

Nitrate reductase enzyme activity as a nutritional indicator in maize under two irrigation systems

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ABSTRACT

Objective: Maize (*Zea mays* L.) is one of the most economically significant cereal crops worldwide due to its substantial socioeconomic value. This study aimed to evaluate the enzymatic activity of nitrate reductase in the leaves of maize hybrids subjected to two different irrigation systems.

Design/methodology/approach: Key parameters assessed included nitrate reductase activity, nitrogen concentration, biomass accumulation, and crop yield. A completely randomized design was employed, with plots divided over time, incorporating two treatments and six composite replications. The treatments comprised two irrigation methods: surface (gravity) irrigation and subsurface drip irrigation using tape. The yellow maize hybrid P1382 was utilized in both treatments.

Results: Findings revealed that nitrate reductase activity declined progressively throughout the crop's phenological development. This reduction corresponded with a decrease in foliar nitrogen concentration, ultimately leading to diminished enzymatic activity during the later growth stages.

Limitations/implications: The results underscore the pivotal role of nitrate reductase in the nitrogen biotransformation process within the plant, highlighting the importance of temporal analysis to better understand the dynamics of nitrogen metabolism.

Findings/conclusions: Regarding the irrigation systems evaluated, subsurface drip irrigation proved more efficient in nutrient utilization, achieving yields comparable to gravity irrigation despite lower fertilizer input. Nevertheless, balanced fertilization strategies could further enhance productivity.

Key words: *Zea mays* L., enzymatic processes, nitrogen, nitrate reductase.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops globally, forming a staple component of the human diet due to its high energy value and ease of cultivation (García & Serna, 2019). Worldwide, maize ranks fifth among basic food basket products, attributed



to its nutritional, cultural, and economic significance (Adu *et al.*, 2021; Fisher *et al.*, 2015). Mexico holds the seventh position in global maize production, cultivating approximately seven million hectares and harvesting around twenty-seven million tons (FAO, 2021). The leading maize-producing states in Mexico include Aguascalientes, Baja California, Baja California Sur, Campeche, Coahuila, Colima, Chiapas, and Chihuahua (SIAP, 2022). Achieving high crop yields requires optimal mineral nutrition, which is determined by efficient fertilization management, improved mineral uptake, and productive output. Among the critical factors influencing crop development, water availability plays a pivotal role (Benyezza *et al.*, 2021; Revilla *et al.*, 2021). In agriculture, various irrigation systems are employed. Historically, gravity or furrow irrigation, sprinkler systems, and subsurface drip irrigation using tape have been implemented. Among these, subsurface drip irrigation has demonstrated superior efficiency, as it delivers moisture directly to the root zone, enhancing nutrient uptake and availability (Cakmakci & Sahin, 2021; Nikolaou *et al.*, 2020; Thamer *et al.*, 2021). Nitrogen is a fundamental element in plant nutrition, playing a direct role in foliar development, structural growth, and enzymatic processes (Hu *et al.*, 2023; Moreau *et al.*, 2019; Plett *et al.*, 2020). However, improper nitrogen fertilization can result in leaching and significant nutrient losses (Nilahyane *et al.*, 2018; Sogbedji *et al.*, 2000). The enzymatic activity of nitrate reductase (NR) is crucial in the nitrogen biotransformation process in plants (Kishorekumar *et al.*, 2020; Oaks, 1994). This enzyme performs multiple functions, primarily facilitating the initial step in nitrate assimilation (Rodríguez *et al.*, 2020). Specifically, NR catalyzes the two-electron reduction of nitrate (NO_3^-) to nitrite (NO_2^-), a reaction dependent on the NADPH coenzyme within the plant (Kaiser *et al.*, 2018). Further studies by Gheysari *et al.* (2009) and Muhammad *et al.* (2022) have identified water and nitrogen-based fertilizers as primary determinants of crop performance. Research indicates that NR enzymatic activity is directly involved in nitrogen metabolism, particularly in the leaves of maize plants, where nitrogen plays a vital role in optimal plant development (Li *et al.*, 2022; Wu *et al.*, 2018). Nonetheless, most investigations into nitrate reductase activity in maize have focused on a single irrigation system. Therefore, it is essential to conduct new studies that contrast enzymatic dynamics under varying irrigation regimes. In this context, the present study aimed to evaluate the temporal enzymatic activity of nitrate reductase in the leaves of yellow maize hybrids under two distinct irrigation systems.

MATERIALS AND METHODS

Experimental locations

The present experiment was conducted on two plots located within the agricultural region of Cuauhtémoc, Chihuahua, Mexico. The first plot covered an area of 2 hectares (ha) and was irrigated using a subsurface drip system with tape, located at coordinates 28° 38' 18" N and 106° 56' 18" W. The second plot spanned 10 hectares and employed gravity-based irrigation, situated at coordinates 28° 36' 36" N and 106° 59' 13" W. According to meteorological data from the Fruit Growers Union of the State of Chihuahua (UNIFRUT), the region experiences an average minimum temperature of 5 °C and a

maximum of 22.7 °C, with an annual mean precipitation of 392.3 mm. The agricultural basin sits at an elevation of 2,000 meters above sea level (CONAGUA, 2010).

Plant material

Both sites utilized the yellow maize hybrid P1382, a commercial variety developed by Pioneer, specifically adapted to the climatic conditions of the region. This hybrid is characterized by a plant height ranging from 2.30 to 2.70 meters, an intermediate to early growth cycle, a harvest window between 175 and 185 days, and high yield potential.

Crop management

Sowing was carried out on April 26 and 27, 2022, with a planting density of 95,000 seeds per hectare in both plots. The first plot was irrigated using a gravity-fed furrow system controlled by gates, while the second plot employed a subsurface drip tape system installed at a depth of 40 cm below the soil surface. A composite soil analysis was performed, consisting of five samples, to determine the physicochemical properties of the soils (Table 1).

Fertilization

The fertilization dose applied to the gravity-irrigated plot was 272-1-18 of N-P-K. This was achieved through the application of 230 kg of a fertilizer blend (Table 2) at planting,

Table 1. Physicochemical characteristics of the soils of the studied plots.

Parameter	Gravity Irrigation	Subsurface Drip Irrigation	Unit	
Texture	Sandy clay loam	Sandy clay loam		
pH	8.77	8.81		
EC	0.694	0.758	dS m ⁻¹	
CEC	14.98	27.39	meq 100g ⁻¹	
OM	0.93	1.71	%	
Nitrogen	17.28	10.5	mg kg ⁻¹	
Phosphorus	48.49	39.67		
Potassium	258.4	771.77		
Calcium	2294.82	3792.8		
Magnesium	240.4	298.95		
Iron	49.66	20.54		
Zinc	2.67	6.03		
Manganese	42.81	18.9		
Sulfur	53.16	60.76		
Boron	1.58	1.8		
Calcium	77.87	74.71		%
Magnesium	13.6	9.81		
Potassium	4.38	7.6		
Sodium	4.14	7.8		

Table 2. Fertilizer mixture used in the fertilization of the irrigated plot in a flooded irrigation system.

Fertilizer	Amount	Unit
K-Mag [®] (Potassium sulfate and magnesium sulfate)	74	kg ha ⁻¹
Ammonium sulfate	74	kg ha ⁻¹
Zinc sulfate	20.5	kg ha ⁻¹
Granubor [®] (Boric acid)	12	kg ha ⁻¹
Caudillo Naranja (16-00-10+Zn and B)	20.5	kg ha ⁻¹
Micronutrient complex (Microcultivos)	27	kg ha ⁻¹

and 550 kg of urea, which was applied in two stages during the leaf development and ear formation phases.

For the plot irrigated by drip tape, a fertilization dose of 134-2.8-3.8 units of NPK was applied, distributed in seven applications during the crop cycle, for which the fertilizers listed in Table 3 were used.

Experimental design and evaluated treatments

Based on the irrigation systems used, a completely randomized design with split plots over time was employed. The main plot factor was the irrigation system gravity irrigation and subsurface drip irrigation with tape while the subplot factor consisted of temporal measurements. Each treatment included six replications, with five plants per replicate. Four sampling events were conducted throughout the crop cycle for both irrigation systems at 35, 50, 85, and 140 days after sowing (DAS).

Evaluated variables

Nitrate reductase enzymatic activity

Enzymatic activity was determined following the protocol described by Jaworski (1971). Leaf discs (taleolas) of 5 mm in diameter, totaling approximately 100 mg of plant tissue, were extracted and placed in 10 mL of buffer solution (100 mM potassium phosphate, pH

Table 3. Fertilizers used in the plot irrigated with drip tape.

Product	Application Number		Product	Application Number		Product	Application Number		Total
	1	2		1	2		1	2	
Agri NS [®]	5	10	Agri NS [®]	5	10	Agri NS [®]	5	10	
Agri APP [®]	5	5	Agri APP [®]	5	5	Agri APP [®]	5	5	
Súper K30 [®]	2	2	Súper K30 [®]	2	2	Súper K30 [®]	2	2	
Maxi Zinco [®]	0.5	—	Maxi Zinco [®]	0.5	—	Maxi Zinco [®]	0.5	—	
Super Traza [®]	1.5	—	Super Traza [®]	1.5	—	Super Traza [®]	1.5	—	
Booster Root [®]	0.25	—	Booster Root [®]	0.25	—	Booster Root [®]	0.25	—	
Super Bora [®]	0.5	—	Super Bora [®]	0.5	—	Super Bora [®]	0.5	—	
Supa Link [®]	0.5	—	Supa Link [®]	0.5	—	Supa Link [®]	0.5	—	
Urea [®]	—	—	Urea [®]	—	—	Urea [®]	—	—	

7.5, containing 1% propanol). Vacuum infiltration (0.8 bar) was applied for 10 minutes. The enzymatic assay was conducted at 30 °C in darkness for one hour, after which the reaction was halted by incubating the samples at 100 °C for 15 minutes. The reaction product (NO_2^-) was quantified spectrophotometrically at 540 nm using a reaction mixture composed of 1 mL enzymatic extract, 2 mL of 1% sulfanilamide, and 2 mL of 0.02% N-1-naphthyl-ethylenediamine (N-NEDA). Two reactions were performed per sample to measure both endogenous NR activity (without NO_3^-) and NO_3^- -induced NR activity ($\text{NR} + \text{NO}_3^-$). The latter was assessed using a modified incubation buffer containing 50 μM KNO_3 . Results were expressed as $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ FW h}^{-1}$.

Nitrogen concentration

Nitrogen concentration was determined through four sampling events during the crop's phenological cycle, using the Kjeldahl method (Guebel *et al.*, 1991), conducted in the Soil, Water, and Plant Laboratory at INIFAP-RASPA.

Plant biomass (dry weight)

To assess plant dry weight, aerial biomass was sectioned and placed in kraft paper bags, then oven-dried at 75 °C for 24 hours using a forced-air drying system. The samples were subsequently weighed using a precision OHAUS digital analytical balance.

Yield

Grain yield was obtained using mechanized harvesting when the grain moisture content reached 14%. The entire plot area was harvested, and results were expressed in tons per hectare (t ha^{-1}).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using a split-plot over time model, with irrigation type as the main plot and time as the subplot factor. Each variable was analyzed using a non-linear model fitted to the trend observed over time, based on variance analysis. When treatment-by-time interactions were significant, mean comparisons between irrigation types at each time point were performed using Tukey's test. Statistical analyses were conducted using IBM SPSS Statistics 25 and SigmaPlot 12.0 software.

RESULTS AND DISCUSSION

Nitrate reductase (NR) enzymatic activity

In the present study, endogenous nitrate reductase (NR) activity exhibited its highest enzymatic response at 30 days after sowing (DAS) (Figure 1). Following this peak, average decreases of 46.27% were recorded at 50 DAS and 91.97% at 85 DAS. However, from this point onward, a significant increase of 300% was observed by 140 DAS. The data showed strong model fitting, with R^2 values of 0.92 for gravity irrigation (GI) and 0.83 for subsurface drip irrigation (SDI).

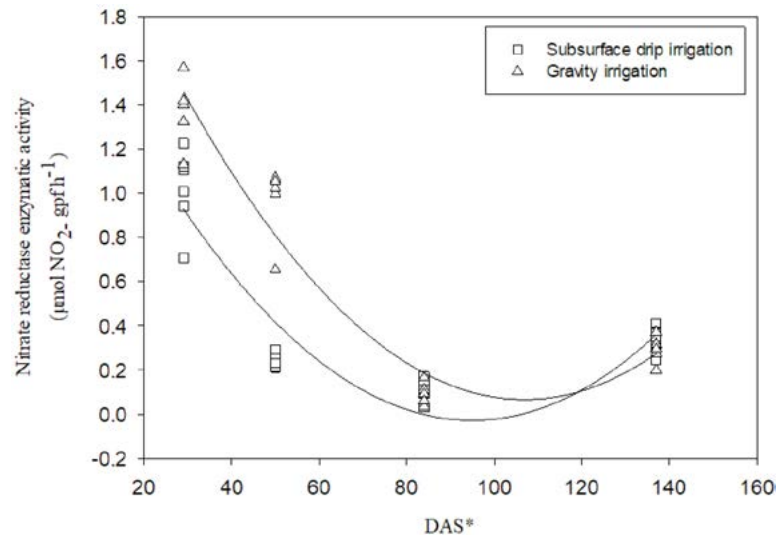


Figure 1. Endogenous nitrate reductase (NR) activity in yellow maize hybrid P1382 under two irrigation systems. DAS: days after sowing; □: subsurface drip irrigation; △: gravity irrigation.

Regarding the irrigation systems evaluated, both demonstrated similar temporal behavior, with their enzymatic peak occurring at 30 DAS. Nevertheless, gravity irrigation resulted in a significantly higher enzymatic response 63.61% greater compared to subsurface drip irrigation, indicating a more pronounced stimulation of NR activity under this condition.

In contrast, nitrate-induced NR activity exhibited an opposite trend to that of endogenous activity. In both gravity and subsurface drip irrigation systems, NR activity was lowest between 30 and 50 DAS (Figure 2). However, as the crop’s physiological cycle progressed, activity levels increased, reaching their peak between 120 and 140 DAS. This

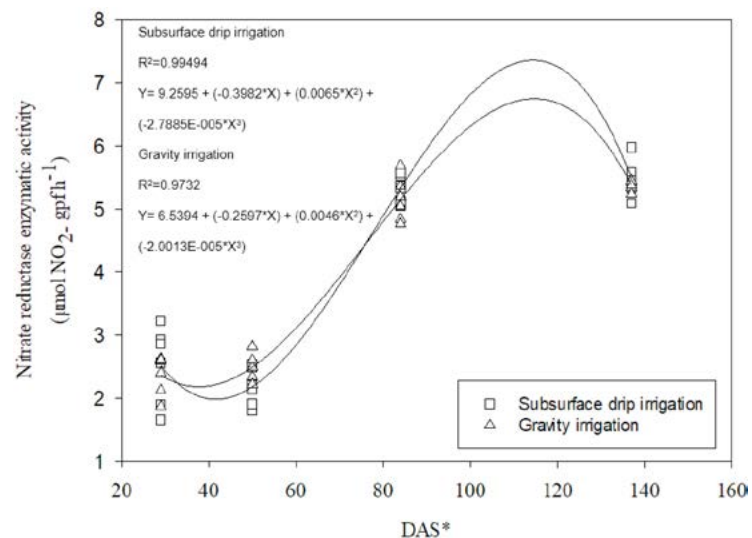


Figure 2. Nitrate-induced nitrate reductase (NR) activity in yellow maize hybrid P1382 under two irrigation systems. DAS: days after sowing; □: subsurface drip irrigation; △: gravity irrigation.

pattern may be attributed to initially elevated nitrate concentrations in the leaves, resulting from the initial fertilization and the smaller size of the plants early in the cycle.

As the crop entered flowering and grain-filling stages, nitrate demand increased significantly for assimilation processes, which in turn reduced the leaf nitrate content and led to a corresponding decline in endogenous NR activity (as shown in Figure 1). Consequently, when NR was externally induced with nitrates, a marked increase in enzymatic activity was observed, reflecting the plant's physiological capacity to respond to nitrate availability during later developmental stages.

NR enzyme activity governs the reduction of nitrate to nitrite, facilitating its subsequent assimilation into the plant and conversion into nitrogen-derived metabolic products (Maldonado *et al.*, 2013; Palacio-Márquez *et al.*, 2021). The results obtained in this study are consistent with previous findings, which report a gradual decline in NR activity as the crop progresses through its developmental stages. Peak enzymatic activity typically occurs during vegetative growth, a phase characterized by higher nitrate accumulation in the leaves due to active biomass expansion (Hernández-Cruz *et al.*, 2015; Wang *et al.*, 2018; Wencomo-Cárdenas, 2019). These observations align with those of Hammad *et al.* (2020), who noted that grain development and filling are largely dependent on nitrogen availability and water uptake. Consequently, NR activity and nitrogen assimilation are elevated during these critical growth phases. Similarly, Muhammad *et al.* (2022) emphasized that nitrogen fertilization at specific developmental stages significantly influences NR activity. Proper nitrogen supplementation during critical periods enhances the activity of key enzymes responsible for nitrogen assimilation.

Xu and Yu (2006) further indicated that post-flowering, leaves become the primary nitrogen source for the grain, causing significant variability in nitrogen levels and consequently affecting both photosynthetic efficiency and NR enzymatic activity. Regarding the irrigation systems evaluated, Muhammad *et al.* (2022) reported contrasting results, finding higher NR activity in maize under low irrigation combined with variable nitrogen doses. Meanwhile, Khawla *et al.* (2019) observed significant differences in NR activity across irrigation systems, identifying subsurface drip irrigation as the most effective in promoting enzymatic response.

Nitrogen concentration

Significant differences in foliar nitrogen content were found in this study (Figure 3), following a pattern similar to that observed in endogenous NR activity (Figure 1). Nitrogen concentration peaked at 30 DAS for both irrigation systems, with average decreases of 17.18%, 38.16%, and 45.75% at 50, 85, and 130 DAS, respectively. When comparing irrigation methods, gravity irrigation yielded an average increase of 14.83% in nitrogen content relative to subsurface drip irrigation.

Leaf nitrogen concentration is one of the most critical parameters for assessing the physiological performance of plants, as it is strongly correlated with photosynthetic activity, protein synthesis, and the concentration of photosynthetic pigments (Schlemmer *et al.*, 2013). The results obtained in this study align with those reported by Ciampitti and Vyn (2011), who observed reductions of up to 28.57% in nitrogen content in maize

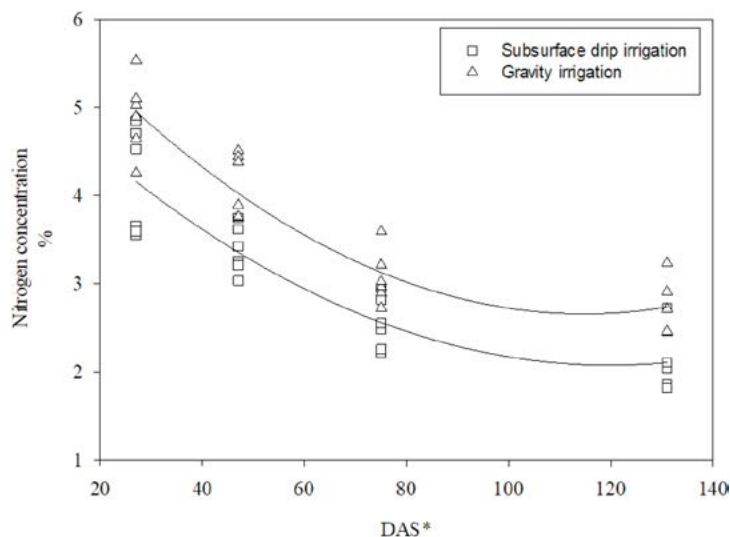


Figure 3. Nitrogen concentration in yellow maize hybrid P1382 under two irrigation systems. DAS: days after sowing; □: subsurface drip irrigation; △: gravity irrigation.

hybrids under varying planting densities and nitrogen fertilization levels as the crop advanced through its vegetative stages. Similarly, Meng *et al.* (2021) documented both decreases and increases in nitrogen content depending on the maize growth stage and treatment conditions. Foliar nitrogen content and its efficient use are influenced by several factors, including soil nitrogen availability, CO₂ fixation (which provides key precursors in the nitrogen assimilation pathway), and the crop's developmental stage. Notably, after flowering, the leaf becomes the primary nitrogen source for grain filling (Anas *et al.*, 2020; Archontoulis *et al.*, 2014; Xu & Yu, 2006).

The nitrogen concentration results obtained across irrigation systems differ from those reported by Muhammad *et al.* (2022), who observed comparable increases using low-flow irrigation systems in maize cultivars under various nitrogen fertilization regimes. Conversely, Wang *et al.* (2017) found that nitrogen accumulation in maize plants declined under water stress or inadequate irrigation an observation that may explain the trends seen in the present study.

Biomass production

In terms of biomass production, the results demonstrated a natural upward trend as the crop progressed through its phenological stages, with significant differences between the irrigation systems evaluated (Figure 4). Gravity irrigation consistently produced greater biomass across all sampling periods when compared to subsurface drip irrigation. By the end of the crop cycle, gravity irrigation led to a 19.82% increase in biomass accumulation relative to the tape irrigation system.

Biomass production is a crucial indicator when evaluating crop development, as final yield is closely linked to the plant's capacity to accumulate biomass (Márquez-Prieto *et al.*, 2022). The findings of this study align with those reported by Orozco-Vidal *et al.* (2016), who observed significant increases in biomass production when assessing two maize

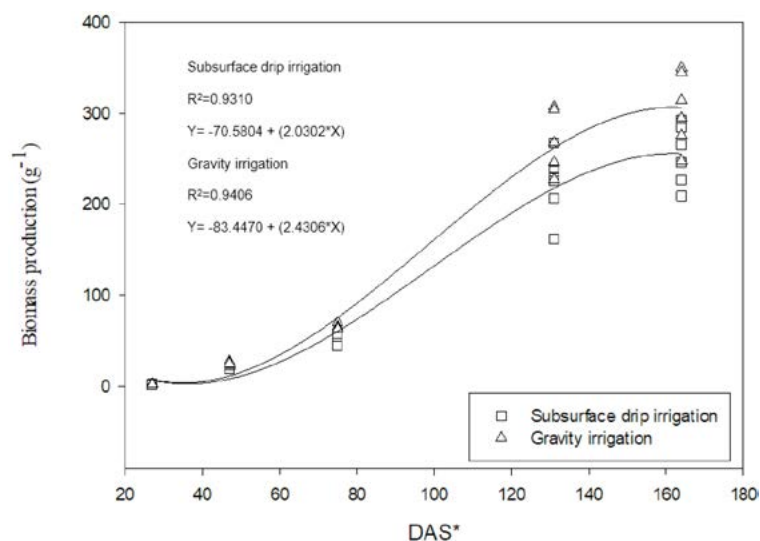


Figure 4. Biomass production in yellow maize hybrid P1382 under two irrigation systems. DAS: days after sowing; □: subsurface drip irrigation; △: gravity irrigation.

cultivars at different phenological stages. Previous studies have reported conflicting results. Muhammad *et al.* (2022) found no statistically significant differences in biomass between two irrigation systems in maize, though slight, non-significant increases were observed under a low-flow irrigation system. In contrast, Khawla *et al.* (2019) reported significant differences when evaluating various irrigation methods, with subsurface drip irrigation outperforming other systems. Unlike the present study, their results showed lower biomass production under surface (flood) irrigation, whereas in this study, gravity irrigation achieved the highest biomass values.

Yield

The yield data in this study revealed no statistically significant differences between treatments (Figure 5). Overall, both irrigation systems achieved yields above the Mexican national average of 3.9 t ha⁻¹, as reported by the Secretaría de Información Agroalimentaria y Pesquera (SIAP, 2022). When comparing treatments, gravity irrigation produced a non-significant yield increase of 10.04% relative to subsurface drip irrigation. These results follow the same trend observed for the other evaluated variables, where gravity irrigation consistently demonstrated superior overall performance.

Water use efficiency is a key determinant in maximizing maize yield, as water deficits can severely impact productivity depending on the intensity and phenological stage at which they occur (Giménez, 2012). The yields recorded in this study exceeded those reported by Biasutti *et al.* (2016), who evaluated various hybrids under semi-arid conditions, achieving an average of 8.3 t ha⁻¹. However, these findings contrast with several other studies, which indicate that drip or precision irrigation systems typically outperform conventional methods in maize production (Khawla *et al.*, 2019; Muhammad *et al.*, 2022; Wang *et al.*, 2017). These contrasting results may be attributed to the lower fertilization rates applied in the subsurface drip irrigation treatment compared to the gravity irrigation treatment.

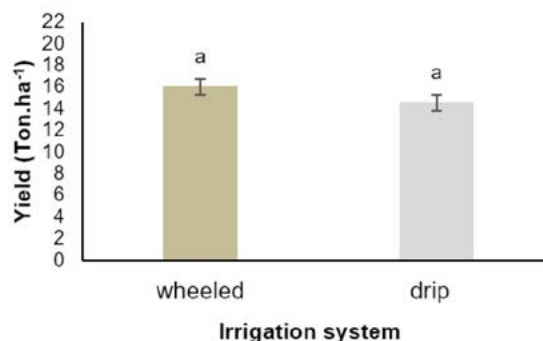


Figure 5. Yield of yellow maize hybrid P1382 under two irrigation systems.

Consequently, it can be inferred that fertilization was insufficient in the drip-irrigated plot. In this context, Ponce and Ramírez (2023) reported that achieving a yield of 14 t ha⁻¹ requires nutrient extraction of approximately 266-84-182 units of N-P-K. Therefore, the fertilization strategy used for the drip irrigation system in this study may have been suboptimal.

CONCLUSIONS

In conclusion, the results related to NR enzymatic activity suggest its potential as an indicator of the physiological status of maize crops, given its parallel behavior with leaf nitrogen concentration a widely recognized parameter for assessing plant nutritional status. Furthermore, NR activity can help identify critical periods for nitrogen fertilizer application. Low NR activity was associated with reduced foliar NO₃⁻ content, while nitrate induction significantly increased enzyme activity. This implies that targeted nitrogen application during these stages could enhance nitrogen assimilation and the synthesis of nitrogen-related metabolic products. Regarding the irrigation systems evaluated, subsurface drip irrigation demonstrated greater nutrient-use efficiency, achieving yields comparable to gravity irrigation with a lower fertilization input. However, balanced fertilization could further improve yield outcomes in drip-irrigated systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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