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 –
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GLOBAL CHARACTERIZATION OF THE *Quercus suber* ECTOMYCORRHIZAL TRANSCRIPTOME USING 454 PYROSEQUENCING

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In temperate forests, trees live in symbiosis with fungi and depend on them for their survival. Species from the Fagaceae family, including oaks, establish a symbiotic relationship in their roots with basidiomycetous fungi, called ectomycorrhizas (ECM). ECM symbiosis is essential for the life and health of trees in temperate and boreal forests where it plays a major role in nutrient cycling and in functioning of the forest ecosystem. Trees with well developed ECM root tips are more tolerant to environmental stresses, such as drought, and biotic stresses such as root pathogens. There is a general agreement that ECM fungi increase plant survival and productivity. Cork oak is well adapted to water scarcity typical of the Mediterranean summer, due to a root system that can reach several metres in depth, and to the abundance of roots at the soil surface associated with ECM. The development of ECM symbiosis is characterized by the successive development of three structural components: a mantle of fungal tissue that encloses the root, the intraradical network of hyphae (Hartig net), where plant and fungus exchange metabolites, and the extraradical mycelium, which extends into the soil and is responsible for nutrient and water uptake. All these processes are highly regulated and are accompanied by alterations on gene expression in both partners. The current project is integrated in the effort for covering the transcriptome of *Quercus suber* and is focused on the identification of ESTs involved in the development of ECM symbiosis in cork oak. Since only 236 ESTs from *Quercus suber* are currently known, the use of genomics approaches for gene discovery or functional studies is far from being achieved for this plant species.

For establishing cork oak ECMs a *Pisolithus tinctorius* strain, isolated from a naturally occurring basidiocarp associated with a cork oak tree in Alentejo, was used. *P. tinctorius* was grown in vitro in a solid substrate in order to obtain fungal inoculum for ECM production. ECM cork oak plants were established in nursery by transplanting seedlings (2 months old) to *P. tinctorius* inoculated substrate. Non-mycorrhizal control plants were established in parallel. The development of the symbiotic process was followed by visual inspection and microscopic observation of the roots (Fig. 1).

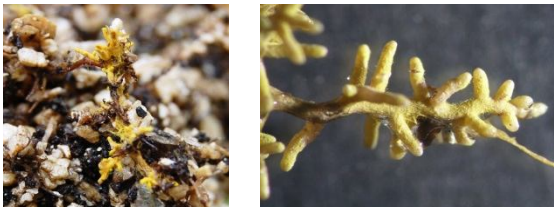

Figure 1. *Q. suber* mycorrhizal roots

Total RNA was obtained from ECM and control roots using the Hot Borate method and was used to generate the three cDNA libraries employed for pyrosequencing runs: i) a normalized

cDNA library from ECM roots, ii) a non-normalized cDNA library from ECM roots and iii) a non-normalized cDNA library from control roots. A full plate sequencing run was performed for each sample, using a Genome Sequencer GS FLX Titanium (Roche), at BIOCANT Advanced Sequencing Services Unit (Portugal). Base calling, quality trimming and size selection (less than 40 nt) of reads were performed by the 454 software. Adaptor sequences were removed using a custom script and Poly-A was masked using MIRA. Preliminary transcriptome assembly and annotation of the *Q. suber* ectomycorrhizal transcriptome was performed as illustrated in Fig. 2

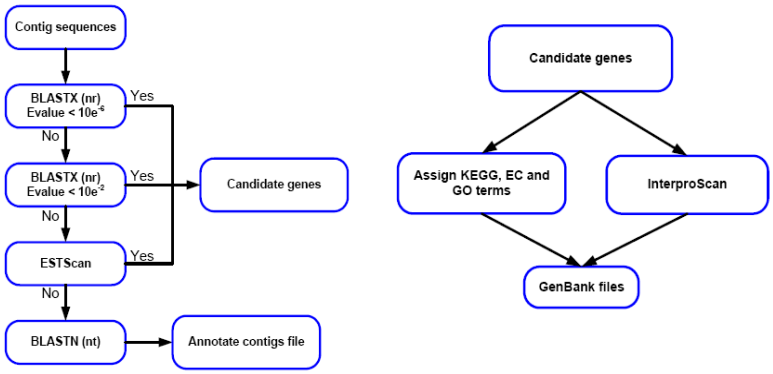

Figure 2. Annotation scheme used for salt-, drought- and oxidative stressed *Q. suber* transcriptome assembly and annotation.

The number of ESTs obtained from all libraries (table 1) will allow extending the global transcriptome of *Quercus suber*. An extensive collection of EST sequences from cork oak will facilitate future gene discover and support an important resource for the genomics of this important plant species for Portuguese economy. Differences on the transcript levels of mycorrized and control roots will allow the singling out of genes that could display an important role on this developmental process.

Table 1. Summary of the annotation performed for normalized (NL) and non-normalized (NNL) libraries.

	NL	NNL	
	Mycorrized roots	Mycorrized roots	Control roots
Number of contigs	60,665	67,655	75,340
Total number of Unigenes	53,872	56,914	61,913
Amino acid sequence assigned to GO terms	16,251	25,786	27,414
Amino acid sequence assigned InterPro terms	25,635	38,799	41,635
Amino acid sequence not assigned InterPro terms (from blastx E<1e-6)	28,383	13,202	14,494
Amino acid sequence not assigned InterPro terms (from blastx E<0.01)	3,476	2,975	3,143
Amino acid sequence not assigned InterPro terms (from ESTScan)	3,710	2,582	2,823

Keywords: *Quercus suber*, ectomycorrhiza, pyrosequencing, transcriptome