Can we improve the efficiency of biostimulants for nitrogen fertilization by studying their biological effects ?

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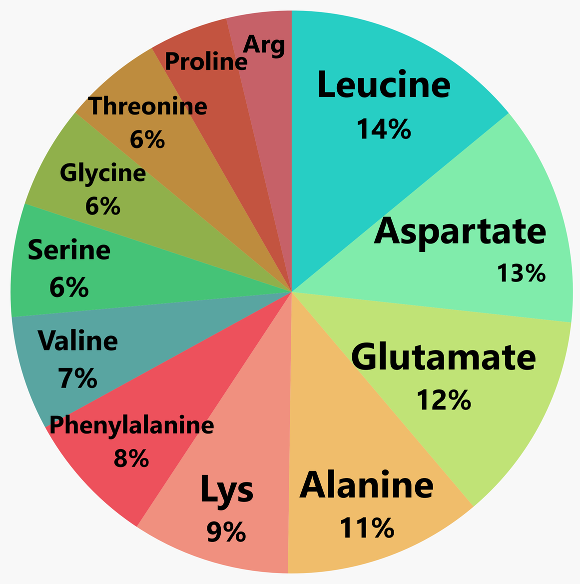
In the current situation, agriculture meets demand to feed the growing world population in a changing climate. The most used fertilizers are composed of inorganic nitrogen (N) which is an essential macro-element for plants. For most of them, N is taken up as inorganic N source from the soil (nitrate or ammonium) but their availability can largely vary in soils. However, N production and addition in fields have a strong polluting impact on the environment. There is thus an urgent need for strategies allowing a better N use efficiency (NUE) in crops. The use of biostimulants like protein hydrolysates (PH) is one of them. They have been developed to improve nutrient use efficiency, storage and remobilisation of nutrient elements in crops along with resistance to stresses[1, 2]. This project aims to better understand how a PH, which contain amino-acids, can improve N fertilization.

All experiments were carried out using Arabidopsis plants grown on vertical plates *in vitro* with 5mM NO_3^- and 1% sucrose. Root growth was measured after 12 days in culture and omics analyses were performed after 14 days in culture. PH is manufactured by the Fertinagro company.

1 Composition of PH (protein hydrolysates):

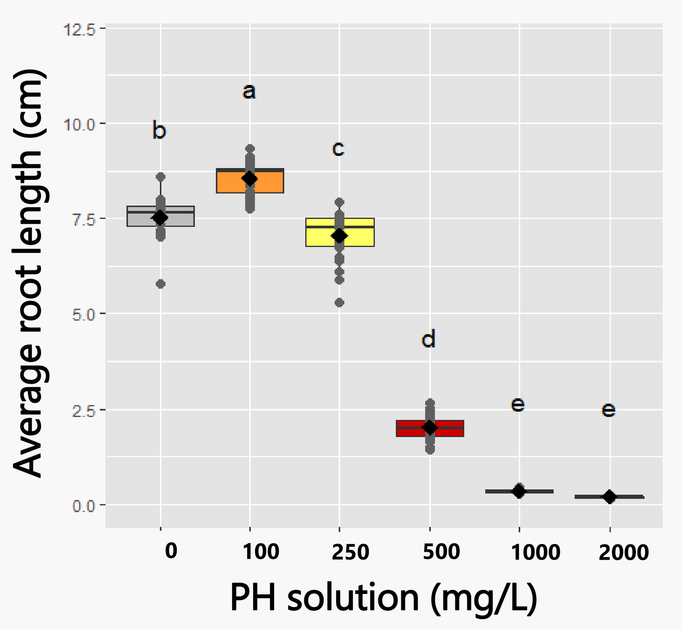
55% free amino-acids + 20% peptides

Leucine, aspartate, glutamate, alanine represent 50% of the PH solution.



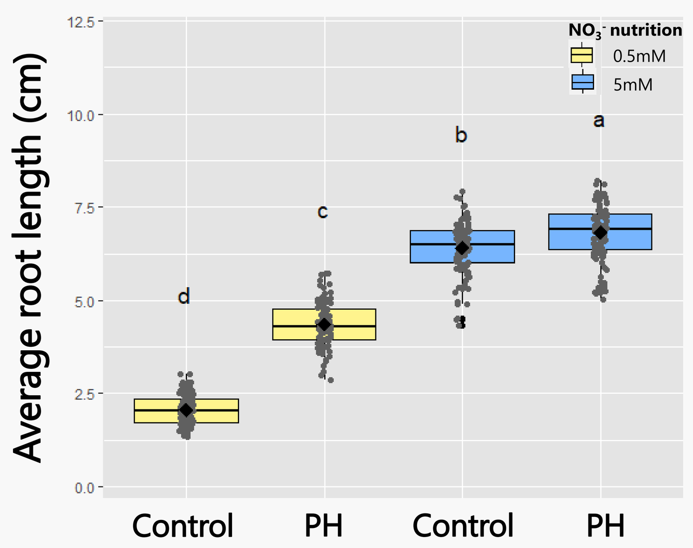
Analyzed by GC-MS
(G. Clément, IJPB PO Platform chemistry/metabolism)

2 Effect of PH on Arabidopsis root growth



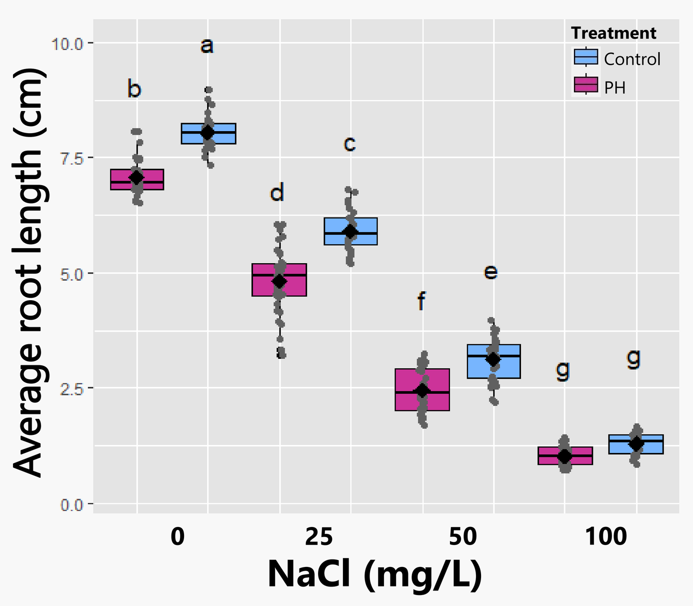
- Root growth is stimulated by low concentrations of PH whereas high concentrations have a inhibitory effect

→ PH stimulating concentration chosen for further experiments:
[PH] = 100 mg/L



- Effect of NO_3^- supply:
Stimulating effect of PH at all NO_3^-concentrations but stronger effect of PH in nitrogen deficiency

→ Next: metabolomic and transcriptomic analysis



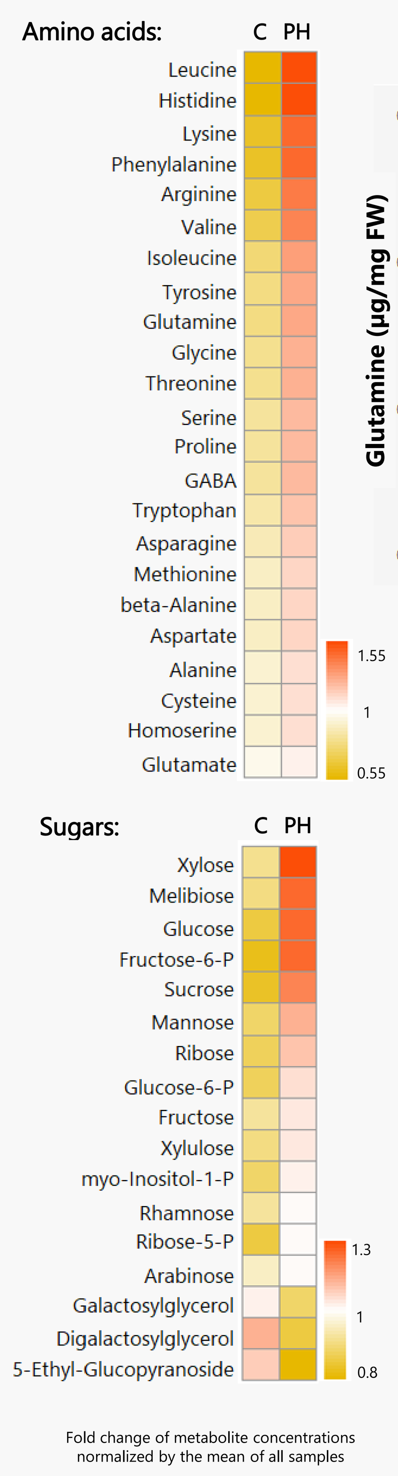
- Effect of salt stress:
Stimulating effect of PH is maintained in moderate NaCl stress

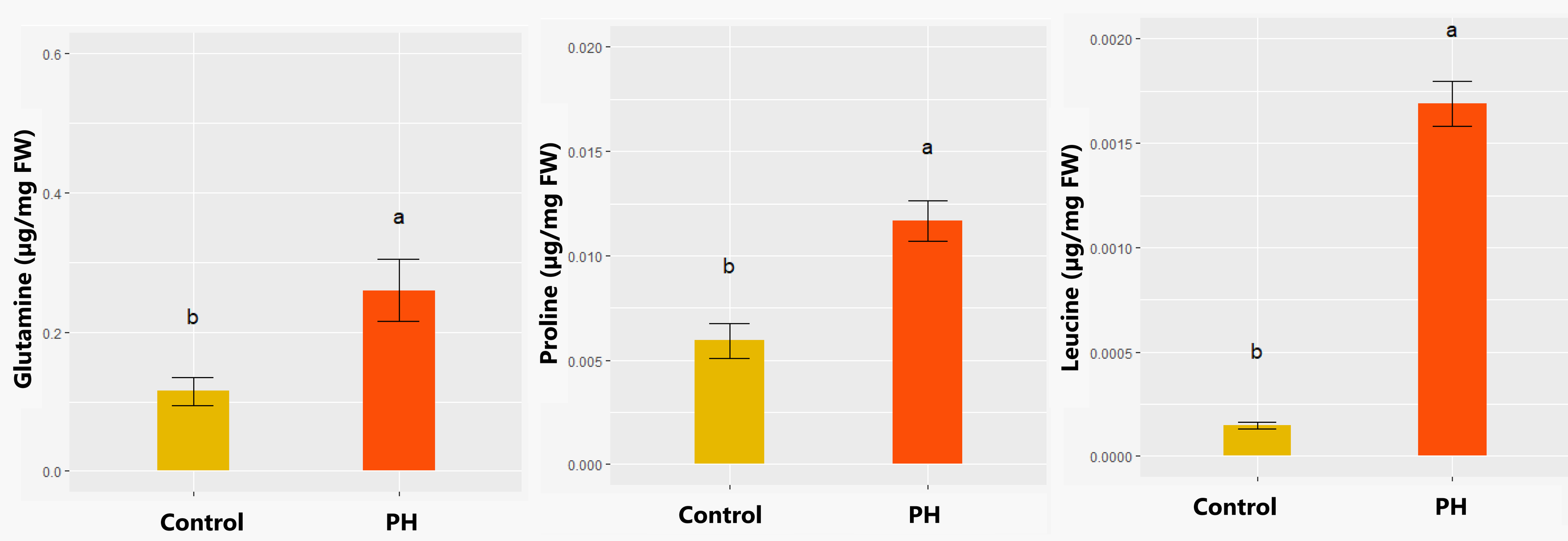
→ Next: characterization of abiotic stress response with PH treatment

ANOVA test (16<n<20 seedlings per condition). Letters indicate a significant difference with the control condition.

3 Omic analyses on Arabidopsis seedlings treated with PH

Metabolomic analysis





Analyzed by GC-MS (G. Clément)

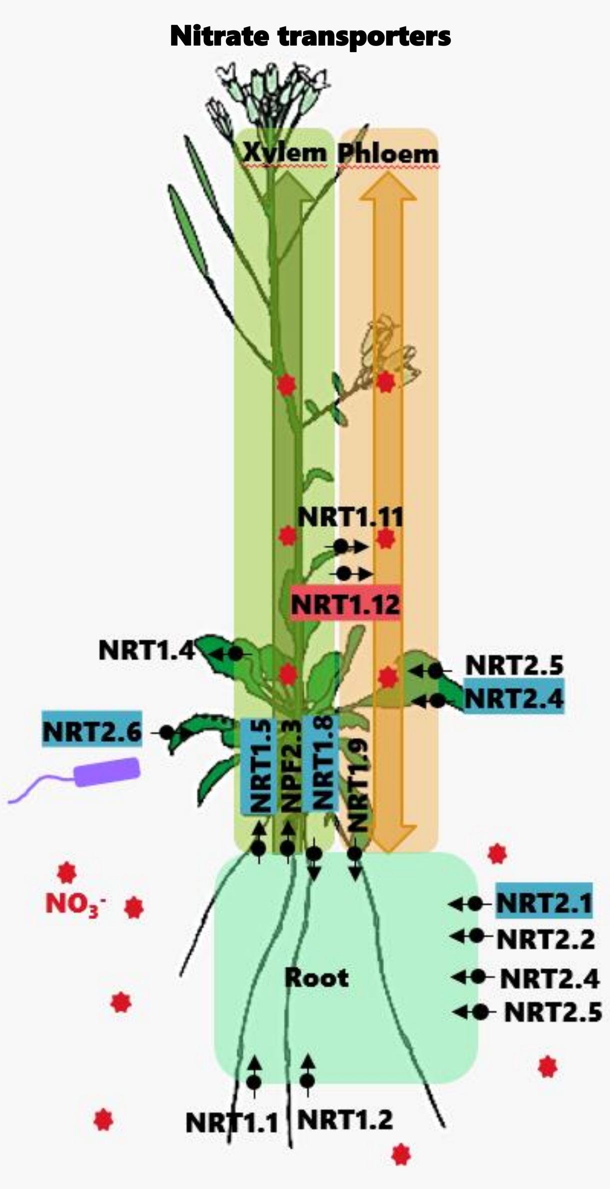
With PH treatment :

- **Accumulation of amino acids** like leucine and arginine which are present in the PH product
- **Accumulation of glutamine** that is not found in PH ➡ Stimulation of N metabolism?
- **Accumulation of some sugars** like xylose, glucose, sucrose ➡ Stimulation of C metabolism?

Transcriptomic analysis

Number of genes differencially expressed with PH treatment compared to control condition

1254 up-regulated | 1287 down-regulated



Adapted from Guan, *Front. Plant Sci*, 2017

Amino acid transporters

ID	name	role
AT3G28070	UmamiT46	nodulin MtN21-like transporter family protein
AT3G28080	UmamiT47	nodulin MtN21-like transporter family protein
AT1G44800	UmamiT18	nodulin MtN21-like transporter family protein
AT1G75500	UmamiT05	nodulin MtN21-like transporter family.
AT5G40780	LHT1	transporter aa
AT4G28040	UmamiT33	nodulin MtN21-like transporter family protein

Log2(FoldChange) -0.5 0 0.5 1

- Some amino acid transporters are differentially regulated by PH

Genes related to biotic stress

ID	name	role
AT5G26170	WRKY50	Jasmonic acid pathway
AT2G34600	JAZ7	Jasmonic acid pathway
AT4G23810	WRKY53	member of WRKY TF
AT1G72450	JAZ6	Jasmonic acid pathway
AT5G 42650	AOS	Jasmonic acid pathway
AT1G75040	PR5	Salicylic acid pathway
AT1G19180	JAZ1	Jasmonic acid pathway
AT3G25770	AOC2	Jasmonic acid pathway
AT2G43590	AT2G43590	PR-3 like gene
AT4G19810	CHI-C	Chitinase
AT1G32640	ATMYC2	MYC activator
AT1G70700	JAZ9	Jasmonic acid pathway
At2g03760	ATSOT1	Salicylic acid pathway
AT2G43570	AT2G43570	Putative basic chitinase
AT3G01970	WRKY45	member of WRKY TF
AT5G44420	PDF1.2a	Jasmonic acid pathway

Log2(FoldChange) -1.5 -1 -0.5 0 0.5

- Down-regulation of NO_3^- HATS transporters

→ Next: measurement of NO_3^- uptake

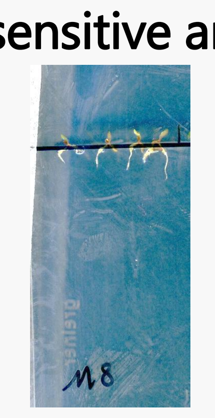
- PH influences expression of biotic stress markers

→ Next: characterization of biotic stress response with PH treatment

4 Screening of Arabidopsis mutants hypersensitive or resistant to PH

Inhibitory effect of 750mg/L PH on Arabidopsis root growth ➡ Basis for a screen to identify mutants using a 200 double haploid EMS collection ➡ We have identified 4 hypersensitive and 7 resistant mutants

Capilla-Perez et al., *Front. Plant Sci*, 2018



➡ Next : determine target genes

Better understanding of PH and amino-acids transport and signalling

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