Impact of K+ and Na+ fertilization on the leaf transcriptome of *Eucalyptus grandis* submitted to water deficiency

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Eucalyptus trees are well adapted to various soils and climate environment. However its growth varies strongly according to these factors [1]. Tropical cultivated surfaces are mainly located in water deficient regions during dry season, and there soils are often deficient in potassium (K) [2], an essential nutrient [3] improving plant growth [4] and drought resistance [5]. Sodium (Na) could partially replace it [4, 6]. However, the respective role of K and Na on plant water regulation has still to be established [7]. Here, we present an transcriptomic analysis on *Eucalyptus grandis* leaves, submitted to normal or reduced rainfalls, and K or Na fertilization. We develop a strategy to analyze gene expression with a multifactor model (fertilization, rainfall regime and interaction of both factors). Then we validate our procedure by analyzing specifically the genes expressed in response to the reduced rainfall regime. Biological processes and genes linked to water deficiency were successfully identified.

EXPERIMENTAL FIELD

Randomized design: one very productive *E. grandis* clone (Suzano Company) [8].

Treatments: 2 rainfall regimes with 100% (+H2O) or 66% rainfalls (-H2O) with +/- rainfall exclusion system, crossed with 3 fertilizations KCl (K) or NaCl (Na) or without KCl and NaCl (C).

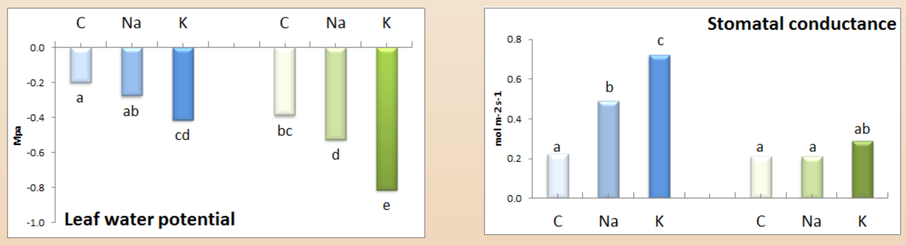

Figure 2: Leaf water potential and stomatal conductance of 2 year-old-tree leaves (blue=100% rainfalls, green=66% rainfalls)

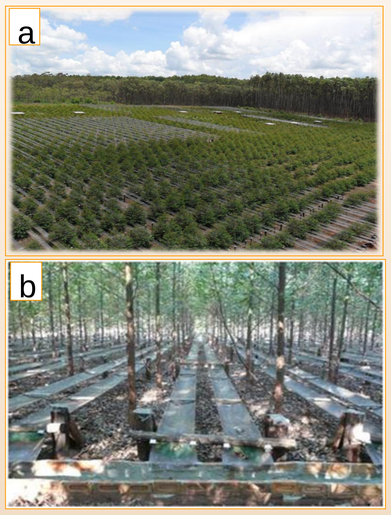

Figure 1: a- 6 month-old experimental field (USP/Esalq experimental station of Itatinga, SP, Brazil); b- Rainfall exclusion system

METHODOLOGY

Leaf sampling: collect on 4 trees/treatment and 6 treatments: K, Na or C with 100% or 66% rainfalls.

RNA extraction: according to Zeng and Yang (2002),

Library preparation: TrueSeq RNA Sample Prep Kit (Illumina).

Sequencing: Illumina HiSeq2000.

Assembling and alignment: TopHat.

Differential expression analysis: DESeq2 (R package), model selection procedure based on likelihood ratio (LR) test with FDR correction (<0.01) and multiple testing to select differentially expressed (DE) genes.

Annotation and enrichment test: Blast2GO at *p-value*<0.01.

RESULTS

Differentially expressed genes

4,159 DE genes, from 36,376 genes sequenced, belonging to 4 groups: 964 genes "fertilization", 2568 genes "rainfall regime", 326 genes "fertilization + rainfall regime" , 301 genes "fertilization x rainfall regime" .

Statistical analysis

Discrimination of the 6 treatments by PLS-DA (Fig.3) firstly according to the rainfall regimes, then to the fertilizations.

Selection of the most significant genes over-expressed for the 2 rainfall regimes with LR test.

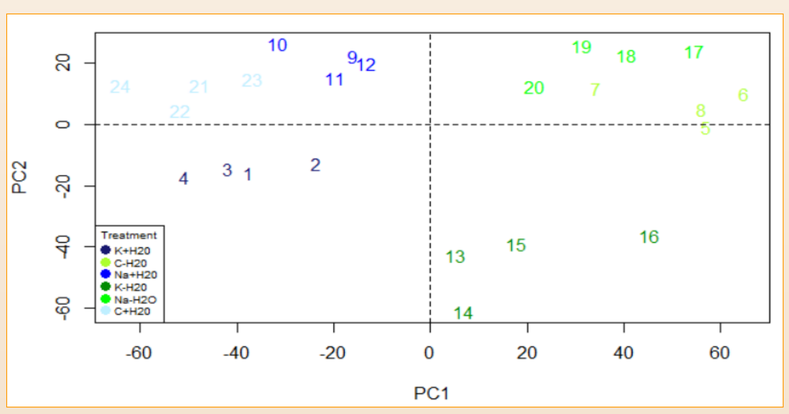

Figure 3: PLS-DA of DE genes

GO enrichment analysis of genes "rainfall regime"

Over-represented GO terms are identified for +H2O (cation transport) and -H2O (biosynthesis of organic substance, development, rRNA processing) (Fig.4).

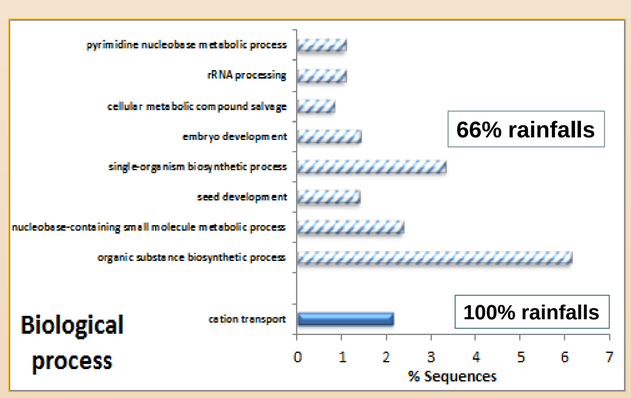

Figure 4: Percentage of sequences in significant GO terms for the 2 rainfall regimes

Example of genes in "Organic Substance Biosynthetic Process" GO term (*p-val*=4e-7): ABA signaling, response to water deprivation (Water deficiency); xanthophyll, phytochromobilin (Photosynthesis); pentose-phosphate shunt, starch catabolism (Carbon metabolism)

DISCUSSION

Leaf ecophysiological parameters on 2-year-old E.grandis demonstrate the significant impact of the 2 factors applied (Fig.2), the fertilization (F) crossed with the rainfall regime (R).

We develop a strategy to analyze the leaf transcriptome in response to the 2 factors F and R.

- **Identification of DE genes** by applying a selection model procedure dissecting each factor of "F"+"R"+"F+R"+"FxR": 4 groups of DE genes identified expressed only according to each term of the model.
- **Validation of the procedure**: the identified DE genes discriminate the 6 treatments by PLS-DA (Fig.3).
- **Identification of significant genes** by univariate analysis (LR) to discriminate the 2 rainfall regimes.
- **Analysis of the significant GO terms** (Fig 4): over-represented GO terms in -H2O with genes known to be expressed in response to water deficiency.

The strategy used here successfully identifies biological processes involved in response to water constraint, and highlights potential gene markers. Following the same process, deeper analysis have to be performed to understand the role of K and Na fertilizations on the response of Eucalyptus to water deficiency.

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