Phosphite plant biostimulant mode of action: gene expression, phytohormone levels, enzyme activity

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Extent of work

Scope

- Over 15 years work on phosphite plant biostimulant properties and mode of action
- With 15 MSc and 2 PhD scientists in 2 departments
- Across arable crops (Wheat, Oilseed rape) and the model plant *Arabidopsis thaliana*
- Within Germany
- And collaboration with University of Nottingham

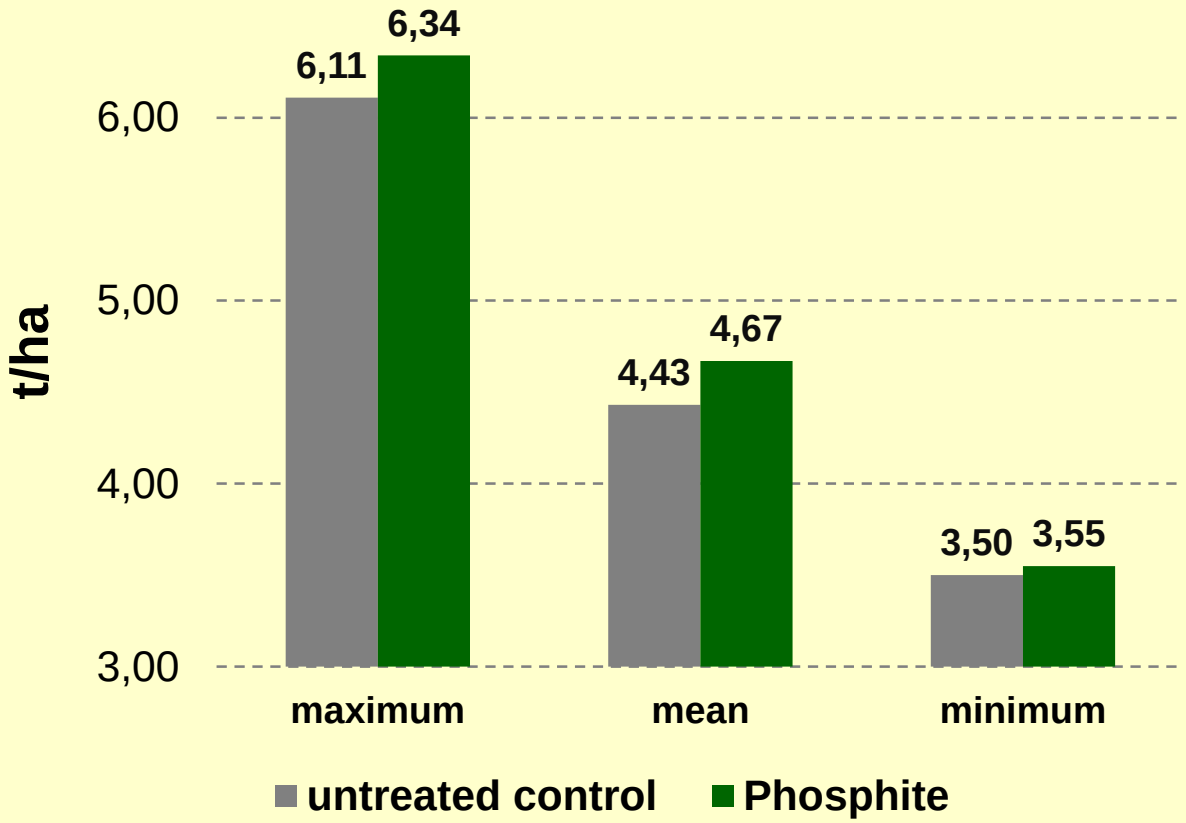
Science and metrics

- Laboratory analysis
- Glasshouse cultivation
- Replicated small-plot field trials
- Nutrient uptake
- Root and shoot weights
- Phytohormone activity
- Enzyme activity
- Gene expression

Phenotype – visible effects

root growth, nutrient use efficiency and yield

15-year oilseed replicated field trials

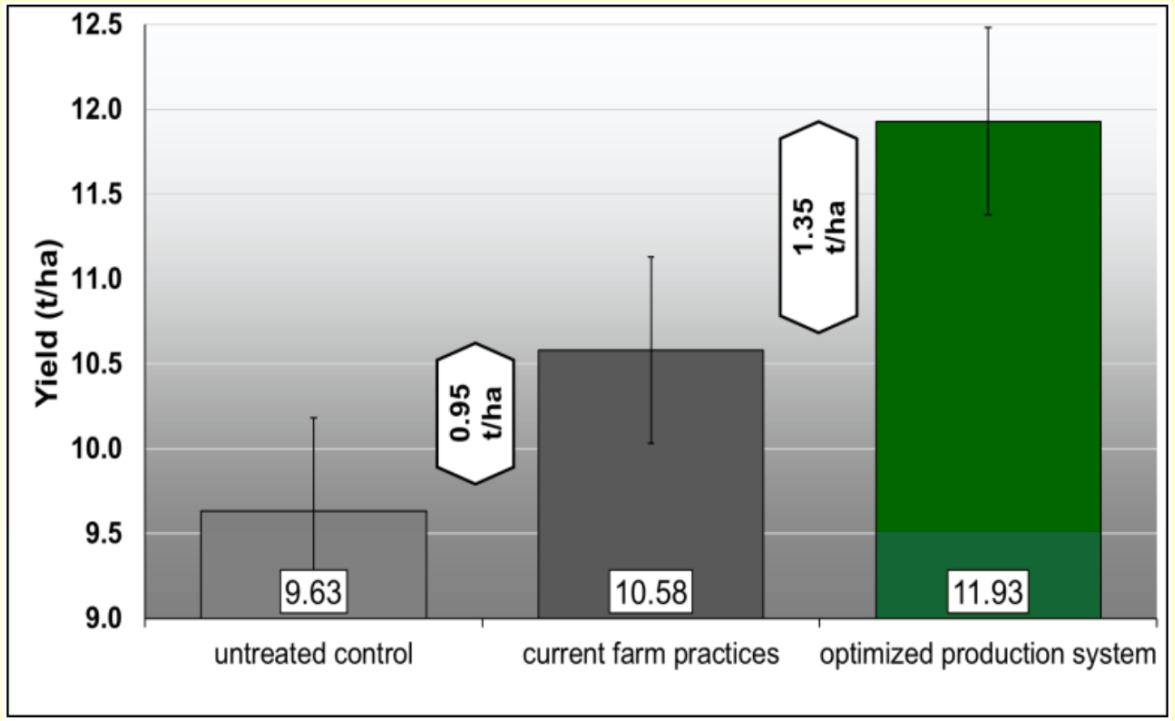


- **2 x 0.5 l/ha foliar phosphite** applications at 2-6 leaf (autumn) and early stem extension (spring)
- equates to **less than 500 g PO_3/ha** in total
- average of **22 trials** over **15 years**

Results

- **0.25 t/ha (+ 5.6 %)** average increase in yield
- **8.21 kg/ha** increase in nitrogen withdrawal (rapeseed grain) could additionally be mobilized and harvested (a very important aspect in the context of the fertilizer ordinance)
- **+ 5.5 %** improved nitrogen use efficiency
- **+ 56 €/ha** increase in farm income

Wheat – comparison of growth regimes



Results

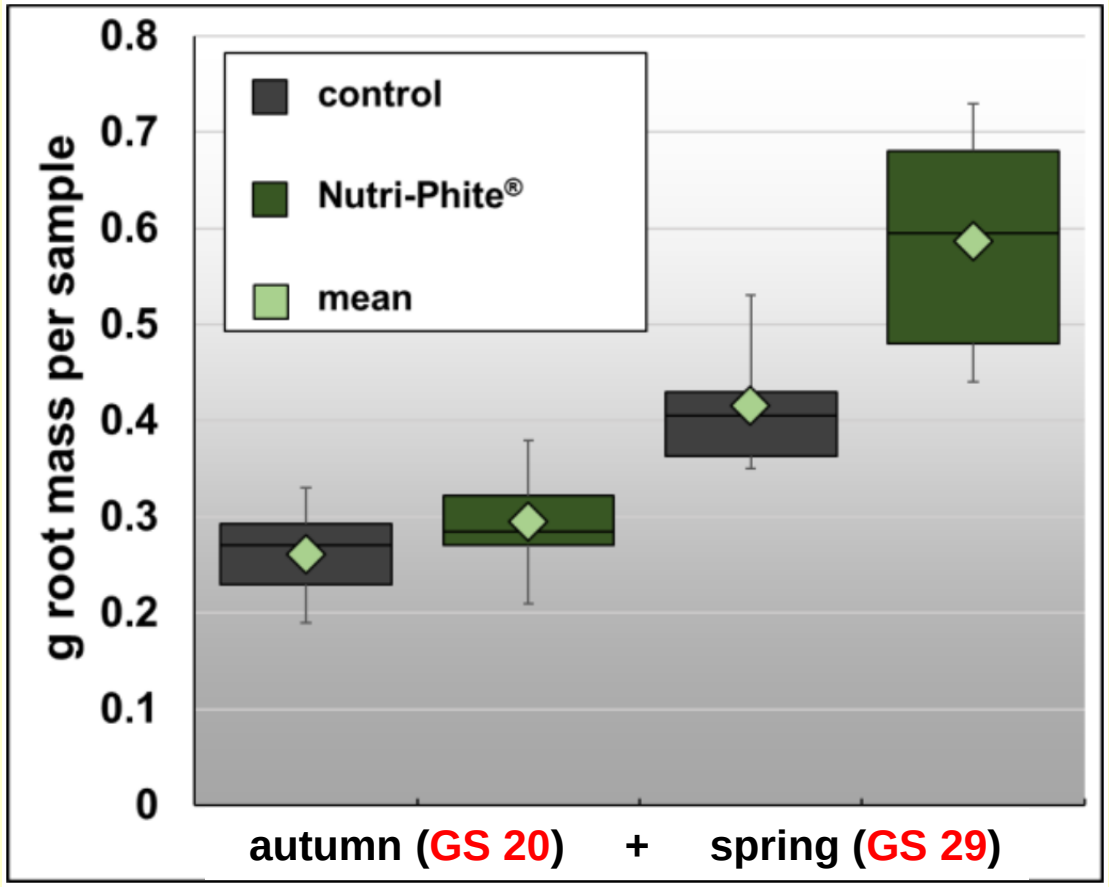
- **+ 1.35 t/ha** increase in yield using 40 kg/ha less nitrogen fertilizer
- **+ 20.7 kg N/ha** (grain) in nitrogen withdrawal could additionally be mobilized and harvested harvested (a very important aspect in the context of the fertilizer ordinance!)
- **+ 32 %** improved nitrogen use efficiency
- **+ € 186/ha** increase in farm income

Control = no phosphite, normal nitrogen level (180 kg/ha)

Current farm practice = as above + pesticides

Optimized production system = plus phosphite (Nutri-Phite®), lower nitrogen level (- 40 kg/ha) and less pesticide

Phosphite seed treatment - wheat root growth



30ml phosphite (Nutri-Phite®) / 100kg seed

Results

- **7.4 %** mean increase in root mass at **GS20** (December)
- **50 %** mean increase in root mass at **GS29** (March)

Due to the proven increase in yield and the increased root growth by Phosphite (PO^{3-}_3), we have investigated the root causes on the plant-physiological and genetic basis.

First time the genes of oilseed rape and the model plant *Arabidopsis thaliana* were analyzed by the ´Whole genome transcriptome analysis´ - NGS (next generation sequencing) technology - with regard to the genetical basic effects of Phosphite (PO^{3-}_3).

mode of action

gene expression, phytohormone levels, enzyme activity

Effects of phosphite on global gene expression

Analysis

- To gain insight into molecular mechanisms involved in the stimulation effects, we analysed effects on global gene expression in plants.
- We applied the so-called “whole genome transcriptome analysis” using NGS (next generation sequencing) technology.
- Both oilseed rape and the model plant *Arabidopsis thaliana*, their roots and leaves in 3 different stages (3, 6 and 9-day post treatment) were analysed.
- Genes and their expression levels as well as related physiological processes, affected by phosphite were identified.

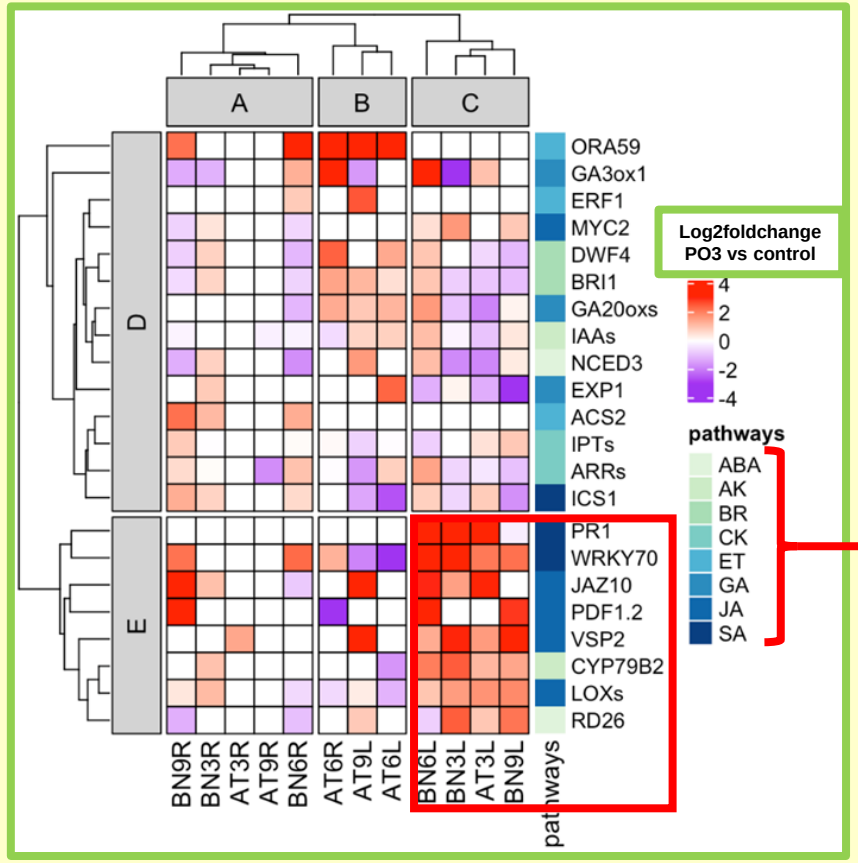
Main results

- A large number of genes are affected by Phosphite treatment.
- Several molecular and physiological processes are positively affected.
- Both enhanced and suppressed gene regulations suggest a complex stimulation process.

Gene expression: increase in phytohormones

Phosphite stimulates production of essential phytohormones in the plant metabolism

- **Auxins** promote elongation, root and shoot growth
- **Cytokinins** promote cell division
- **Gibberellins** promote elongation of stem axis and flower formation
- **Abscisic acid** promote seed and fruit formation
- **Jasmonates** regulate plant growth and development
- **Brassinosteroids** regulate plant development and growth
- **Ethylene** is a stress hormone

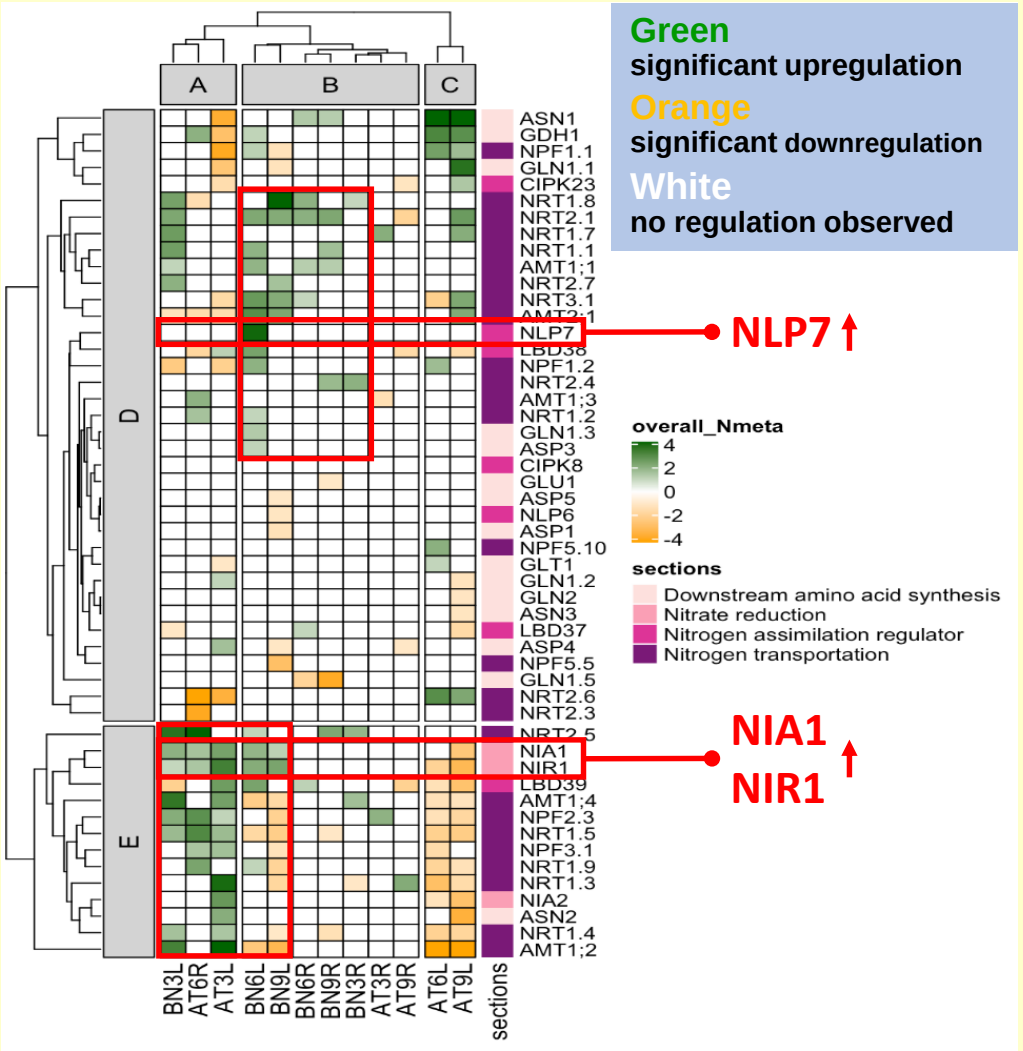


ABA, Abscisic acid
AK, Auxins
BR, Brassinosteroids
CK, Cytokinins
ET, Ethylene
GA, Gibberellins
JA, Jasmonates

Red = significant upregulation
Purple = significant downregulation
White = no regulation observed

´Whole genome transcriptome analysis - NGS (next generation sequencing) technology´

Gene expression: nitrogen assimilation



Three genes key to nitrogen transport and assimilation were significantly upregulated by the application of phosphite:

- **NIA1** encodes nitrate reductase (**NR**)
- **NIR1** encodes nitrite reductase
- **NLP7** regulates nitrate assimilation

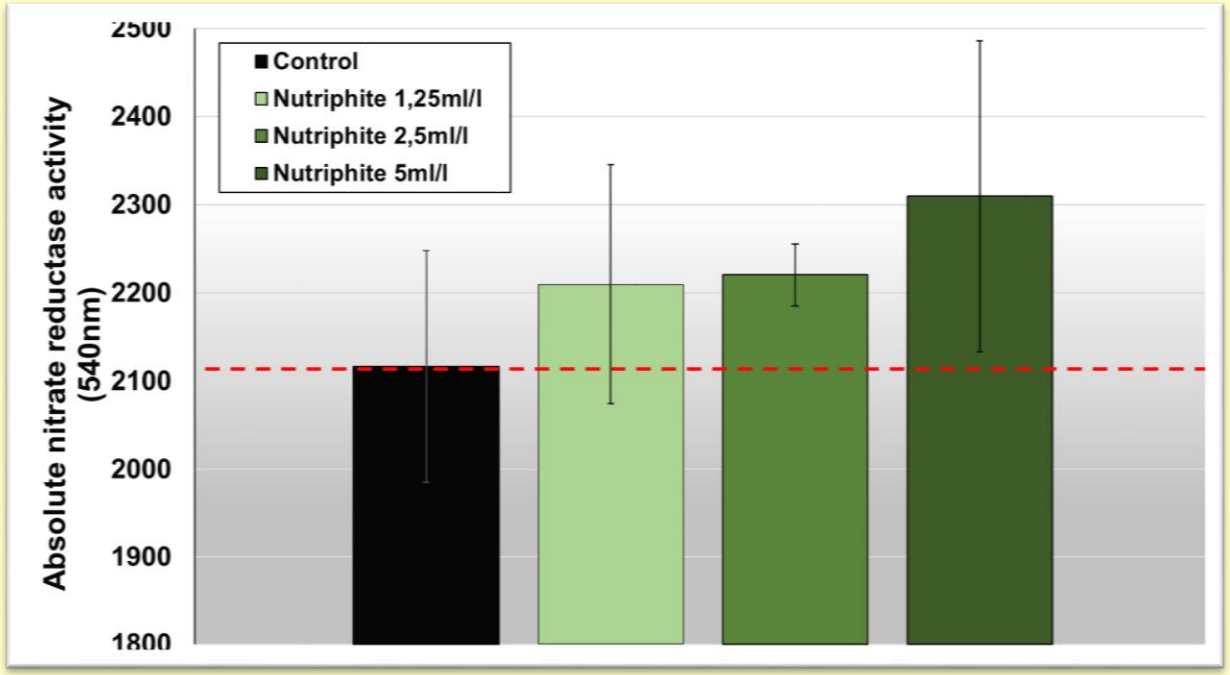
*Nitrate reductase (**NR**) is a key enzyme within the nitrogen assimilation process which is critical to plant metabolism and growth*

nitrate → nitrite → ammonium → glutamine synthetase → amino acids → proteins

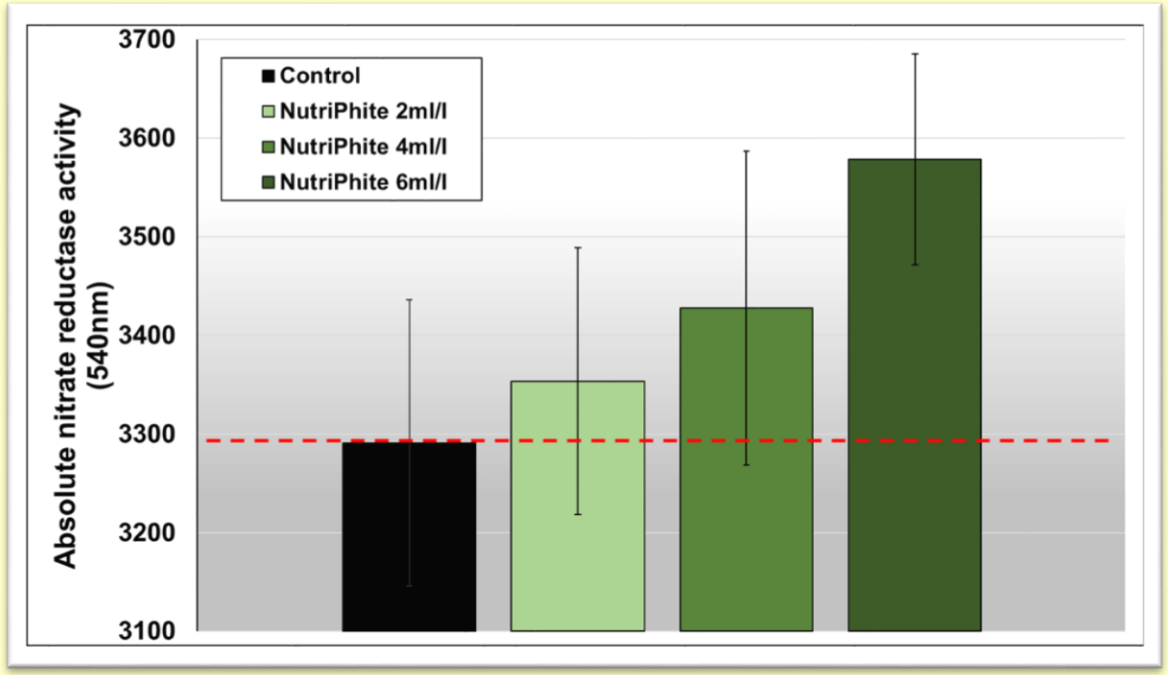
**´Whole genome transcriptome analysis
NGS (next generation sequencing) technology´**

Enzyme activity - nitrate reductase (NR)

Oilseed: **8%** **increase in nitrate reductase (NR) activity within 9 days of Phosphite (Nutriphite) treatment**



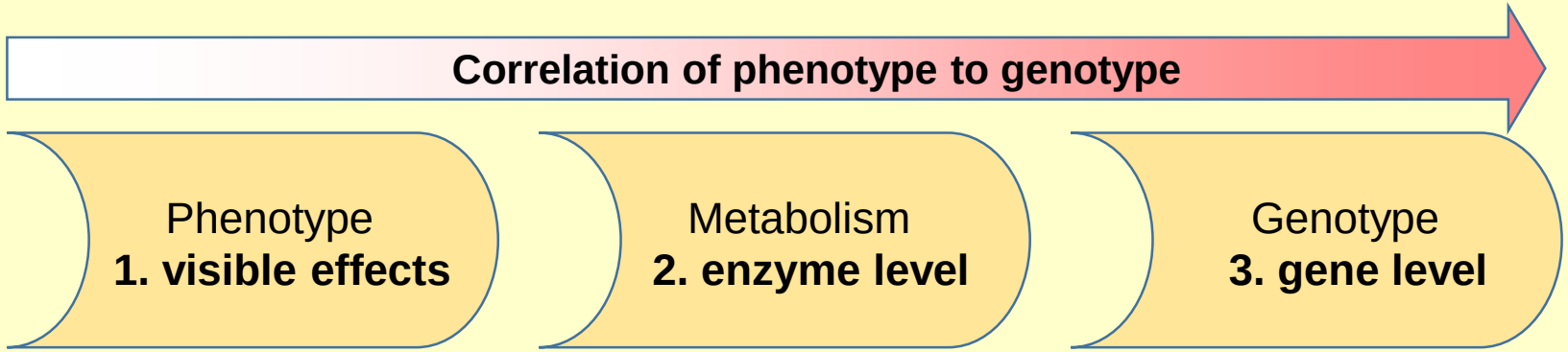
Wheat: **10%** **increase in nitrate reductase (NR) activity within 9 days of Phosphite treatment**



- **Nitrate reductase has a decisive influence on the increased availability of nitrogen compounds in the plant.**
- **Accordingly, an increased nitrate reductase activity leads to an increased assimilation of inorganic N to build up plant organs (root, stalk/stem, leaf, grain/seed).**

Summary

Scientific evidence of phosphite as a plant biostimulant



- **Improved nutrient efficiency**
- **Increased root growth**
- **Increase in yielding structure factors**
- **increased yield**

- **Signal-induced root growth**
- **Stimulating nitrate reductase**

- **Increased gene expression in the important NRT1 gene (nitrate transporter, nitrate reductase)**
- **Increased gene expression in NIA1 & 2 (nitrate reductase)**
- **Increased NLP6/7 (nitrate assimilation regulator)**
- **Increased NIA1/2, NIR1 (nitrate assimilation)**
- **Transporter genes are upregulated**

The results presented that based on the

- ***Phenotype effects*** **(increase in root growth and yield) and**
- ***Enzyme level*** **by stimulating the nitrate reductase activity with increasing of nitrogen mobilization and**
- ***Genetic level*** **(next generation sequencing) with an upregulating and therefore intensifcation of the key genes (NIA1 & 2, NLP6/7, NIR1) with an increasing of nitrate transporter, nitrate reductase and nitrate assimilation regulator**

clearly document that

Phosphite (PO^{3-}_3)

has to be regarded as a Plant-Biostimulant !