

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

Océane Cassan¹, Sophie Lèbre^{2,3}, Antoine Martin¹

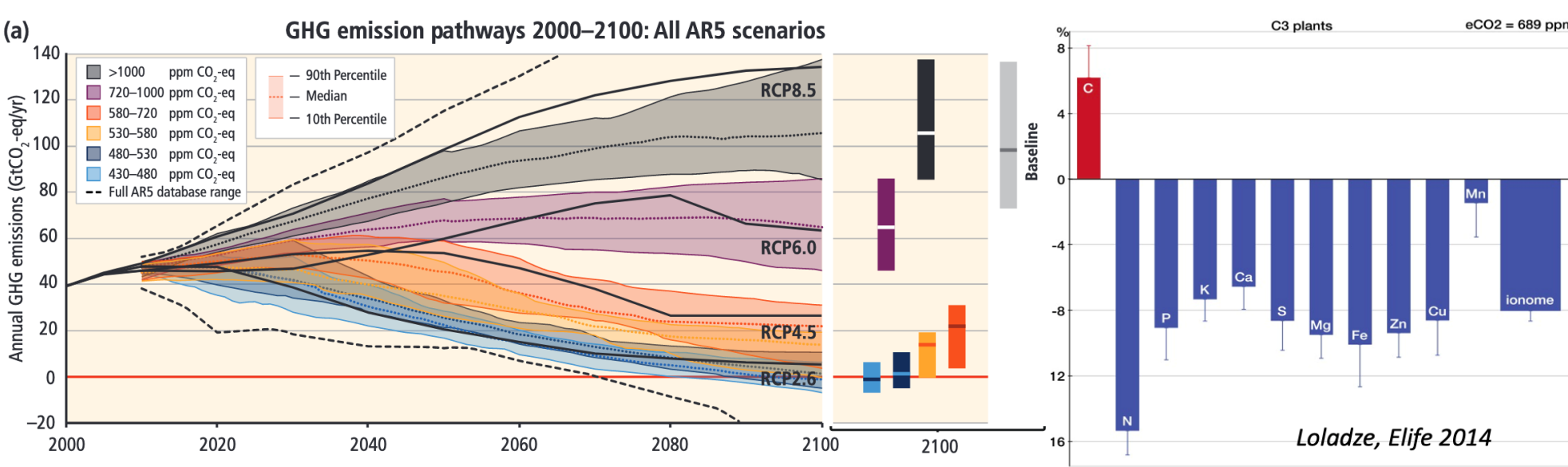
EMBO workshop, April 25 & 26 2021

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

ANTHROPIC 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]



Intergovernmental Panel on Climate Change predictions, 2014 : according to the most likely scenario, CO2 emissions could be doubled in 2100

Relative change in C3 plants ionomes under elevated CO2

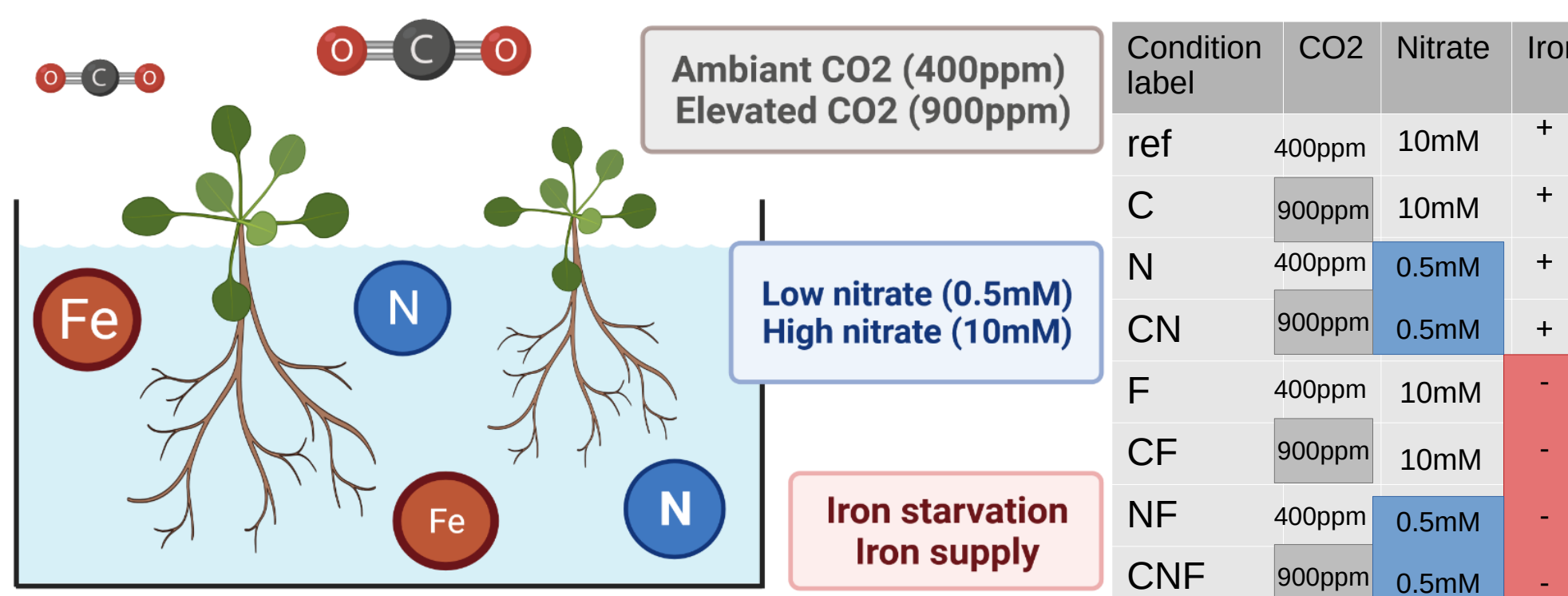
UNDERSTANDING REGULATORY MECHANISMS INVOLVED IN MINERAL STATUS DEPLETION

- 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

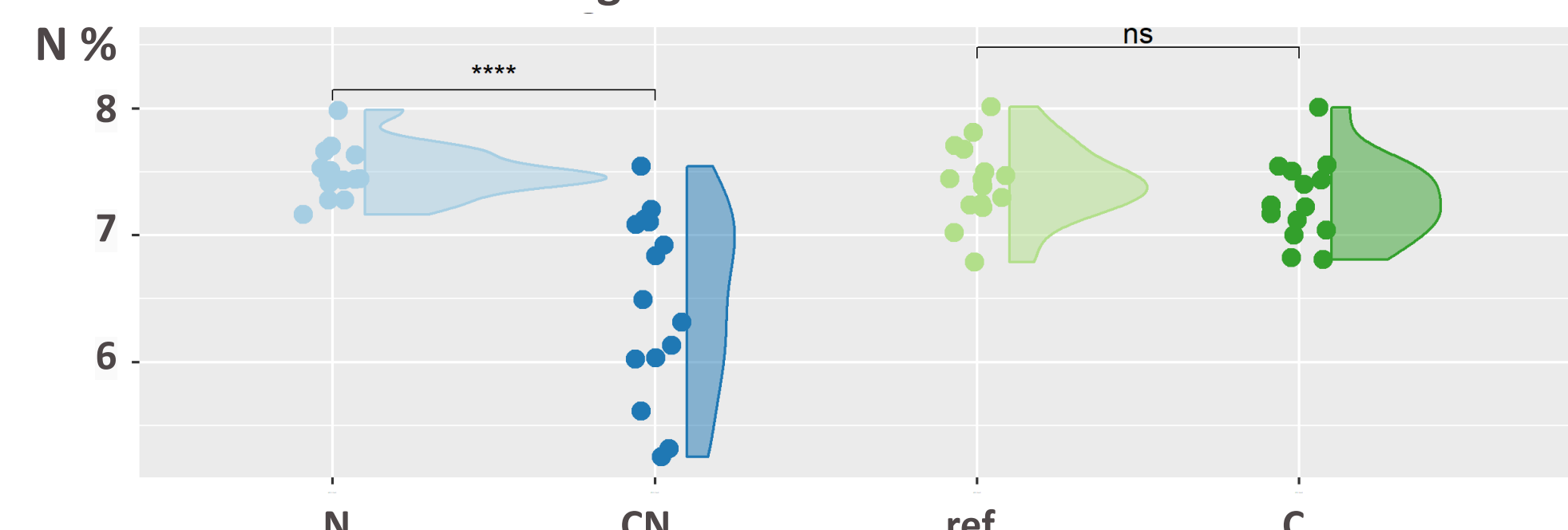
Arabidopsis plants were exposed to elevated CO2, **low nitrate** conditions and **iron starvation** in a combinatorial fashion.



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



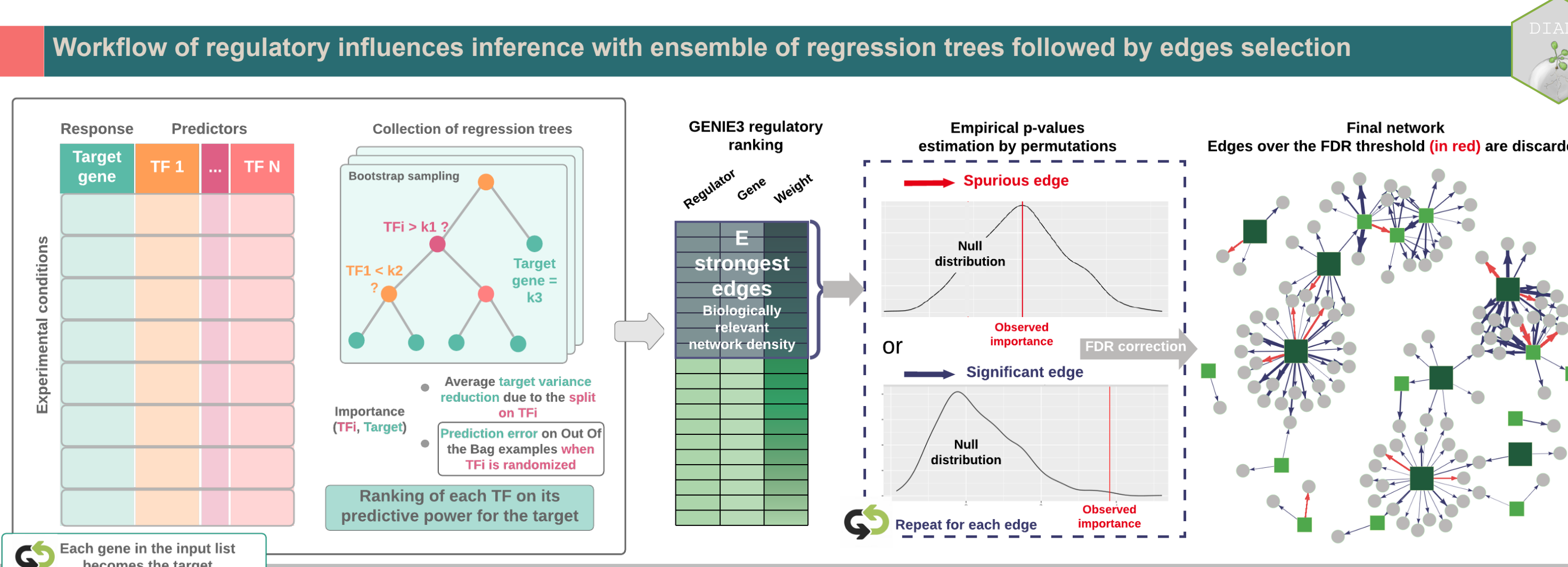
Nitrogen percentage in shoots for the ref, C, N and CN conditions.

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.



AUTHORS AFFILIATIONS

- ¹ BPMP, CNRS, INRAE, Institut Agro, Univ Montpellier, Montpellier, France
- ² IMAG, Univ Montpellier, CNRS, France
- ³ Univ Paul Valéry Montpellier 3, France

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

MAIN REFERENCES

- [1] Long et Al (2006) Food for Thought: Lower-Than-Expected Crop Yield Stimulation with Rising CO2 Concentrations, Science
- [2] Loladze, I. (2014). Hidden shift of the ionome of plants exposed to elevated CO2 depletes minerals at the base of human nutrition. *elife*, 3:e02245.
- [3] Myers et Al (2017). Climate change and global food systems: potential impacts on food security and undernutrition. *Annual review of public health*, 38.
- [4] Bloomet AIB. (2010). Carbon dioxide enrichment inhibits nitrate assimilation in wheat and arabidopsis. *Science*, 322(5780):999-1003.
- [5] Bloom et Al (2002) Root development and absorption of ammonium and nitrate from the rhizosphere. *Journal of Plant Growth Regulation*, 21(4):416-431.
- [6] Zhu et al. (2018). Carbon dioxide (CO2) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest-dependent countries. *Science advances*, 4(5):eaq1012.
- [7] Brooks et Al (2020) ConnectF: A platform to integrate transcription factor-gene interactions and validate regulatory networks. *Plant Physiology*.
- [8] De Graaf et Al (2006) Interactions between plant growth and soil nutrient cycling under elevated CO2: a meta-analysis. *Global Change Biology*

CONTACT

oceanecassan@cnrs.fr

PARTNER INSTITUTIONS



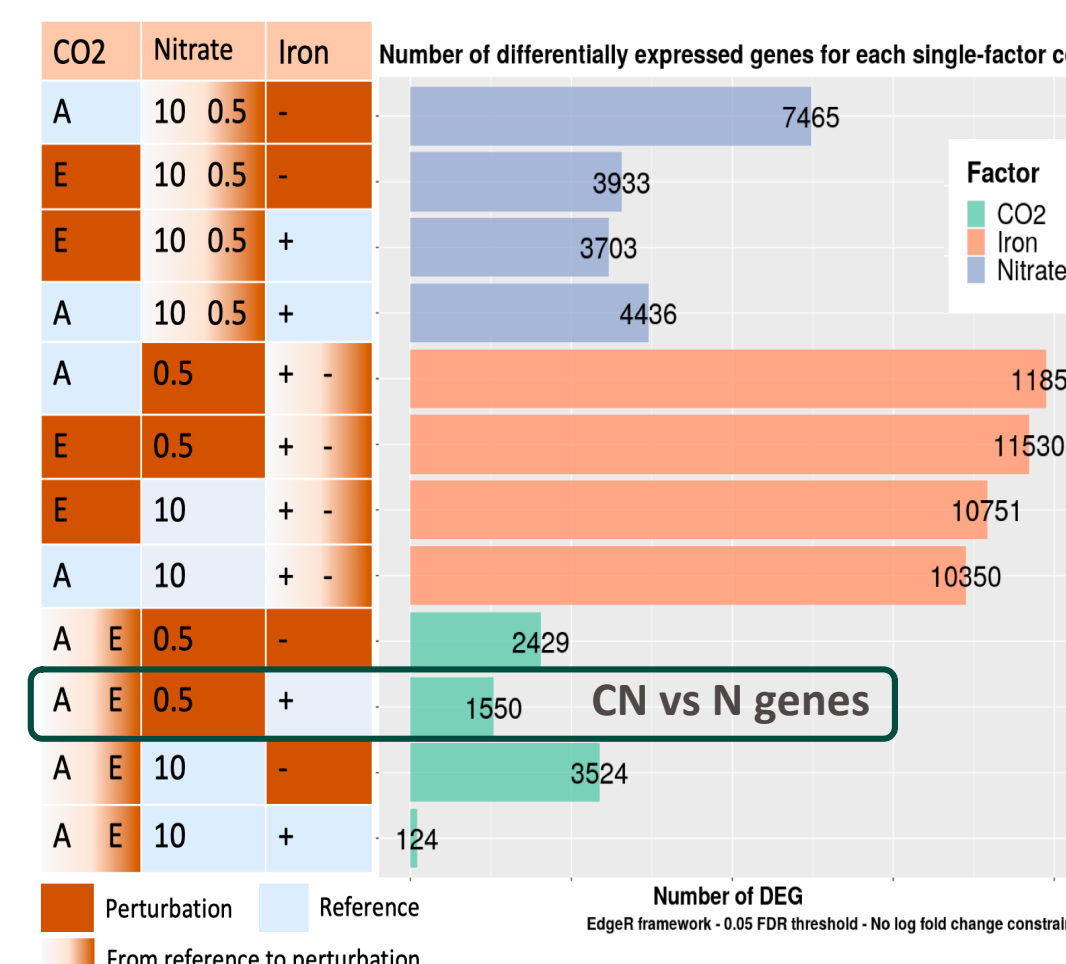
03. Elevated CO2 deregulates the response to nutritional starvations: clustering and network inference

GLOBAL TRANSCRIPTOME ANALYSIS

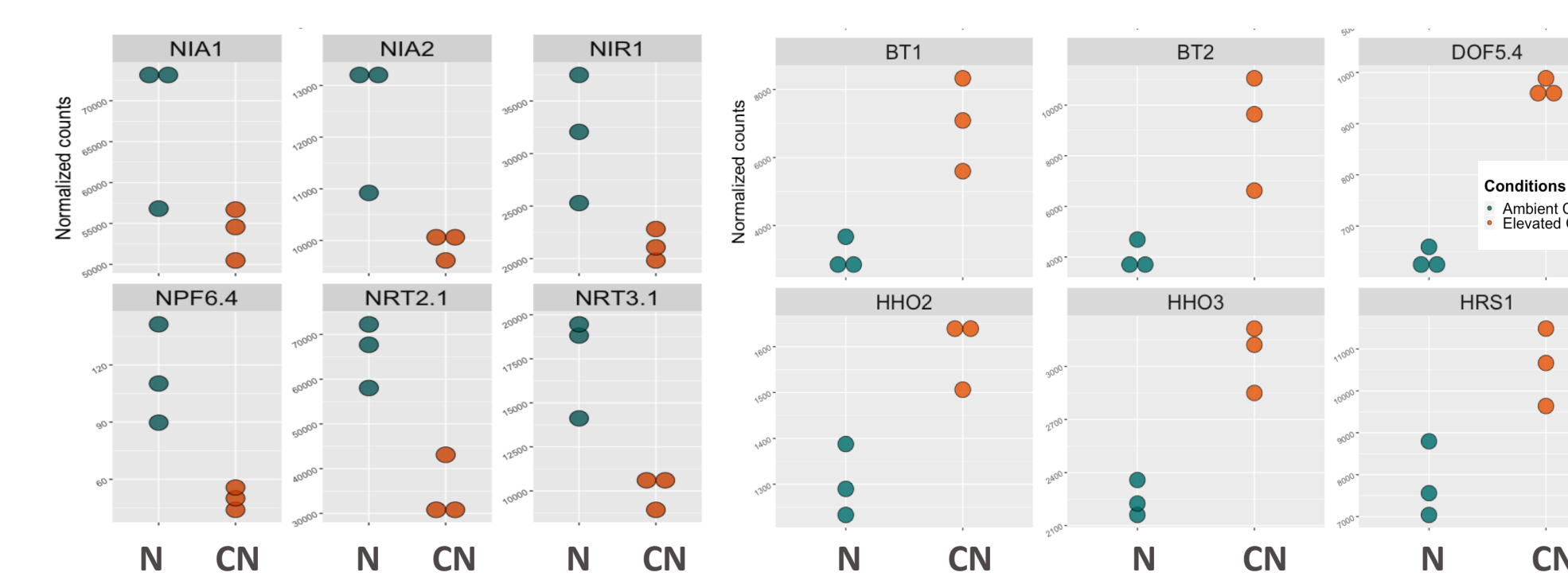
Differential expression analyses were carried out to quantify the impact of each simple and combined perturbation on gene expression

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



Genes involved in nitrate uptake, transport and assimilation are repressed under elevated CO2 and low nitrate, while a number of their known repressors are upregulated

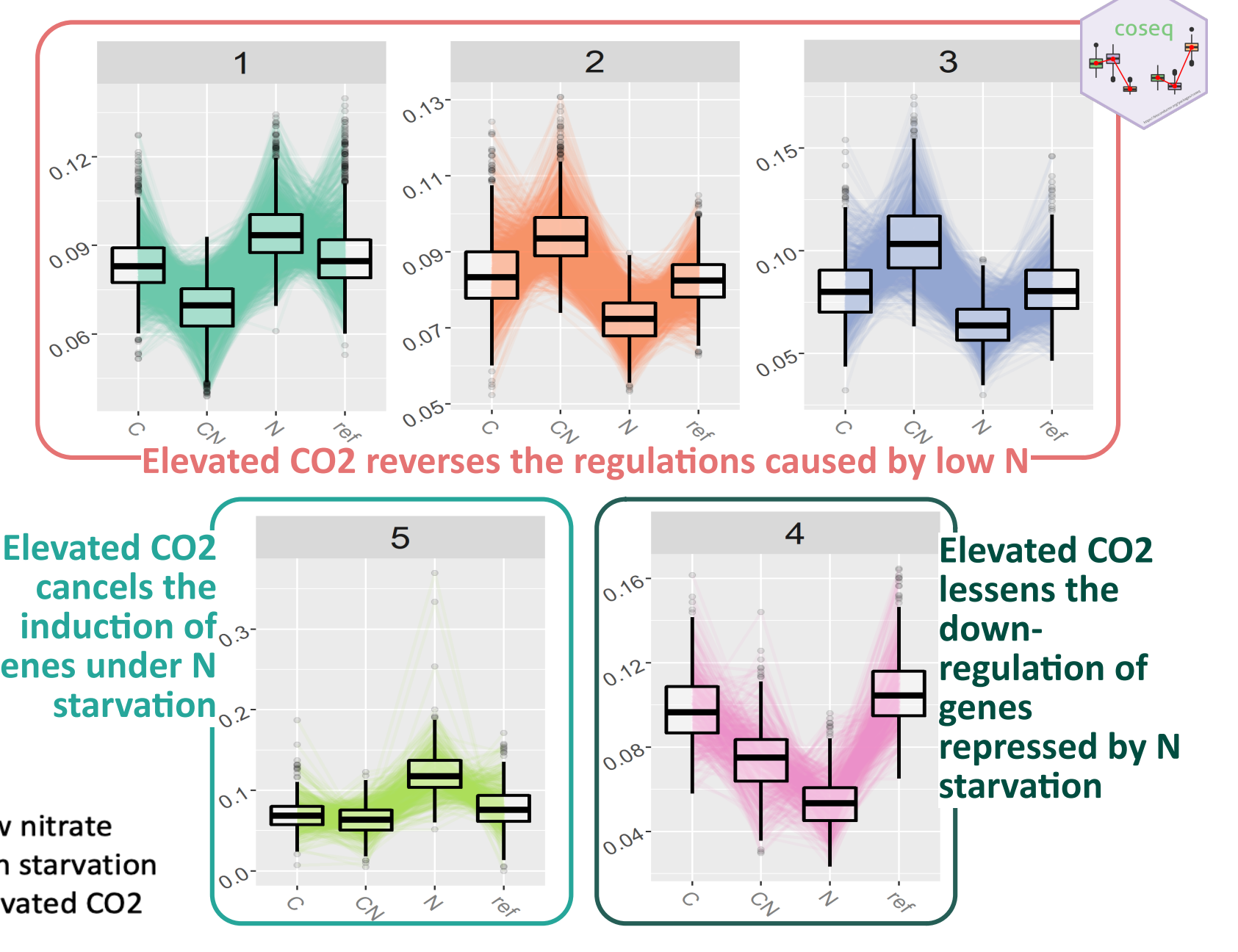
BEHAVIOR OF GENES DIFFERENTIALLY EXPRESSED BY ELEVATED CO2 UNDER LOW NITRATE

Poisson Mixture Models were adjusted via the Coseq R package to group genes with similar expression profiles across perturbations of nitrate and CO2

ELEVATED CO2 AND LOW NITRATE

Elevated CO2 reprograms gene expression in an **opposite direction** as observed in the regulations at stake in simple nitrate starvation.

Similar disruptions of the response to iron starvation were observed by running gene clustering on the conditions ref, C, F and CF.

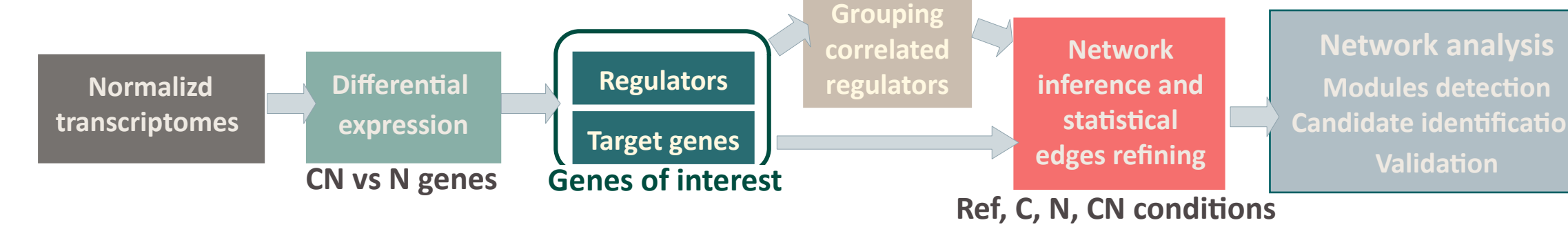


Expression profiles obtained by clustering the CN vs N genes on the conditions ref, C, N and CN

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

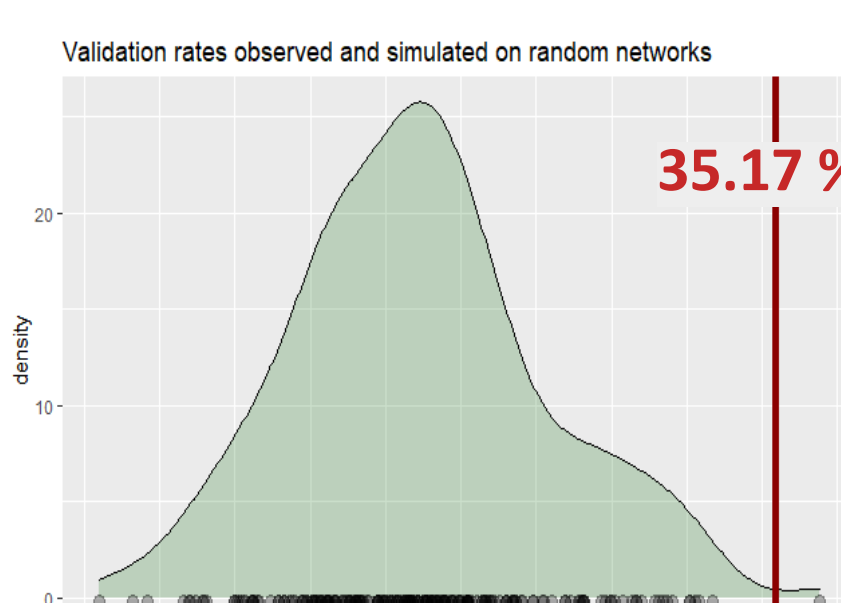
Machine learning techniques described in section 04 were used on the CN vs N genes, to infer transcriptional dependencies between regulators and targets based on their expression profiles across perturbations of nitrate and CO2

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 **connectF**[7], a recent database of regulatory interactions from in vitro and in vivo binding, as well as in planta regulation experiments.



Validation rate : 35.17 %

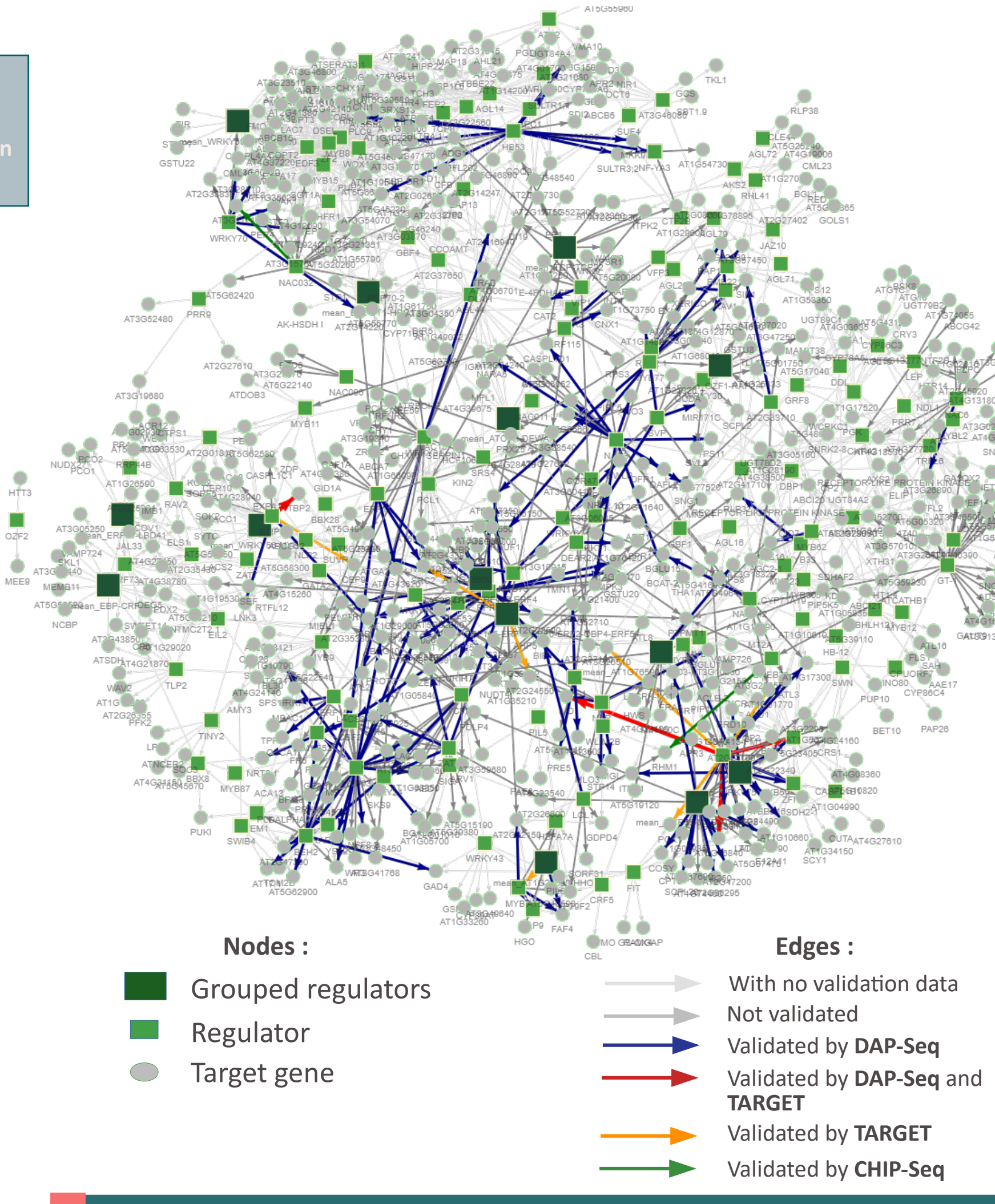
Our inference technique has a significant added value in terms of **prediction precision** as compared to randomness.

Validation rates were computed for 200 random networks, and compared in an empirical statistical test to the observed validation rate, $p = 0.005$

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**.



Predicted regulatory network of the response to elevated CO2 under low nitrate, with edges colored from connectF validation database