Role of phosphite in plant growth and development

White paper

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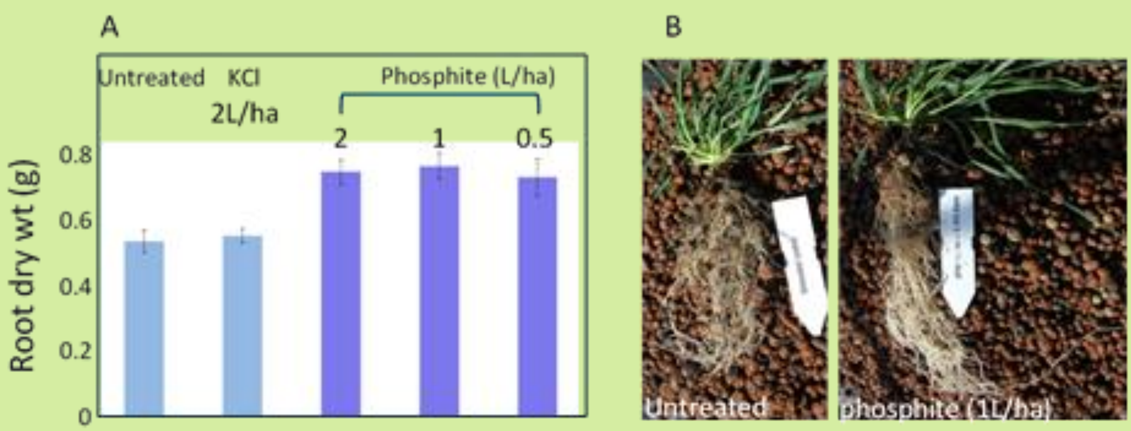
Executive Summary

Global food security is one of the biggest challenges facing world agriculture. Significant improvements in crop yields are urgently required to meet the predicted 50% increase in world population by 2050[1-2]. There have been several studies and reports that suggest that improvement in root architecture can have profound impact in improving crop productivity and resource use efficiency.

Phosphite represents a reduced form of phosphate that belongs to a class of crop growth promoting chemicals termed biostimulants. Foliar application of phosphite enhances root growth and development in a range of plant species, typically increasing biomass by around 30% (Figure 1)[3-7].

Figure 1: Phosphite promotes root growth



Wheat plants were treated with potassium phosphite based formulation at stage GS12 and harvested at GS23 and root dry weight measured. Root dry weight only increased upon phosphite (not K) treatment. Figure reproduced from: [4]Rossall S, Qing C, Paneris M, Bennett M and Swarup R. 2016 A 'growing' role for phosphites in promoting plant growth and development. Acta Hortic 1148, 61-67

Phosphite cannot be converted to phosphate, so does not enhance plant growth via a nutritional mechanism[8]. As part of a BBSRC LINK grant we have been investigating the effect of phosphite on plant growth. Using a systematic approach involving a combination of cell biology, plant physiology, biochemistry and X-Ray imaging techniques, we have tested the effect of phosphite in wheat and oilseed rape.

In addition, we have six industrial partners with an excellent track record in research, development and marketing of a range of biostimulant products, including phosphite. Their studies on biostimulant properties of phosphites in glasshouse and field conditions complement the physiological studies done under more controlled conditions in Nottingham. They have been testing different formulations, doses and their effect in a range of crops and in different agro-climatic conditions in several different countries including UK, Spain, Italy, Germany, Czech Republic, Finland, Canada and Brazil.

This white paper summarises key findings of the BBSRC-LINK grant.

- Foliar application of phosphite (typically 1L of formulation/ha) enhances root biomass by an average 30% (Figure 1). Both in oilseed rape and wheat we find that some varieties are more responsive to phosphite treatment. We also find that wheat response to phosphite is more robust than oilseed rape.
- Both in wheat and oilseed rape, the effect of phosphite is more pronounced under mild stress (restricted water condition or under reduced nutrient strength).
- Phosphite treatment in wheat enhances root system architecture, as revealed by X-ray CT imaging.
- Foliar application of phosphite typically improves carbon assimilation and leaf water use efficiency (carbon gained per unit water loss).
- At the low doses used, phosphites have no effect on foliar diseases of wheat; septoria leaf blotch and powdery mildew, and thus not acting as fungicides.
- Phosphites did not elicit plant defense when both *Phaseolus* and *Vicia* beans were inoculated with the ubiquitous fungal plant pathogen *Botrytis cinerea*.
- In collaboration with Kiel University, we show that phosphite treatment results in increase in Nitrate Reductase-a key enzyme in N assimilation.

Our results show that low doses of phosphite promotes root growth and improve resource use efficiency and thus is likely to have a direct impact on farm income leading to improved nutritional, financial and social stability. This facilitates entry to a new area of precision farming where traits may be deliberately manipulated via application of non-harmful chemicals.

Full Report

Phosphites represent a reduced form of phosphate that belongs to a class of crop growth promoting chemicals termed biostimulants. Foliar application of phosphite enhances root growth and development in a range of plant species, typically increasing biomass by around 30%.

Introduction

The green revolution brought dramatic increases in food production through the development of high yielding, dwarf varieties of rice and wheat and the application of large quantities of inorganic fertilizer, pesticides, and irrigation water. Unfortunately, this increased agricultural productivity has had a deleterious environmental impact increasing soil salinity, ground water pollution and using ~8 % world oil output. In addition, the world population is estimated to reach ~8.3 Bn by 2030 with the majority of that increase occurring in the developing world[1,2]. The need to feed this growing population, sustainably, and against the significant threats to food crop harvests arising from climate change, could not be more pressing. With no more agricultural land available, increases in food production of between 40-50% must be achieved through a sustainable intensification of agriculture. There have been several studies and reports that suggest that improvement in root architecture can have profound impact in improving crop productivity and resource use efficiency[9-11].

Biostimulants are emerging as a class of crop growth promoting chemicals and could play an important role in securing yields and increasing efficiency, which could become a key component in integrated crop production[12]. Biostimulants are not nutrients, nor fertilizers, nor pesticides. Biostimulants affect plant growth and plant development in a variety of ways throughout the life cycle of the crop, from seed germination to plant maturity. These include: Improving the efficiency of the plant's metabolism to improve crop quality, yield and tolerance and to abiotic influences. Biostimulants facilitate plant recovery from abiotic stress, improve nutrient and water acquisition and distribution, improve the quality of plant produce including sugar content, colour etc. They also, can help improve physicochemical properties of the soil and promote plant microbe interaction.

This white paper summarizes the work done at the University of Nottingham, as part of a BBSRC-LINK grant with six industrial partners, on the biostimulant properties of Phosphites.

Phosphites are reduced form of phosphate and several studies have reported positive effects of phosphites in plant growth and development[3-7]. Phosphite cannot be converted to phosphate, so does not enhance plant growth via a nutritional mechanism.

Some phosphite salts have prior registration as pesticides. At high doses they can inhibit some oomycete, spoil-borne pathogens, such as Phytophthora. This is probably mediated by induction of host defence gene expression. We will present data to show phosphites, applied at low levels with no fungicide activity, can also act as biostimulants, mediating enhanced root development in crops ([3]**Swarup *et al*** (2015) New Ag International Nov/Dec, 76-77; [4]**Rossall *et al*** (2016) *Acta Hortic* **1148**, 61-67).

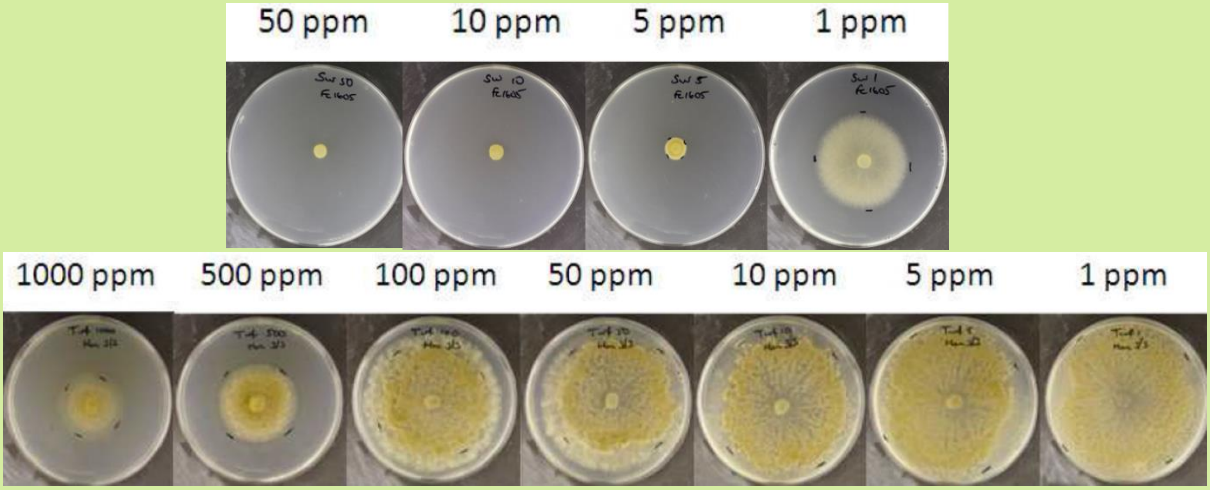
These findings can be summarized as below:

- Foliar application of phosphite enhances root growth and development in a range of plant species, typically increasing biomass by 30% (Figure 1).
- At the doses used, phosphites has no effect on foliar diseases of wheat; septoria leaf blotch and powdery mildew.
- Phosphites did not elicit plant defense when both Phaseolus and *Vicia* beans were inoculated with the ubiquitous fungal plant pathogen *Botrytis cinerea*.
- In amenity turf, phosphites were shown to reduce symptoms of *Microdochium* disease (snow mould, a root disease). Independent work undertaken at FERA (York, UK) concluded that the phosphite used had little or no fungicidal activity against this true fungus (Figure 2).

Figure 2: Phosphites show little or no fungicidal activity

a

b



Microdochium nivale growth in presence of (a) conventional fungicide ipridione (EC 50 1PPM) and (b) phosphite (EC50 500 ppm)

We did, however, confirm that foliar applied phosphite significantly enhanced root growth in ryegrass. Thus, the phenotypic disease symptom suppression is likely to be disease escape, as the grass would have sufficient healthy roots to tolerate the infection.

BBSRC-LINK Grant

As part of a Research Council UK (BBSRC) LINK grant with six industrial partners, we are investigating the mechanism(s) through which phosphites promote root growth and how this impacts above ground physiology, as well as nutrient and water use efficiency. In addition, our partners are involved in a series of studies on biostimulant properties of phosphites in glasshouse and field conditions and thus complement the physiological studies done under more controlled conditions in Nottingham. They continue to test different formulations, doses and their effect in a range of crops and in different agro-climatic conditions in several countries including UK, Spain, Italy, Germany, Czech Republic, Finland, Canada and Brazil.

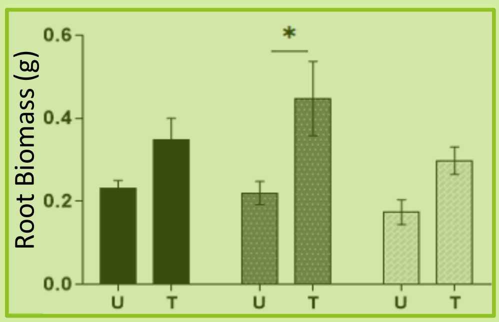
Key findings

We show that phosphite treatment improves resource use efficiency (Figure 3). Detailed root trait analysis by 2D germination paper (Figure 4) and non-invasive 3D X-ray CT (Figure 5) imaging show that phosphite treatment improves root traits.

Figure 3: Phosphite treatment improves resource use efficiency

A. Nutrient use efficiency

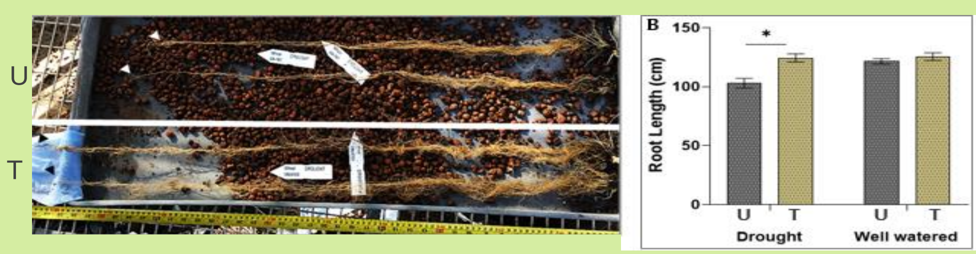
Phosphite treated (T) plants show an increase in root biomass under different nutrient strength of a commercial soluble fertilizer (NPK: 20-8-20) compared to untreated (U) plants.



% standard nutrients **100% 75% 50%**

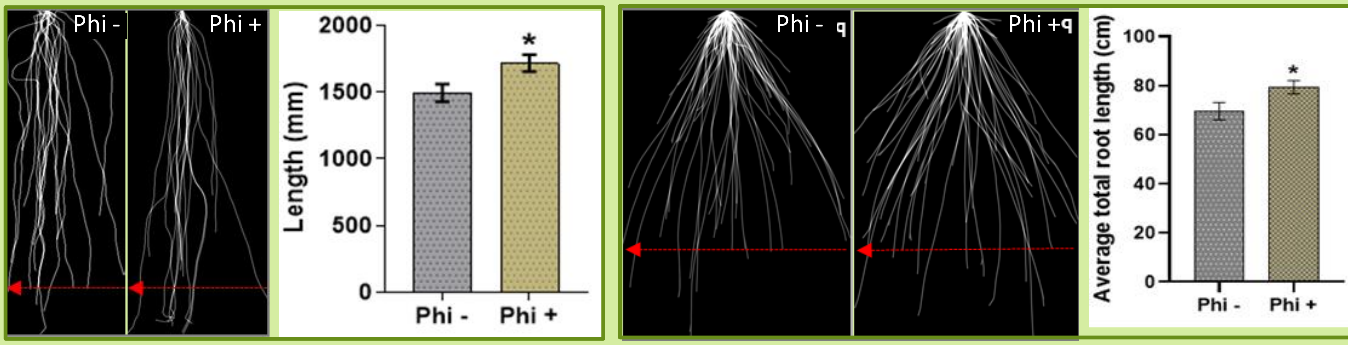
B. Water use efficiency

Phosphite treated (T) plants show an increase in root length under drought (restricted water 7 day after sowing)



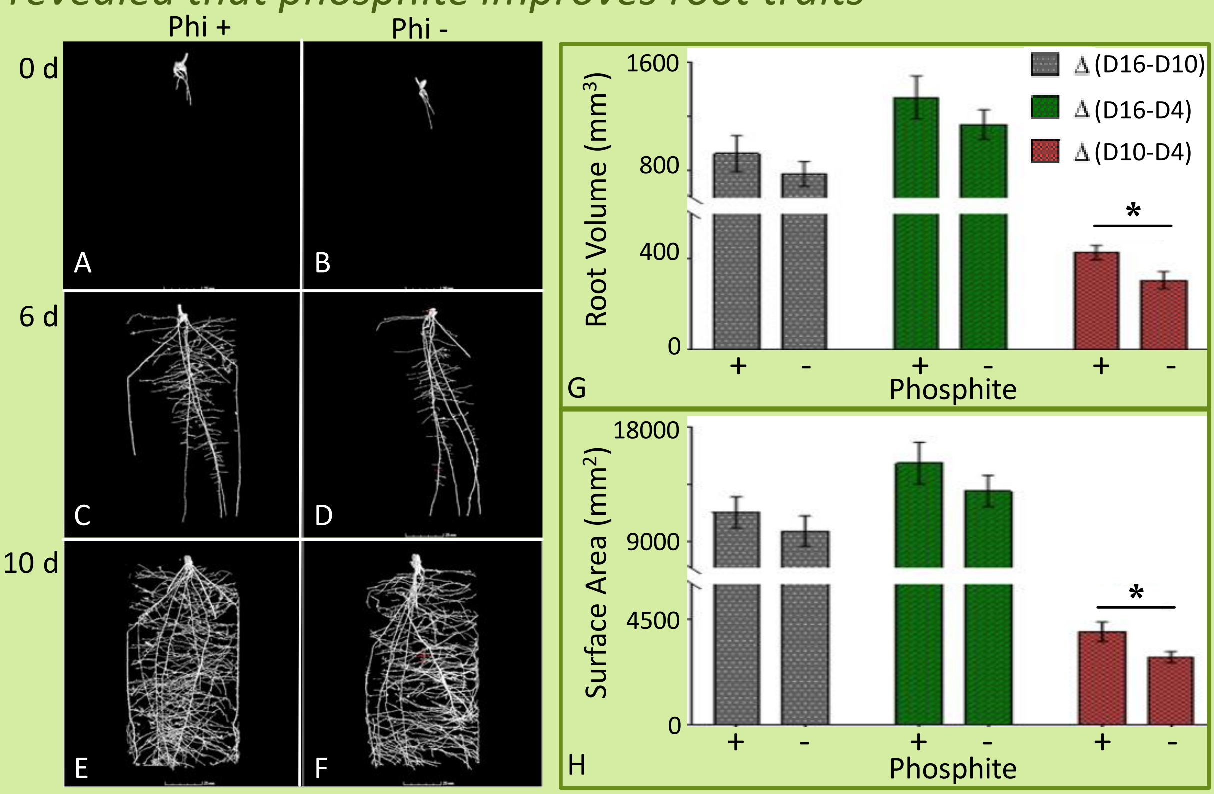
Wheat grown in nutrient solution. Phosphite application GS12-GS16; Growth assessment 30-45 days after treatment

Figure 4: Phosphite treatment improves root traits



*Superimposed 2D root images of young seedlings grown in hydroponics showing improvement in primary root length at 7 days post phosphite treatment in Oilseed rape (**A**) and Wheat (**B**). Pre-imbibed seeds were grown on germination papers, treated with phosphite and growth assessment was done 7 days post treatment. Images were analysed using imageJ.*

Figure 5: High resolution X-ray CT imaging in wheat revealed that phosphite improves root traits

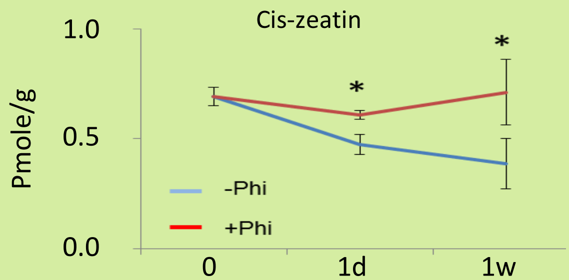


*X-ray CT imaging (**A-F**) reveals that phosphite treatment results in increase in root volume and root surface area (**G. H**). Wheat (variety siskin) seeds were sown in sandy loam soil in small columns (7.5 x 17cm). 4 day old seedlings were treated with a potassium phosphite based formulation and X-RAY CT imaging was performed at 0, 6 and 12 d post application. All values are means ±SE (n= 10); P≤0.05.*

Phosphite: Mode of Action

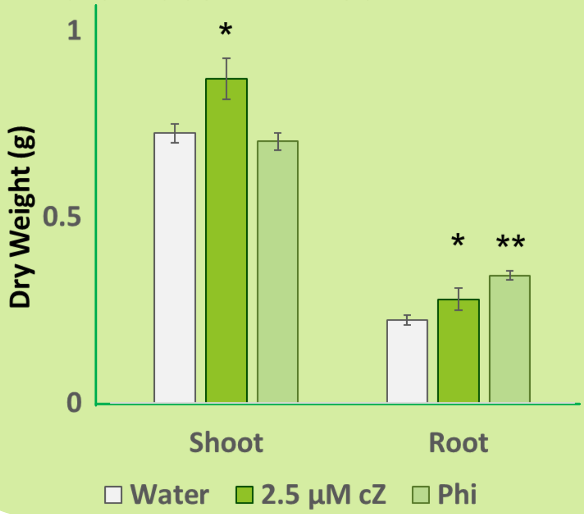
Plant hormones represent key factors regulating root growth and development. To test if there is a role for plant hormones in regulating root architecture, we performed detailed profiling of key plant hormone classes (plus their precursors and degradation products). One class of cytokinin termed cis Zeatin (cZ) showed a significant increase in root tissues across all time points (Figure 6). Cytokinins play a vital role during lateral root (LR) organogenesis in directing the flow of auxin in the new LR primordia[13]. This suggests a possible mode of action, through which foliar phosphite application, promotes cZ production in shoots, this is then transported to LR primordia where it promotes downstream process such as LR emergence. We do find that direct cZ application improves root and shoot biomass in wheat (Figure 7) further supporting our hypothesis. Currently experiments are underway to determine if the effects of phosphites are mediated via cZ.

Figure 6: Phosphites enhance cis-zeatin levels



Hormone profiling shows a significant increase in cis-zeatin levels in roots upon phosphite treatment (students t test P=<.05).

Figure 7: Direct application of cis-zeatin improves root and shoot biomass in wheat



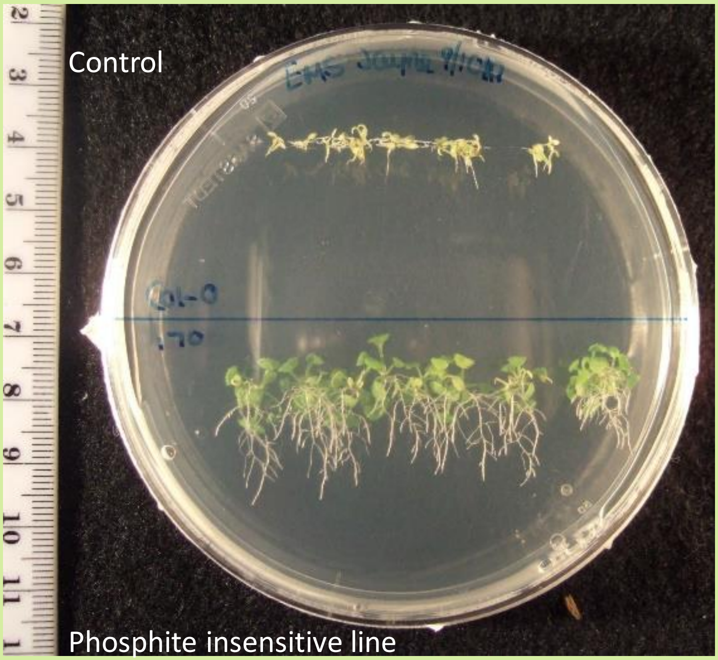
Phosphite treated (T) plants show an increase in root biomass upon cis-zeatin treatment.

Application GS12; Growth assessment 2 weeks post treatment.

Phosphite Signal Transduction Pathway

To identify novel components of the phosphite response pathway, we have performed an unbiased forward genetic screen using a homozygous EMS population[14] (INRA Versailles). This population has over 300 lines with 100-400 independent mutations per line with a high probability of a mutation in every *Arabidopsis* gene. This population has been grown in presence of inhibitory concentration of phopshite and 14 lines have been identified that do not respond to the phosphite treatment and thus may have defect in phosphite response pathway (Figure 8). Characterisation of these genes using a bulk segregation approach and next generation sequencing is likely to shed light on phosphite response pathway.

Figure 8: Forward genetic screen to identify phosphite response pathway components

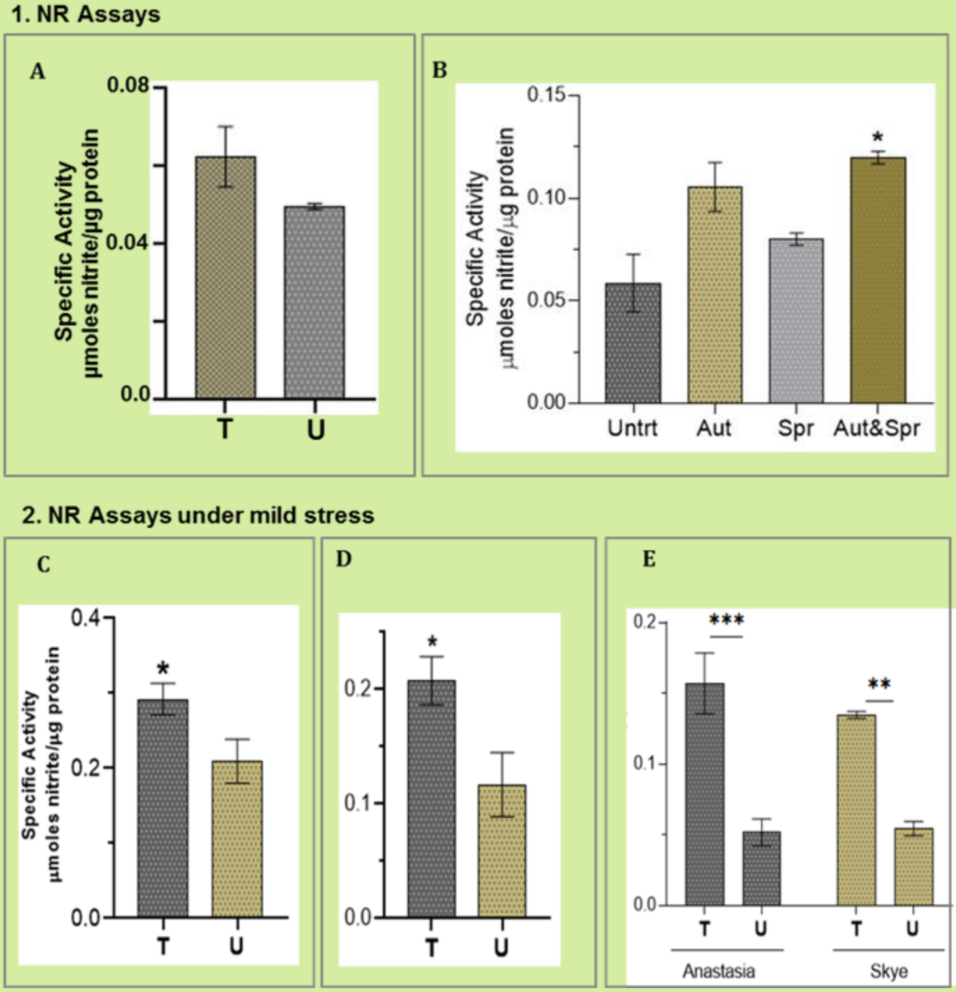


Arabidopsis seeds were sterilised and plated directly on plates containing inhibitory concentration of phosphite. Growth assessment was done 5-7 days post treatment. Phosphite resistant seedlings were taken to next generation and their phosphite tolerance was further validated in the next generation.

Phosphite treatment results in increased Nitrate Reductase Activity

Researchers in Institute of Phytopathology, Kiel University have observed that phosphite treatment results in the induction of Nitrate Reductase (NR), a key enzyme involved in N assimilation[6-7]. These findings have been validated at Nottingham in both wheat and Oilseed Rape (Figure 9).

Figure 9: Phosphites enhance Nitrate Reductase activity



A. Wheat (variety siskin) grown in field with different time of phospite application
B. oilseed rape (variety Skye).

***C**. Oilseed rape (skye)*
***D**. Wheat (siskin)*
grown under reduced nutrient (50% strength).
E. Soil grown and drought stressed OSR commercial varieties; Skye and Anastasia

Effect of phosphite on nitrate reductase activity is more pronounced under mild stress.
Growth assessment was done 9 and 19 days post treatment, respectively.
All values are means ±SE (n= 5 - 25); P≤0.05.

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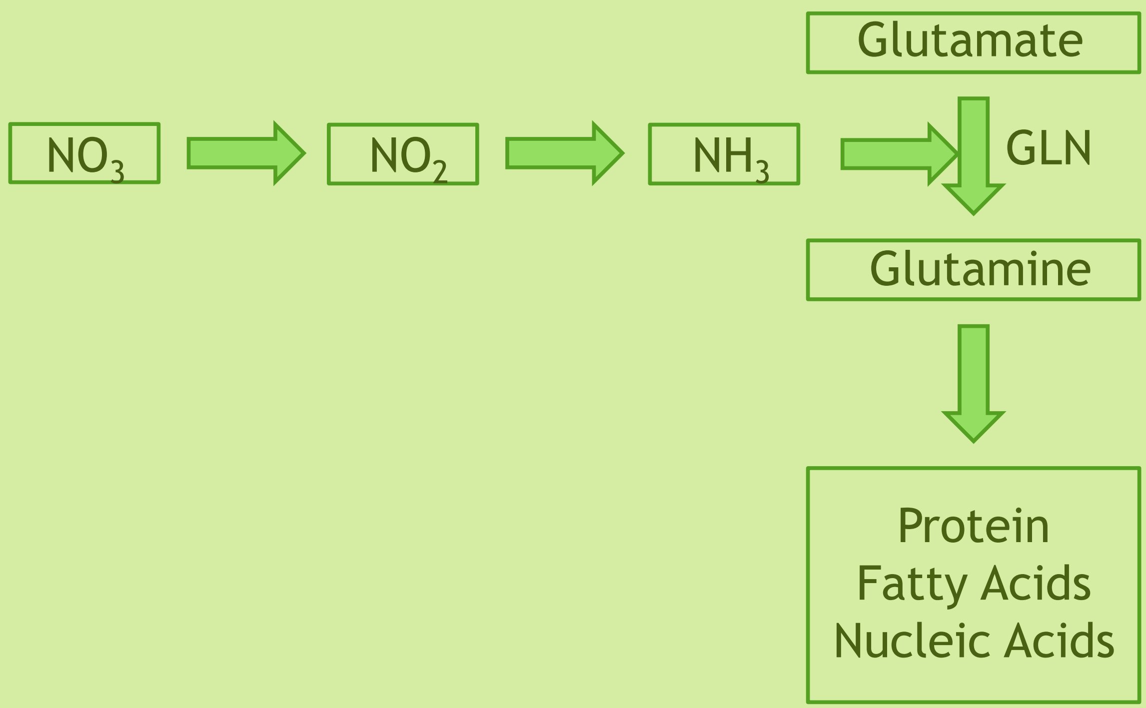
Relevance of Nitrate Reductase

Nitrate reductase (Figure 10) is a key enzyme in N metabolism and catalyses nitrate to nitrite conversion[15]. The nitrite formed is reduced by the enzyme nitrite reductase to ammonium, which then reacts with glutamate to form glutamine. The latter serves as the amino group donor for the synthesis of amino acids. The total nitrogen flux from the nitrate to the amino acids is limited by the activity of the first enzyme nitrate reductase. In plants, the enzyme nitrate reductase is part of the extremely important nitrogen metabolism and helps to provide reduced, metabolizable nitrogen for the synthesis of organic matter. Thus 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).

Figure 10: Nitrate reductase a key enzyme in N metabolism



Nitrate reductase catalyses nitrate to nitrite conversion. The nitrite formed is reduced by the enzyme nitrite reductase to ammonium, which then reacts with glutamate to form glutamine by glutamine synthetase. The latter serves as the amino group donor for the synthesis of amino acids, proteins and Nucleic acids.

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Impact

Social Impact

Global food security is one of the biggest challenges facing world agriculture. By having a better understanding of the role of phosphite in improving root architecture, this research project is likely to have a direct impact in improving resource use efficiency and plant fitness in a number of commercially important horticultural and cereal crops. With crops yielding better returns due to improved resource use efficiency, this research is likely to have a direct impact on farm income leading to improved nutritional, financial and social stability.

Environmental Impact

The increases in food production to feed the growing population must be achieved through a sustainable intensification of agriculture. Improvement in root architecture is likely to promote low input agriculture and this will have a direct environmental impact.

Phosphites: An impact case study

At the Institute of Phytopathology at Kiel University, Joseph Verreet and colleagues found a very strong economic impact case for phosphites as biostimulants (Vereeet et al, 2019, 3rd Biostimulant Congress, Barcelona[7]). Their research spanning over 14 years and involving 21 field trials in oilseed rape show that phosphite treatment results in improved N use efficiency, yield and farm income. They estimate an average yield increase of 0.245 t/ha equating to a net profit of 57.25 €/ha (Vereeet et al, 2019[7]).

Verreet et al[7] also report increase in net yield and farm income in wheat. They report that phosphite treated seeds results in an average yield increase of 0.97t/ha. The yield increase was even more pronounced when seed treatment was supplemented with foliar phosphite treatment and resulted in an average yield increase of 2.3 t/ha compared to the untreated control. Significantly, the increase in yield of 2.3t/ha is with the same fertilization amount, indicating an improved N-efficiency.

Based on N content determination in the grain, Verreet et al, 2019 calculated that phosphite treated samples accumulated 38.3 kg more N in the grain per hectare.

As phosphite treatment improves N mobilization, they designed experiments to test if N application can be reduced without compromising the yield. A combination of seed treatment and foliar phosphite treatment resulted in a yield increase of 1.35 t/ha even by using 40 kg N/ha less of nitrogen fertilizer. This suggests that a higher nutrient efficiency can be obtained on a reduced fertilizer level resulting in a net increase of 186 €/ha (assuming a wheat price of 160 €/t).

Conclusion

The conclusion from data presented in this white paper is that phosphites do act as potent, cost-effective biostimulants of root growth, when applied at low doses. We have made good progress in understanding the molecular basis of this observation and suggest registration of phosphites as biostimulants should be accepted alongside the prior pesticide registration.

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