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Melisande Blein-Nicolas, Sandra Negro, Thierry Balliau, Claude Welcker, Llorenç Cabrera-Bosquet, et al.. Genome wide association study for protein expression under normal and water deficit conditions in maize leaves. Spectrométrie de Masse, Métabolomique et Analyse Protéomique (SMMAP) 2017, Oct 2017, Marne-la-Vallée, France. . hal-03879943

**HAL Id: hal-03879943**

**<https://hal.science/hal-03879943>**

Submitted on 30 Nov 2022

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# Genome wide association study for protein expression under normal and water deficit conditions in maize leaves

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## Objective and strategy

Maize is one of the main crops worldwide (160 Mha, 753 Mt/year). But its yield can be severely affected by drought, particularly at flowering. Drought tolerance in maize is genotype-dependent, indicating that this trait can be genetically improved. To decipher the molecular basis for drought tolerance, we used a systems genetics approach whereby causal variants of traits of interest and their molecular effects were inferred through integration of genomic data with proteomic data in a context of natural diversity (Fig. 1).

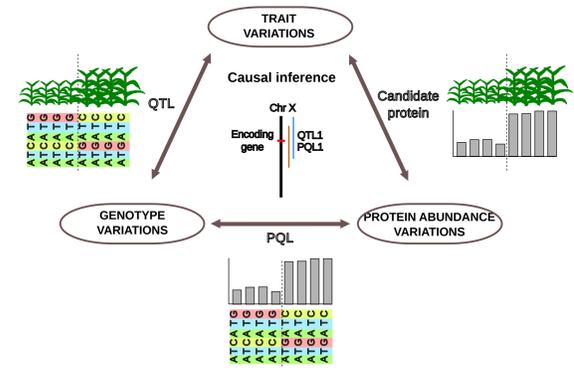


Fig. 1: Schematic representation of the systems genetics approach

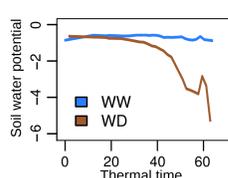
## Experimental pipeline



251 genotypes grown in PhenoArch (Montpellier) (2 conditions x 2 replicates)



1004 leaf samples taken at the pre-flowering stage



Ecophysiological parameters measured during plant growth



Shotgun proteomics analysis using a QExactive (Thermo)



Protein identification and inference using X!TandemPipeline [1]



Peptide quantification using MassChroQ [2]



GWAS using FaST-LMM [3] (758,860 SNPs tested)



Data analyses using R

## Water deficit has deeply impacted regulation of protein expression

- A total of 1950 proteins were quantified in the two watering conditions (973 by XIC, 977 by peak counting). Of them, 70% responded significantly to water deficit indicating that environmental change deeply impacted the proteome (Fig. 2).

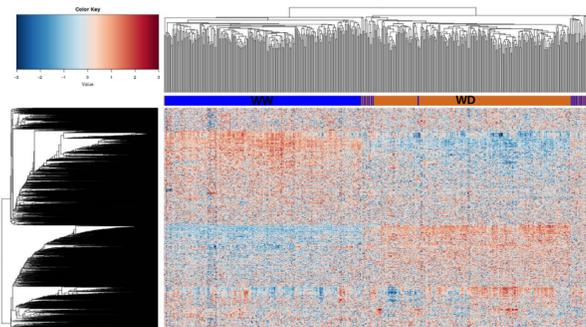


Fig 2: Heatmap representation of protein abundances showing that the proteome was deeply remodeled in response to water deficit. Each line corresponds to a protein and each column to a genotype x environment combination. For each protein, abundance values were scaled and represented by a color code as indicated by the color-key bar

- GWAS was performed for 3900 molecular phenotypes (1950 proteins x 2 environments). A total of 27151 pQTLs were detected for 95% of the molecular phenotypes (13716 in WD; 13435 in WW). Less than 10% of the pQTLs were conserved between the two environments, indicating that water deficit has deeply impacted the regulation of protein expression (Fig. 3).

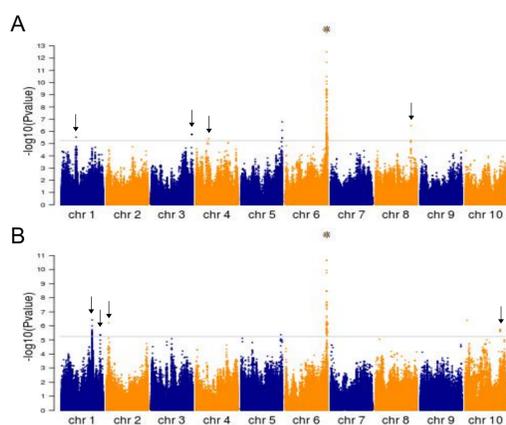


Fig 3: Manhattan plot showing the pQTLs detected for a protein in the WW (A) and WD (B) conditions. Black arrows indicate pQTLs detected in only one of the two conditions. Stars indicate the position of the protein encoding gene

## Co-regulation networks are partially preserved between environments

- Co-expression networks were built from protein abundances in each environment, showing that stress proteins were co-expressed specifically under WD. In both environments, modules correlated to traits of interest were identified (Fig. 4).

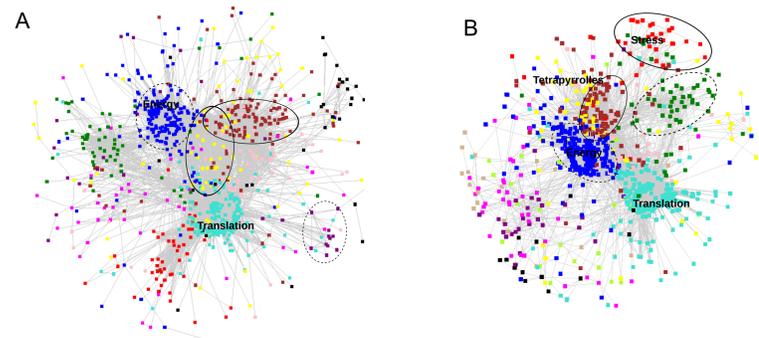


Fig. 4: Representation of the co-expression networks in WW (A) and WW (B). Several modules were significantly enriched for specific functional categories. Modules positively or negatively correlated to plant phenotypes such as biomass or leaf area are indicated by plain and dotted circles.

- Genomic regions controlling the abundances of more than 20 proteins were identified, representing potential hotspots of regulation. Several of these regions colocalized with QTL for co-expression modules indicating that the proteins within these modules are co-regulated. Regions controlling the abundance of ribosomal proteins were detected in both environments. These regions were not the same in the two conditions indicating that regulation of translation is affected by water deficit (Fig. 5).

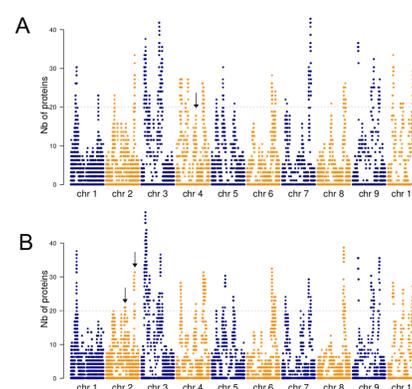


Fig. 5: Detection of hotspots of regulation in WW (A) and WD (B). Black arrows indicate genomic regions controlling the abundances of ribosomal proteins.

## Conclusions

Analyzing a thousand samples in proteomics: it is doable! Nearly 2000 proteins were quantified, hundreds of them were affected by mild water deficit and many of them showed genetically variable responses. pQTLs were detected for most proteins confirming the high genetic variability of their abundances. Altogether, these results show the potential of high-throughput quantitative proteomics to decipher the determinants of protein abundance regulation and to discover candidate genes and proteins potentially involved in the variation of plant phenotypic traits.