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WHITE PAPER

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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. 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 over the next two decades.

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.

Biostimulants are emerging as a new 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. 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.

Investigating the role of Phosphite in Plant development

This white paper summarizes the work done in the Institute of Phytopathology, Kiel University, Germany on the biostimulant properties of Phosphites.

Phosphites are reduced form of phosphate and several studies and patents have reported positive effects of phosphites in plant growth, flowering and fruiting and yield increase. Phosphite cannot be converted to phosphate, so does not enhance plant growth via a nutritional mechanism.

Long term field trials show Phosphite treatment in *oilseed rape* results in improved N use efficiency, yield and farm income

At the Institute of Phytopathology at Kiel University, we have been working on phosphites for several years our work clearly shows that phosphites are biostimulants. Our results over 14 years involving 21 field trials in oilseed rape find that phosphite treatment results in improved N use efficiency, yield and farm income.

The key findings (Table 1) are summarized below:

Phosphite treatments result in

- an increase in pod number per plant (increased number of pods on the main shoot by 2, in the first by-shoot by 4 and in the second by-shoot by 5 pods per plant).
- all 21 trials showed a positive yield increase (0.04 – 0.59 to/ha)
- an average increase in yield of 0.25 t/ha in phosphite treated plants compared to untreated plants
- increase in farm income by 56 €/ha (assuming a rapeseed price of €350/t)

Table 1: Effect of Nutri-Phite Magnum S on yield and net profit over a 14 year periods involving 21 trials

		Mean	**Minimum**	**Maximum**
Untreated Control	t/ha	4.43	3.50	6.11
Nutri-Phite Magnum S (0,5 l/ha in autumn + spring)	t/ha	4.67	3.55	6.34
Yield difference	t/ha	**0.25**	0.41	0.59
Net profit *(35.00 €/dt rape, 28.50 €/L Nutri-Phite Magnum S)*	€/ha	56	-15	177

Phosphite (Nutri-Phite®Magnum S) was applied at 2-6-leaf stage in autumn and at beginning of stem elongation in early spring at a dose of 0.5l/ha.

In addition to the increase in yield, phosphite treatment also resulted in an improved nitrogen utilization (Table 2).

Table 2: Effect of Nutri-Phite Magnum S on yield and nitrogen balance over a 14 year period involving 21 trials **in oilseed rape**

Main Crop	Yield t/ha	N-withdrawal grain* kg/ha
Untreated Control	4.43	148.30
Nutri-Phite Magnum S (0,5 l/ha in autumn + spring)	4.67	156.51
Difference	**+ 0.25**	**+8.21**

Phosphite (Nutri-Phite®Magnum S) was applied foliar at 2-6-leaf stage in autumn and at beginning of stem elongation in early spring at a dose of 0.5l/ha.

*based on N contents in rapeseed 33.49 kg/t (Source: Bayerische Landesanstalt - Website, January 2019)

Phosphite promotes root growth in wheat

In addition to the effects observed in oilseed rape, we also find that phosphite promotes root growth in wheat (Figure 1). Wheat seeds were treated with Nutri-Phite® Magnum S and a positive effect of phosphite treatment on early root development was seen (Figure 1). The determination of the root mass revealed an increase of 7.4% at GS 20 (Dec 2018) and 50% at GS GS 29 (Mar 2019), respectively.

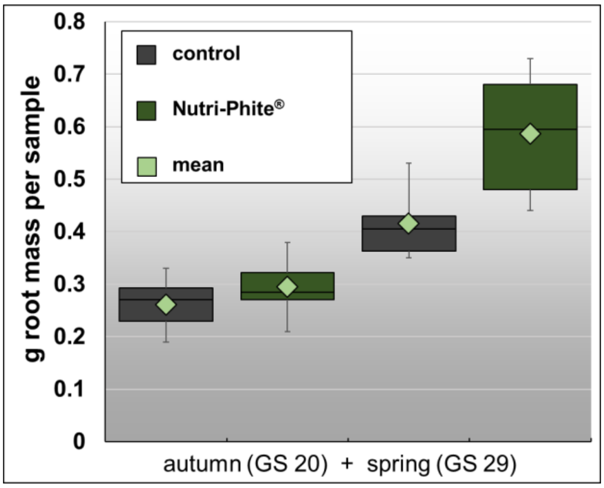

Figure 1: Phosphite promotes root growth in wheat.
Wheat seeds were treated with Nutri-Phite® Magnum S (30 ml/100kg seeds) and applied together with adjuvant Kantor® (45 ml/100kg seeds) and root biomass was measured at GS20 (Dec 2018) and GS29 (Mar 2019).

Phosphite treatment improves grain yield in wheat

To test if improved root growth also improves grain yield in wheat, a series of experiments were performed comparing different treatment regime including seed treatment and foliar treatment.

As shown in Figure 2, single seed treatment of Nutri-Phite® Magnum S (PO^{3-}_3) + Kantor® (30 + 45 ml/100kg seeds) resulted in an additional yield of 0.97 t / ha compared to the untreated control (= current farm practice).

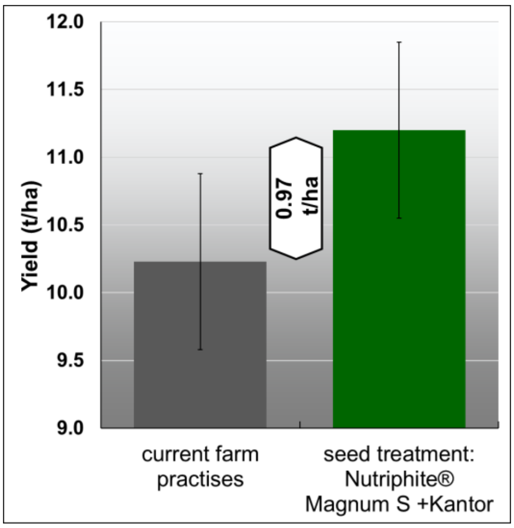

Figure 2: Experiments using phosphite treated seeds revealing 0.97 t/ha increase in mean wheat grain yield compared to untreated control (2018-19). Seeds were treated with Nutri-Phite® Magnum S (PO^{3-}_3) + Kantor® (30 + 45 ml/100kg seeds).

The yield increase was even more pronounced when seed treatment was supplemented with foliar phosphite treatment that resulted in an average yield increase of 2.3 t/ha compared to the untreated control (Table 3). 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 it can be calculated that phosphite treated samples accumulated 38.3 kg more N in the grain per hectare compared to untreated controls. It is conceivable that net N mobilization is much higher (>2) than 38.3 Kg N/Ha in the treated samples when we also take into account the N content in the straw. The increase in yield and the higher N-uptake indicates an improved N-efficiency in the sense of "nutrient efficiency" and is an important factor in the "nutrient balance" for nitrogen.

Table 3: Wheat grain yield (t/ha), crude protein (%) and calculated N-withdrawal of grain (kg/ha) after application of Nutri-Phite® Magnum S (foliar without and seed application with Kantor®) compared to the untreated control (*Phosphite - Nutri-Phite®Magnum S - was applied as seed dressing and also as foliar spray at GS* 12-14, GS 25-31, GS 37-39 *at a dose of 0.35l/ha)*

	Grain Yield t/ha	Crude Protein (%)	N-withdrawal kg/ha
Untreated Control	9.6	11.6	167.70
Common Practice	10.6	11.3	180.4
Optimized System	11.9	11.5	206.0
Difference (Optimized System - Untreated Control)	2.3	-0.1	+38.3
Difference (Common Practice - Untreated Control)	1.0	-0.1	+12.7
Difference (Optimized System - Common Practice)	1.4	+0.2	+26.6

Phosphite treatment improves nutrient use efficiency and improves yield even under low N

As phosphite treatment improves N mobilization (Table 3), experiments were designed to test if N application can be reduced without compromising the yield.
For these experiments we compared three different production systems as shown in table 4 and summarized below:.
Control- *no phosphite treatment; no fungicide treatment; Normal N*
Current farm practices-*no phosphite treatment; optimized fungicide application with Kantor; Normal N*
Optimized production system- *includes an optimized fungicide application with Kantor® and 3 foliar applications with Nutri-Phite®Magnum S (GS 12-14, GS 25-31, GS 37-39; application rate*

0.35l/ha) and an additional seed treatment with Nutri-Phite® Magnum S (PO^{3-}_3) + Kantor® (application rate 30 + 45 ml/100 kg seeds). In addition, in the optimized production system, a reduced nitrogen fertilization of 140 kg N/ha was used as compared to 180 Kg N/Ha used for control or the current farm practices.

Table 4: Comparison of three different growth regimes

	N (Kg/Ha)	**Standard Fungicide application**	**Nutri-Phite Magnum S**
Control	180	No	No
Current Farm Practice	180	Yes	No
Optimised Production System	**140**	**Yes**	**Yes**

Phosphite (Nutri-Phite®Magnum S) treatment included 3 foliar applications with Nutri-Phite®Magnum S (PO^{3-}_3) (GS 12-14, GS 25-31, GS 37-39) and an additional seed treatment with Nutri-Phite® Magnum S (PO^{3-}_3) + Kantor®.

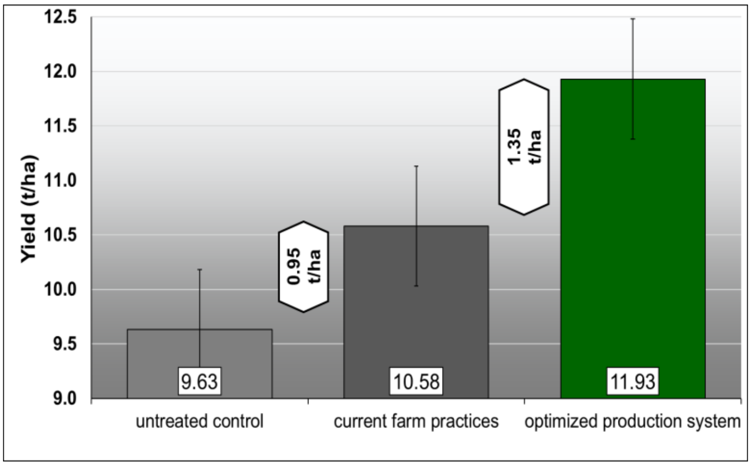

Figure 3: Grain yield (t/ha) in the untreated control (1), current farm practices (2) and the optimized production system (3) with fungicides combined with Kantor®, foliar applications of Nutri-Phite® Magnum S (PO^{3-}_3) and seed treatment Nutri-Phite® Magnum S (PO^{3-}_3) + Kantor® in winter wheat in 2018/19.

As shown in Figure 3 the difference between the current farm practices and the optimized production system resulted in a yield increase of 1.35 t/ha even by using 40 kg N/ha less of nitrogen fertilizer. The data suggest 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.

Mode of Action:

Phosphite-treated plants show higher nitrate reductase activity compared to the untreated control in both oilseed rape and wheat

The decisive question now arises as to how phosphite treatment results in improved N-efficiency, N mobilization and yield. Kiel group has recently shown that both in oilseed rape and wheat phosphite treated plants have higher nitrate reductase activity compared to un-treated plants (Figure 4 & 5).

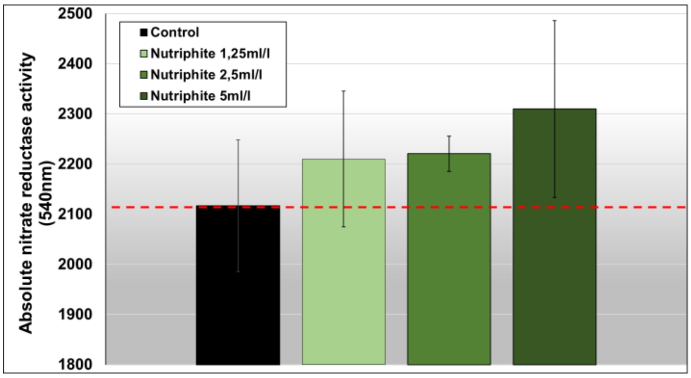

Figure 4: Phosphite treatment results in increased Nitrate Reductase activity of up to 9% in oilseed rape.
Oilseed rape plants were treated with Nutri-Phite® Magnum S and nitrate reductase activity measured 9 days after application.

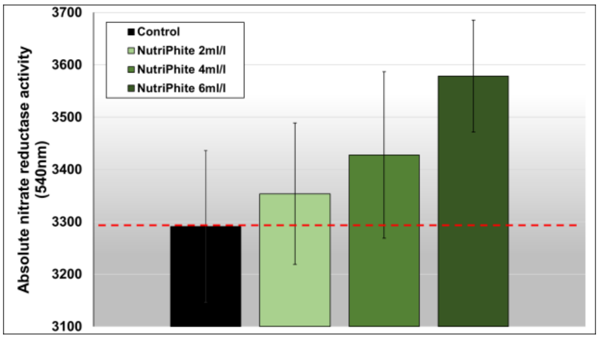

Figure 5: Phosphite treatment results in increased Nitrate Reductase activity of up to 10% in wheat.
Wheat plants were treated with Nutri-Phite® Magnum S and nitrate reductase activity measured 9 days after application.

Nitrate reductase (NR) (Figure 6) is a key enzyme in N metabolism and catalyses nitrate to nitrite conversion. The nitrite formed is reduced by the enzyme nitrite reductase (NiR) 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).

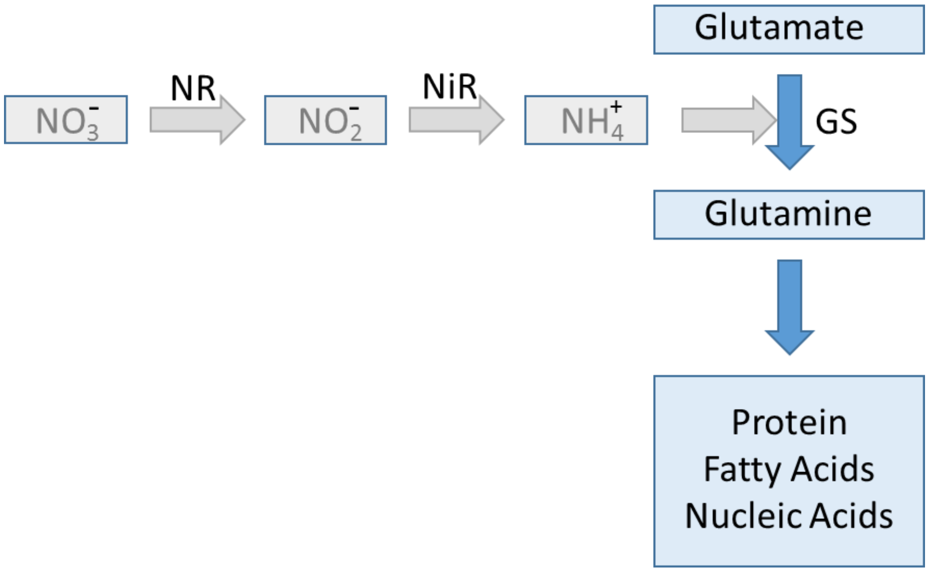

Figure 6: Nitrate reductase (NR) is a key enzyme in N metabolism and catalyses nitrate to nitrite conversion. The nitrite formed is reduced by the enzyme nitrite reductase (NiR) to ammonium, which then reacts with glutamate to form glutamine by glutamine synthetase (GS). The latter serves as the amino group donor for the synthesis of amino acids, proteins and Nucleic acids.

Effect of phosphite (Nutri-Phite® Magnum S = (PO^{3-}_3) on the genes controlling nitrate reductase in the genetic model plant ´*Arabidopsis thaliana*´

Detection: Gene signaling of nitrate reductase

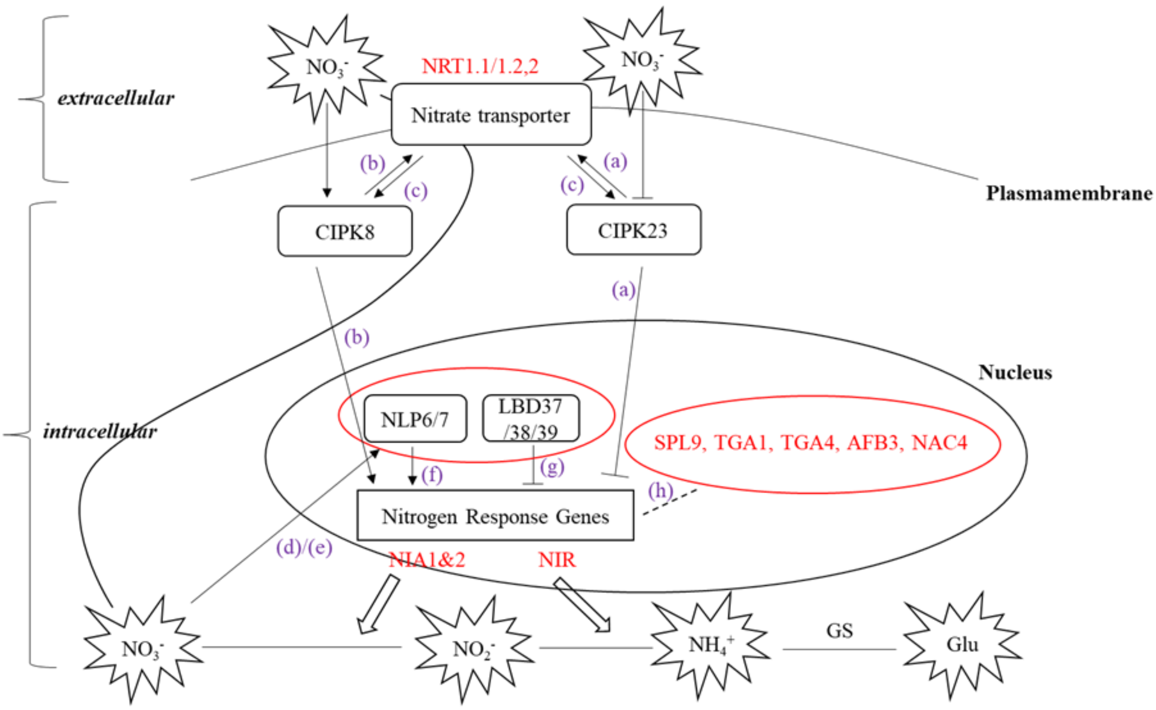

Figure 7: Scheme of the genes influencing nitrate reductase

1. CIPK family

CIPK(CBL-Interacting Protein Kinase) 8 and 23 serve as sensor to nitrate concentration in extracellular space, positively and negatively regulate expression of downstream NRG (Nitrogen Response Genes) respectively, such as NRT(Nitrate Transporter) genes, NIA genes (Nitrate reductase), NIR genes (Nitrite reductase). In low concentration of nitrate, CIPK23 would play a major role to phosphorylate nitrate transporter in plasmamembrane and change it into high-affinity mode (a), concurrently repress NRGs expression, whereas in high concentration, CIPK8 would be dominant to switch transporter into low-affinity and induce downstream gene expressions(b). In turn, Nitrate transporters also regulate the production of these two enzymes(c).

2. Transcriptional Factors (mainly NLP6/7, LBD37/38/39, assumably SPL9, TGA1, TGA4, AFB3, NAC4)

Those TFs are mainly regulated by intracellular nitrate concentration, which would be changed by property of nitrate transporter located in plasmamembrane. For NLP6 and 7, high concentration of nitrate will trigger the their transmission and maintainance in nucleus (d) which would boost the expression of NRGs (f). Also nitrate concentration will affect the production of LBD37/38/39 in transcriptional level (e), who in turn repress the expression NRGs (g). Through system biology tools, some TFs (SPL9, TGA1, TGA4, AFB3, NAC4)have been identified related to nitrate assimilation but still need to be confirmed and figured out how they get involved in process by further experiments (h).

3. Downstream pathways

NIA1&2 and NIR genes, which encode nitrate reductase and nitrite reductase respectively, are regulated by factors mentioned above and crucial genes in nitrate assimilation pathway in plant.

Results: Upregulated expression of both genes responsible for nitrate reductase; NRT1.2 ; NIA1

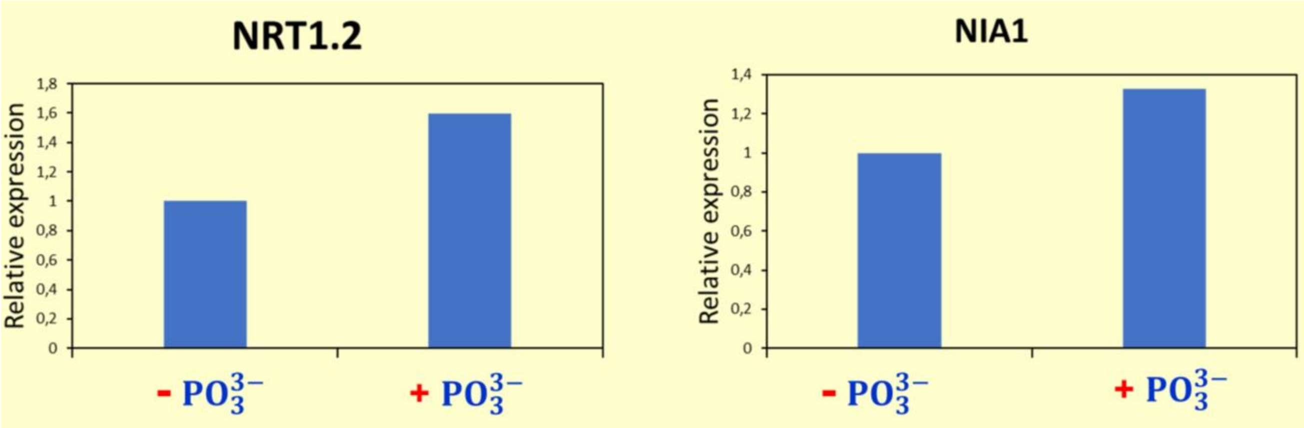

Figure 8: Influence of Nutri-Phite® Magnum S (PO^{3-}_3) on both genes responsible for nitrate reductase (NRT1.2, NIA1)

The expression of both nitrate reductase regulation genes NTR1.2 and NIA1 is positively influenced by the Nutri-Phite® Magnum S treatment. The nitrate reductase activity is upregulated by the treatment with Nutri-Phite® Magnum S (PO^{3-}_3) .

Summary:

1) Phosphite [Nutri-Phite® Magnum S (PO^{3-}_3)] has a very high positive influence on the key plant enzyme, nitrate reductase (NR). This effect is demonstrated for the first time.

2) Phosphite [Nutri-Phite® Magnum S (PO^{3-}_3)] is positively influencing the expression of the key nitrate reductase genes NRT 1.2 and NIA1. This effect is demonstrated for the first time.

3) Biostimulating effects of phosphite [Nutri-Phite® Magnum S (PO^{3-}_3)] are reflected by increased root growth, increased nutrient efficiency, increased nitrogen mobilization, increased yield structure factors and increased yield.

4) Phosphite has been scientifically proven as a biostimulant according to EU regulation 2019/1009.