Metabolomic responses of Q*uercus ilex* seedlings to wounding simulating herbivory.

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Plants defense themselves against herbivore attack at several levels, being one of them the synthesis of inducible chemical defenses. The study of plant metabolic changes and processes related with herbivore attack has been mostly limited to the identification of single compounds or families of metabolites. We think that an ecometabonomic approach would provide a wider view of the phenomena, since that approach considers the metabolome of the plant, i.e. the total number of metabolites, and their shifts in response to an environmental change or, as in our case, in response to a plant-herbivore interaction [1,2]. We have studied the metabolic changes of plant leaves after a wounding treatment simulating herbivore attack in the Mediterranean sclerophyllous tree *Quercus ilex* by a NMR-based metabolomic study [3]. There is no much reported work regarding the response to wounding in trees by metabolomics. We aimed to discern whether mechanical wound injury in leaves changes its metabolome; for that we have chosen the widespread Mediterranean tree, *Quercus ilex* L. (holm oak), one of the most abundant species in Mediterranean forests.

METHODOLOGY

Plant treatment:

· Control (leaves before clipping) *vs* wounding (leaves from same tree after clipping, i.e. simulation of wounding)

Samples: 2 leaves per each tree (3 trees)

Sample preparation for NMR analyses:

· Extraction of polar and nonpolar metabolites

50% $CHCl_3$ + 50% H_2O/MeOH (1:1)

· Drying

· polar extracts: lyophilization
· non-polar extracts : round-bottom evaporator

· NMR samples preparations (detuerated solvents + internal standard)

· polar extracts samples: D_2O + TSP
· non-polar extracts samples: $CDCl_3$ + TMS

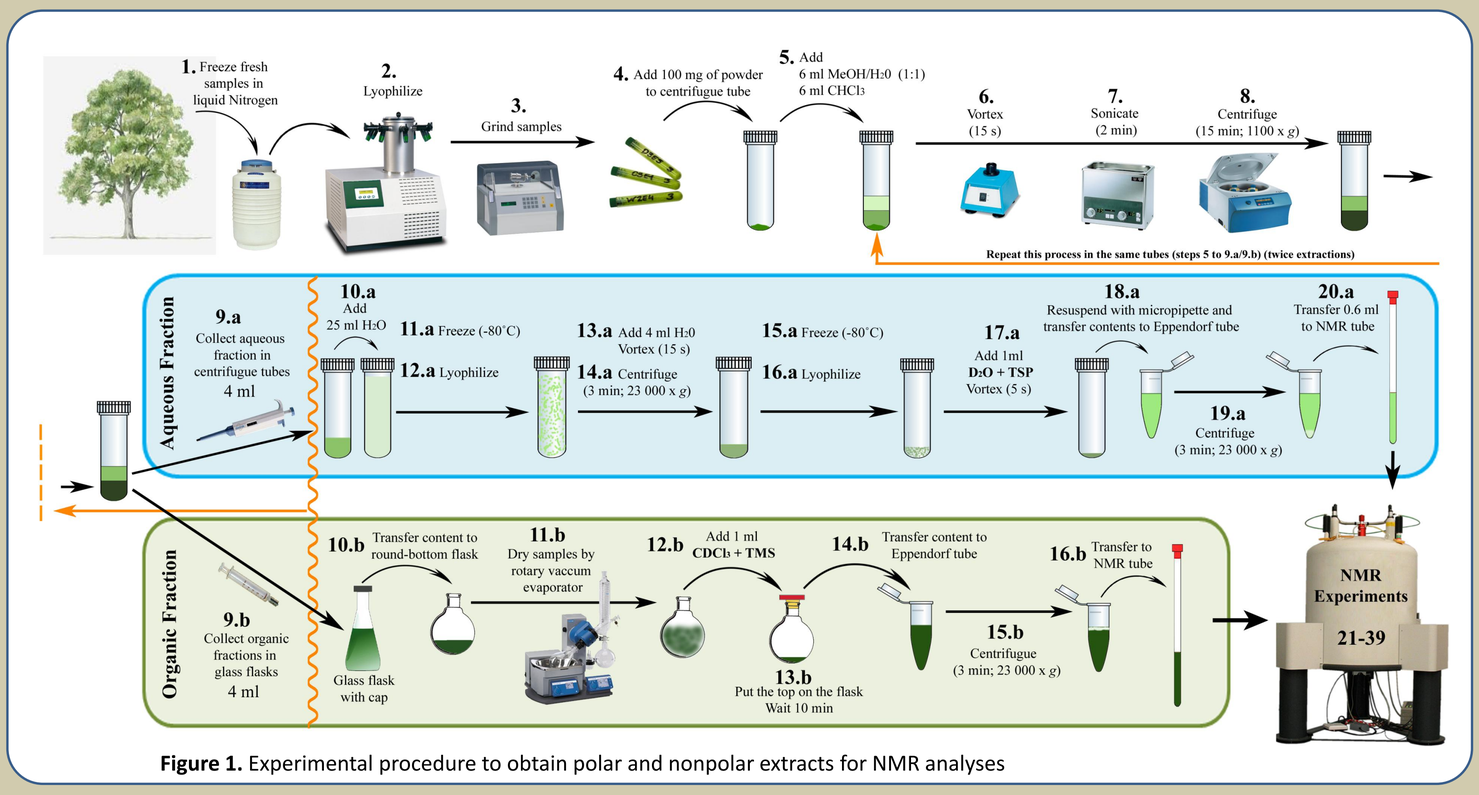

Figure 1. Experimental procedure to obtain polar and nonpolar extracts for NMR analyses

RESULTS & DISCUSSION

Results obtained showed an increase of the concentration of glucose, which is related to higher levels of photosynthetic assimilation rates (increase of 19%) and in the actual photosynthetic efficiency in wounded leaves. That suggests and increase of carbon uptake after wounding.

Lower concentrations of leucine and isoleucine have been observed in wounded leaves compared to control, which presented a higher asparagine concentration. Asparagine is a general nitrogen-transporter and has a key role in transamination process [4]. Thus, results suggest a shift of nitrogen (N) from isoleucine and leucine to asparagine and choline. The increase in choline concentration in wounded leaves is related to the increase in the synthesis of membrane components after membrane injure [5]. Previous studies in *Arabidopsis thaliana* observed that wounding induces choline synthesis by the jasmonic acid signal transduction pathway [6]. That effect has been observed not only in the wounding site but also in the undamaged area of wounded leaves [7]. The accumulation of N-rich soluble molecules has been suggested as a mechanism to store N and C after wounding, helping to prevent N losses during the wound response [8]. Thereafter, these wound-induced reserves could serve as a source for a new growth after wound-recovery phase.

This study also confirms that quinic acid and quercitol are present in high concentrations in wounded leaves of genus *Quercus* plants. Acid quinic is a metabolite related to metabolic response (inducible defense) to biotic stress [9].

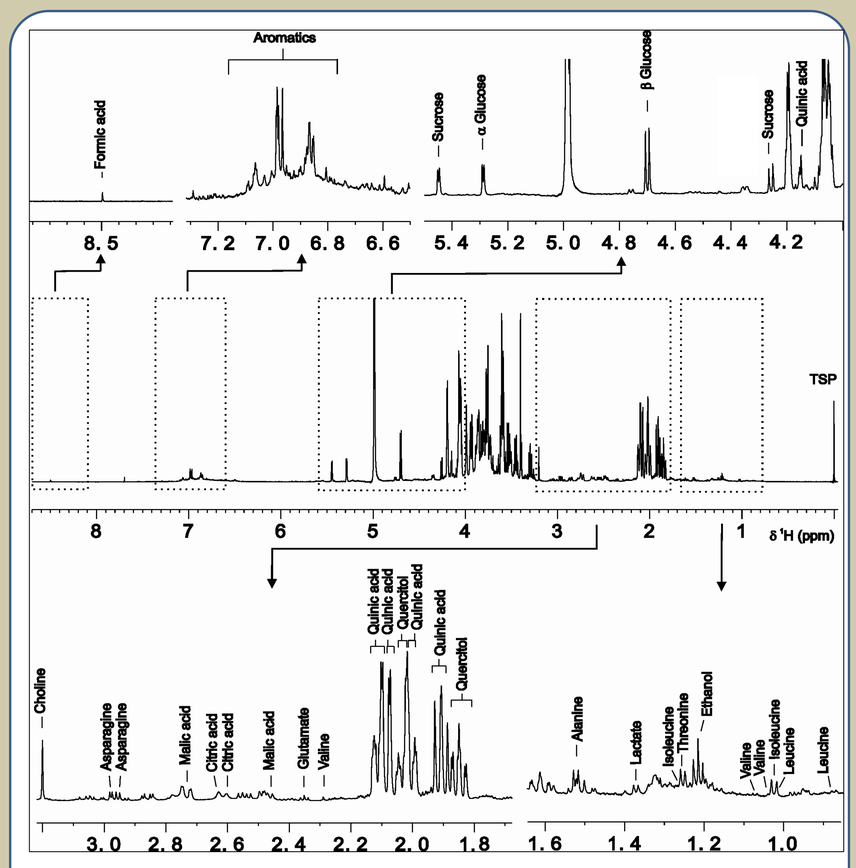

Figure 2. Example of the ^{1}H NMR metabolic profile of the polar (water–methanol 1:1) extract sample of *Q. ilex* leaf. Assignments of the main peaks are indicated. The sample was dissolved in CD_3OD-D_2O 1:1 (pH 6.0) and referenced to TSP.

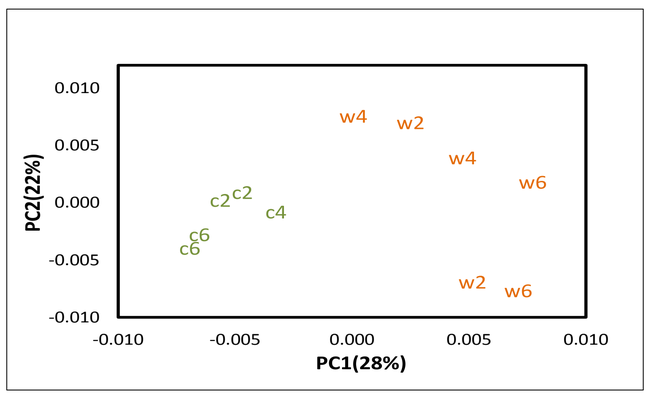

Figure 3. Biplot of the second principal component (PC2) versus the first principal component (PC1) scores resulting from PCA conducted with ^{1}H NMR metabolomic variables from polar extracts of *Q. ilex* leaves.

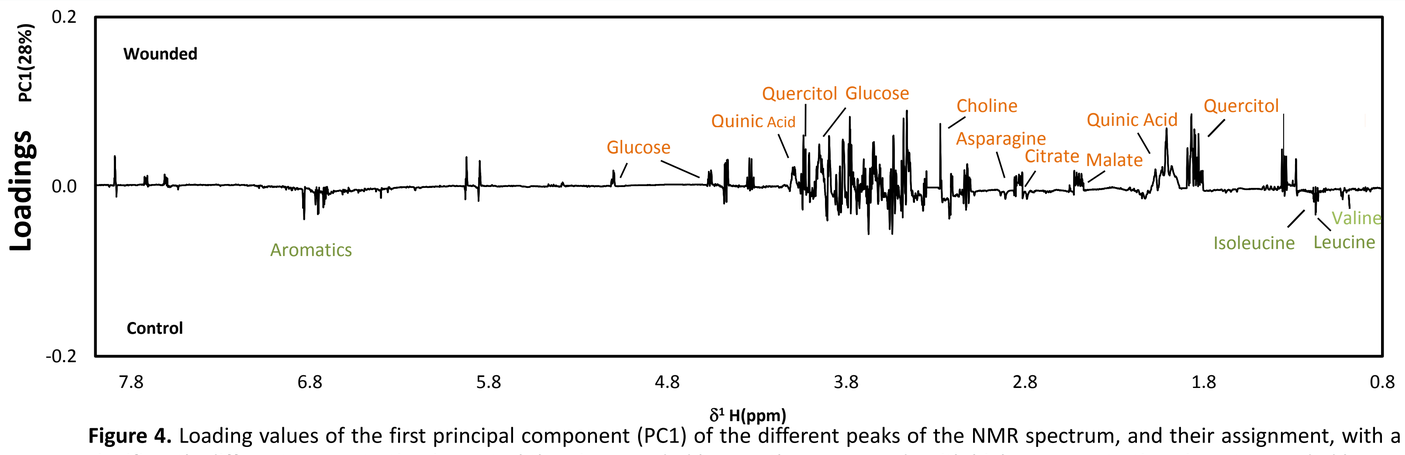

Figure 4. Loading values of the first principal component (PC1) of the different peaks of the NMR spectrum, and their assignment, with a significantly different concentration in control than in wounded leaves; the compounds with higher concentrations in not wounded leaves (control) are green colored and those with higher concentrations in wounded leaves are red colored.

CONCLUSIONS

Results show that the metabolome of *Quercus ilex* shifted after wounding presenting a significant increase in C-rich secondary metabolites quinic acid and quercitol, which are related to the shikimic acid metabolic pathway [2]. An increase in photosynthetic activity, related to a glucose production, and a shift in N from leucine and isoleucine to asparagine and choline (involved in mechanisms against biotic stress such as membrane reparation and N conservation) were observed. Results also states the suitability of ^{1}H NMR-based metabolic profiling to detect and study the global metabolome shifts after a biotic stress in tree leaves.

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