



Advances in biology through agronomy, aquaculture, coastal and environmental sciences

— Volume II —

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**Advances in biology through
agronomy, aquaculture, coastal and
environmental sciences**
Volume II



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Advances in Biology through Agronomy, Aquaculture, Coastal and Environmental Sciences, Volume II, is an e-book that continues the work of the first version (<https://editorapantanal.com.br/ebooks/2023/advances-in-biology-through-agronomy-aquaculture-coastal-and-environmental-sciences/ebook.pdf>), published in 2023 by Leandris Argente!-Martínez and Ofelda Peñuelas-Rubio.

Our e-book compiles 25 manuscripts that present scientific results and reviews based on biological aspects. All of them contribute to explaining the responses of living organisms to agricultural, aquaculture, and coastal ecosystems, as well as those facing fragility or threat of climate change. This e-book was edited by Leandris Argente!-Martínez, Ofelda Peñuelas-Rubio, and Joe Luis Arias-Moscoco, members of the Consolidated Academic Group ITVAYA-CA-3.

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The reduction in chemical fertilization combined with PGPM under a controlled deficit irrigation system for maize

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ABSTRACT

This study was conducted under open field experimental conditions on a Vertisol soil located in Block 611 of the Yaqui Valley, Sonora, Mexico. The objective of the trial was to evaluate the effect of a 50% reduction in nitrogen fertilizer on the phenology and biomass yield of the Hipopótamo maize hybrid. The timing of phenophases, chlorophyll content, normalized difference vegetation index (NDVI), and biomass yield were evaluated in two treatments: T1: application of 100% of the nitrogen dose, and T2: a 50% reduction in nitrogen fertilizer with the aid of the bacteria *Enterobacter cloacae*, applied 24 hours before planting as a pre-germination treatment. The 50% reduction in nitrogen fertilizer did not cause a significant variation in phenology compared to T1. Although chlorophyll content and NDVI increased significantly in T2 during growth stages V1 and V2, no differences were observed between T1 and T2 from V3 to V12. However, during reproductive phenophases, T1 maintained higher chlorophyll and NDVI values, as well as a longer duration of phenophase occurrence. Biomass yield was not affected by the reduction in nitrogen fertilizer, reaching an average of 218 t ha⁻¹ in both treatments. The experiment demonstrated that with the aid of *Enterobacter cloacae*, it is not necessary to apply 400 kg ha⁻¹ of nitrogen for Hipopótamo corn cultivation in the Yaqui Valley.

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INTRODUCTION

In recent years, high doses of nitrogen fertilizers have been applied in maize production systems in northwestern Mexico, primarily in Sinaloa and Sonora, to ensure high maize yields (Santillano-Cázares, 2021). This practice aims to provide a greater amount of nitrogen to the plants from the initial stages of development, due to the low organic matter content (Parra-Cota et al., 2018). In most of these production systems, high yields are obtained due to these practices, but production costs are also high (Delgado & Rodríguez, (2019). At the same time, the application of high doses of fertilizer can cause soil salinization and contaminate aquifers.

In these regions, rainfall is coincidentally very low, and the availability of water for irrigation is reduced (González et al., 29026), leading producers to try to provide the crop with fertilizers to ensure faster field closure and prevent water loss through evaporation (Plata-Rocha et al., 2026). The use of pressurized and controlled deficit irrigation systems is an important alternative a better water use efficiency (Zou et al., 2021), even when combined with fertilization to reduce application rates. Meanwhile, the use of microorganisms that promote plant growth and contribute to the availability of essential minerals has proven to be a scientifically validated ecological and agronomic alternative for ensuring production and improving soil health (Xing et al., 2025, Krasilnikov et al., 2022.). Some genera, such as *Enterobacter* make a significant contribution to the physiological and agronomic performance of crops and contribute to tolerance to abiotic conditions such as drought (Azizoglu, 2025).

Based on the above, an open-field study was conducted to evaluate the effect of a 50% reduction in nitrogen fertilizer with the aid of the bacterium *Enterobacter cloacae* on the phenology, chlorophyll content, NDVI, and biomass yield of the commercial corn hybrid Hipopótamo, compared to when 100% of the nitrogen fertilizer is applied without the use of microorganisms.

MATERIALS AND METHODS

Study site

The work was carried out at an experimental site in the Yaqui Valley, Sonora, Mexico. During the winter-spring period, the soil was a vertisol that had been cultivated for more than 30 years, primarily with wheat and maize. The experimental area covered 0.12 ha (35 x 35 m²). The crop was planted from east to west, with a spacing of 0.11 m between plants and 0.8 m between rows (Figure 1).



Figure 1. Image of the experiment.

Climate variability

Edaphoclimatic variables were evaluated in situ at the experimental area. Ambient temperatures and relative humidity were measured by means of a digital hygrometer (EMA) placed at the central part of the plots, placing the sensor at 2.4 m of height. During the experimental period, the climate exhibited temperature variations (Figure 2). The lowest minimum temperature recorded was 10.3°C in January; all other months-maintained temperatures above 12°C, so there was no risk of frost damage to the crop. Temperatures remained below 36°C, except for the planting date, which reached 38.1°C.

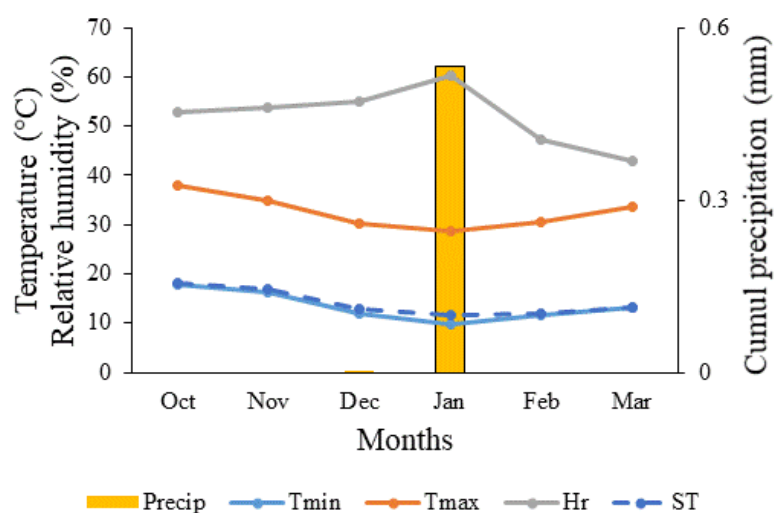


Figure 2. Climatic conditions during the crop cycle Oct 2025 - Mar 2026.

Relative humidity remained between 50% and 55% in most months, except for January, which saw only 0.55 mm of precipitation. The lowest relative humidity was observed in February and March due to reduced irrigation in the region. Soil temperature was measured by means of a FST100-2006A soil temperature sensor, placed at the central part of each plot. The minimum soil temperature consistently remained between 12°C and 18°C.

Plant material

The commercial hybrid Hipopótamo, provided by ASGROW, was used as an experimental model. This hybrid is one of the best adapted to the climatic conditions of northwestern Mexico and possesses relative tolerance to drought, salinity, and high temperatures (Argentel-Martínez et al., 2024). Its biological cycle is 154 days, with flowering occurring at 70–73 days when cultivated under four supplemental irrigations with an average water depth of 16 mm. Its average yield for northwestern Mexico is 215 t ha⁻¹ of biomass, and its grain yield can exceed 16 t ha⁻¹.

Plant growth promotion microorganism used

For the experiment it was used *Enterobacter cloacae* which is a non-hemolytic endophytic rhizobacteria highly producer of siderophores, and in average production of indolacetic acid of 26.7 µL mL⁻¹. Also, this bacterium has the ability of phosphate solubilization index of 3.14 (Azizoglu et al., 2025).

Variables evaluated

Plant phenology (Phenophase occurrence time) was measured at each treatment when more than 51% of the plants presented the characteristics related to each of the phenophases described by the phenological scale used by Ibarra et al. (2020) for the northwest of Mexico.

For the chlorophyll content (SPAD units), a portable SPAD-type portable device was used. The measurements were performed on a total of 10 leaves in the central part, aiding the central nervature. These measurements were carried out during flowering phenophase in completely well-exposed leaves to sunlight between 12:00 h and 14:00 h (Li et al., 2024).

The normalized difference vegetation index (NDVI) ($1 > \text{NDVI} > -1$) was determined during each crop phenophase. The measurement was carried out by using a portable Greenseeker-type device (Trimble™) (Govaerts and Verhulst, 2010). All measurements were taken at 11:00 a.m., with the device positioned 0.6 m from the plant leaf area (Prakasha et al., 2020).

Biomass and grain yield evaluation

The biomass yield (BY: kg ha⁻¹) was assessed for a total of 10 plants per treatment when they were in the R3 phenophase (Figure 3). These plants were cut at the base of the stem and weighed separately to determine the average weight. This value was multiplied by the number of plants in each treatment and finally extrapolated to one hectare.



Figure 3. Image of the Hipopótamo maize Hybrid at R3 phenophase ready for harvest.

Statistical analysis

The theoretical assumptions of normality and homogeneity of variances for the evaluated variables were verified. Subsequently, a t-Student test for continuous quantitative variables was used, employing the t-test for significance levels of 1% (Gosset, 1917). These analyses were performed using the STATISTICA professional statistical package, version 15.0 for Windows.

RESULTS AND DISCULTS

Phenophase occurrence time

The phenology of the commercial hybrid Hypopótamo did not significantly differ between the vegetative phenophase and the first two reproductive phenophases. From grain filling onward, the differences were highly significant (Figure 4). Since the same irrigation regime was applied, it is assumed that the extension of the reproductive phenophases was due to the effect of the microorganism used, which has high indolacetic acid synthesis capacity, promoting greener plants and reducing the likelihood of senescence.

Phenological studies are extremely important for crop plants. In maize, in particular, a shortening of the typical phenology alters the mobilization rates of elaborate substances and decreases the efficiency of carbon nutrition, even in the reproductive stage (Irmak et al., 2024).

When plants spend more time in a reproductive phenophase, although their biological cycle is delayed, it offers more possibilities for active metabolism and the translocation of elaborate substances, resulting in higher-quality fruits (Singh et al. 2025).

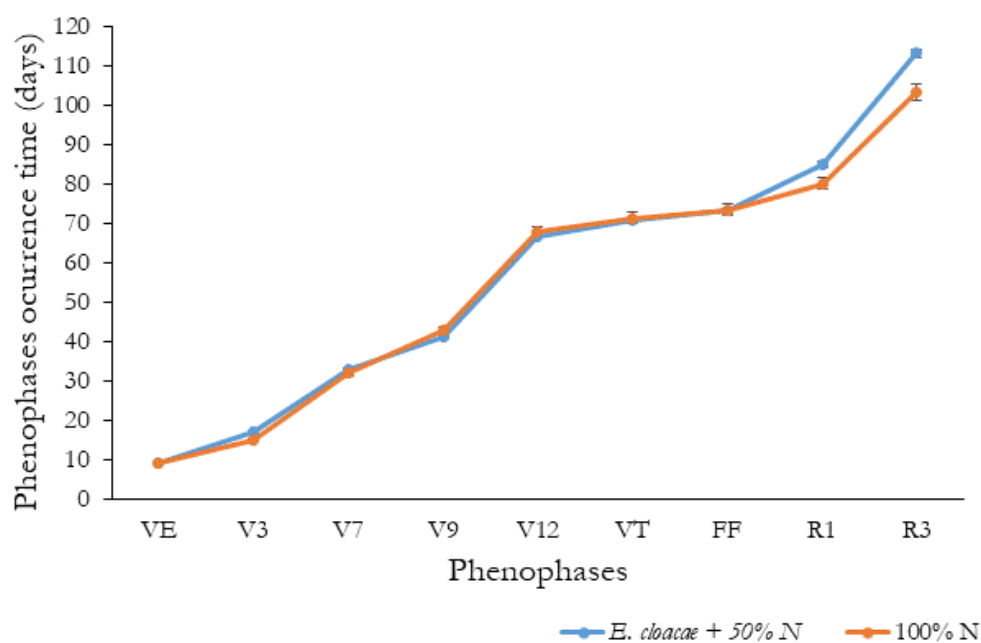


Figure 4. Phenophase occurrence time (days) of maize at the different phenophases: VE: emergence, V3; V7, V9; V12: development of the third, seventh, ninth and twelfth leaves; VT: male flowering; FF: female flowering; R1: grain formation; R3: full and milky grain.

In some regions of the world, the low biomass and grain yields of maize are due to inadequate management of nitrogen fertilizer application rates and untimely application. A high fertilizer rate applied inappropriately during phenological development alters assimilation patterns and nitrogen fertilizer use efficiency (Yadete et al., 2024).

In 2024, in a highly productive maize region of Ethiopia, the effects of low nitrogen fertilizer rates (0, 46, 92, 138, and 184 kg N ha⁻¹) and three application times (full application at planting, half at planting + half at the 5-leaf stage, and one-third at planting + one-third at the 5-leaf stage + one-third at the heading stage) were tested. The authors reported that the application rate significantly affected the phenological and agronomic variables. The study demonstrated that the highest average grain yield (9.2 t ha⁻¹) was obtained at planting, during the V5 phenophase, and at flowering when a dose of 184 kg ha⁻¹ of total nitrogen was used (Yadete et al., 2024). This fertilization scheme was implemented in the treatment where only 50% of the nitrogen fertilizer was applied.

The normalized difference vegetation index (NDVI)

The NDVI in the initial phenophases of development was highly significantly different, with the indicator being highest in the treatment in which 50% of the nitrogen fertilizer was applied. This response is possibly due to the increased concentration of IAA in the plants, which promotes plant vigor (Figure 5). From the VT and FF phenophases onward, no significant differences were observed; therefore, the 50% difference in nitrogen fertilizer did not alter the NDVI in crucial phenophases such as flowering.

With respect to reproductive phenophases, the NDVI decreased significantly but more markedly in the treatment in which 100% nitrogen was applied. Perhaps the high concentration of fertilizer in the soil caused the NDVI to decrease more rapidly because of the reduction in the osmotic potential generated in the soil, consequently reducing the soil water potential and the plants' ability to absorb water.

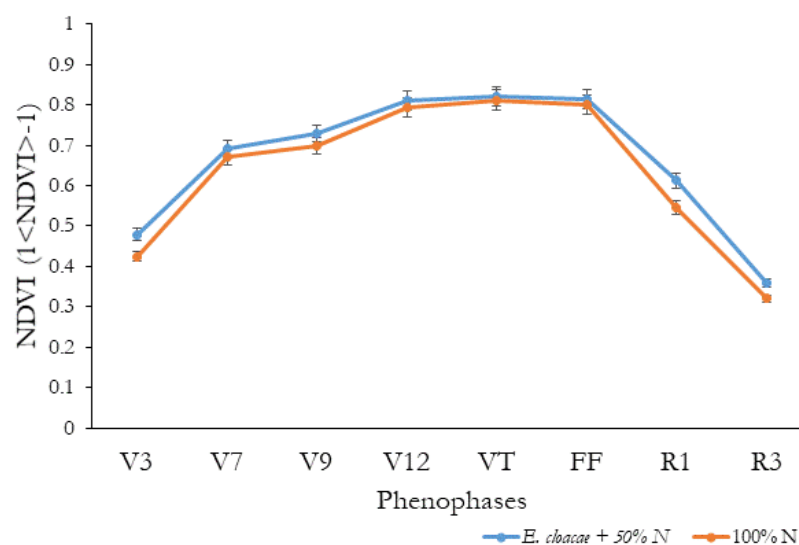


Figure 5. Normalized difference vegetation index (NDVI) in maize phenophases: VE: emergence, V3; V7, V9; V12: development of the third, seventh, ninth and twelfth leaves; VT: male flowering, FF: female flowering; R1: grain formation; R3: full and milky grain; R5: fully full and dry grain.

In both treatments, the NDVI in the VT, FF, and R1 phenophases remained above 0.8, with the highest values throughout the entire crop cycle. This value indicates very good nitrogen nutrition, which manifests as a very good physiological state (Zainuddin et al., 2025).

In a recent study conducted on maize under diverse climatic and soil conditions, testing nitrogen fertilizer doses revealed that compared with the control treatment (dose above 300 kg ha⁻¹), 120–180 kg ha⁻¹ of nitrogen yielded the best nutritional levels for maize, significantly increasing the grain yield by +5 t ha⁻¹. The statistical analyses performed demonstrated a strong correlation between the NDVI values and yield, indicating the practical utility of the NDVI measurements for predicting maize yield. These authors reaffirmed that NDVI-based monitoring is a highly effective remote sensing alternative not only for optimizing N management but also for supporting specific fertilization strategies for improving maize productivity and nutrient use efficiency (Illés et al., 2025).

Chlorophyll content

The chlorophyll content did not vary significantly during any of the vegetative growth stages or in the reproductive growth stage (VT) (Figure 6). From this stage to R3, the differences were significant. An important finding was that the chlorophyll values in both treatments exceeded 50 SPAD units during the most critical growth stages (V7 to R1). During these stages, the nitrogen demand of plants is high,

primarily because important processes such as pollen formation are highly dependent on nitrogen for plant viability. Even during the R3 growth stage, the chlorophyll content remained considerably high, indicating that the photosynthetic ability of the corn to continue grain filling increased; however, in this study, the corn was harvested for fresh consumption at the R3 growth stage.

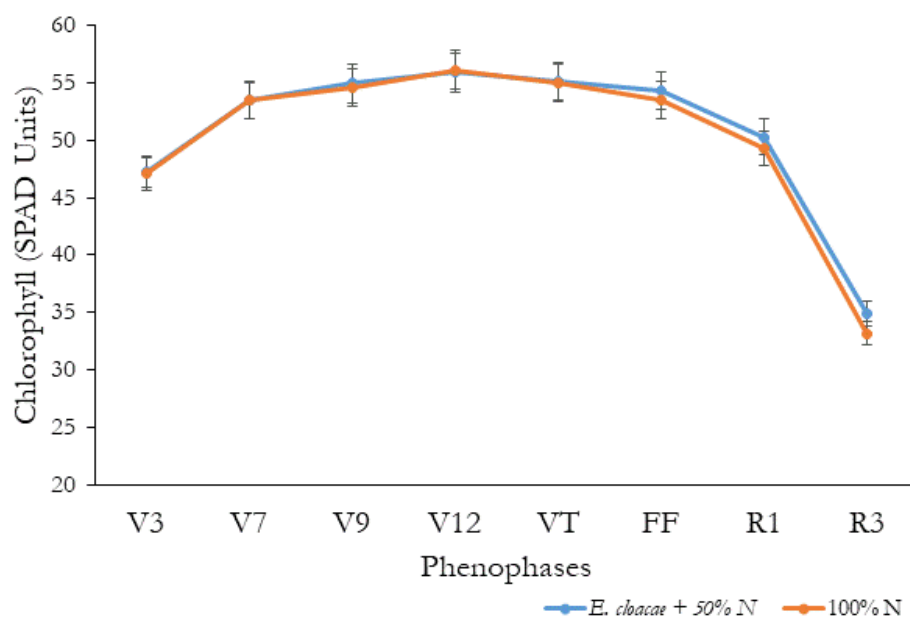


Figure 6. Chlorophyll content at the phenophases: VE: emergence, V3; V7, V9; V12: development of the third seventh, ninth and twelfth leaves; VT: male flowering, FF: female flowering; R1: grain formation; R3: full and milky grain.

The chlorophyll content is the most important variable related to nitrogen metabolism in maize (Shivashankar et al., 2025). This plant species is particularly indicative of nitrogen deficiency, as its chlorophyll concentration decreases significantly when nitrogen availability declines. This study demonstrated that 50% nitrogen fertilizer (180 kg ha^{-1}) is sufficient to achieve normal physiological and agronomic development, representing fertilizer savings and a reduction in soil pollutant load from excess nitrogen application (Ali & Bijay-Singh, 2025).

Biomass yield

When the biomass yield at the R3 phenological stage (full, tender kernels for consumption as corn on the cob) was analyzed, no significant differences were found between treatments. However, the numerical trend tended to increase in the treatment with *E. cloacae* and with only 50% nitrogen fertilizer (Figure 7). These results demonstrate that applying the regionally recommended dose does not increase the yield of the commercial hybrid used. Greater variability was observed in the data compared with the mean of the treatment with 100% nitrogen fertilizer, even though the sample size was similar. The lack of significant differences confirms the feasibility of using the growth-promoting microorganisms

employed in this research and the corresponding reduction in the nitrogen fertilizer application rate, which could be applied to other crops to avoid excess nitrogen fertilizer in the soil.

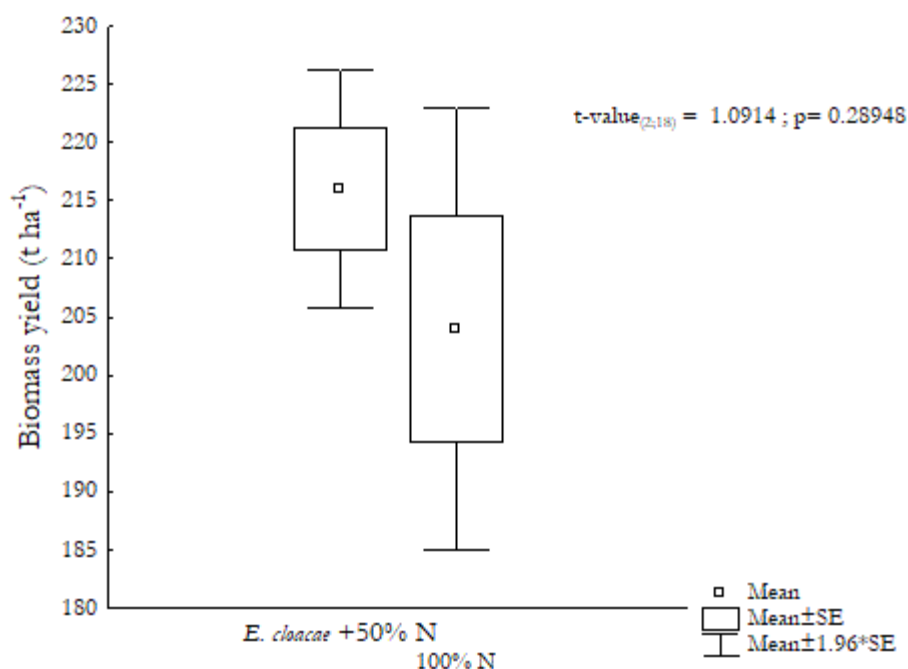


Figure 7. Biomass yield (t ha⁻¹) at R3 phenophase in maize under *E. cloacae*+50% N fertilizer and 100% N fertilizer. [t value: calculated t-student value. P: probability].

It is well known that nitrogen deficiency in the soil is among the factors that most affects the production of various crops, especially maize (Gehl et al., 2005). Reports support the idea that maize can yield close to the genetic potential of the planted material with a total nitrogen dose of 200 kg ha⁻¹, primarily with the aid of organic components or microorganisms (Yang et al., 2024). Even in soils supplemented with growth-promoting and remineralizing microorganisms, high biomass and grain yields can be obtained. Other reports have demonstrated that the increase in maize grain yield is not correlated with the increase in nitrogen dose (Patil et al., 2026).

The amount of nitrogen applied in some maize-producing regions can reach 400 kg N ha⁻¹, which is equivalent to one ton of urea. This is a very expensive fertilizer and has very adverse effects because of the environmental pollution generated by its excessive use, which can cause environmental degradation and resource depletion. In this context, low doses of nitrogen induce the overexpression of important genes in maize that increase yield (Nazir et al., 2016).

The excessive use of nitrogen fertilizers by farmers to increase productivity can also contribute to global warming and other types of environmental pollution from greenhouse gas nitrogen (Srikanth et al., 2016). Recent studies, some reported as early as 2016 (Wen et al., 2016), have shown that maize cultivars differ in their nitrogen absorption and metabolism capacity. Currently, the development of

maize cultivars that are tolerant to low levels of nitrogen fertilizers is the subject of research and is undoubtedly among the best ways to reduce pollution (Zhang et al., 2018).

CONCLUSIONS

Compared with the high doses recommended in the Yaqui Valley (up to 400 kg ha⁻¹ of nitrogen), the 50% reduction in nitrogen fertilizer with the aid of *E. cloacae* did not cause a significant reduction in the phenology of the Hypopótamo maize hybrid. The high values obtained for the physiological variables chlorophyll content and NDVI demonstrated that when 50% of the nitrogen fertilizer was applied in the Yaqui Valley for a maize crop, a good nutritional status of the plants and a high biomass yield at R3 (for sweet corn) could be achieved when plant growth-promoting microorganisms with a high capacity for indolacetic acid synthesis, such as *E. cloacae*, were used. This work contributes to sustainable production with the consequent incorporation of microorganisms into the soil and a reduction in the pollutant load from excess nitrogen.

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