ECOMETABOLOMIC STUDY OF PLANT SHOOTS/ROOTS RESPONSES TO DROUGHT

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Ecometabolomics aims to analyse the metabolome of the plant, i.e. the complete set of metabolites, and their shifts in response to environmental changes [1,2]. Ecometabolomics has recently been used to monitor the phenotypic changes of a particular genotype in response to the drivers of global change, particularly shifts in temperature [3,4,5]. When a stress treatment - or different stresses occurring simultaneously like in real field conditions - is applied to a plant, plant organs can responds differently. Shoots are autotrophic and roots heterotrophic organs of plants with different physiological functions. Roots and shoots can responds asymmetrically, as has been observed at the morphological level, e.g. shifts in the shoot/root biomass and growth-rate ratios occur when the availability of soil water changes [6]. Shoots essentially have a photosynthetic function, whereas roots take up water and nutrients. Shoots and roots may thus compete for the resources that a plant acquires. The responses of plants to drought are crucial because drought is one of the most important environmental stressors for plants related to climate change[7]. Do plant shoots and roots have different metabolomes? Do their metabolisms respond differently to environmental changes such as drought? We used metabolomics and elemental analyses to answer these questions.

METHODOLOGY

Experimental design:
Samples from the cases (2 species × 2 organs (leaf blades and fine roots) × 2 sampling dates (July and September) × 3 precipitation manipulations × 15 plots) -> 400 individuals (shoots and roots)

Sample preparation (Fig. 1)

Chemical Analysis:
1D 1H NMR [600.13 MHz; 298 K]
HPLC-MS [ESI (±), Orbitrap]
Elemental Analysis [ICP/OES, Elemental Analysier]

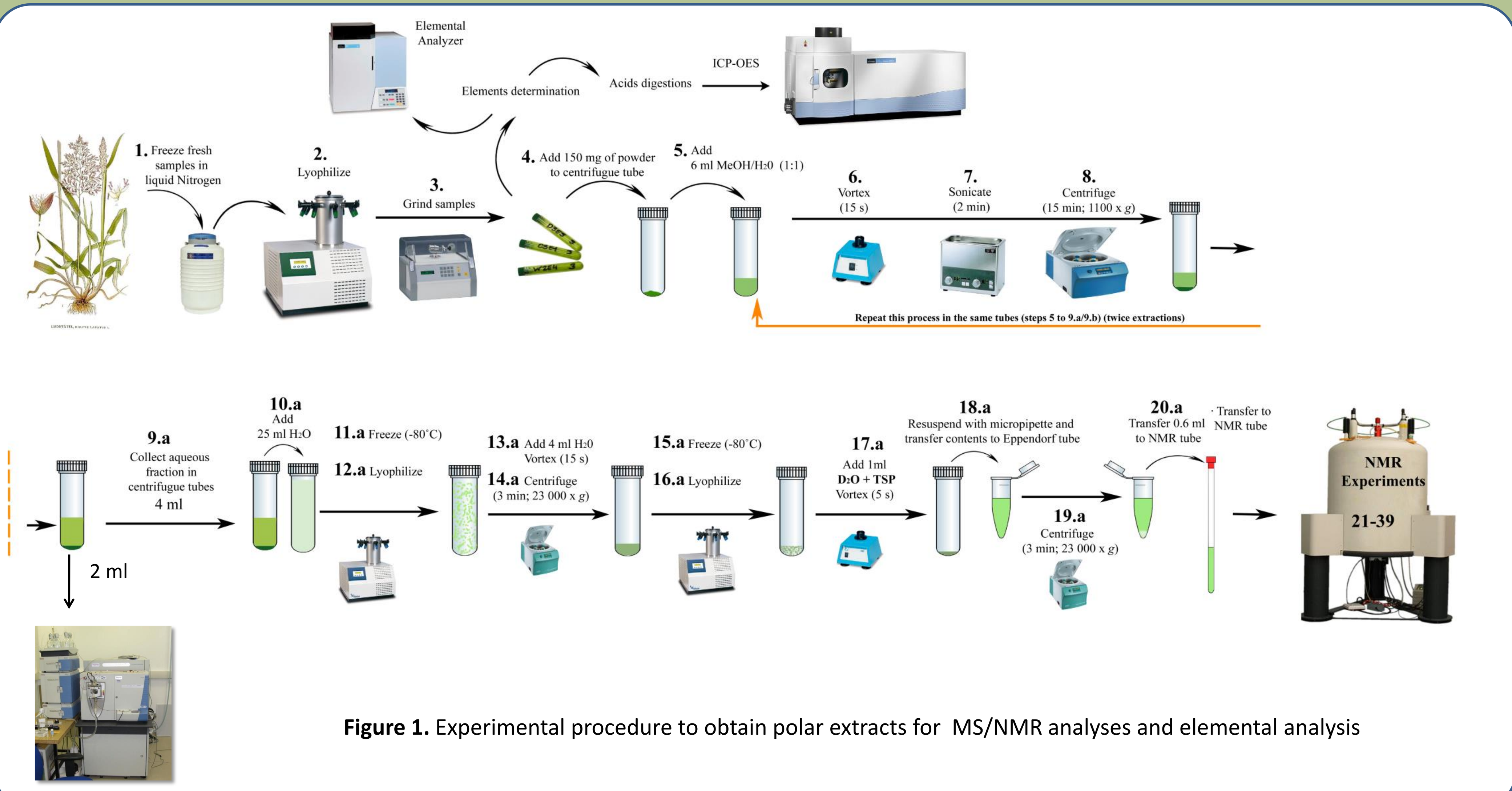

Figure 1. Experimental procedure to obtain polar extracts for MS/NMR analyses and elemental analysis

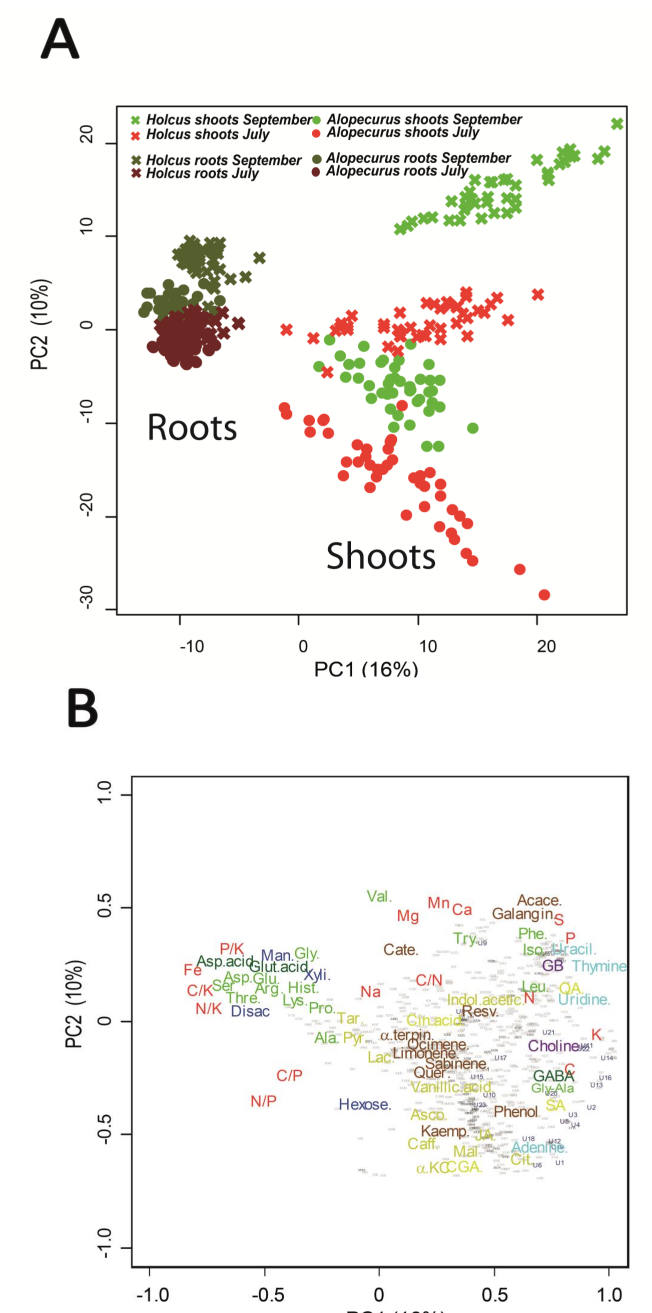

Figure 2. Plots of cases and variables in the PCA conducted with the elemental, stoichiometric and metabolomic variables in *Holcus lanatus* and *Alopecurus pratensis* using PC1 versus PC2. ((A) The cases are categorised by season and organ. Seasons are indicated by different colours (green, September; red, July). The two species are indicated by geometric figures (circles, *A. pratensis*; crosses, *H. lanatus*). Dark green and red colours are for roots, and light green and red colours are for shoots. (B) Loadings of the various elemental stoichiometric and metabolomic variables in PC1 and PC2.

RESULTS & DISCUSSION

- Differences between the two species and between the two seasons of sampling (July and September)
- Differences between shoots and roots at both the metabolic and elemental concentration levels (Fig. 2).
- The most significant changes in metabolomic structure were between shoots and roots, which were separated along the first PC axis (Fig. 2).
- The variability of the metabolome was lower in root samples than in shoot samples (Fig. 2).
- The metabolome and stoichiometry of shoots and roots of both plant species responded to drought in opposite ways (Figs. 3).
- The concentrations of choline and glycine betaine, which are involved in osmotic protection[8], and of gamma-aminobutyric acid and primary metabolites decreased in the shoots, and terpenes and metabolites related to anti-stress mechanisms increased in shoots under drought (Fig. 3).

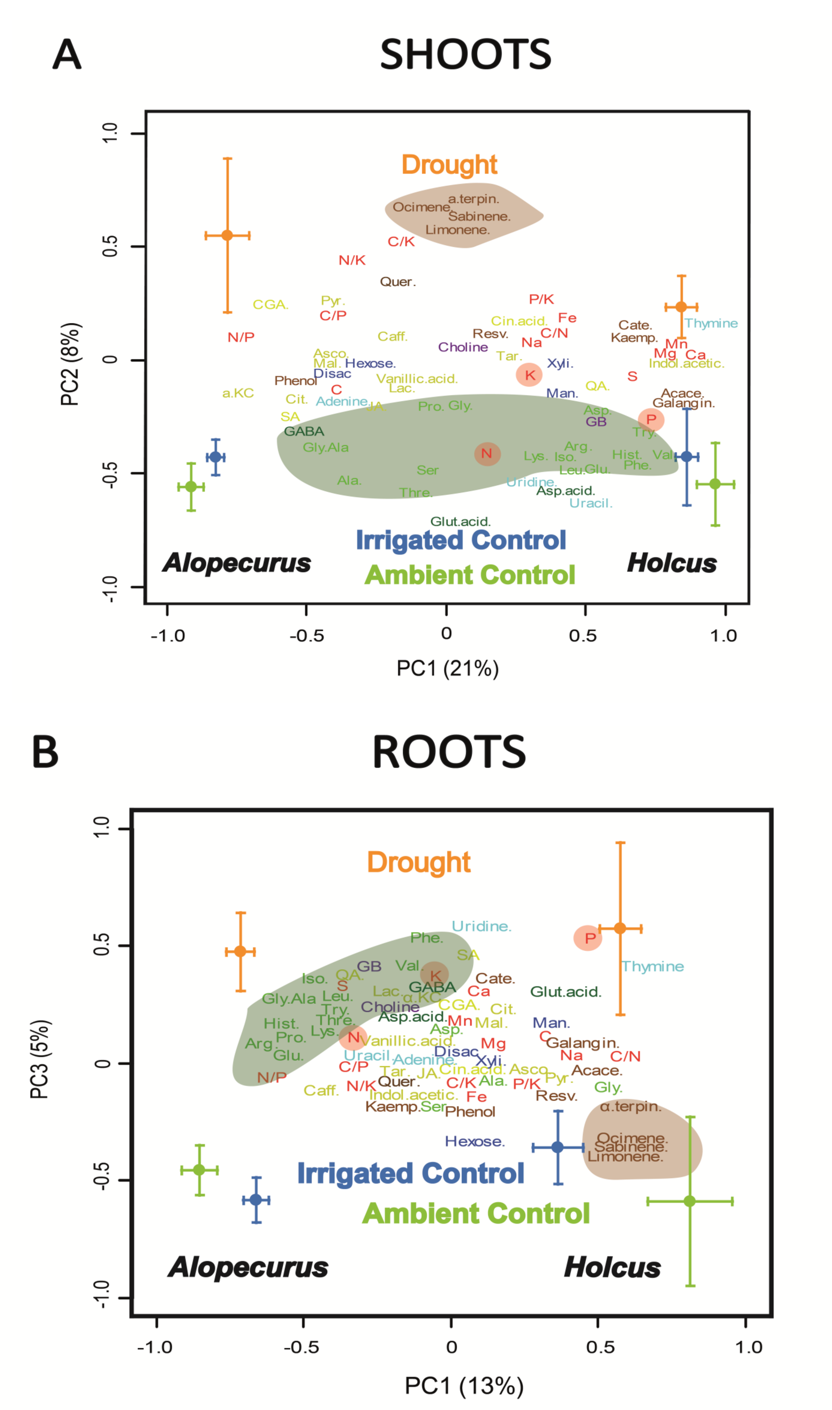

Figure 3. Plots of cases and variables in the PCAs conducted with the elemental, stoichiometric, and metabolomic variables in plants sampled in September. (A) Plot of cases and variables for shoots. (B) Plot of cases and variables for roots.

CONCLUSIONS

- Plants have a high capacity to modulate and vary the allocation of nutrients and the relative activities of different metabolic pathways for producing biomass in both shoots and roots.
- Under drought conditions, the plasticity of the plants allow a shift to increased synthesis or allocation of several primary metabolites, N, P, and K to roots while decreasing allocation to shoots. When water is a limiting factor, metabolites involved with energy production and growth (especially sugars and amino acids) are shifted from shoots to roots.
- The simultaneous ecometabolomic analysis of roots and shoots can provide a complete view of the entire plant, including the response of different organs to environmental changes, the global phenotypic response, and the metabolic mechanisms underlying these responses.
- Shoots and roots have different metabolomes and nutrient concentrations, the shoot metabolome is much more variable than the root metabolome, and roots and shoots respond to drought with opposite metabolic changes.

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