Leaf metabolomic fingerprinting of *Eucalyptus grandis* on field submitted to water constraint and K^+ or Na^+ fertilization

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Fast growing 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]. K is an essential nutrient [3] improving plant growth [4] and drought resistance [5]. Sodium (Na) could partially replace it [4, 6]. However, research needs to establish the respective role of K and Na on plant water regulation [7]. Here, we present an untargeted metabolomic analysis of leaves from trees under normal or reduced water regime and K or Na fertilization. We aim to highlight differential metabolite patterns between treatments and select significant metabolites.

Figure 1: 6 months old Eucalyptus experimental field (USP /ESALQ experimental station of Itatinga,SP, Brazil)

Material

Experimental design

Completely randomized design of *E. grandis* clone (Suzano Company) with 2 water regimes x 3 fertilization treatments (Fig 1 and 2) [8]

- 66% (+H_20) or 100% (-H_20) water regimes (+/- rainfall exclusion system)
- KCl (+K) or NaCl (+Na) or without KCl and NaCl (C)

Leaf sampling

- at the end of the rainy season (2 year-old trees)
- 4 biological replicates (tree) /treatment
- 20 leaves (1 month-old) per trees

Figure 2: Rainfall exclusion system of Eucalyptus experimental field at Itatinga station (SP, Brazil)

Methodology

Metabolomic analysis

- 2 independent extractions of 50 mg of leaf/tree
- Solvents: Methanol/Chloroform [9]
- Apparatus: UPLC-ESI-QTOF-MS, positive mode (Waters, UK)
- Injections: Each extract analyzed 3 times

Statistical analysis

- Preprocessing with MarkerLynx (Waters, UK)
- Data cleaning with R software (version 3.0.2)
- Selection and clustering: Sparse Partial Least Square Discriminant Analysis (sPLS-DA) with "mixOmics" R package
- Analyze of direct relation between metabolites: Gaussian Graphical Model (GGM) with "GGMselect" and "network" R packages

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Results

- Generation of 9600 metabolites after preprocessing
- Selection of 25% of metabolites after cleaning process
- Selection and clustering of the 200 most significant metabolites by sPLS-DA:
  - Identification of 2 groups of metabolites "black" and "red" clustered on the 1st and 2nd components respectively (Fig 3)
  - Discrimination of leaf samples according to water regime and fertilization on the 1st and 2nd components respectively (Fig 4)
- Analysis of direct correlation between metabolites inside groups "black" and "red" by GGM
  - Detection of numerous metabolites directly correlated in both groups "black" and "red" (Fig 5)
- Representation of relative expression between treatments of 2 correlated metabolites belonging to the cluster "black" (n°11 and 14)
  - Expression profil of metabolites mainly related to water regime (Fig 6), as expected for cluster "black"

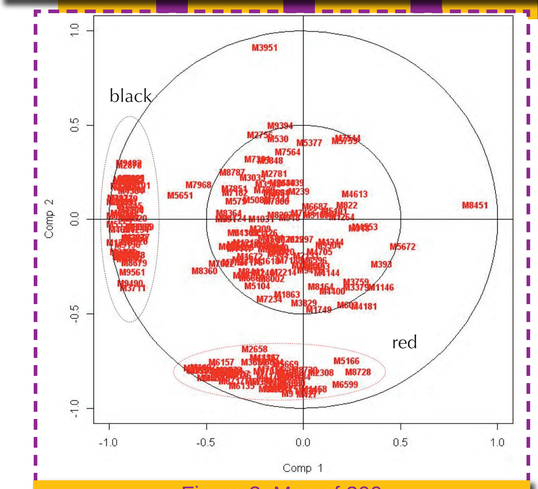

Figure 3: Map of 200 variables by sPLS-DA on PC1-PC2 plan

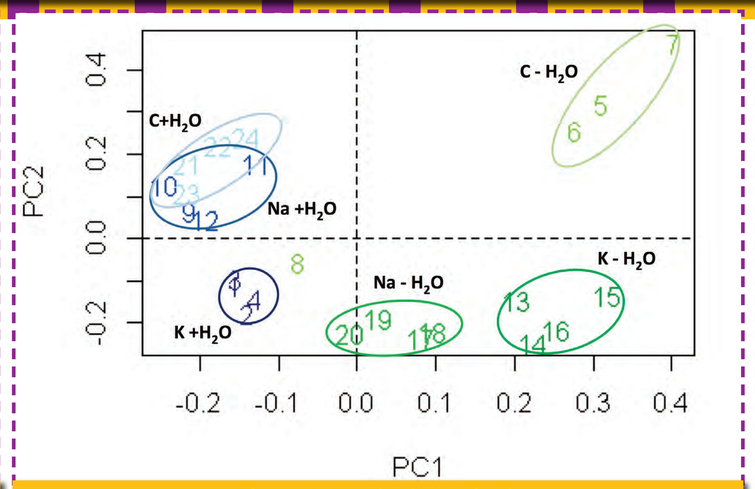

Figure 4: Graph of samples by sPLS-DA on PC1-PC2 plan

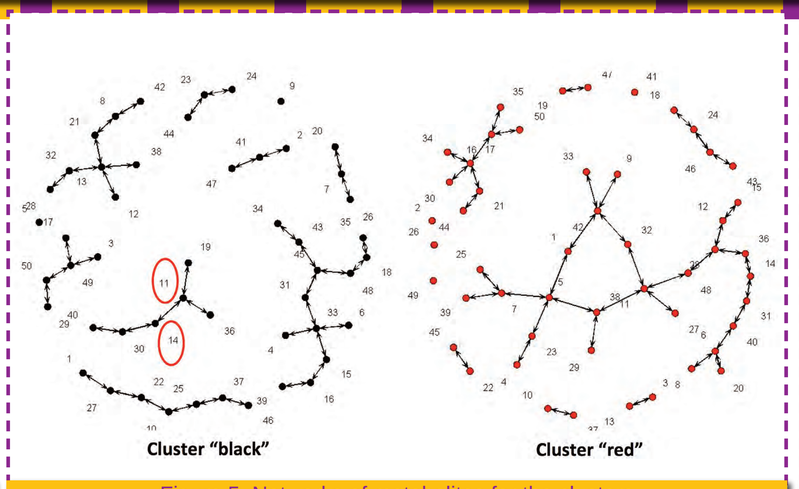

Figure 5: Networks of metabolites for the clusters "black" (PC1) and "red" (PC2) detected by sPLS-DA

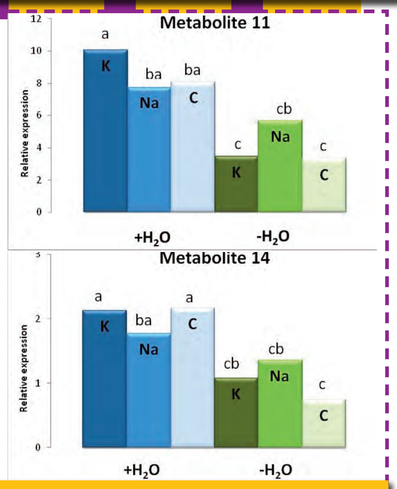

Figure 6: Relative expression for 2 correlated metabolites (cluster "black"). Mean comparison by Tukey test

Conclusions et perspectives

Data analysis of untargeted metabolomic is limited because of metabolite identification unreliability. The study presented here detected leaf metabolite pattern according to water regime and fertilization treatment, and allowed to select 2 groups of significant metabolites. Inside each group, we detected numerous relationships between metabolites (direct correlation), with specific expression profile related to the treatments. Metabolites mapped on the network are expected to be more likely biologically relevant, while "false" metabolites should be distributed randomly [10]. This selection could be useful to identify metabolites more reliably and to develop validation methods. Moreover, the statistical approach applied here could be used for integrative analyses.

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