatty acids from membrane lipids. Here we have used plants overexpressing (OE) or silenced (AS) for *pPLAIIα*, to investigate the role of this enzyme in total fatty acid composition under control and drought conditions. To evaluate the physiological impact of membrane lipids changes we calculated photochemical parameters based on OJIP fast fluorescence kinetics. Results show that well watered plant leaves from the three lines display very similar fatty acid composition, except for a higher content of oleic acid (C18:1) in OE. In response to progressive drought OE and wild-type plants are able to increase the content of linolenic acid (C18:3), whereas AS plants do not display a significant 18:3 increase. Linolenic acid is the major polyunsaturated fatty acid present in leaves, with a fundamental role in maintaining membrane fluidity. Decreases in C18:3 contents are generally associated to membrane damage, while increases might be related to stress tolerance. The photochemical performance also indicates that AS plants are more affected by drought, namely the maximum quantum efficiency of photosystem II (Fv/Fm) and the performance index (PIABS). The specific energy fluxes per reaction center particularly show limited electron transport (ETo/RC), without changes in absorption (ABS/RC) and trapping (TRo/RC). In conclusion our results indicate that pPLAIIα impacts membrane fatty acid composition and contributes to drought tolerance. Analysis of the different lipid classes will contribute to further investigate the changes observed in total fatty acids.

Contribution of Multiple Alternative Oxidase Isoforms to Arabidopsis Leaf Respiration Under Progressive Drought

Matos AR*, Pinxteren D, Bernardes da Silva A, Coito JL, Marques da Silva J, Casimiro A, Arrabaça MC, Arrabaça JD

4BIOFIG – Centro para a Biodiversidade, Genómica Integrativa e Funcional, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal 1749-016
* e-mail: armatos@fc.ul.pt

Plant mitochondria have an alternative oxidase (AOX), which accepts electrons from ubiquinol, lowering ATP yield. AOX forms with alternative NADH dehydrogenases (ND) alternative respiratory chains, preventing the over-reduction of the electron transport chain. We have investigated the contribution of AOX to leaf respiration using Arabidopsis plants expressing AOX1a in antisense (AS-AOX1a). In response to, imposed by withholding irrigation in soil-grown plants, an up-regulation of AOX capacity was observed in WT and, to a lower extent, in AS-AOX1a. In contrast, total respiration, measured as O2 consumption or CO2 efflux rates, remained unchanged under water deficit, although control and stressed ASAOX1a plants released less CO2. Besides *AOX1a*, also *AOX1c*, *AOX1d* and *AOX2*, were upregulated by drought in both genotypes. The external *NDB2* and the internal *NDA2* shared similar expression profiles with *AOX1a*. AS-AOX1a had higher biomass under control conditions but a more significant reduction of leaf fresh weight upon drought. Control ASAOX1a had lower proline content but an increase with drought was seen for both lines. The inverse pattern was observed for starch.

Our results indicate that although total respiration remains unchanged under water deficit, there is an increase in AOX capacity and transcripts levels. Several AOX isoforms seem to be involved in the drought response but the decreased *AOX1a* expression in AS-AOX1a impacts plant metabolism and growth and negatively affects drought tolerance.

.Membrane Tolerance in Coffea Species in Response to Environmental Constraints

Scotti-Campos P [1]*, Pais IP [1], Fortunato AS [2], Lidon FC [3], Ramalho JC [2]

1 Unidade de Investigação em Biotecnologia e Recursos Genéticos, Inst. Nac. Inv. Agrária e Veterinária, I.P., Qta do Marquês, Av. República, 2784-505 Oeiras, Portugal.
2 Grupo Interações Planta-Ambiente, Centro Ambiente, Agricultura e Desenvolvimento, Inst. Inv. Científica Tropical, I.P., Qta do Marquês, Av. República, 2784-505 Oeiras, Portugal.
3 Cicege, DCT, Fac. Ciências e Tecnologia, UNL, Qta da Torre, 2829-516 Caparica, Portugal.
* e-mail: paula.scotti@iniav.pt

Coffee is a tropical crop that constitutes the social and economic basis of many tropical developing countries, as it is one of the most agricultural traded commodities worldwide. In the context of climate changes and global warming, low water availability episodes are expected to occur more often, strongly affecting plant physiology and yield. Cellular membranes are main targets of environmental constraints, playing a major role in plant stress acclimation, when able to undergo biophysical and biochemical modifications. The aim of this work is to evaluate drought responses of 2 Coffea genotypes, representing agronomic valuable species (C. arabica L. cv. Icatu and C. canephora Pierre Ex A. Froehner cv. Apoatã), as regards photosynthetic capacity (Amax), membrane cellular damage (MDA) and changes in chloroplast lipid composition (Total Fatty Acids, TFA; Double Bond Index, DBI).

Drought was induced in potted plants by gradually reducing irrigation, until three treatments were established: well irrigated (Ctr), moderate drought (MD) and severe drought (SD), registering midday relative water contents (RWC) close to 86, 76 and 69% MPa, respectively. These values were kept for 2 weeks before measurements were carried out. Under MD, the potential photosynthetic activity (Amax) was affected (by 21%) only in Apoatã, whereas under SD both genotypes were significantly affected (25-30%), still reflecting a high potential. Furthermore, 3 days after rewatering only Icatu presented a total Amax recovery.

Well watered plants presented similar TFA amounts. Yet, opposite responses were observed under drought. TFA reductions were observed under MD (13%) and SD (40%) in Apoatã, whereas increases occurred in Icatu, mainly under MD (58%). DBI remained unaltered in Apoatã, while it increased in Icatu, linked to changes in major FAs (decreases in C16:0 and C18:0, and increases in C18:3). Also, MDA values increased 150% (Apoatã) and 58% (Icatu) under MD conditions. Under SD, MDA further rose (57%) in Apoatã but not in Icatu, suggesting a higher membrane preservation that might have contributed to the quicker photosynthetic recovery upon rewatering.

Acknowledgments: This work was supported by Portuguese national funds through Fundação para a Ciência e Tecnologia, under the scope of the project PTDC/AGRPRO/ 3386/2012 and the grant SFRH/BPD/47563/2008 (A.S. Fortunato), the latter cofinanced through the POPH program subsidized by the European Social Fund.

Physiological Evaluation of Drought Tolerance in *Triticum Durum* Genetic Resources

Scotti-Campos P[1]*, Semedo JN[1], Pais IP[1], Oliveira MM[1], Passarinho JA[1], Santos M[1], Ramalho JC[2], Almeida AS[1], Costa AR[1], Pinheiro N[1], Coutinho J[1], Maçãs B[1].

1 Unidade de Investigação em Biotecnologia e Recursos Genéticos, Inst. Nac. Inv. Agrária e Veterinária, I.P., Qta Marquês, 2784-505 Oeiras / Apartado 6, 7350-591 Elvas, Portugal.
2 Grupo Interações Planta-Ambiente & Biodiversidade, Centro Ambiente, Agricultura e Desenvolvimento, Inst. Inv. Cient. Tropical, I.P., Qta Marquês, 2784-505 Oeiras, Portugal.
* e-mail: paula.scotti@iniav.pt

Drought is a major cause for yield reduction of many important food crops, such as wheat. Under Mediterranean conditions, climate changes associated to decreasing water availability urge to implement adaptation strategies for agriculture, and germplasm characterization is needed to assist Portuguese wheat breeding programs. The aim of this work was to identify traits that may indicate an adaptation to drought during grain filling period. Durum wheat (Triticum durum L.) varieties were selected from different evolutive and/or breeding groups: traditional varieties (Castiço, Cocorit), modern currently used varieties (Celta) and advanced lines resulting from recent breeding work (Gediz, Minimus, Ajaia and Arment). Seeds were sown in 60 L containers, filled with clay loam soil collected from the field and maintained in a greenhouse, under environmental semi-controlled conditions (mean daily values: temperature 23-26ºC; relative humidity 50-60%). Drought was imposed by withholding irrigation for ten days, after visual assessment of anthesis (ca. 89 DAS). According to water potential (Ψw) values, well irrigated (Ψw ≈ -0.65 MPa) and droughted (Ψw ≈ -1.3 MPa) plants were compared concerning gas exchanges, instantaneous water use efficiency (iWUE) and membrane integrity. Plant water status was also evaluated through relative water content (RWC) and osmotic potential (Ψs). At anthesis, drought reduced net photosynthesis (Pn) in all varieties, and chlorophyll values decreased in Gediz. Stomatal conductance (gs) was particularly reduced in advanced lines, and remained stable in Castiço. Under drought Cocorit presented the highest iWUE and unaltered membrane integrity, the latter also observed in Celta and Arment. To evaluate yield components, plants were kept under control or drought conditions until harvest. Grain yield (g/m2) decreased in droughted plants of all varieties, but 1000 grain weight was maintained. Implications of water regime in physiological responses and yield will be discussed focusing genotypes adaptability to dry environments.

Acknowledgments: This work was funded by the Portuguese PRODER program, Med. 2.2.3.1. Genetic Resources, PA 18998.

Differences among Grapevine Cultivars in their Water Use Strategy Under Progressive Water Stress

Bota J.*, Tomás M.*, Medrano H, Flexas J. and Escalona JM

Research Group on Plant Biology. Balearic Islands University. Ctra Valldemossa Km 7,5. 07122. Palma de Mallorca. Spain
*These authors contributed equally to this study
* e-mail: j.bota@uib.es

Soil water deficit is the main environmental constraint for viticulture in Mediterranean regions. In this context, evaluation of different mechanisms adopted by grapevine cultivars to deal with drought periods is of major importance. On the other hand, the choice of the plant material is a key issue for crop adaptation to the future viticulture. In this report a large number of cultivars (23), including local and foreign grape varieties, are studied under field conditions in order to identify the different behaviours in response to water deficit and how it can affect plant water use efficiency. stem was used to assess plant water status. Although these cultivars share a common environment, a high variability was found in photosynthetic parameters and stomatal response. The results demonstrate that a better stomatal control allows increasing WUEi. This kind of behaviour can be mostly found in the local cultivars demonstrating that some old local varieties can be good candidates for the actual and future viticulture.

Application of Thermal Imaging to Assess the Physiological Status of Papaya Crop (Carica papaya L.) Under Different Deficit Irrigation Regimes

Campostrini E*[1], Lima RSN[1], García-Tejero I[2], Costa JM[3], Lopes TS[1], Vaz M[4], Chaves MM[3]

1 Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brasil.
2 IFAPA-Centro 'Las Torres-Tomejil'. Ctra. Sevilla-Cazalla Km. 12,2. 41200. Alcalá del Río,Sevilla, Espanha.
3 Plant Molecular Ecophysiology Laboratory (LEM), Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República – EAN, 2780-157, Oeiras, Portugal.
4 Departamento de Biologia Instituto de Ciências Agrárias e Ambientais Mediterrânicas ICAAM, Universidade de Évora, Portugal
* e-mail: campostenator@gmail.com

Papaya is one of the most important crops in Brazil, with an annual yield of 1.9 t and a total surface close to 35,000 ha. Although papaya is generally considered to be drought sensitive, it is necessary to define the best irrigation deficit strategies able to keep a sustainable crop water status and hence marketable yields. In this line there are many tools to control the physiological status of a crop when this is subjected to water stress. Thermography emerges as a remote and non-destructive option tool to assess the crop water status in many crops and even support the irrigation scheduling under limiting irrigation conditions. However, to optimize the use of thermal information (leaf T or thermal indexes) in field conditions, it is in many cases necessary to the define the main relationship with other more studied physiological variables such as stomatal conductance (gs) or net photosynthesis (An).

The aim of this work was to study the physiological response of young plants of Papaya subjected to different deficit irrigation regimes. The trial was conducted in a greenhouse, in the Northern Rio de Janeiro State University from February, Brazil, to April of 2012. Four irrigation treatments were defined: i) a full irrigated treatment (Control), keeping the soil at field capacity; ii) a partial root-zone drying (PRD), which received a 50% of total water applied in Control to only one side of the root system, alternating sides each 7 days; iii) a deficit irrigation (DI), in which was applied the 50% of Control, in both sides of plant; iiii) and a nonirrigated treatment (NI). Seven days before of the end of the experience, NI treatment was recovery, being supplied the same irrigation water than Control for NI. Periodical measurements of gs, An, and transpiration (E) were taken every two days. Additionally, canopy temperature was monitored at the beginning, at the end and in the middle of experience, and at the end corresponding to the moment of maximum stress, using a Flir ThermaCam i50 (Flir Sys AB, Sw).

Significant relationships were found between canopy temperature (Tc) and the studied physiological variables, defining the threshold values of Tc according to the found functions. Taking into account these results, it can be concluded that Tc and hence, thermal imaging could be a promising technique to monitor the physiological status in this crop.

Influence of Soil and Irrigation Management on the Quality of Seedless Crimson Table Grapes.

Coelho R[1]*, Agulheiro C[1], Shahidian S[1], Vaz M[1], Rato A[1], Vieira F[2], Valverde P[2].

1 ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal
2 Universidade de Évora, 7002-554 Évora, Portugal
* e-mail: rcoelho@uevora.pt

The market for table grapes is moving into mass production of specialty seed-less grapes in covered areas, aiming at obtaining premium prices with early or late production of high quality products. Production of quality seedless grapes is not straightforward since it is requires the correct combination of various independent characteristics, such as color, sugars, size and quantity at the right moment for successful harvesting and marketing. The present study was carried out at the two largest Portuguese producers located in Alentejo, and has the objective of studying the effect of irrigation management strategies and two different soils on the various relevant parameters for successful production and marketing. The management strategies were the application of ten day stress at the end of the cycle, in order to promote early maturing of the grapes.

Three different timings of the stress were applied. Soil moisture, sap flow, bark thickness, as well as leaf water potential, stomatal conductance and chlorophyll content were measured regularly during the production season. The results indicate that the roots explore a rather large soil volume and the plants can successfully withstand reasonable periods of drought without significant changes to the plant physiology. Additionally late rains can mask the effect of any farmer applied drought and invalidate any farmer induced stress to the plants. Water-logged soils tend to cause early onset of maturity, but cause the ripening stage to extend over a longer period of time, and thus, in effect result in a delay in the harvest date. Topography also has some effect on the ripening, since hot air tends to accumulate under the plastic at the higher areas of the field.

This work is funded by PRODER, 4.1, within the scope of project MORECRIMSON

Response to salinity in young olive trees of three Iberian varieties

Coelho R[1]*, Sousa A[1], Rato A[1], Vaz M[1]

[1] ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal
* rcoelho@uevora.pt

Soil salinity is becoming a major issue due to the decrease in good quality irrigation water. This paper reports a research on tolerance to salinity in two year-old olive trees (*Olea europaea* L.) of three Iberian varieties: Arbequina, Cobrançosa and Galega Vulgar.

Plants were grown in 10 L plastic pots containing approximately 9 Kg of a sandy granitic soil, on a greenhouse. Plants were subject to three levels of salinity in the irrigation water, 0 mM, 80 mM or 200 mM NaCl (6 plants per salinity level in a total of 18 plants of each variety). After 3 months, several parameters were assessed, of the soil, water content and salinity, and on the leaves, stomatal conductance (g_s), mid-day water potential (□), percentage of water, relative water content, specific leaf area (SLA), chlorophyll content and hyperspectral leaf signature. Soil electric conductivity and soil water content were significantly higher on salt-irrigated pots. Salt irrigation decreased significantly stomatal conductance (g_s). Plants of Cobrançosa showed in general higher g_s then the two other varieties. Mid-day water potential (Ψ) was significantly lower on salt-irrigated plants but simultaneously slightly higher Cobrançosa. Percentage of water, relative water content and specific leaf area were not greatly affected by salt irrigation during this three month exposure to salt, neither was the chlorophyll content of leaves.

In general, hyperspectral reflectance indexes did not show good correlations with salt irrigation, except for the Photochemical Reflectance Index (PRI) which was clearly lower on plants of all three varieties irrigated with salt. Frequently, plants of Cobrançosa showed vegetation indexes different from the other two varieties, higher Moisture Stress Index (MSI), Normalized Phaeophytinization Index (NPQI), Normalized Difference Vegetation Index (NDVI) and Normalized pigment chlorophyll ratio index (NPCI), but lower Normalized Difference Infrared Index (NDII), Water Index (WI), Structure Insensitive Pigment Index (SIPI), Carotenoid Reflectance Index (CRI_2), Anthocyanin Reflectance Index (ARI_2) and Plant Senescence Reflectance Index (PSRI).

The differences between Cobrançosa and the other two varieties are discussed.

Testing four methods to assess leaf area in young olive trees

Coelho R[1]* , Dambiro J[2], Carlos R[2], Pirata M[2], Campos T[2], Queirós R[2], Carrilho J[2], Barreto S[2], Tufik C[2], Mestre T[2]

1 ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal
2 Master students 2013-14, Universidade de Évora, 7002-554 Évora, Portugal
* rcoelho@uevora.pt

Assessment of leaf area is of fundamental importance to calculate diverse physiological parameters such as the total rate of transpiration or photosynthesis of a plant or its leaf area index.

This paper reports the determination of total leaf area in 3 year-old olive trees of two varieties, Arbequina and Cobrançosa, using five different methods, leaf counting, photograph analysis, ceptometer, Hemisfer software and finally by measuring plant transpiration and leaf conductance.

Leaf area was determined on 7 plants of each variety, Arbequina and Cobrançosa, by thoroughly counting the total number of leaves on each plant and then multiplying by the average leaf area for each variety. This procedure gave a value for each plant's total leaf area with less than 10% error.

Leaf area of the same plants was then estimated by (1) the analysis, with software ImageJ, of a photo of each plant from a side, (2) the crown light scattering measured with a ceptometer in the ground at 10 and 20 cm from the trunk, (3) the analysis with software Hemisfer of a photo of the plant from the top, with a white background, and finally (4) by the determination of the rate of transpiration (E) of each plant over a 2 hour period by a weighing method and dividing this value by the mean leaf conductance (g_s) of each plant, measured on 10 leaves with a porometer.

Average leaf area (n = 7 × 30) was 0,238 × 10^{-3} m^2 and 0,264 × 10^{-3} m^2 for Arbequina and Cobrançosa, respectively. Total leaf area of each plant varied from 3,38 m^2 and 4,85 m^2 for Arbequina and 0,86 m^2 and 3,99 m^2 for Cobrançosa.

Both the ceptometer (AccuPAR Model LP-80) and the Hemisfer software give a value of leaf area index (LAI). In order to obtain the leaf area, it was necessary to attribute a value for the projection of the crown. This proved to be quite difficult and inaccurate as the crown shape and pattern of branching was quite variable from tree to tree.

On the other, leaf area assessment by the analysis of the plant photo from a side and also by dividing E by g_s were reasonable accurate.

Screening Chickpea (*Cicer arietinum)* Accessions on the Basis of Leaf Traits: Temperature, Gas Exchange and Hormones

Costa JM[1,2]*, Pinheiro C[1,3]*, Simões N[4], Toureiro A[4], Duarte I[4], Ribeiro IC[1], Machado A[1], Müller M[5], Pintó-Marijuan M[1,5], Munné-Bosch S[5], Chaves MM[1]

1 Instituto de Tecnologia Química e Biológica, UNL, Apartado 127, 2780-901 Oeiras, Portugal.
2 LEAF, Instituto Superior de Agronomia, UL, Tapada da Ajuda, 1349-017 Lisboa, Portugal.
3 Faculdade de Ciências e Tecnologia, UNL, 2829-516 Caparica, Portugal
4 INIAV Apartado 6, 7350-901 Elvas, Portugal.
5 Dep.Biologia Vegetal, Facultat de Biologia, Univ. de Barcelona, Av. Diagonal, 645, E-08028 Barcelona, Spain.
**These authors contributed equally*
* e-mail: miguelc@itqb.unl.pt; pinheiro@itqb.unl.pt

Novel genotypes of *Cicer arietinum* L. (chickpea), with high environmental plasticity (more resistant to drought and heat, in particular during the reproductive stage) but without compromising seed yield are needed. Trials carried out in South Portugal (Elvas) in 2009 and 2010 revealed that grain yield of two genotypes (ILC588 and ELIXIR) were stable across multiple environments, while four others (Eldorado, FLIP87-008C, FLIP03-046C and ILC3279) were well adapted to favorable environmental conditions but performed badly under drought. In 2011, we have characterized this group of six genotypes under rainfed conditions. Plants were observed at two phenological stages: 1) beginning of flowering (26 April 2011; BF) and 2) end of flowering (14 June 2011; EF). Soil and plant water status, and several leaf traits (temperature, gas exchange, Chl a fluorescence, hormones) and grain yield were measured. In all genotypes we observed an increase in leaf temperature in parallel with a decrease in leaf stomatal conductance to water vapour and net assimilation from BF (no stress) to EF. This was more pronounced in FLIP87-008C, which also presented a marked decrease of ΦPSII at EF contrary to the other genotypes. Principal components analysis (PCA) including data on water status, leaf gas exchange, and hormones, showed that BF and EF stages are discriminated along the first axis (66% of variation explained). When BF and EF are considered separately (in two distinct datasets) Eldorado significantly differed from the remaining genotypes at EF, while ELIXIR and ILC588 group very close. This distinct pattern suggests metabolic differences among genotypes in the response to stress with potential impact on crop production.

Key-Words: leguminous, phenotyping, water stress, stomata, metabolites, yield

Drought Indicators in Pinus Radiata Seedlings

Diego N[1,2], Pérez-López U[3], Saiz-Fernández[2], Muñoz-Rueda A[3], Moncalean P[4], Lacuesta M[2]*.

1 Centre of the Region Hana´ for Biotechnological and Agricultural Research, Palacký University, Czech Republic.
2, 3 Department of Plant Biology and Ecology, 2 Faculty of Pharmacy, 3 Faculty of Science and Technology. University of Basque Country UPV/EHU Spain.
4 Department of Plant Production and Protection, NEIKER-Tecnalia. Spain.
* e-mail: maite.lacuesta@ehu.es

Drought is one of the abiotic stresses that has a greater influence in forest health. Water stress influences the growth, survival and distribution of forest trees species and the selection and production of drought tolerance seedlings becomes a decisive factor. To identify drought tolerance mechanisms, four month plants from six geographical separated plantations of Pinus radiata D. Don (O1-O6) were evaluated after exposed stress until 50% of plants for each plantation showed external symptoms of stress as apical curvature and epinastic needles (after 54 days in O1 and 80 days in O5). In our study, different behaviours among plantations were observed depending of water availability and developed different strategies for matter allocation. More vigorous plantations with higher dry matter presented less tolerance than plantations with low mass accumulation. Besides, vigorous plants normally focused the matter allocation to shoots while the less vigorous one accumulated in root. When seedling showed external symptoms, all plants had water potential values lower than -2.8 MPa. WUE was the most adequate marker to detect more tolerant seedlings, and O5 was the most tolerant one. For this reason, plants from this plantation could be perfect to include in breeding programs.

Acknowledges: This research was supported by MEC-INIA and Fondo Europeo de Desarollo Regional (FEDER) (grant RTA2010-00041-CO2-02), Grupo GV-IT326-10, MSMT (CZ.1.07/2.3.00/30.0004). Iñigo Saiz Fernández has a fellowship from the UPV/EHU.

Microclimatic Conditions and Gas Exchange in a Vineyard Under Plastic Cover

Esteves BS[1]*; Sousa EF[1]; Lousada LL[1]; Pessanha DP[1]; Bressan-Smith RE[1]; Campostrini E[1]; Fernández JE[2]*

1 Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brasil.
2 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Avenida Reina Mercedes n.º 10. 41012-Sevilla
* e-mail: barbbarase@gmail.com; jefer@irnase.csic.es

Brazil is a major producer and consumer of fruits. This explains the interest for viticulture, withnumerous vineyards in central and south areas of the country. Plastic covers are being used in an increasing number of vineyards to avoid problems derived from high radiation and rainfall levels. The effects of this new management system on microclimatic, gas exchange and crop water demand are not well known. In this context, the objective of this study was to analyse possible changes in soil moisture, microclimatic variables and gas exchange in a vineyard (cv. Niágara Rosada, for table consumption) under transparent plastic cover. The experiments were performed in São Fidelis, Rio de Janeiro State, Brazil, on a growing cycle (March to June 2013). The crop was cover at the top with 160 µm thick low density polyethylene film. Within a commercial vineyard (1 ha) we had two plots (covered and uncovered, 0.035 ha each), each one instrumented with a meteorological station. Soil humidity was determined by gravimetry. Stomatal conductance (gs) and net photosynthesis (AN) measurements were made at 08.00- 10.00 am, in 5 plants of each treatment and on day 42, 56, 68, 91 and 106 after pruning. Greater soil moisture and reduced photosynthetic active radiation were registered in the plot with plastic cover. Both the maximum and average air temperatures, as well as the vapour pressure deficit increased under the cover. Minimum air temperature, however, was not affected. Measurements all along the growing cycle showed no differences on AN between treatments. For gs, this happened on day 42 after pruning only. For the rest of the cycle, greater gs values were observed in the plants under cover.

Chloride Nutrition Regulates Water Balance in Plants

Franco-Navarro JD[1], Brumós J[2], Rosales MA[1], Vázquez-Rodríguez A[1], Sañudo BJ[1], Díaz-Rueda P[1], Rivero C[1], Talón M[2], Colmenero-Flores JM[1]

1 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNASE). Consejo Superior de Investigaciones Científicas (CSIC). Avda. Reina Mercedes 10, 41012-Sevilla, Spain.
2 Instituto Valenciano de Investigaciones Agrarias. Centro de Genómica. Carretera Moncada - Náquera, Km. 4,5. 46113-Moncada (Valencia), Spain.
* e-mail: chemacf@irnase.csic.es

Chloride (Cl-) is one of the 16 elements essential for plant growth. Because it is supposedly needed in small quantities for healthy growth of higher plants (<50-100 mM in the nutrient media), Cl- is considered a micronutrient (Johnson *et al.*, 1957; Terry, 1977; Whitehead, 1985). Usually non-halophytic plants present critical deficiency contents below 0,2 mg/g shoot dry weight (reviewed in Flowers, 1988; Marschner, 1995; Xu *et al.*, 2000; White and Broadley, 2001), under which, deficient plants show a significant decrease in leaf area as a result of a reduction in cell division rates (Terry, 1977). Chloride is considered however to be an strange micronutrient since actual Cl- concentration in plants is typical of the content of a macronutriente (10-60 mg/g, about 50-300 times higher than the content required as essential micronutrient; Marschner, 1995; Brumós *et al.*, 2010; Franco-Navarro *et al.*, 2012; Franco-Navarro *et al.*, 2013). This is relevant provided that all the other mineral micronutrients (B, Cu, Fe, Mn, Mo, Ni, Zn) are present at much lower concentrations in plant tissues (10-4-10-1 mg/g) and their accumulation to higher levels result in plant toxicity (Hänsch and Mendel, 2009). Besides the micronutrient functions, Cl- is an osmotically active solute in the vacuole. Participation of Cl- in cell osmotic regulation has been largely argued through its involvement in the regulation of cell turgor processes like stomatal movement, and the activity of motor cells controlling nastic movements (Leigh and Wyn Jones, 1985; Flowers 1988 and references therein; Marschner, 1995; Iino *et al.*, 2001; Shabala and Lew 2002). Thereby we proposed and demonstrated that Cl- plays a biological role in controlling water balance and plant water relations (to be published). We observed that in the low millimolar range Cl- promotes plant growth and improves developmental and water relation parameters like leaf expansion, cell elongation, the relative water content, tissue osmolarity, water-use efficiency (WUE) and drought tolerance (Franco-Navarro *et al.*, 2012; Franco-Navarro *et al.*, 2013). But there were left many unsolved questions, i.e., the response of plants to increasing concentration of anions and the correlations with different water parameters, including a complete leaf water/osmotic/turgor potential measurement.

In order to elucidate these issues, two independent experiments were done. Tobacco plants were grown under well-irrigated semi-hydroponic conditions for 8 weeks subjected to different treatments: basal nutrient solution (BS); BS supplemented with different concentrations of Cl- salts (CL); BS supplemented with different concentrations of NO3- salts (N); BS supplemented with different concentrations of SO4 2- + PO4 3- salts (SP). All treatments (CL, N and SP) contained the same concentration of charge-balancing cations. Data concerning correlations between anions content *vs.* water balance parameters will be presented.

Effects of Climate Change (Elevated CO2, Elevated Temperature and Water Deficit) on Growth, Water Status, and Yield Quality of Two Grapevine (*Vitis Vinifera* L.) Cultivars

Kizildeniz T[1], Mekni I[1], Pascual I[1], Irigoyen JJ[1*], Morales F[1,2]

1 Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC, EEAD, Zaragoza e ICVV, Logroño. Facultades de Ciencias y Farmacia, Universidad de Navarra, Irunlarrea 1, 31008, Pamplona.
2 Estación Experimental de Aula Dei (EEAD). CSIC. Dpto. Nutrición Vegetal. Apdo. 13034. 50080. Zaragoza.
* e-mail: jirigo@unav.es

Climate change is associated to atmospheric [CO2] increases, enhanced temperatures and scarce water availability, at least in the Mediterranean area, limiting seriously crop yield and quality. Therefore, it is urgent to investigate the effects of climate change scenarios. In the present work, we studied the effects of elevated CO2, elevated temperature and water deficit, acting individually and/or interacting, on vegetative and reproductive growth, substrate and plant water status, and must quality in fruit-bearing cuttings of two grapevine (Vitis vinifera L.) cultivars (Red and White Tempranillo). In four temperature gradient greenhouses, eight different treatments were applied, from fruit set to maturity: CO2 level (400 versus 700 □mol mol-1), temperature (ambient versus ambient +4ºC), and water availability (well irrigated versus cyclic drought). In most cases, Red Tempranillo produced more vegetative and reproductive matter than the White one. Drought, especially under higher temperature, drastically reduced vegetative growth, bunch fresh and dry weight in both cultivars, and elevated CO2 attenuated the negative effect of drought. In general, grape yield was less affected by the treatments than vegetative growth. The effects of climatic factors on production were not associated to a worse water status of the vegetative or reproductive organs. The combination of elevated CO2, temperature and drought reduced total polyphenol index (TPI), malic acid and color density, but did not modify anthocyanins concentration and acidity in the red cultivar. In white Tempranillo, climate change modified only tartaric acid, although drought increased TPI under ambient temperature, regardless of CO2 level.

According to the results, Red Tempranillo appeared to be more productive than the White cultivar. Drought combined with high temperatures were the main factors reducing grapevine plants performance, and CO2 seemed to prevent such deleterious effect.

Acknowledgments: Asociación de Amigos de la Universidad de Navarra (T. Kizildeniz PhD Grant). IAMZ CIHEAM (I. Mekni Master Grant). European project (INNOVINE Call FP7-KBBE-2011-6, Proposal Nº 311775), Spanish Ministry of Economy and Innovation (MINECO BFU2011-26989). Many thanks to M. Oyarzun, A. Urdiain and H. Santesteban by the great technical work.

Comparative Assessment of Water Requirements and the Physiological and Agronomical Response of Strawberry Cultivars (*Fragaria* X *Ananassa* Duch.) to Water Shortage.

Martínez-Ferri E [1]*, Medina JJ[3], Muriel Fernández JL[2], Ariza MT[1], Miranda L[3], Domínguez P[3], Soria C[1].

1IFAPA. Centro de Churriana. Cortijo de la Cruz s/n, 29140 Churriana, Málaga, Spain.
2 IFAPA. Centro Las Torres-Tomejil. Ctra. Sevilla-Cazalla, Km. 12,2, 41200 Alcalá del Río, Sevilla. Spain.
3 IFAPA. Centro de Huelva. C/ Julio Caro Baroja s/n. 21002 Huelva. Spain.
* e-mail: josel.muriel@juntadeandalucia.es

Strawberry production requires the input of large amounts of water and its cultivation under plastic tunnels, relies entirely on water provided by irrigation during the entire production cycle and at high frequency, since soils are very poor and with low water holding capacity (sandy soils mostly). In these systems, water management is not easy and growers tend to use excessive and inadequately supplied water, which can have negative impact on the natural environment. This is especially tough at the Huelva region (the most important cultivation area of Europe), were water is scarce and strawberry cultivation is on the surrounding of the Doñana Natural Park. To achieve sustainable agriculture practices with no impact on crop yield and quality, an accurate irrigation water management must be done. It requires the knowledge of water requirements of the main competitive strawberry cultivars of the region and their physiological and agronomical response to possible restrictions of water supply. In this sense, no information pertaining to water requirements, water use efficiency (WUE) and to the relative tolerance to water shortage of the main strawberry cultivars currently growing at Huelva is available. A relatively more efficient or tolerant cultivar could contribute to increase water productivity and economic benefit of strawberry cultivation but keeping its environmental sustainability. Two experimental designs were carried out for the comparative evaluation of water requirements of seven strawberry cultivars (*Fragaria* x *ananassa* Duch.) and of their physiological and growth response to water shortage (70% ETc).

Main results showed that water consumption differ substantially among cultivars and these differences were associated with differences in the biomass partitioning into the harvest product (i.e. harvest index) and in the transpiration efficiency (TE) closely related with intrinsic water use efficiency (A/T). Cultivars were segregated on the basis of the relationship between both parameters, which integrate the differences among cultivars at the physiological (chlorophyll fluorescence, gas exchange, SPAD index, etc) and at the growth response (fruit production, patterns of carbon allocation, LMA) levels in both water treatments. It is concluded that breeding for greater leaf-level water use efficiency (A/T) in horticultural crops, such strawberry, is not always an agronomical advantage.

Physiological Comparison Behaviors between Two Cultivars of *Vitis Vinifera* (Grenache and Tempranillo Cvars.)

Martorell S[1]*, Díaz-Espejo A[2], Tomás M[1], Pou A[1], El Aou-uad H[1], Escalona JM[1], Vadell J[1], Flexas J[1], Ribas-Carbó M[1], Medrano H[1].

1 Research Group on Plant Biology under Mediterranean Conditions, Departament de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain
2 Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain
* e-mail: sebastia.martorell@uib.es

Knowledge about regulation of stomatal conductance is necessary to improve grapevine water use efficiency. The rich amount of grapevine varieties let us the opportunity to choose the better enhanced cultivars to global warming changing conditions. A comparison between two reputed cultivars (Tempranillo and Grenache) with contrasted water use efficiency was performed during two years in a field experiment. Water relations, leaf gas exchange and ABA were measured in different stages of grapevine growing season. A clear difference in regulation of leaf water relations parameters under water stress were observed between both cultivars. Results indicated that there is a clear relationship between hydraulic conductance (*K*h) and stomatal regulation. Abcisic Acid (ABA) can excert a changing role on the stomatal control during different stages of grapevine growth period. The combination of both signals, ABA and *K*h, control the stomatal aperture in a different way leading to have differential water use efficiency between cultivars.

Influence of Sustained Deficit Irrigation on the Phenolic Composition and Quality of Grape Berries (*Vitis Vinifera* L.) Cv. Tempranillo and Graciano

Niculcea M[1], Martínez-Lapuente L[2], Guadalupe Z[2], Sánchez-Díaz M[1], Ayestarán B[2], Antolín MC[1*]

1 Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño). Facultades de Ciencias y Farmacia, Universidad de Navarra, c/ Irunlarrea 1, 31008, Pamplona, Spain
2 Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, Gobierno de La Rioja y CSIC) c/Madre de Dios 51, 26006 Logroño, Spain
* e-mail: cantolin@unav.es

Changes in vine water status affect polyphenol levels, indicating that irrigation practices can be used to influence berry composition (Chaves 2010, Niculcea et al. 2013). Therefore, the aim of this study was to analyze skin phenolic composition during development and ripening of berries by characterizing the response of *Vitis vinifera* L. cvs. Tempranillo and Graciano to sustained water-deficit irrigation. The study was carried out using container-grown grapevines grown under controlled conditions in a greenhouse. Two irrigation treatments were imposed: control (well-watered) and sustained deficit irrigation (SDI). Twenty-eight phenolic compounds, including anthocyanins, flavonols and monomeric flavan-3-ols (catechins) as well as phenolic acids derivatives have been identified in the extracts prepared from the berry skins at physiological maturity. For both varieties, water deficit reduced leaf area and leaf area to crop mass ratio, and decreased berry size. However, there were no changes in juice total soluble solids, pH or total polyphenolic content.

Water deficit resulted in decreased must titratable acidity in Graciano berries. In Tempranillo, water limitation reduced total anthocyanins and flavonols, and increased hydroxycinnamic acids. In Graciano, water deficit resulted in increased flavonols and reduced catechins. Altogether, we conclude that under water-deficit irrigation, Graciano grapes presented a differential composition of phenolic compounds that could result in improved fruit quality.

References: Chaves et al. 2010. Ann. Bot. 105, 661-676. Niculcea et al. (2013). J Plant Growth Regul, 32: 551-563.

Acknowledgments: Project funded by Ministerio de Ciencia e Innovación (MCINN BFU2011-26989) of Spain. Fellowships: Asociación de Amigos de la Universidad de Navarra.

Comparison of Potential Irrigation Strategies to Confront Water Restriction Periods in Lemon Trees Grown in Semi-Arid Regions

Perez-Perez JG[1,3]*, Robles JM[1], Garcia-Sanchez F[2,3], Garcia-Oller MI[1], Cordoba F[1], Arques E[1], Frutos JM[1], Quinto V[1], Botia P[1,3].

1 Department of Citriculture, Instituto Murciano de Investigacion y Desarrollo Agrario y Alimentario (IMIDA), c/ Mayor s/n, 30150 La Alberca (Murcia) Spain.
2 Centro de Edafologia y Biologia Aplicada del Segura (CEBAS). CSIC. Campus Universitario de Espinardo. Espinardo. 30100.Murcia. Spain.
3 Unidad Asociada al CSIC de Fertirriego y Calidad Hortofruticola (IMIDA-CEBAS), Spain.
*e-mail: juang.perez@carm.es

Lemons are mainly grown in the Mediterranean regions, with a semi-arid climate characterized by low rainfall and high evapotranspiration - which can produce long periods of water restriction. The scarcity of good water resources forces lemon growers to use alternative water sources, such as ground water of high salinity, or to optimize water use by using regulated deficit irrigation (RDI) strategies. Under this context, it is not known which of these two strategies would be better for the citrus crops; thus, the aim of this work was to compare, from the physiological and agronomic points of view, the responses to two irrigation strategies, RDI and the controlled use of saline water, of 15-year-old 'Fino 49' lemon trees (*Citrus limon* (L.) Burm. fil,) grafted on *Citrus macrophylla* Wester. The experiment was carried out over two years in an experimental orchard located in Torre Pacheco (Murcia, Spain). Three treatments were applied: Control, well-irrigated (100% ETc of non-saline water - 0 mM NaCl); RDI, regulated deficit irrigation (25% ETc of 0 mM NaCl), and RSI, regulated saline irrigation (145% ETc of saline water – 40 mM NaCl). Both the RDI and RSI treatments were maintained along the crop season, except during the period of high evapotranspiration (corresponding to phase II of fruit growth – cell elongation) when the irrigation dose was 100% ETc of non-saline water in both treatments.

During the application of the RDI and RSI treatments, the soil water deficit imposed by the RDI treatment decreased the soil water content (θv), while in the RSI treatment it was higher than in the control soil. Differences in soil water status were reflected in the plant water status, the midday stem water potential being reduced only in RDI trees. However, the net CO2 assimilation rate (*A*) was decreased in both the RDI and RSI treatments during the stress periods, stomatal closure being one of the main factors limiting *A*, although high leaf Cl- concentration could also have been responsible for reduction of *A* in the RSI treatment. RDI and RSI produced similar effects on vegetative growth, decreasing pruning rest compared with the control trees. RDI maintained water use efficiency (WUE) similar to that of the control trees, because yield reduction was related with the irrigation water saved (31% less).

However, the WUE of RSI trees was significantly reduced due to the extra irrigation dose of saline water during the stress periods (145% ETc – corresponding with the leaching fraction), that did not minimize the salinity effects on yield. RSI did not affect fruit quality significantly, but RDI delayed fruit maturation, based on lower fruit diameter and juice content and higher titratable acidity and total soluble solids than in fruits of control trees.

Influence of the Irrigation System on the Suitability of Trunk Diameter Reference Lines for Irrigation Scheduling in Lemon Trees

Perez-Perez JG*, Robles JM, Garcia-Oller MI, Quinto V, Botia P.

Department of Citriculture, Instituto Murciano de Investigacion y Desarrollo Agrario y Alimentario (IMIDA), c/ Mayor s/n, 30150 La Alberca (Murcia) Spain
*e-mail: juang.perez@carm.es

The aim of this work was to assess the suitability of trunk diameter reference baselines for irrigation scheduling in surface (SURF) and subsurface (SUB) drip irrigation systems, in 18-year-old 'Fino 49' lemon trees (Citrus limon (L.) Burm. fil.) grafted on Citrus macrophylla Wester. The experiment was carried out over two consecutive years in an experimental orchard located in Torre Pacheco (Murcia, Spain). Well-watered trees for each irrigation system were maintained by applying irrigation water independently, maintaining in both systems the soil water content of the root zone at ≈ 80% of the amount of water available and the midday stem water potential (Ψmd) > -1.3 MPa. The results indicate that reference equations obtained from pooling data of the midday stem water potential (Ψmd) and climatic parameters (daily crop reference evapotranspiration, ET0; daily mean air vapour pressure deficit, VPD; daily mean air temperature, T) were not influenced by the irrigation system.

However, the reference equations generated from maximum daily trunk shrinkage (MDS) and the ET0, VPD and T parameters were different for each irrigation system, although there were no significant differences in the slopes of the MDS reference equations - indicating that the prediction potential of MDS was similar in both systems. In both cases, the environmental variable that best correlated with Ψmd and MDS was T. The relationship between MDS and Ψmd was also influenced by the irrigation system, the slope of SUB being significantly decreased. Thus, based on these results, MDS measurements can be suitable for adjustment of the irrigation scheduling of lemon trees, but differences found between irrigation systems in the MDS reference baselines show the necessity of determining the baselines for each irrigation system.

Xylem Anatomy, Vulnerability to Drought-Induced Embolism and Hydraulic Safety Margins in Roots of *Q. suber* and *Q. ilex*

Pinto C [1], David JS [2,3,] Caldeira MC [2,3], Quilhó T [4], Henriques MO [3], David TS [1,3]

1 Instituto Nacional de Investigação Agrária e Veterinária I.P., Quinta do Marquês, Av. da República, 2780-159 Oeiras, Portugal
2 Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
3 Centro de Estudos Florestais, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
4 Centro das Florestas e Produtos Florestais, Instituto de Investigação Científica Tropical, Tapada da Ajuda, 1349-017 Lisboa, Portugal
* e-mail: clara.pinto@iniav.pt

Quercus suber and Q. ilex trees are major elements of Mediterranean landscapes, which are threatened by increasing water deficits. We compared the vulnerability to xylem embolism and the anatomical properties of surface roots of these oaks. Data were collected in mature trees at two sites, in central/coastal and southern/inland Portugal. Results of a previous work showed that current-year shoots of both species did not differ in vulnerability to drought-induced embolism (Pinto et al., 2012). The air-injection technique was used to assess vulnerability of root xylem to drought-induced embolism. In situ safety margins to hydraulic failure were evaluated from long-term predawn leaf water potential records. Mean vessel diameter, hydraulically weighted mean diameter and the mean diameter of the vessels that account for 95% of the predicted hydraulic conductivity were estimated from anatomy data. Roots of Q. suber and Q. ilex had similar vulnerability to drought-induced xylem embolism, with a 50% loss in hydraulic conductivity (Ψxyl,50PLC) at xylem water potentials of -0.86 and -1.15 MPa, respectively. Mean vessel diameter was higher in roots of Q. ilex (113.88 µm) than of Q. suber (96.94 µm). In roots of both species, a small number of vessels were partially or completely blocked by tyloses. The hydraulic safety margins, for both species, showed that even during the driest periods, both oaks lived above the most critical embolism thresholds. However, in the driest site, the safety margins were smaller. Compared to shoots (Pinto et al., 2012), roots showed higher vulnerability and smaller safety margins under water stress. This suggests that roots are the weakest point of the conducting system, in what concerns hydraulic failure potential. Results are relevant for the understanding of survival, growth, and functional behavior of Mediterranean evergreen oaks under recurrent/seasonal drought conditions.

Post-Harvest Pear Maturation is Influenced by the Orchard Irrigation Regime

Silvestre S[1*], Elias E[1], Antunes R[2], Bernardes da Silva A[1], Marques da Silva J[1].

1 Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Vegetal, Center of Biodiversity, Functional and Integrative Genomics, Campo Grande, 1749-016 Lisboa, Portugal.
2 FRUTOESTE - Cooperativa Agrícola de Hortofruticultores do Oeste, CRL, Estrada Nacional 8, Carrascal, 2665-009 Azueira, Portugal.
* e-mail: smsilvestre@fc.ul.pt

Pyrus communis L. var. “Rocha”, a Portuguese pear variety with a Protected Designation of Origin, is the main kind of fresh fruit exported from Portugal. Its long shelf life and a superior resistance to handling opens up the possibility of supplying markets for a long period of time, while maintaining a constant quality. Post-harvest maturation was studied in pears grown in irrigated (Ir) and non-irrigated (NIr) orchards and stored under normal (NA) and controlled (CA) atmosphere conditions. Total chlorophyll content (Chl a+b) and the minimal (Fo) and maximal (Fm) fluorescence and the maximum photochemical efficiency of PSII reaction centers (Fv/Fm) of pears’ skin were measured, as well as the pulp firmness and the total content of soluble solids (ºBrix), both usual methods to evaluate fruit maturation. At harvest, Ir and NIr fruits had similar firmness but after 4 months of cold storage, Ir fruits were firmer than NIr fruits when stored under CA. In NA, no differences between the irrigation regimes were found. The total content of soluble solids increased in a parallel way in both groups in CA, while in NA ºBrix was higher in NIr fruits. Total chlorophyll content was similar in both irrigation treatments, decreasing in a similar way in both storage conditions. All fluorescence parameters decreased in Ir and NIr fruits, being Fo and Fm higher in Ir fruits when stored under CA. On the contrary, in NA, Fo was higher in NIr fruits, while Fm was not affected by the irrigation treatment. Fv/Fm always showed the same trend as Fm. These results suggest that the fruit from Ir “Rocha” pear orchards is able to stand longer post-harvest conservation periods under CA.

Interaction of Light and Water Stress on the Ecophysiological Response of *Nothofagus Antarctica* (G. Forster) Oerst.

Bahamonde HA[1,2]*, Sánchez-Gómez D[4], Gyenge J[1,3], Peri PL[1,2,3], Aranda I[4]

1 Instituto Nacional de Tecnología Agropecuaria, INTA, Buenos Aires, Argentina.
2 Universidad Nacional de la Patagonia Austral, Unidad académica Río Gallegos, UNPA UARG, Argentina
3 Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Buenos Aires, Argentina
4 Intituto Nacional de Investigaciones y Tecnologías Agrarias y Alimentarias, Centro de Investigación Forestal (INIA-CIFOR), Carretera de La Coruña km 7.5 Madrid, España
* e-mail: bahamonde.hector@inta.gob.ar

Nothofagus antarctica (G. Forster) Oerst. (ñire) occupies a latitudinal range from 36° 30´ to 56° 00' S, and extends from sea level to 2,000 m.a.s.l. It's the species of higher ecological amplitude in *Nothofagus spp.* from south-America. In Patagonia Sur Argentina, ñire forests extend across 360,000 ha through a wide range of environmental conditions. Its main presence is between the *N. pumilio* forests and steppe where water conditions are limiting for other forest tree species. Productivity and different ecological issues of ñire forests have been previously assessed by different approaches, though specific studies related to the functional response to water stress of species are scarce. Thus, the main subject of present study was to evaluate the functional response and growth of ñire seedlings under the interaction of water stress and two light treatments. Ten seedlings were grown for 6 months in a climatic chamber under a photoperiod 14/10 hours of light/darkness, temperature range 25ºC day / 20ºC night, and 65% relative humidity. One shoot on each seedling was shaded from the beginning of the experiment with a shading mesh (transmittance of 5 % of full light). Rest of plant received at the top a PPFD of 800 µmolm-2s-1. After five months of well-watering, half of seedlings were submitted to a water stress cycle by one additional month. By the end of experiment, diameter growth at the base of stem seedling was measured. In addition different leaf functional parameters were recorded: specific leaf área (SLA), net photosynthesis (An), stomatal conductance to water vapour (gwv), and different parameters from building P-V curves: osmotic potential at maximum and zero turgor (Π100; Π0), relative water content at zero turgor (RWC0), maximum modulus of elasticity (Emax), and dry/full-hydrated weight ratio (DW/TW). Plant water status was recorded from measuring predawn water potential (Ψpd). Light and water stress impinged in most leaf functional parameters, and with different patterns according to the considered trait that ranged from synergic to antagonistic impacts.

The Use of Laser-Induced Chlorophyll Fluorescence (LIF) as a Rapid and Non-Destructive Method to Investigate Water Deficit in Arabidopsis

Gameiro C[1]*, Utkin AB[2,3], Cartaxana P[1], Marques da Silva J[4], Matos AR[4]

1 Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal;
2 INOV – INESC Inovação, Rua Alves Redol 9, 1000-029 Lisboa, Portugal;
3 ICEMS, IST, Universidade Técnica de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal;
4 BIOFIG – Centro para a Biodiversidade, Genómica Integrativa e Funcional, Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.
* e-mail: clgameiro@fc.ul.pt

Chlorophyll fluorescence measurements have been widely applied, as non-destructive methods, to study the photosynthetic efficiency of plants, under control or stress conditions. Comparing to most protocols of Pulse Amplitude Modulation (PAM), Laser-Induced Chlorophyll Fluorescence (LIF) has the advantage of being a much faster method, allowing the analysis of a great number of individual plants in a short time period. Such analyses are extremely useful, for instance when doing large phenotyping screens of *Arabidopsis thaliana* mutants or ecotypes. Water deficit is a major abiotic stress compromising plant growth and productivity. Arabidopsis has been adopted as the main model organism in plant sciences and has contributed to a better understanding of stress response mechanisms, namely drought. However, the use of LIF techniques in this model plant is scarce. Here we report the use of LIF to investigate changes in chlorophyll *a* (Chl*a*) fluorescence signature under progressive drought of potted Arabidopsis plants (slow stress) and under fast dehydration of detached leaves (rapid stress).

Results show that the two dehydration methods cause distinct modifications on the red/far-red Chl*a* fluorescence ratio (F685/F735) and on the wavelength of Chl*a* fluorescence peaks maxima. Those differences are likely related to distinct changes in water content, pigment composition and other metabolic adaptations which are differently regulated in slow and rapid stress. Of particular interest are Ch*a* emission fluorescence changes which take place, under progressive drought, before a measurable decrease in leaf water content. Additionally, the influence on LIF emission spectra, of the leaf size and the leaf surface (adaxial *vs* abaxial) was addressed. Similar LIF emission spectra were found in fully expand and young leaves whereas different spectra results were obtained for the two leaf surfaces. We conclude that LIF is a rapid and nondestructive tool suitable for high throughput Arabidopsis phenotyping under water deficit.

Ecophysiological Studies on *Cistus Palhinhae* Ingram and *Cistus Ladanifer* L. in Southern Alentejo

Mattioli M, Pereira IP*, Santos-Silva C

ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal
* e-mail: ipp@uevora.pt

Cistus palhinhae Ingram is an endemic plant of Portugal, with a non-consensual taxonomical position. Some authors consider it as a variety or sub-specie of *Cistus ladanifer* L. Until now, the knowledge about *C. palhinhae* is limited, so we pretend to compare ecophysiologicaly and anatomically both species in the same area. The study was performed in 3 sampling sessions in Cape Sardão region, where the two species occur. Water potential, leaf histology, plant cover structure, radiation interception and leaf typology based on leaf index were analyzed. *C.palhinhae* has xeromorphic features and cushion shape, differing from *C. ladanifer* a semi-deciduos sclerophyll shrub. The ecophysiologicals processes are different for the studied species, specially the ones associateds with xeric characteristics of *C. palhinhae* habitat.

Plant/Leaf traits and adaptive strategies of *Cistus* species to Mediterranean drought and insolation in southern Portugal

Fonseca E[1], Simões MP[1],Gazarini L[1], Cochard H[2] ,Vaz M[1]*

1Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)/Dep. Biologia, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal
2INRA, UMR 547 PIAF, F-63100 Clermont-Ferrand, France
*e-mail:mvaz@uevora.pt

The effects of climate change can result in dramatic consequences, particularly in very specific ecosystems such as montado, that are seriously threatened by the reduced natural regeneration of cork and holm oaks. Significant shrub species in montado belong to the Cistus genera, which may be associated with tree regeneration, since soil patches beneath shrub canopies may be areas of high fertility. In this context, it was compared the life strategies of Cistus ladanifer L., C. monspeliensis L., C. populifolius L. and C. psilosepalus Sweet, in order to determine their potential ability to adapt and survive to the climate change expected for the Mediterranean region. Leaf water potential and xylem vulnerability curves were measured in the four Cistus species. Very significant differences in ΨlPD and xylem vulnerability curves were found between Cistus species. The results indicate that C. monspeliensis is extremely resistant while C. psilosepalus is less tolerant to drought. These different strategies and responses to drought may be important for laying down guidelines for montado shrub management.

Author index