# GC-TOF-MS Metabolite Profiling of Drought Tolerant *Quercus ilex*

**Ana M. Rodrigues, Jesús Rodríguez-Calcerrada, Luis Gil, Carla António**

## INTRODUCTION

Holm oak (*Quercus ilex* L.) is one of the dominant tree species in natural forest ecosystems of the Western Mediterranean Basin. This species is well adapted to summer droughts but might not be able to cope with future increases in drought severity, duration, and/or frequency as the climate becomes warmer and the water availability decreases [1]. To better understand the mechanisms underlying drought tolerance in *Q. ilex*, a metabolite profiling analysis was performed with gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) using leaves of 2-year-old *Q. ilex* seedlings subjected to increasing drought severity.

## RESULTS & DISCUSSION

### GC-TOF-MS

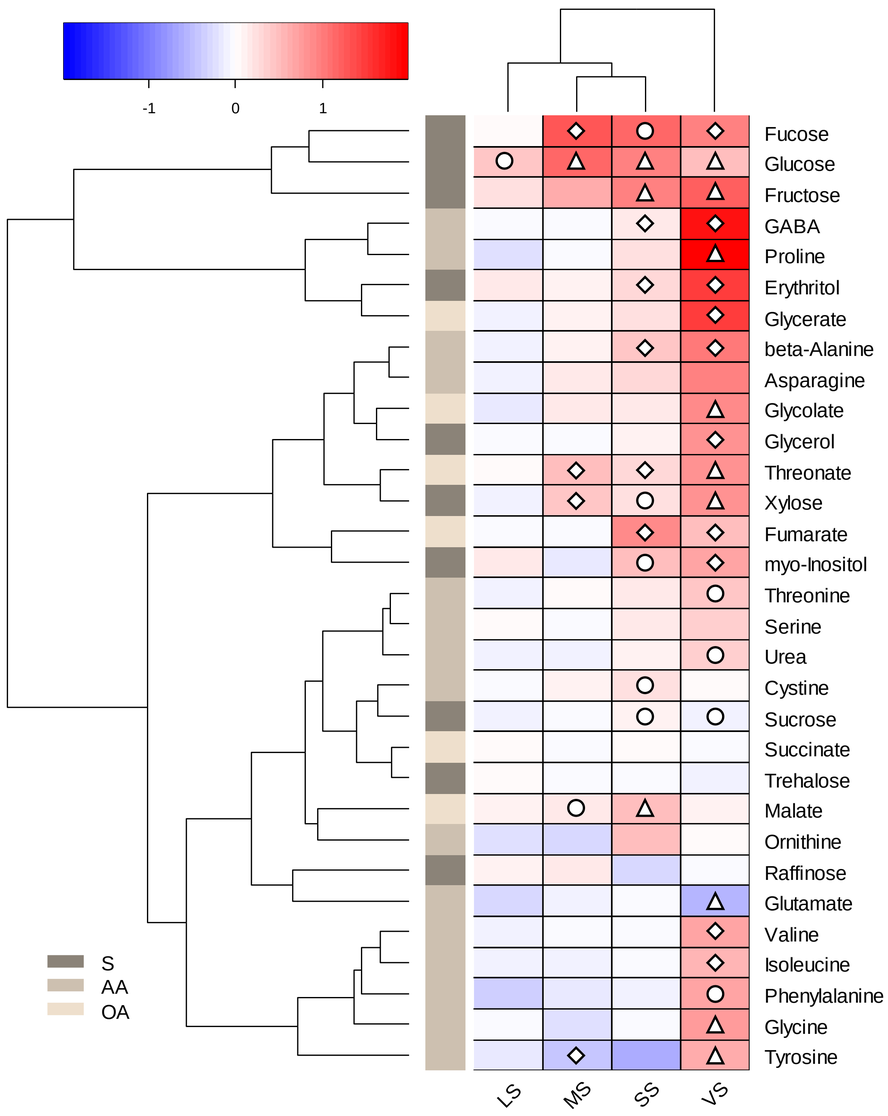


**Fig. 1.** Heat map of fold changes in leaf primary metabolite relative values in *Quercus ilex* 2 year-old seedlings subjected to increasing levels of drought stress (mild, LS; moderate, MS; severe, SS; and very severe, VS) in relation to their well-watered controls. Relative values (as means ± SE of four independent measurements) were normalized to the internal standard (ribitol) and fresh weight of the samples; false color imaging was performed on $\log_{10}$-transformed GC-TOF-MS metabolite data. Values corresponded to means ± SE of four independent measurements. Significant changes using Student's *t* test are indicated as ○ $p < 0.05$; ◊ $p < 0.01$; △ $p < 0.001$, with respect to controls. Metabolites grouped in sugars & sugar alcohols (S), amino acids & derivatives (AA) and organic acids (OA). Heat maps and clustering were performed in R software [3] using the "heatmap.2" function from "gplots" package [4]

- ✓ A set of **31 primary metabolites** was detected in *Q. ilex* leaves.
- ✓ **Amino acids** and derivatives were the most abundant metabolites with a total of 15 chemical species, followed by **sugars** and **sugar-alcohols** (seven and three chemical species, respectively) and six **organic acids**.
- ✓ **Mild water stress** caused most **sugars** and **sugar alcohols** to **increase** through moderate to very severe water stress conditions, suggesting a role of these metabolites in stress signaling and osmoregulation.
- ✓ At **very severe water stress** conditions most **amino acids** dramatically **increased**, especially γ-aminobutyric acid (**GABA**) and **proline**, which have a role in protection against oxidative damage.

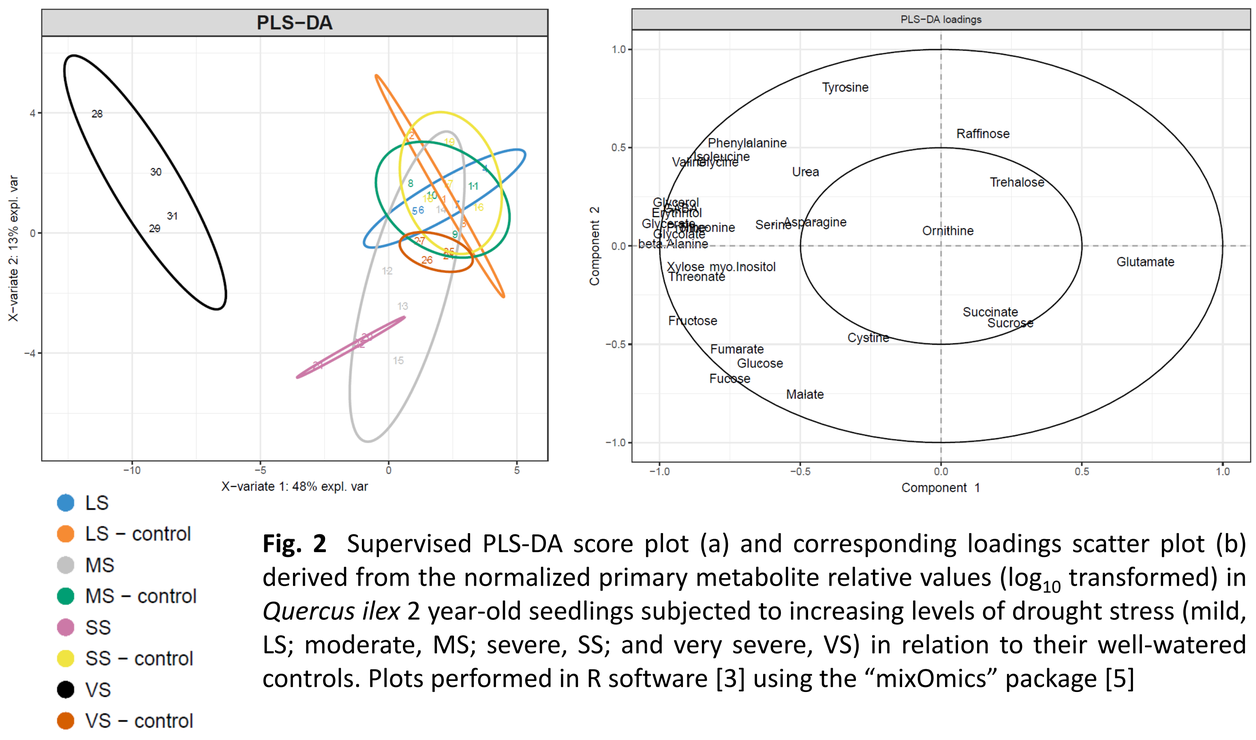


**Fig. 2** Supervised PLS-DA score plot (a) and corresponding loadings scatter plot (b) derived from the normalized primary metabolite relative values ($\log_{10}$ transformed) in *Quercus ilex* 2 year-old seedlings subjected to increasing levels of drought stress (mild, LS; moderate, MS; severe, SS; and very severe, VS) in relation to their well-watered controls. Plots performed in R software [3] using the "mixOmics" package [5]

- ✓ **Very severe stress** group was clearly separated from the others.
- ✓ The characteristics of PLS-DA score plot and respective loadings scatter plot were consistent with the result of cluster analysis in the heat map (Fig. 1).

The high drought tolerance of *Q. ilex* might rely on **early water stress signaling** and **osmoregulation** by hexoses and polyols, and enhanced **protection against oxidative damage** by amino acids at severe water stress. *Q. ilex* has shown mechanisms of acclimation to drought, which can be useful for its persistence under a future drier climate.

## METHODS

- ❑ Leaf sampling from 2 year-old *Q. ilex* seedlings was carried out at mild (**LS**), moderate (**MS**), severe (**SS**) and very severe (**VS**) drought stress conditions. Stress conditions were established as function of predawn ($\Psi_{pd}$) and midday ($\Psi_{md}$) leaf water potential as **LS**: $-0.5 < \Psi_{pd} < -1$ MPa and $-2 < \Psi_{md} < -2.5$ MPa; **MS**: $-1 < \Psi_{pd} < -4$ MPa and $-2.5 < \Psi_{md} < -5$ MPa; **SS**: $-4 < \Psi_{pd} < -7$ MPa and $-5 < \Psi_{md} < -7.5$ MPa; **VS**: $\Psi_{pd} < -7$ MPa and $\Psi_{md} < -7.5$ MPa.
- ❑ Primary metabolites were extracted and analysed using a well-established protocol for metabolite profiling as in [2]. Statistical analyses were performed using R software [3-5].

## REFERENCES

[1] A Barbeta, J Peñuelas, *Plant Ecol Divers* (2016) DOI:10.1080/17550874.2016.1212288
[2] J Lisec, *et al.*, *Nat Protoc*. 1 (2006) 387-396
[3] R Core Team (2016) https://www.R-project.org
[4] GR Warnes, *et al*. (2016) https://CRAN.R-project.org/package=gplots
[5] KA Le Cao, *et al*. (2016) https://CRAN.R-project.org/package=mixOmics

The authors are thankful to Guillermo G. Gordaliza and Meng Li. A.M. Rodrigues acknowledges FCT for the PhD fellowship (PD/BD/114417/2016) and the ITQB NOVA International PhD Programme "Plants for Life" (PD/00035/2013). J. Rodríguez-Calcerrada acknowledges funding by the project OLMOS (AGL2012-35580). C. António acknowledges FCT Investigator Programme (IF/00376/2012/CP0165/CT0003), ITQB NOVA R&D GreenIT 'Bioresources for sustainability' (UID/Multi/04551/2013), and LabMet metabolomics facility at CTBE (Campinas, Brazil) for GC-TOF-MS metabolite profiling services.