

## PHYSIOLOGICAL AND AGRONOMIC RESPONSE OF MAIZE TO FOLIAR APPLICATION OF UTRISHA N: EVIDENCE FROM REAL FARM FIELD TRIALS IN WESTERN ROMANIA

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### Abstract

*The present study evaluated the effectiveness of the biological product Utrisha™ N (Methylobacterium symbioticum) on maize cultivated under real commercial farming conditions, without major changes to standard crop management practices. The experiment was conducted in 2024 at the Neudorf experimental field (Arad County, Western Romania) using a paired strip design consisting of four independent strip pairs (Utrisha N vs. untreated control), each covering 1 ha. Statistical analysis was performed using a paired t-test (n = 4). Application of Utrisha N resulted in a significant mean grain yield increase of +461 kg ha<sup>-1</sup> (+7.0%, p < 0.001). This yield response was associated with a significant increase in chlorophyll index (+3.05 AT Leaf units, p = 0.007), indicating enhanced photosynthetic activity. The physiological benefit confirmed by chlorophyll measurements suggests a measurable and biologically relevant mechanism, supporting the scientific credibility of the treatment. Grain moisture at harvest was slightly higher in the treated variant (+0.45 percentage points, p = 0.016), indicating a marginal delay in physiological maturity. Overall, the results confirm the effectiveness of Methylobacterium symbioticum in improving maize yield performance and physiological status under the pedoclimatic conditions of Western Romania.*

**Key words:** biofertilizer, chlorophyll index, *Methylobacterium symbioticum*, Utrisha N.

### INTRODUCTION

The increasing global demand for agricultural products, together with pressures generated by climate change, soil degradation, and the need to reduce chemical inputs, necessitates the identification of sustainable solutions for intensifying agricultural production. Maize (*Zea mays* L.), one of the most important cereal crops worldwide, is highly dependent on nitrogen supply, and improving nitrogen management efficiency represents a major priority for modern agriculture (Sheoran et al., 2021). In this context, biological nitrogen fixation and the use of microbial biostimulants have attracted growing scientific and practical interest. Biological nitrogen fixation (BNF) is a natural process through which diazotrophic microorganisms reduce atmospheric nitrogen into forms assimilable by plants. Although this mechanism is well established in legumes, its extension to cereal crops was long considered limited (Guo et al., 2023). However, recent research has

demonstrated that bacteria associated with cereal roots can be genetically or agronomically manipulated to express nitrogenase activity under controlled conditions (Ryu et al., 2020; Bloch et al., 2020). These findings have opened new perspectives for integrating BNF into conventionally fertilized agricultural systems (Wen et al., 2022). In parallel, the development of agricultural biostimulants has expanded significantly. Biostimulants are defined as substances or microorganisms which, when applied to plants or soil, stimulate natural processes to enhance nutrient use efficiency, stress tolerance, and crop quality (Calvo et al., 2014; Shahwar et al., 2023). Recent meta-analyses indicate that the application of biostimulants can lead, on average, to significant yield increases, although crop responses depend on species, pedoclimatic conditions, and the type of product used (Li et al., 2022). Of particular interest are bacteria belonging to the genus *Methylobacterium*, which are recognized for their ability to colonize leaf surfaces and the

rhizosphere, solubilize phosphates, and interact with plant metabolism (Agafonova et al., 2013; Chibeba et al., 2025). Certain strains, such as *Methylobacterium symbioticum*, have been developed as foliar inoculants capable of supplying biologically fixed nitrogen to maize under field conditions (Corteva Agriscience, 2022). However, the results obtained are variable: while some studies report increases in productivity and nitrogen recovery (Santos & Bundt, 2025), others indicate limited effects or responses dependent on nitrogen fertilization rates (Rodrigues et al., 2024; Quemada et al., 2026). The interaction between nitrogen nutrition and plant metabolism is closely linked to the role of free amino acids, which function both as structural molecules and as metabolic signals during seed development and vegetative growth (Amir et al., 2018; Ryu et al., 2020). By influencing nitrogen availability, biostimulants and nitrogen-fixing microorganisms can alter these metabolic fluxes, with direct implications for yield and crop quality. The adoption of microorganism-based technologies is also influenced by agronomic and digital factors. Monitoring crop nitrogen status using optical sensors provides valuable tools for optimizing the timing and rates of biological input applications (Gabriel et al., 2019). At the same time, the integration of digital twin models enables the simulation of agricultural yields and the assessment of the impact of new technologies under variable environmental conditions (Chitu et al., 2024).

The effectiveness of microbial biostimulants is further conditioned by the dynamics of crop-associated microbiota. Recent studies show that inoculants can modify the structure of soil and rhizosphere bacterial communities throughout the growing season, indirectly influencing nutrient uptake and plant health (Kruščić et al., 2025). These effects must be evaluated in the context of climate change and water resource availability, factors that significantly affect agricultural productivity in many regions (Șmuleac et al., 2020).

At the European level, the use of nitrogen-fixing microbial products is increasing; however, their adoption remains heterogeneous, being influenced by agricultural policies, farmer perceptions, and associated economic risks (Visser & Babiker, 2021; Sitnicki et al., 2024).

In this context, the development of next-generation biostimulants, supported by genomic sequencing strategies and microbial engineering, represents a promising direction for sustainable agriculture (Garg et al., 2024). Overall, the current literature highlights the considerable potential of biological nitrogen fixation and microbial biostimulants in maize cropping systems. Nevertheless, the variability of observed responses underscores the need for integrated approaches that combine plant genetics, nutrient management, digital technologies, and adaptation to local pedoclimatic conditions.

## MATERIALS AND METHODS

The experiment was carried out in 2024 in Neudorf village, Arad County (Western Romania), under real commercial farming conditions. Maize was sown on 15 April 2024 and harvested on 20 September 2024.

A paired strip design was used, consisting of eight parallel strips of 1 ha each (total area: 8 ha), arranged alternately as Utrisha N treated / untreated control. This layout generated four independent experimental pairs ( $n = 4$ ), which were used as the statistical unit for inferential analysis. The study was conducted on a medium-fertility brown argic soil with a loam-clay texture, neutral reaction, and good water-holding capacity. Particle size distribution indicated approximately 42% sand, 32% silt, and 26% clay. Soil pH ranged from 6.8 to 7.0, with low calcium carbonate content ( $\sim 0.9\%$ ).

Organic matter content was moderate ( $\sim 2.0\%$ ), and total nitrogen was approximately 0.14%, indicating moderate natural fertility. The cation exchange capacity was around 17 me 100 g<sup>-1</sup> soil, with base saturation close to 80%. Available phosphorus showed medium to good levels, while available potassium was well supplied. Field capacity was approximately 28%, with plant-available water estimated at 15–16%. Micronutrients (Fe, Mn, Zn, Cu) were present at adequate levels.

The biological product Utrisha™ N (*Methylobacterium symbioticum*) was applied foliarly on 20 May 2024, at the V6 growth stage, at a rate of 333 g ha<sup>-1</sup> diluted in 200 L water ha<sup>-1</sup>. The control received the standard crop technology without biostimulant application.

Measurements: grain yield (kg ha<sup>-1</sup>): determined at harvest for each strip; grain moisture (%): measured at harvest; chlorophyll index (AT Leaf): measured on the 4th and 5th leaves of 10 randomly selected plants per strip; strip means were used for analysis. All inferential analyses were performed using a paired t-test (n = 4). Results are presented as mean ± SEM with 95% confidence intervals. Statistical significance was set at p < 0.05.

## RESULTS AND DISCUSSIONS

### *Grain production and crop response to the application of Utrisha N*

The results obtained in the experiment carried out in 2024 in Neudorf, Arad County, highlight a positive and constant agronomic response of the corn crop to the application of the biological product Utrisha N. The analysis of the yields (Table 1) carried out in the four experimental pairs shows that, in all cases, the treated strips exceeded the control variants, confirming the efficiency of the treatment under real farm conditions. The average values of grain production, presented in Table 1 and Figure 1, indicate a level of 7067 kg/ha for the Utrisha N variant, compared to 6606 kg/ha for the control variant.

Table 1. Influence of the application of Utrisha N on maize grain production (Neudorf, 2024)

| Variation | Yield (kg/ha) |
|-----------|---------------|
| Utrisha N | 7067 ± 115 a  |
| Control   | 6606 ± 102 b  |

The values represent the average of four experimental bands ± the standard deviation. The values followed by different letters (a, b) differ statistically significantly at p < 0.05.

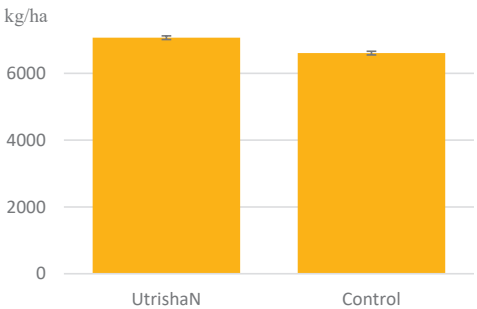


Figure 1. Yield (kg/ha) - paired t-test (n = 4 pairs)  
UtrishaN: 7067 ± 63 SEM;  
Control: 6606 ± 55 SEM  
Difference: +461 kg/ha (95%CI [428.2, 494.3]),  
 $t(3)=44.44, p<0.001$   
Relative Change: +7.0% (95% CI [6.6, 7.4])

The average production increase was +461 kg/ha, which corresponds to an increase of about 7%. This result is particularly relevant from a practical point of view, as it was obtained without changes in the basic culture technology, but only by introducing a biological product applied foliar in the V6 phenophase.

An important aspect to emphasize is the consistency of the response. The production gains were not the result of a single high-performance belt, but manifested uniformly in all four experimental pairs. This uniformity indicates a stable effect of the product, independent of small local variations of soil or microclimate, and suggests a good adaptation of the bacterium *Methylobacterium symbioticum* to the pedoclimatic conditions in western Romania.

From an agronomic perspective, a production increase of 400-500 kg/ha is significant, especially in the current context, characterized by the increase in input costs and the pressure to reduce the use of chemical fertilizers. Even in years with average production potential, such an increase can make the difference between profitability and the break-even point of the crop.

### *Grain moisture at harvest and technological implications*

In addition to the increase in production, the data obtained indicate a slight increase in the moisture of the grains at harvest in the variant treated with Utrisha N. The average values were 14.64% for Utrisha N, compared to 14.19% for the control. The difference, of about 0.4-0.5 percentage points, is small but constant and was statistically significant.

Table 2. Influence of the application of Utrisha N on corn kernel moisture (Neudorf, 2024)

| Variation | Moisture at harvest (%) |
|-----------|-------------------------|
| Utrisha N | 14.64 ± 0.24 a          |
| Control   | 14.19 ± 0.12 b          |

The values represent the average of four experimental bands ± the standard deviation. The values followed by different letters (a, b) differ statistically significantly at p < 0.05.

This moderate increase in humidity can be interpreted as a minor delay in physiological ripening, associated with a longer period of metabolic activity of the plants. In the case of biostimulants with a physiological effect, this phenomenon is common and reflects a prolongation of the grain filling phase, in which the plant continues to translocate assimilated to cobs.

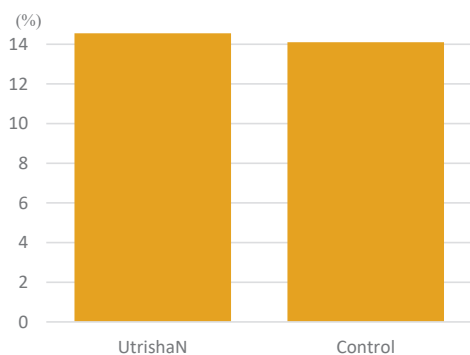


Figure 2. Humidity (%) - paired t-test  
Difference: +0.45 pp (95%CI [0.2, 0.7]),  $t(3)=4.93$ ,  $p=0.016$

From a practical point of view, the moisture level of the grains remains within normal limits for harvesting and does not imply significant additional costs for drying. Moreover, this slight delay in maturation can be considered beneficial, as it is associated with a greater accumulation of dry matter in the grains, which explains the production increases obtained.

### Chlorophyll index as an indicator of plant physiological status

The determinations of the chlorophyll index (AT Leaf) provided essential information on the physiological state of the plants and the mechanism of action of the product Utrisha N. The values obtained on leaf 4 and leaf 5 were constantly higher in the treated variants compared to the control, the differences being statistically significant (Table 3 and Figure 3).

Table 3. Effect of applying Utrisha N on chlorophyll index (AT Leaf) in maize (Neudorf, 2024)

| Variation | Analyzed leaf | Chlorophyllin Index (AT Leaf) |
|-----------|---------------|-------------------------------|
| Utrisha N | Leaf 4        | 44.9 ± 2.3 a                  |
| Control   | Leaf 4        | 41.9 ± 2.1 b                  |
| Utrisha N | Leaf 5        | 45.8 ± 2.0 a                  |
| Control   | Leaf 5        | 42.5 ± 2.2 b                  |

The values represent the average of the determinations made on 10 plants/strip, in four treated strips and four control strips. The values followed by different letters (a, b) differ statistically significantly at  $p < 0.05$ .

For leaf 4, the chlorophyll index reached an average value of 44.9 AT Leaf in the treated variant, compared to 41.9 AT Leaf in the control. Similar differences were observed on leaf 5, where the mean values were 45.8 AT Leaf at Utrisha N and 42.5 AT Leaf at control. These

differences, of about 3 units of AT Leaf, exceed the natural variability expressed by the standard deviation and indicate a clear treatment effect.

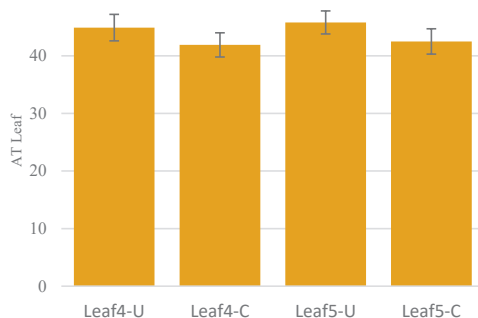


Figure 3. Chlorophyll Index (AT Leaf) - band averages; paired t-test

Leaf 4:  $\Delta = +2.77$  (CI95% [0.3, 5.3]),  $t(3)=3.50$ ,  $p=0.039$   
 Leaf 5:  $\Delta = +3.32$  (CI95% [2.2, 4.5]),  $t(3)=9.36$ ,  $p=0.003$   
 Average leaf 4&5:  $\Delta = +3.05$  (CI95% [1.6, 4.5]),  $t(3)=6.68$ ,  $p=0.007$

The increase in chlorophyll index reflects an improvement in plant nitrogen nutrition, given the central role of nitrogen in the structure of the chlorophyll molecule. In the case of Utrisha N, the bacteria *Methylobacterium symbioticum* colonize the tissues of the plant and fix atmospheric nitrogen, making it available to the plant in a gradual way, correlated with physiological requirements.

This continuous supply of nitrogen helps keep leaves green for a longer period, delaying senescence and supporting photosynthesis in critical phases for production formation. The high values of the chlorophyll index observed on leaf 5, a fully developed and intensely photosynthetically active leaf, confirm this mechanism.

### The relationship between photosynthesis and production accumulation

The correlation of chlorophyll index data with grain yield data shows a direct relationship between plant physiological status and final crop yield. In the variants treated with Utrisha N, higher levels of chlorophyll index were associated with consistent production increases, suggesting that improved photosynthesis is the main mechanism by which the product exerts its effect.

Intensified photosynthesis leads to a higher production of assimilates, which are

subsequently translocated to cobs. In the grain filling phase, this increased availability of assimilates allows the accumulation of a larger amount of dry matter, which is reflected in the increase in the mass of the grains and, implicitly, in the total production.

In addition, maintaining high photosynthetic activity for a longer period can contribute to a more efficient use of water and nutrient resources, especially under moderate stress. Thus, the application of Utrisha N can play an important role in stabilizing productions, not just increasing them.

The results obtained in this experiment have a high practical relevance for farmers, as they demonstrate the efficiency of a biological product in a commercial production system, on a hectare scale. Unlike testing in microplots, where conditions are strictly controlled, the present experiment reflects the reality on the farm, with all its variables.

The application of Utrisha N at a dose of 333 g/ha, in phenophase V6, proved to be an effective solution for increasing maize production and improving the physiological state of plants. The benefits observed are not the result of a direct supply of mineral nitrogen, but of an optimization of the plant's metabolism, which makes the product compatible with modern strategies to reduce chemical inputs.

In the current context, marked by rising prices for nitrogen fertilisers and pressure to adopt more sustainable agricultural practices, the use of nitrogen-fixing bacteria is a promising direction. The results obtained in Neudorf confirm that such solutions can generate concrete economic benefits without compromising the quality of production.

## CONCLUSIONS

The application of the biological product Utrisha N resulted in a significant increase in grain production compared to the control variant. The mean of the experimental pairs was  $7067 \pm 63$  SEM kg/ha in the treated version, respectively  $6606 \pm 55$  SEM kg/ha in the control, resulting in an average increase of  $+461$  kg/ha (95%CI [428, 494]),  $p < 0.001$ .

The values of the grain moisture at harvest were slightly higher in the treated version ( $+0.45$  percentage points, 95%CI [0.2, 0.7],  $p = 0.016$ ),

indicating a slightly delayed maturation, but without negative impact on recovery.

The determinations carried out on leaf 4 and leaf 5 showed a significant increase in the chlorophyll index in the treated version. The mean on the band indicates an increase of approximately  $+3.05$  AT Leaf units (95%CI [1.6, 4.5],  $p = 0.007$ ), confirming the intensification of photosynthetic processes, which indicates a real and measurable agronomic mechanism.

The results obtained attest to the potential of Utrisha N to improve the efficiency of nitrogen use and optimize crop performance in real farm conditions. Overall, the experimental data show that the application of the biological product Utrisha N led to a significant improvement in the physiological status of maize plants, reflected by higher chlorophyll index values, and to a stable and reproducible production increase. The correlation of the physiological parameters with the productive ones supports the hypothesis that the main effect of the product is a physiological one, manifested by the intensification of photosynthesis and the prolongation of the period of activity of the foliar apparatus.

These results confirm the usefulness of Utrisha N as an effective tool in modern maize cultivation technologies and provide farmers with a practical solution for increasing productivity in real farm conditions.

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