



Statistical inference of a gene regulatory network in *Arabidopsis* under the combination of climate change and nutritional starvations

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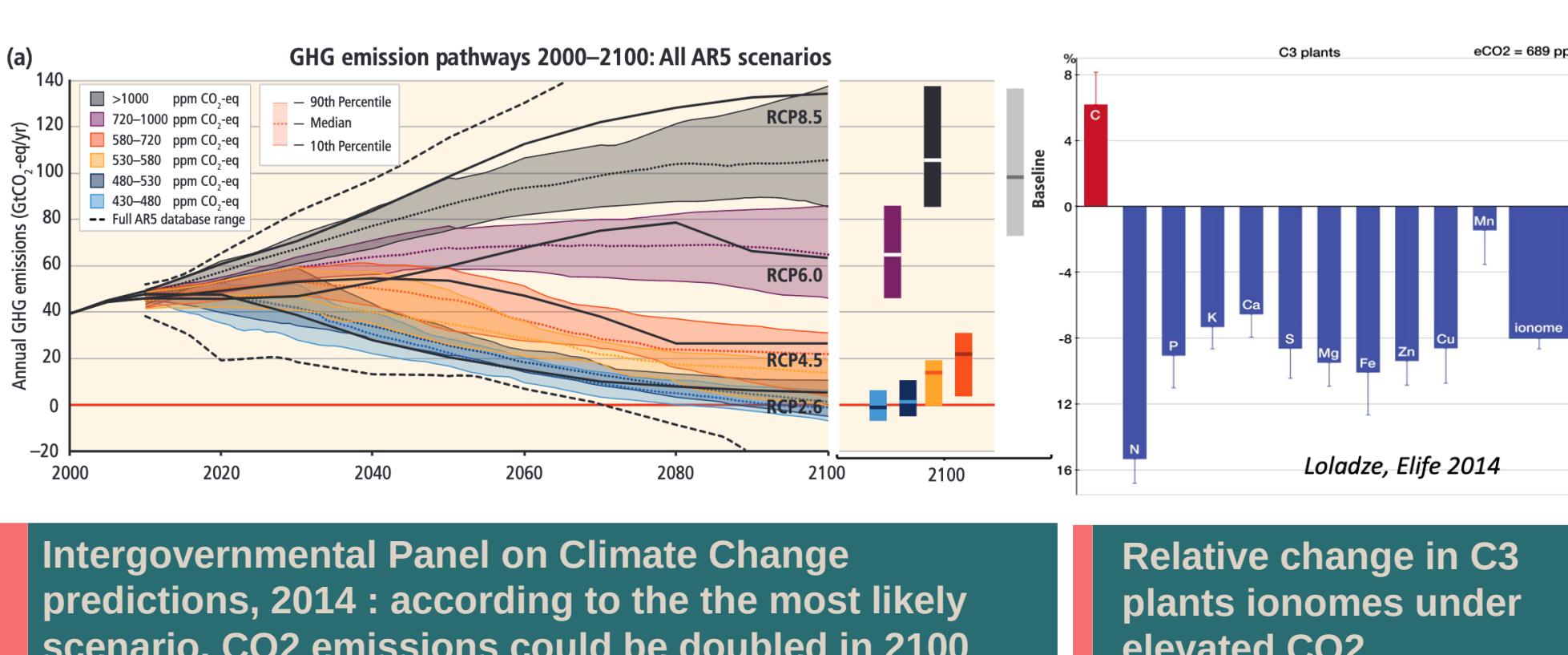
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01. Elevation of CO₂ levels : a major threat to human health

ANTHROPOIC CO₂ EMISSIONS AS A CHALLENGE TO AGROECOLOGY

Two main deleterious repercussions of atmospheric CO₂ elevation on plants:

- CO₂ acclimation : biomass increase is lower than predicted^[1]
- Depletion of the content of most nutrients in crops, deteriorating their nutritional value^[2]. This exposes populations relying on primary agriculture to deficiencies in the future, and threatens world-wide food security.^[3]



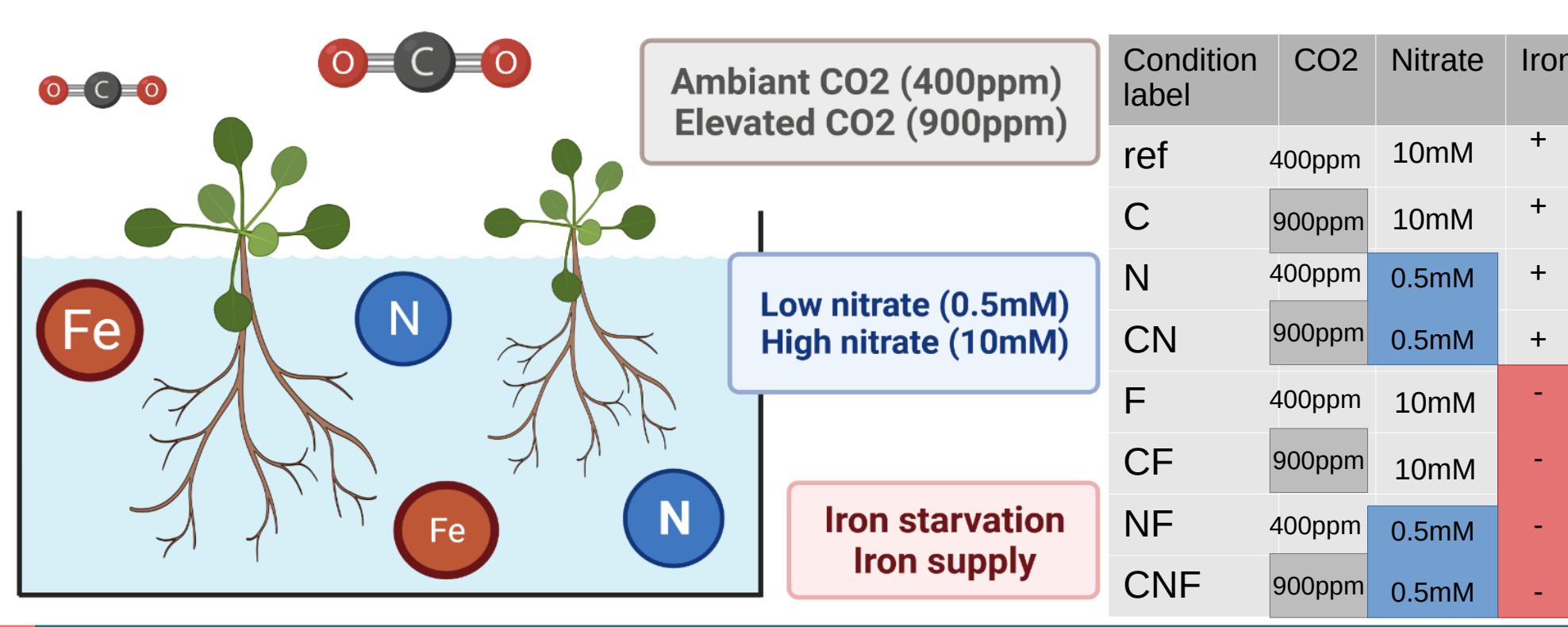
UNDERSTANDING REGULATORY MECHANISMS INVOLVED IN MINERAL STATUS DEPLETION

- CO₂ and mineral nutrition maintain a complex interplay^[4,5,8], but the molecular processes driving them remain unclear.
- Genetic variation was observed in the response of the mineral status to elevated CO₂^[6], but no key regulator genes have been discovered yet
- We present here an experimental design and a panel of statistical analyses to uncover pathways orchestrating nutrient uptake in roots under high CO₂, in a context of sustainable agriculture with low inputs.

02. Combinatorial dataset

EXPERIMENTAL DESIGN OF A TRANSCRIPTOMIC DATASET

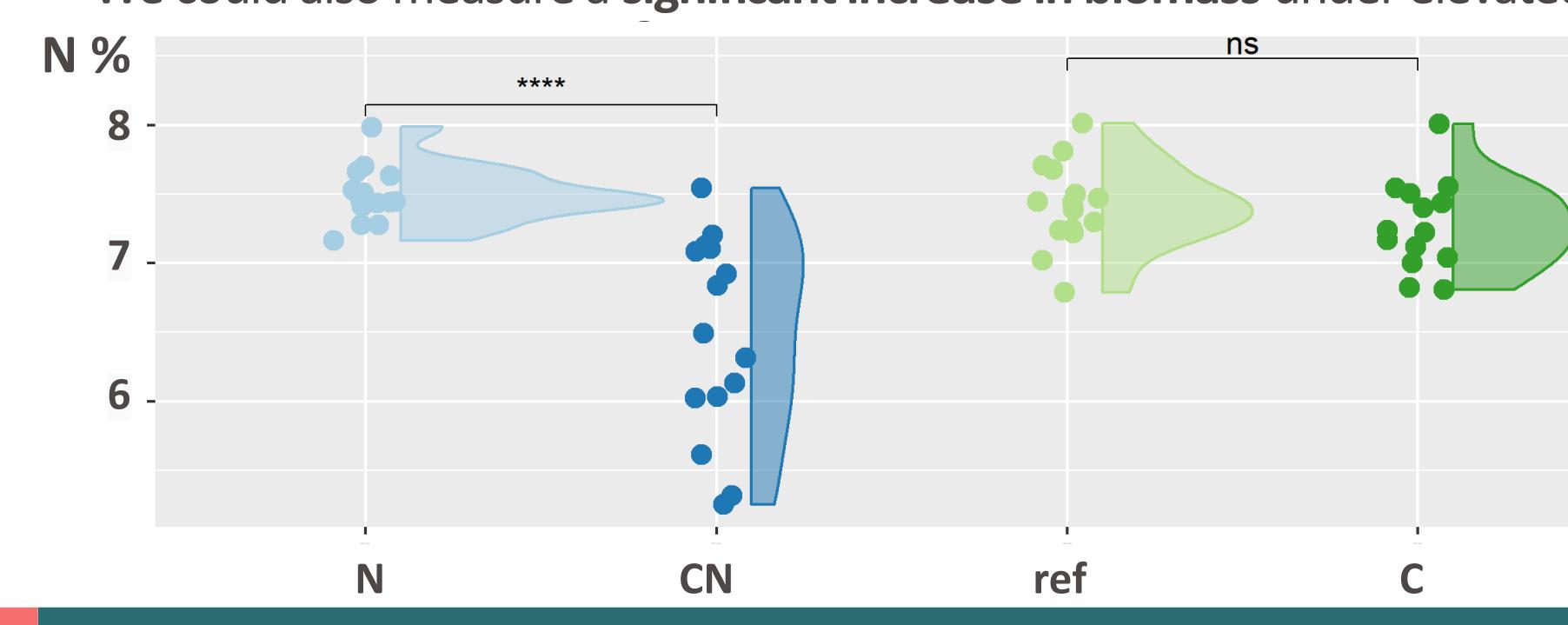
Arabidopsis plants were exposed to elevated CO₂, low nitrate conditions and iron starvation in a combinatorial fashion.



Schematic view of the combinatorial hydroponic experiment and conditions labels

PHENOTYPIC OBSERVATION OF NITROGEN STATUS DEPLETION

- Significant decrease in nitrogen percentage under elevated CO₂ in foliar tissues, when nitrate nutrition is low (N vs CN).
- We could also measure a significant increase in biomass under elevated CO₂

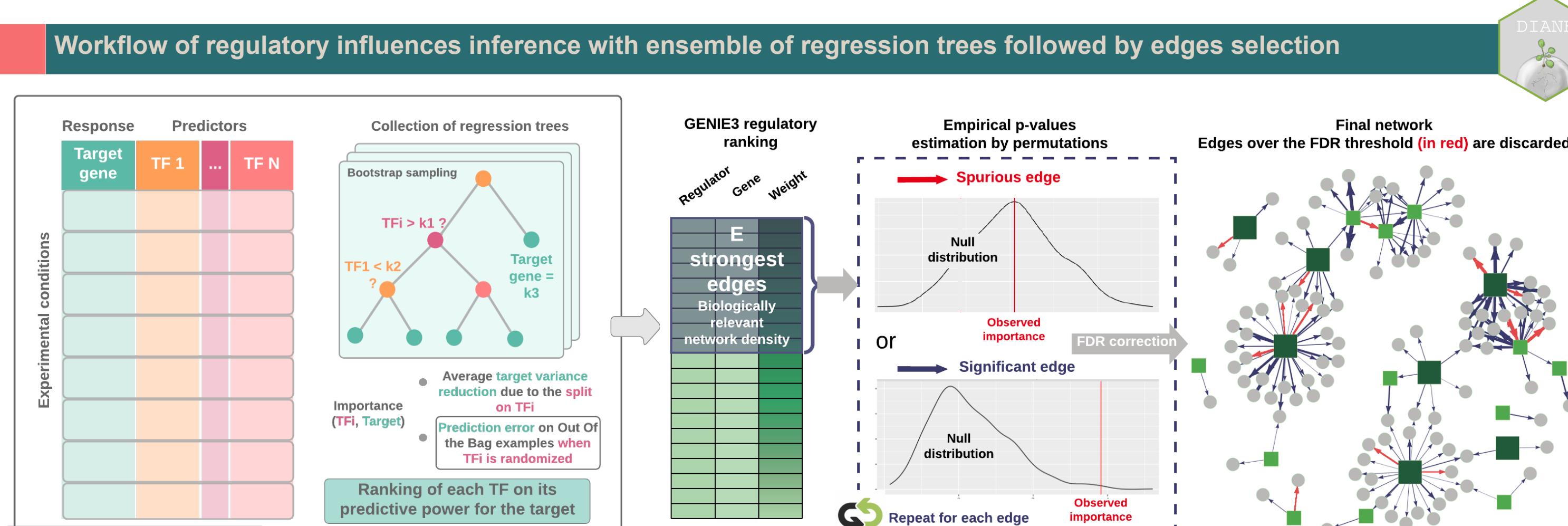


04. Network inference method

HOW CAN WE RECONSTRUCT REGULATORY INTERACTIONS FROM RNA-SEQ EXPRESSION PROFILES ?

The chosen approach, GENIE3, uses Random Forests to infer oriented edges from regulators to targets, weighted by the predictive power of each regulator on the target gene expression.

To select statistically significant pairs in the final network, we designed a procedure of empirical tests on regulatory edges using permutations on Random Forests importance metrics.



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03. Elevated CO₂ deregulates the response to nutritional starvations: clustering and network inference

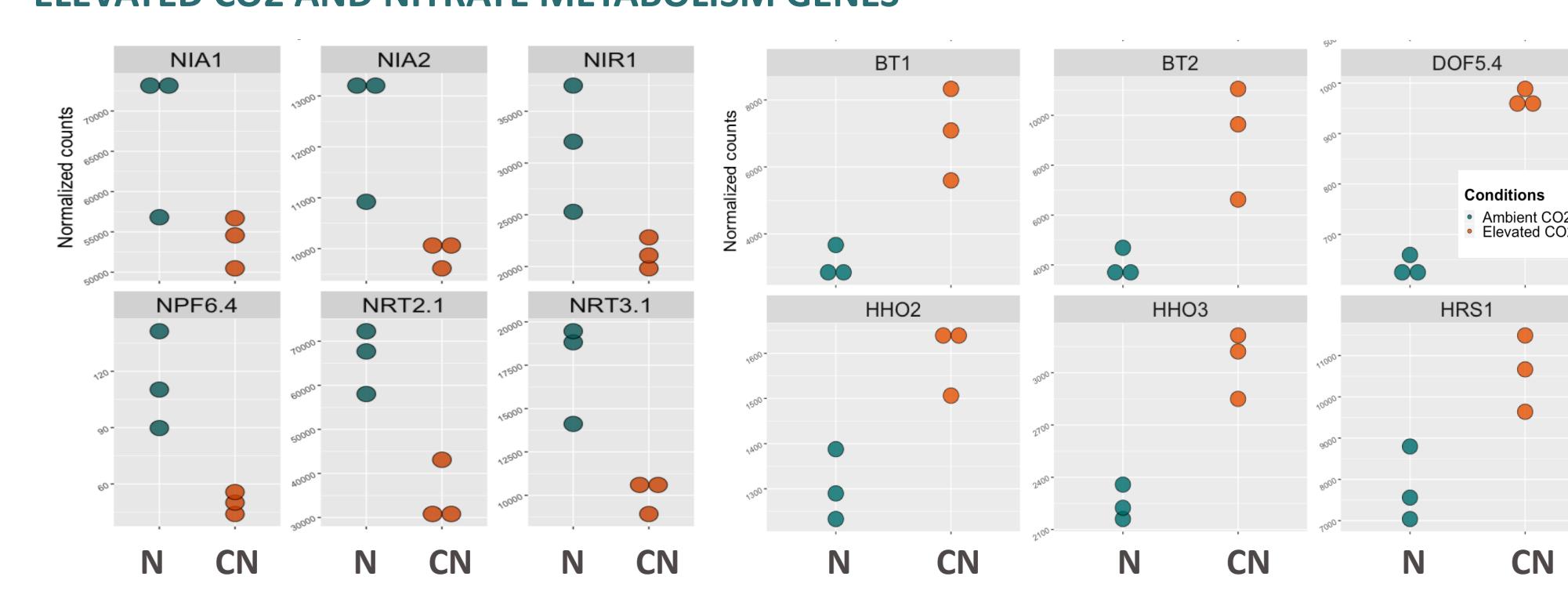
GLOBAL TRANSCRIPTOME ANALYSIS

Differential expression analyses were carried out to quantify the impact of each simple and combined perturbation on gene expression

MAIN OBSERVATIONS

- Iron starvation is the most influent perturbation on gene expression
- The effect of CO₂ is very slim when there is no nutritional starvation
- We chose to focus on the CN vs N genes, that are the genes differentially expressed by elevated CO₂ when the plant are, in both transcriptomes, under low nitrate conditions.

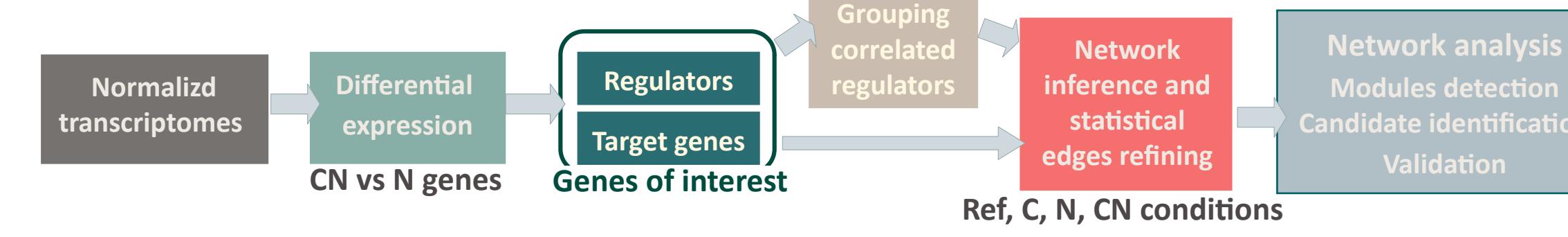
ELEVATED CO₂ AND NITRATE METABOLISM GENES



GENE REGULATORY NETWORK OF THE RESPONSE TO ELEVATED CO₂ UNDER LOW NITRATE

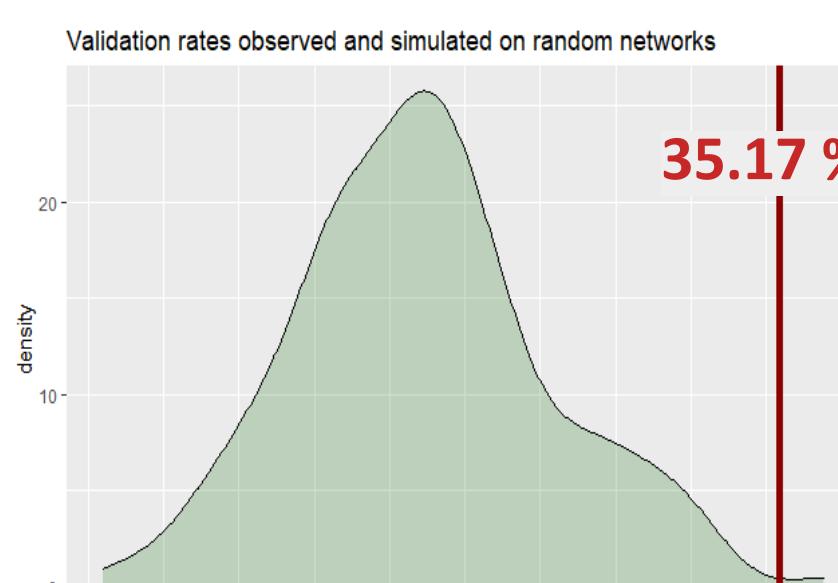
Machine learning techniques described in section 04 were used on the CN vs N genes, to infer transcriptional dependencies between regulators and targets based on their expression profiles across perturbations of nitrate and CO₂

NETWORK RECONSTRUCTION PIPELINE



NETWORK ENRICHMENT AND VALIDATION

- Known associations between nitrate transport genes and some of their regulators (BT1-BT2-HRS1, NRT2.1-NAR2.1...) were found.
- Inference was validated with connectTF^[7], a recent database of regulatory interactions from in vitro and in vivo binding, as well as in planta regulation experiments.



IDENTIFICATION OF CANDIDATE REGULATORS

The top 25 % of most connected regulators were enriched in GO terms linked to gene expression regulation, negative regulation of nitrate metabolism, and root system plasticity.

The most connected genes are potential key regulators, 10 of them are currently under root system and shoots mineral status phenotyping.



05. Conclusions

• There are clear interactions between high atmospheric CO₂ and nutritional starvations

• The phenotypic and transcriptomic effect of elevated CO₂ is almost canceled when plants grow in nutrient-rich media. This supports previous findings in the literature on CO₂ acclimation, lifted by nitrate rich nutrition^[8].

• Disrupted root architecture and nutritional strategies could explain the degradation of nutritional value in plants under climate change

• Experimentally testing candidate genes for their implication in deleterious phenotypic response under high CO₂ is a promising lead for crops improvement in the future.

PERSPECTIVES

- Studying shoot transcriptomes to understand CO₂ effect on photosynthesis efficiency, and suspected shoot to root signals
- Time series under CO₂ exposure could reveal more causal gene interactions and the dynamic of regulations
- GWAS studies could reveal genomic variants influencing plant mineral status under elevated CO₂ and new candidate genes

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