Abstract Book

- *WORKSHOP* -

"Present and Future of Cork Oak in Portugal"

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

– Oeiras - 21 Oct. 2011 –
Instituto de Biologia Experimental e Tecnológica –
– Instituto de Tecnologia Química e Biológica

Scientific and Organizing Committee

M. Margarida Oliveira (ITQB & iBET)
A. Rios de Amorim (Corticeira Amorim)
C. Pinto Ricardo (ITQB)
Célia Miguel (iBET)
Filomena Nóbrega (INRB)
Helena Pereira (ISA)
J. Santos Pereira (ISA)
José A. R. Graça (ISA)
José António Matos (INRB)
José Carlos Rodrigues (IICT)
Nelson Saibo (ITQB)
Sara Pereira (AIFF)

Honor Committee

Representative of the "Secretaria de estado da Floresta e Desenvolvimento Regional"
João Ferreira do Amaral (Presidente da AIFF – Associação para a Competitividade da Indústria da Fileira Florestal)
Edmundo M.R. de Sousa (Coordenador da Unid. de Silvicultura e Produtos Florestais – INRB)

Sponsors

TRANSCRIPTOME OF *Quercus suber* CHALLENGED BY DROUGHT, SALINITY, AND OXIDATIVE STRESSES

Azevedo, H., Oliveira, R., Lino-Neto, T., Tavares, R.M.

Centre for Biodiversity Functional and Integrative Genomics (BioFIG), Plant Functional Biology Centre, University of Minho, Campus de Gualtar 4710-057 Braga, Portugal

The cork oak (*Quercus suber*) forest (the "montado") is a unique and emblematic resource for Portugal, due to its ecological, socio-economic significance, and the commercial value of cork. Plant abiotic stresses, particularly reduced water availability and extreme temperatures, are substantial constraints to agricultural and agro-forestry production. In the particular case of cork oak, the damaging land-use policies, the climate change, and imposition of abiotic stresses related with high light intensities have been threatening the cork oak forests. The adaptation to abiotic stresses comprises a series of morphological, physiological, biochemical and molecular changes controlled by complex molecular networks. Recent advances in increasing plant tolerance were achieved after the identification of specific genes suited for plant genetic engineering. However, as abiotic stress is commonly present in the field as a combination of different stresses, the complex plant response mechanisms are far from being elucidated.

This work is part of a coordinated effort to uncover the transcriptome of *Q. suber* and attention was paid to the identification of expressed sequence tags (ESTs) involved in responses to distinct abiotic stress challenges, namely drought, salt and oxidative stresses. To impose drought and salt stress, cork oak acorns were collected in the Lisbon region. Stratified acorns were germinated in sand/vermiculite, at 23ºC for 3 weeks, in the dark, transferred to peat/vermiculite/sand and cultivated under 125 µmol photon m^{-2} s^{-1}, at 23ºC. For drought stress, two-month-old seedlings were divided into five pools and subjected to the following water regimes: 100%, 90%, 50%, 25% and 10% of field capacity. For salt stress, two-month-old seedlings were switched to a 300 mM NaCl regime for 8h, 24h, 2d, 4d and 8d. To impose oxidative stress, three-year-old plants regularly maintained at 250 µmol photon $m^{-2}s^{-1}$ were divided into three pools and sprayed with 50 µM Paraquat, 50 µM Rose Bengal, or 60 mM aminotriazole and switched to 1000 µmol photon m^{-2} s^{-1}. Leaves were harvested after 6 and 24 hours of treatment. Salt- and drought-stressed roots and leaves and oxidative-stressed leaves were ground in liquid nitrogen and total RNA was purified according to the hot borate method. High-purity and high-integrity total RNA samples were used to generate four cDNA libraries used for pyrosequencing runs: a normalized cDNA library, resulting from a pool of identical total RNA amounts from salt- and drought-stressed leaves and roots and oxidative stressed leaves; three non-normalized cDNA libraries of drought-stressed roots and leaves from seedlings watered with 10%+20%, 50%, and 90%+100% field capacity.

Two full plate sequencing runs were performed, one for the normalized library and another for the three non-normalized libraries, using a *Genome Sequencer GS FLX Titanium* (Roche), at the *BIOCANT Advanced Sequencing Services Unit* (Biocant Company; Cantanhede, Portugal). Base calling, quality trimming and size selection (less than 40 nt) of reads was performed by the 454 software. Adaptor sequences were removed using a custom script and Poly-A was masked using MIRA. Preliminary assembly and annotation of the

transcriptome of salt-, drought- and oxidative stressed *Q. suber* was performed, as illustrated in Fig. 1. Results from the annotation performed for each library are presented in Table 1.

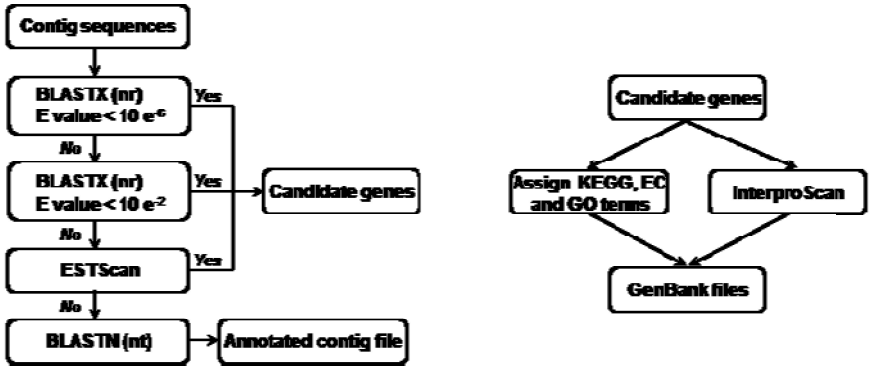

Figure 1. Annotation scheme used for salt-, drought- and oxidative-stressed *Q. suber* transcriptome assembly.

Table 1. Summary of the assembling and annotation results for the *Q. suber* normalized (NL) and non-normalized libraries from seedlings watered with 10%+20% (D10/25), 50% (D50) and 90%+100% (D90/100) field capacity.

	NL	D10/25	D50	D90/100
Number of Reads	657,467	378,923	531,405	494,178
Number of contigs	56,086	38,875	41,656	41,467
Average contig length	493.81	564.5	599.15	569.81
Range of contig length	40-4,376	40-10,156	40-6,109	40-7,758
Number of singletons	3,454	990	1,435	839
Total number of Unigenes	48,732	32,453	34,459	34,092
Amino acid sequence assigned to GO terms	20,563	14,067	15,496	15,021
Amino acid sequence assigned InterPro terms	31,583	21,691	24,213	23,271
Amino acid sequence not assigned InterPro terms (from blastx E<1e-6)	15,268	7,793	8,053	7,858
Amino acid sequence not assigned InterPro terms (from blastx E<0.01)	2,239	1,220	1,213	1,404
Amino acid sequence not assigned InterPro terms (from ESTScan)	2,689	1,636	1,009	1,457

The identified ESTs will contribute to the ongoing coordinated effort of assembling the *Quercus suber* transcriptome. Comparison of non-normalized libraries will be crucial to single out genes putatively important in abiotic stress responses.

Keywords: *Quercus suber*, drought, transcriptomics abiotic stress