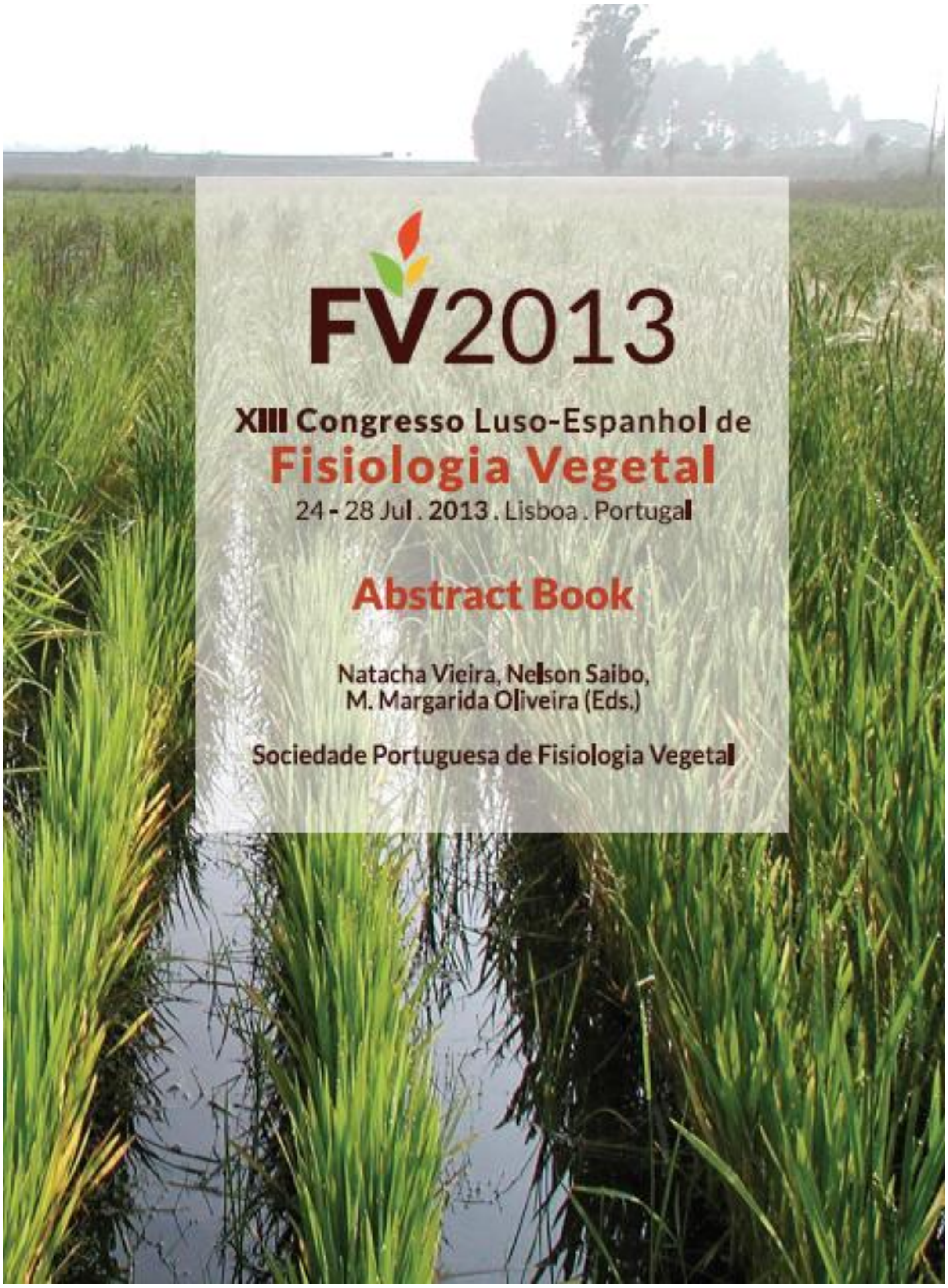

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Abstract Book

Natacha Vieira, Nelson Saibo,
M. Margarida Oliveira (Eds.)

Sociedade Portuguesa de Fisiologia Vegetal

S1A/O3: A PHYSIOLOGICAL AND TRANSCRIPTOMIC APPROACH TO STUDY THE DROUGHT STRESS RESPONSE IN *JATROPHA CURCAS*

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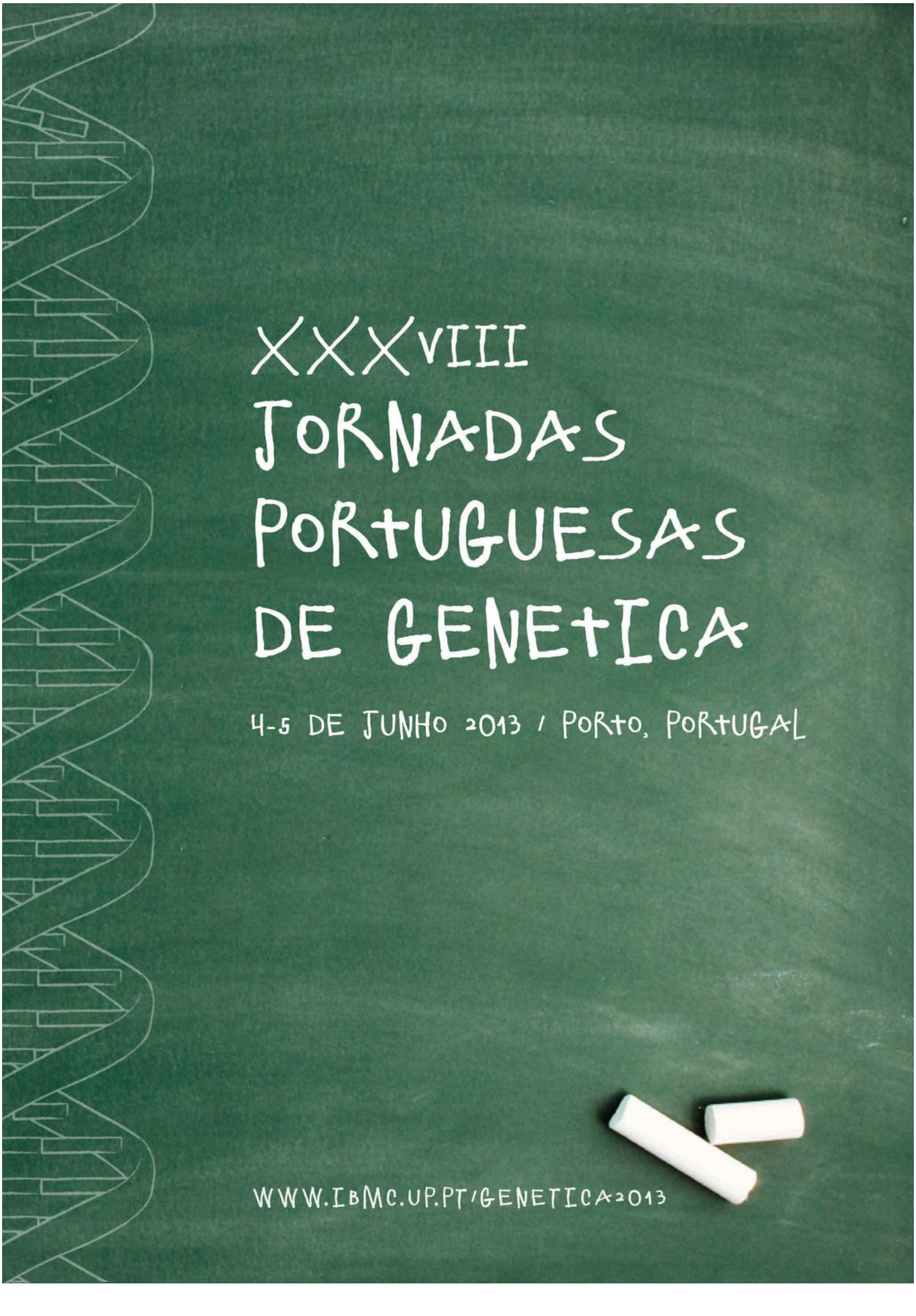
Jatropha curcas is a multipurpose plant that is attracting much attention in the past few years due to its high oil content amenable for biofuel production. *J. curcas* has the advantages of being non-edible and able to grow on poor and marginal soils. Species with high plasticity response to extreme environmental conditions may help to unveil new stress tolerance mechanisms. This is of major importance since abiotic stresses such as drought or salinity are responsible for major crop losses. So far, the reason behind *J. curcas* tolerance to water-limiting conditions has been poorly characterized. To address this, we have studied the response of two *J. curcas* accessions from different climate origins (wet tropical and semi-arid climate) to water stress. One month-old potted plants were subjected to continuous well-watered conditions or to water withholding followed by re-watering. Soil and plant water status, growth and biomass partitioning, leaf gas exchange and chlorophyll a fluorescence were assessed. RNA was collected at several time points along drought (49 days) and re-watering (another 7 days) for both, leaf and root tissues. Using Next Generation Sequencing (RNAseq) we were able to sequence 22 cDNA libraries, resulting in over 2 billion reads of approx. 50 bp in length. Sequences were annotated to the publicly available Jatropha genome database (http://www.kazusa.or.jp/jatropha/) and expression values were normalized with the reads/Kb/Million (RPKM) method. The two accessions were found to behave similarly with a correlation between the expression values ranging between 0.913 and 0.996. This high similarity was confirmed by the morpho-physiological data, both under stress and control conditions. A hierarchical clustering analysis of the transcriptomic data grouped all samples under 3 major clades, leaf samples, root samples and a third cluster containing root and leaf tissues under maximum stress (day 49). Using the two accessions as replicates and focusing in the maximum stress time point, we have found ~4000 (2000 in roots and 2000 in leaves) genes responsive to drought. These genes are described as involved in known pathways of plant response to stress (e.g. carotenoid biosynthesis which includes the abscisic acid synthesis). Further functional studies (quantitative RT-PCR) confirmed the RNAseq data validating a correlation between the expression of some putative genes involved in chlorophyll biosynthesis and breakdown, with the physiological data collected. The drought stress recovery capacity of *J. curcas* was also investigated and we found that after 3 days of re-watering the stressed plants were fully recovered (in growth and physiological parameters) showing a transcriptional profile similar to control plants. Our data suggest that *J. curcas* rapidly responds to drought, withstanding severe water stress and rapidly recovering after re-hydration.

Acknowledgments: Project funding by FCT through Project PTDC/AGR-GPL/101435/2008.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Strategies to uncover the drought tolerance mechanism of the biodiesel plant *Jatropha curcas* (purging nut)

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Jatropha curcas is an emerging source of biodiesel due to the high quality oil content of its seeds. Historically, these plants have been spread around the world by the Portuguese during the discoveries period. *Jatropha curcas* plants are very resilient to low water availability and are amenable for cultivation in areas with poor soil quality. Because the molecular mechanisms of drought stress response of *J. curcas* are still poorly described, we started our studies by comparing the behaviour of two accessions adapted to two contrasting climates (semi-arid and wet tropical). Seedlings of these plants were submitted to drought stress by water withhold in laboratory conditions (49 days), followed by recovery (1 week), and were evaluated for several morphological and physiological parameters[1]. In parallel, samples were collected for RNA isolation and 22 cDNA libraries were constructed and sequenced using Next Generation Sequencing (NGS) technology. Sequences were annotated to the publicly available *Jatropha* genome database (http://www.kazusa.or.jp/jatropha/) and expression values were normalized using the reads/Kb/Million (RPKM) method. Seedlings of both accessions behaved similarly at morpho-physiological level, both under stress and control conditions. However, differences were clear when comparing control conditions versus drought in maximum stress. Hierarchical clustering analysis of the transcriptomic data allowed forming 3 major clades, encompassing leaf samples, root samples and a third group joining root and leaf samples under maximum stress (day 49). For day 49 we found approximately 2000 genes in roots and 2000 in leaves that are responsive to drought. These genes have been described as involved in known pathways of stress response. We have been conducting RT-qPCR for specific genes, and further confirming the RNAseq data. Moreover, we found a very high drought stress recovery capacity, with the plants showing full recovery after only 3 days of re-watering, in terms of growth, physiology and transcriptomic data. We also found interesting changes at the photosynthetic metabolism, with decrease in the chlorophyll *a/b* ratio under severe stress, supported by the transcriptomic and RT-qPCR data. At present, we are further characterizing the drought responsive system of *J. curcas* by analysing changes at the hormonal level. The most recent data will be presented.

Acknowledgments: Quinvita for providing the seeds and Funding by FCT (PTDC/AGR-GPL/101435/2008).

[1] Sapeta H, Costa JM, Lourenço T, Maroco J, van der Linde P, Oliveira MM. (2013) - Environm. Exp. Bot. 85: 76-84.

Plant Environmental Physiology group

Ecophysiology Techniques Workshop

Lisbon, Portugal 10-15th September 2012

Abstract booklet

using both in situ and remote sensing methods. The radiative transfer and energy balance would be modelled using physically based SCOPE model. SCOPE will further be updated with subsurface soil and ground water modules.

Using a process-based model to predict water use in olive

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A process-based stomatal model was used in a hedgerow olive orchard under deficit irrigation for understanding the mechanisms behind the control of transpiration. The hydromechanical model fitted our canopy conductance data (estimated from sap flow data) satisfactorily and allowed us to analyze the physiological parameters obtained.

Comparing drought stress tolerance of two Jatropha curcas ecotypes.

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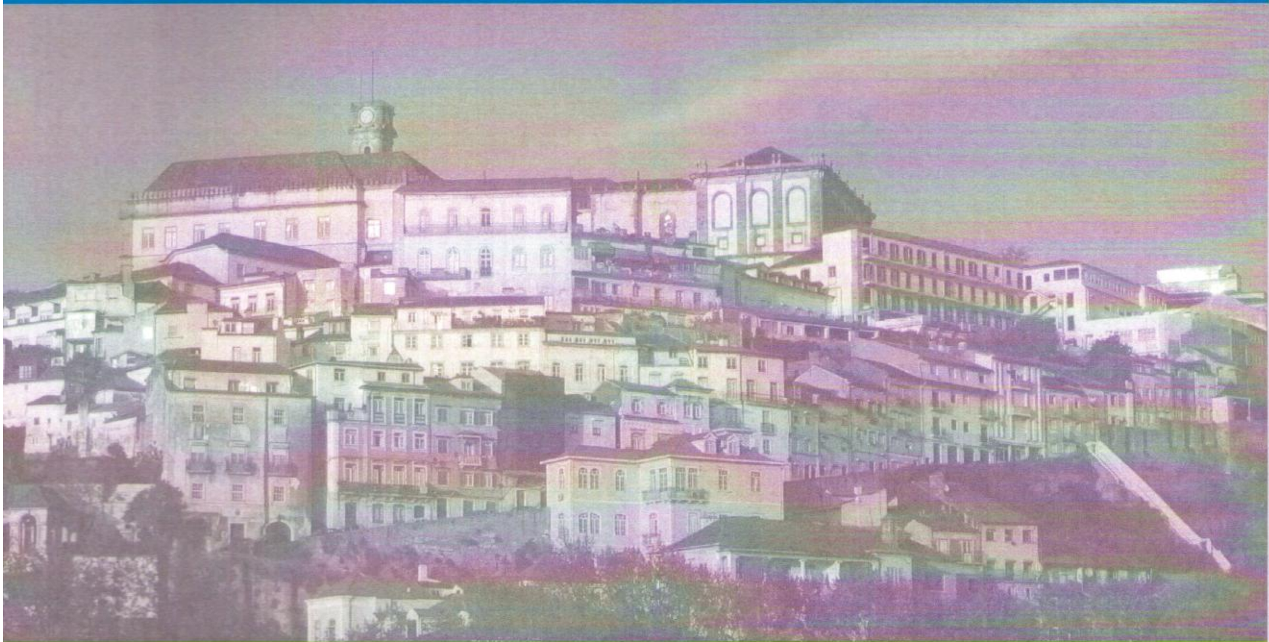
Jatropha curcas is a plant well adapted to semi-arid climates .The focus of our work is to explore the mechanisms of this tolerance. We are studying, at the ecophysiological and molecular levels, the behaviour of two different ecotypes (from arid and tropical regions) when submitted to drought.

Xylem hydraulic conductivity: Methods for improving it measurement

J.M. Torres-Ruiz[1], J.S. Sperry[2] & J.E. Fernández[3]

XXXVI Jornadas Portuguesas de Genética

30 DE MAIO A 1 DE JUNHO 2011



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Drought stress tolerance in *Jatropha curcas*: a comparative study

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Abiotic stress such as drought or salinity is responsible for important crop losses. In this context, species particularly well adapted to extreme environmental conditions may help to unveil new stress tolerance mechanisms, to explore in breeding programs and transfer to specific crops. *Jatropha curcas* is a plant particularly well adapted both to drought conditions and poor soils, although its major interest is due to the quality of its oil-seed for biofuel production. The reason of *J. curcas* tolerance to water-limiting conditions has not yet been explored and is the focus of our work. We are studying, at the physiological and molecular levels, the behaviour of two different ecotypes (adapted to arid or to wet tropical regions) when submitted to drought stress. Seeds from both ecotypes were germinated in a growth chamber with controlled conditions for stress application and periodic analyses. At the physiologically level we have monitored leaf gas exchange, water relationships and growth under water deficit and during recovery. To gain insight into the molecular mechanism of drought tolerance in *J. curcas*, we have targeted putative key genes. In a first approach, we targeted several candidates known from heterologous species as being involved in drought tolerance (regulators of gene expression, regulators of osmolyte accumulation, and signal transduction regulators). Degenerate primers were designed for the target genes and used on cDNA produced from total RNA isolated from control and drought induced tissues to amplify homologous sequences in both ecotypes. The obtained fragments (300-600 bp) were sequenced, confirmed for homology, and extended by RACE-PCR. Specific primers were designed for *Jatropha* sequences and used in expression analyses along the drought-stress treatment. Preliminary experiments suggested discriminating strategies of the two ecotypes to cope with water stress especially after re-hydration. Changes in the gene expression profiles of the target genes are not enough to justify the different plant behaviour. New experiments are under way with more extended treatments and recovery periods. From these studies we are having a more clear idea of the most important time points for RNA collection, aiming to identify genes differentially expressed in the two plant ecotypes and putatively responsible for the differential adaptation.