REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.13.e1896
ISSN 2007-3380

Original article / Artículo original

Integrated carbon and nitrogen assimilation under chlorine dioxide and nano Zn-Mo application in bean plants

Asimilación integrada de carbono y nitrógeno bajo aplicación de dióxido de cloro y nano Zn-Mo en plantas de frijol

Ramírez Estrada, C.A. [1], *Rábago González, Y.* [2], *Sánchez Chávez, E.** [1], *Palacio Márquez, A.* [2], *Pérez Álvarez, S.* [2], *Rivas Lucero, B.A.* [2]

[1] *Laboratorio de Fisiología y Nutrición Vegetal, Centro de Investigación en Alimentación y Desarrollo, Av. Cuarta Sur 3820, Fracc. Vencedores del Desierto, C.P. 33000, Cd. Delicias, Chih. México.*
[2] *Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Km 2.5 Carretera Delicias a Rosales,C.P. 33000, Cd. Delicias, Chih.México.*

ABSTRACT

Sustainable food production is a challenge for modern agriculture. An alternative to increase crop yields is the use of fertilizers that allow a reduction of inputs such as nanofertilizers and foliar sprays with nutrients in gas form. Combinations of nano Zn and Mo, as well as chlorine dioxide (ClO_2), have shown favorable results. However, information on the use of Zn and Mo nanofertilizers and ClO_2, and their effects on primary metabolism and yield in bean plants, is scarce. Therefore, the objective of the present study was to determine the effect of foliar application of nano Zn-Mo and ClO_2 on nitrogen and carbon assimilation and their relationship with bean plant yield. Bean plants (*Phaseolus vulgaris* L.) cv. Strike were grown for 53 days. The application of ClO_2 at 30 ppm, nano Zn-Mo at 4 ppm, and a control without application were compared. The application of nano Zn-Mo increased biomass, yield, chlorophyll index, soluble amino acids, total chlorophyll, and sucrose. The application of ClO_2 favored biomass, photosynthetic rate, and electron transport rate. Finally, the results suggest that carbon assimilation is integrated with nitrogen assimilation, and that foliar application of nano Zn-Mo could boost both metabolisms and improve yield in bean plants.

KEY WORDS: *Phaseolus vulgaris* L., ammonium assimilation, productivity, photosynthesis, nitrate reductase.

Please cite this article as/Como citar este artículo: Ramírez-Estrada, C.A., Rábago-González, Y., Sánchez-Chávez, E., Palacio-Márquez, A., Pérez-Álvarez, S., Rivas-Lucero, B.A. (2025). Integrated carbon and nitrogen assimilation under chlorine dioxide and nano Zn-Mo application in bean plants. *Revista Bio Ciencias*, 13, e1896.
https://doi.org/10.15741/revbio.13.e1896

Article Info/Información del artículo
Received/Recibido: May 30th 2025.
Accepted/Aceptado: December 13h 2025.
Available on line/Publicado: January 26th 2026.

***Corresponding Author:**

Esteban Sánchez-Chávez. Laboratorio de Fisiología y Nutrición Vegetal, Centro de Investigación en Alimentación y Desarrollo, Av. Cuarta Sur 3820, Fracc. Vencedores del Desierto, C.P. 33000, Cd. Delicias, Chih. México. E-mail: esteban@ciad.mx

Revista Bio ciencias
ISSN 2007-3380

RESUMEN

La producción de alimentos de forma sustentable es un reto para la agricultura moderna. Una alternativa para incrementar el rendimiento de los cultivos es la utilización de nanofertilizantes y aspersiones foliares con micronutrientes. Combinaciones de nano Zn y Mo, así como el dióxido de cloro (ClO_2), han mostrado resultados favorables. Por lo tanto, el objetivo del presente estudio fue determinar el efecto de la aplicación foliar de nano Zn-Mo y ClO_2 sobre la asimilación de nitrógeno, carbono y su relación con el rendimiento de plantas de frijol. Plantas de frijol ejotero (*Phaseolus vulgaris* L.) cv Strike se desarrollaron durante 53 días. Se compararon la aplicación de ClO_2 a 30 ppm, nano Zn-Mo a 4 ppm y un control sin aplicación. La aplicación de nano Zn-Mo incrementó biomasa, rendimiento, índice de clorofila, aminoácidos solubles, clorofila total y sacarosa. La aplicación de ClO_2, favoreció la biomasa y la tasa fotosintética. Finalmente, los resultados sugieren que la asimilación de carbono está integrada a la asimilación de nitrógeno y que la aplicación foliar de nano Zn-Mo podría tener la capacidad de impulsar ambos metabolismos y mejorar el rendimiento en plantas de frijol ejotero.

PALABRAS CLAVE: *Phaseolus vulgaris* L.; asimilación de amonio; productividad, fotosíntesis, nitrato reductasa.

Introduction

Beans are the most important legume in the global diet, especially in developing countries, due to their protein, vitamin, mineral, and fiber content. However, food insecurity and low agricultural production lead to malnutrition and other public health problems (Qaim, 2020). Furthermore, it is estimated that by 2050, the world population will reach 10 billion, and the area available for planting will be reduced to one-third (Pardey *et al*., 2014). That said, the growing demand for production can be met with intensification systems. Wich involves increasing the use of fertilizers, inputs such as pesticides, and water (Alexander *et al*., 2015). However, this would result in increased greenhouse gas emissions, soil and water quality deterioration, and biodiversity loss. Therefore, producing sufficient food sustainably is a priority challenge for modern agriculture (Govindan, 2018).

A viable alternative for increasing crop yields is the use of fertilizers that reduce inputs, such as nanofertilizers (Singh, 2017). Due to their particle size (1-100 nm), they enable more effective nutrient delivery and release to plants. Likewise, the quality of resources such as soil and water is maintained because losses of unabsorbed compounds are reduced (Al-Mamun *et al*., 2021). Several authors have reported favorable results in terms of seed germination, growth, and

Revista Bio ciencias
ISSN 2007-3380

plant production through the use of nanoformulations of Cu, Mn, Mo, Zn, Fe, and Si (Subramanian *et al*., 2015).

Another alternative that has been explored is the use of compounds with the ability to facilitate plant absorption, such as chlorine dioxide (ClO_2). ClO_2 is a yellow synthetic gas used as a disinfectant and for post-harvest treatment. It also has the ability to inactivate pathogenic cells attached to inaccessible parts of the plant and is effective with a short treatment time at low concentrations (Sun *et al*., 2019). It is classified as a safe product with restrictions, whose residual effects are minor and whose application does not require special care, due to the low concentrations in which it is used.

On the other hand, the processes of carbon (C) and nitrogen (N) assimilation are key to plant growth and development. N is necessary for the formation of amino acids, chlorophyll, and phytohormones. C, meanwhile, provides reducing agents, cellular energy, and carbon skeletons to integrate the assimilated N (Bloom *et al*., 2015). Thus, there is close interaction between these pathways, and their coordination is vital for the source-sink relationship and production. Therefore, the objective of this study was to determine the effect of foliar application of Zn-Mo nano fertilizer at 4 ppm and ClO_2 at 30 ppm on nitrogen and carbon assimilation and its relationship with bean plant yield (*Phaseolus vulgaris* L.).

Material and Methods

Crop management

The experiment was conducted at the Food and Development Research Center in Cd. Delicias, Chihuahua, Mexico, during September and October 2022, under shade net conditions. Strike variety green bean plants were used. Two plants were grown in 13 L plastic pots filled with a 2:1 (v/v) mixture of vermiculite and perlite. During the experiment, the plants were irrigated with a standard Hoagland nutrient solution (Table 1) adapted to bean physiological needs, with a pH of 6±0.1. Five hundred milliliters of nutrient solution per pot were applied every 48 hours until the flowering stage and 1,000 milliliters until harvest. Traditional agronomic management was applied, and two applications of Imidacloprid sprayed at a dose of 2 mL L were made to control whiteflies.

Experimental design

A completely randomized, single-factor, single-level design was used, comparing the application of ClO_2 at 30 ppm, nano Zn-Mo at 4 ppm, and a control to which distilled water was applied. The treatments were applied weekly starting 15 days after planting, with a total of five applications. In total, there were three treatments and six replicates in each. The experimental unit was one plant per pot (Figure 1).

Revista Bio ciencias
ISSN 2007-3380

Table 1. Composition of the standard Hoagland nutrient solution adapted for growing green beans.

Macronutrients		
Compound	**Chemical formula**	**Concentration (mM)**
Ammonium nitrate	NH_4NO_3	6
Dibasic potassium phosphate	K_2HPO_4	1.6
Potassium sulfate	K_2SO_4	0.3
Calcium chloride	$CaCl_2$	4
Magnesium sulfate	$MgSO_4$	1.4
Micronutrients		
Compound	**Chemical formula**	**Concentration (mM)**
Iron chelate	Fe-EDDHA	5
Manganese sulfate	$MnSO_4$	2
Copper sulfate	$CuSO_4$	0.15
Boric acid	H_3BO_3	3.5
Sodium molybdate	Na_2MoO_4	0.3

Figure 1. Effect of foliar application of chlorine dioxide (ClO2) and nano Zn-Mo on Strike variety green bean plants.

Revista Bio ciencias
ISSN 2007-3380

Total biomass and yield

Once physiological maturity was reached, 53 days after sowing, the plants were harvested for sampling. One plant was randomly selected from each pot and weighed fresh using a compact balance (A&D Co., LTD, EK-120, Tokyo, Japan). The plant was then dissected into leaves, stem, pods, and root, and each organ was weighed fresh. Yield was expressed as the fresh weight of pods per plant (g plant^{-1} FW).

The organs obtained were rinsed three times in distilled water and dried on filter paper at room temperature for 24 h. After this period, the plant material was dried in a 13.9-cubic-foot forced-air laboratory oven (Shel-Lab 1380FX, Oregon, United States) at 70 °C for 24 h. After the samples lost their moisture, they were weighed using an electronic analytical balance (A&D Co., LTD, HR-120, Tokyo, Japan). Total biomass was expressed as the sum of the dry weight of the four plant organs (g plant^{-1} FW). Finally, the samples were ground and stored for quantification of total nitrogen and carbon.

The plant that was not dried was divided into leaves, stem, fruit, and root, and was used in vivo for the quantification of nitrate reductase activity, total chlorophyll, soluble amino acids, and sucrose concentration.

Chlorophyll index

A Minolta SPAD 502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) was used to quantify the chlorophyll index. Healthy leaves with uniform coloration and free of damage were selected. Values were reported as SPAD values.

Nitrate reductase enzyme activity "*in vivo*" (E.C. 1.7.1.1)

Nitrate reductase enzyme activity was quantified using the method described by Sánchez *et al*. (2004). 0.25-0.5 g of leaf discs were weighed and infiltrated with 10 mL of a 100 mM buffer-P solution (solution of 18.21 g of $Na_2HPO_4 \cdot 12H_2O$ dissolved in 1 L of distilled water, adjusted to pH 7.5 with a solution of 13.60 g of KH_2PO_4 issolved in 1 L of distilled water) for endogenous activity and with 100 mM buffer-P, pH 7.5 with 50 mM KNO_3 for NO3-infiltrated activity. The samples were vacuum infiltrated at 0.8 bar (NAPCO 5851 vacuum oven, Winchester, Virginia, USA) for 10 min and incubated in the dark at 30 °C (WIG-50 digital incubator, DAIHAN SCIENTIFIC, Seoul, Korea). After 1 h, the samples were placed in a water bath at 100 °C for 15 min. Next, a 1 mL aliquot was extracted and mixed with 2 mL of 1 % sulfanilamide (1 g of sulfanilamide dissolved in 100 mL of distilled water and HCl in a 4:1 (v/v) ratio). Next, 2 mL of 0.02 % N-(1-naphthyl) ethylenediamine dihydrochloride (20 mg of NNEDA dissolved in 100 mL of distilled water) was added. The reaction mixture was measured by spectrophotometry at a wavelength of 540 nm in a UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYS™ 10S, Wisconsin, United States). The results were expressed as μM NO_2- reducidos · g^{-1} FW · h^{-1}.

Revista Bio ciencias
ISSN 2007-3380

Concentration of soluble amino acids

0.5 g of plant sample from the leaf blade was homogenized with 5 mL of 50 mM phosphate buffer, pH 7, at 4 °C (solution of 6.8 g of K_2HPO_4 dissolved in 1 L of distilled water, adjusted to pH 7 with a solution of 8.81 g of KH_2PO_4, dissolved in 1 L of distilled water). The sample was filtered through 4 layers of gauze and centrifuged at 1000 rpm for 15 min in a centrifuge cooled to 4 °C (Allegra™, 64R Centrifuge, Beckman Coulter, Brea, California, United States). The supernatant was used to determine soluble amino acid concentration using the method described by Yemm *et al*. (1955). The results were expressed as (mg · g^{-1} FW).

Total chlorophyll concentration

The total chlorophyll concentration was determined using the method described by Wellburn (1994). Ten leaf discs with a diameter of 7 mm were weighed and infiltrated with 10 mL of methanol (CH_3OH). The samples were sealed and left to stand in the dark for 24 h. After this time, the absorbance of the samples was measured at wavelengths of 653 and 666 nm for chlorophyll b and chlorophyll a, respectively, using a UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYS™ 10S, Wisconsin, United States). The total chlorophyll concentration represented the sum of chlorophylls a and b and was expressed as µg·cm^2 and calculated using the following formulas (Equation 1 and Equation 2):

1. $$Chl\ a = (15.65 \times abs\ 666) - (7.34 \times abs\ 653)$$
$$\frac{Chl\ a \times V \times P1}{P2 \times (\pi \times r^2) \times n}$$

2. $$Chl\ b = (27.05 \times abs\ 653) - (11.21 \times abs\ 666)$$
$$\frac{Chl\ b \times V \times P1}{P2 \times (\pi \times r^2) \times n}$$

Where, *V*: Final volume; *P1*: Weight per leaf disc; *P2*: Total weight of leaf discs; *r*: Radius of leaf discs; *n*: Number of leaf discs.

Total nitrogen and carbon concentration

An organic elemental analyzer (Thermo Fisher Scientific, FLASH 2000, Massachusetts, United States) was used to quantify total nitrogen and carbon. In addition, the methodology adapted by Krotz & Giazzi (2014) for plant material served as a basis. 0.3 mg of ground plant material (leaf, stem, fruit, and root) was weighed on a soft tin microcontainer on an ultra-microbalance (Mettler Toledo, XP6 Excellence Plus XP, Ohio, United States), to which 9 mg of vanadium pentoxide (V_2O_5) was added and then sealed. The sealed capsules were placed in the automatic sampler carousel for analysis. The results were expressed as a total percentage (%).

Revista Bio Ciencias ISSN 2007-3380

Photosynthetic rate, internal CO_2 concentration, and electron transfer rate

The photosynthetic rate, internal CO_2 concentration, and electron transfer rate were measured in the leaves when the plant reached physiological maturity, between 11:00 a.m. and 12:00 p.m. A portable LI-COR 6400 meter (Lincoln, Nebraska, USA) was used. Healthy leaves with uniform color and free of damage were selected. A concentration of 400 μmol per mol of CO_2 was used in the reference cell, while the sample cell was maintained at approximately 380 μmol per mL of CO_2. The air vapor pressure deficit in the sample chamber was less than 1.5, and the temperature of the block housing the leaf was 25 °C. The photosynthetic rate was expressed as μM CO_2 $m^2 \cdot s^{-1}$. The internal CO_2 concentration was expressed as μM $CO_2 \cdot mol^{-1}$. The electron transfer rate was expressed as μM m^{-2} s^{-1}.

Sucrose concentration

To determine the sucrose concentration, the method described by Irigoyen *et al*. (1992). was used. 0.5 g of fresh plant material from the leaf blade was weighed and homogenized with 5 mL of 96 % ethanol (v/v) (CH_3CH_2OH). The resulting mixture was filtered through four layers of gauze and rinsed twice with 5 mL of 70 % ethanol (v/v). The resulting mixture was centrifuged at 4 °C at 5500 rpm for 10 min in a refrigerated centrifuge (Allegra™, 64R, Beckman Coulter, Brea, California, United States). 0.1 mL of the supernatant was extracted and 3 mL of anthrone reagent (300 mg of anthrone ($C_{14}H_{10}O$) dissolved in 300 mL of 70 % sulfuric acid (v/v)) was added. The samples were immersed in a water bath at 100 °C for 10 min. Finally, the sucrose concentration was measured using a UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYS™ 10S, Wisconsin, United States) at 650 nm against a sucrose standard curve. The results were expressed as milligrams per gram of fresh weight ($mg \cdot g^{-1}$ FW).

Statical analysis

Once the data were obtained, they were subjected to a Shapiro-Wilk test to assess normality. In addition, it was subjected to a Bartlett test to check for homogeneity of variances. Once the assumptions had been checked, the data was subjected to a one-way analysis of variance and a test of separation of means using the LSD-Fisher test. The SAS 9.0 statistical package was used for the statistical analyses. Different letters indicated statistically significant differences according to the LSD-Fisher test ($p \leq 0.05$). Significance level: NS: Not significant; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Results and Discussion

Total biomass and yield

The effectiveness of the foliar compounds applied was observed in biomass accumulation, whose value increased significantly with both chlorine dioxide and nano Zn-Mo compared to the

Revista Bio ciencias
ISSN 2007-3380

control (Figure 2A). Furthermore, although the highest accumulation occurred with the application of ClO_2, with a 29 % increase compared to the control, there was no significant difference compared to the application of nano Zn-Mo. Therefore, both compounds are effective in increasing total biomass accumulation ($p \leq 0.01$).

Pod yield in bean plants was stimulated by the application of nano Zn-Mo (Figure 2B), which showed a significant increase of 145 % compared to the control and 90 % compared to the application of ClO_2 ($p \leq 0.001$).

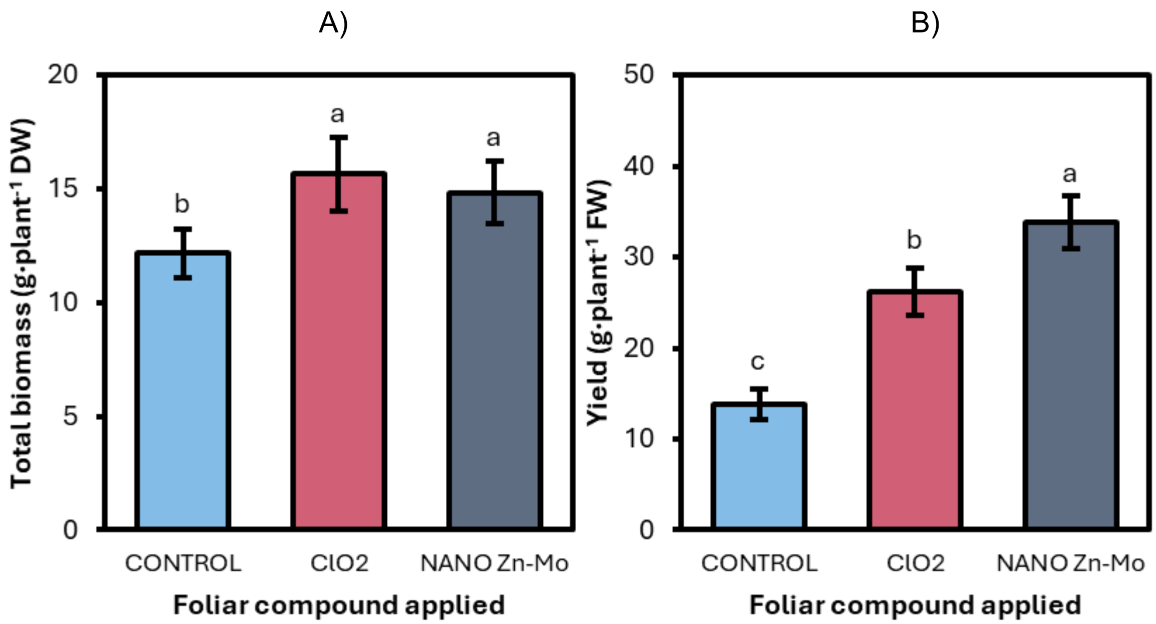

Figure 2. Effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on Strike bean plants on total biomass accumulation (A) and pod yield (B).

Different letters indicate statistically significant differences according to the LSD-Fisher test ($p \leq 0.05$).

Source: Own.

Chlorophyll index

In this experiment, the chlorophyll index measured with SPAD units varied at different levels depending on the measurement date (Table 2 and Figure 3). At 18 dds, which corresponds to the primary leaf formation stage, no significant difference was found between treatments ($p > 0.05$). At 25 dds, when the third trifoliate leaves were formed, the nano Zn-Mo treatment

Revista Bio Ciencias ISSN 2007-3380

yielded the highest SPAD units, with an 8 % increase over the control (p < 0.01). At 32 dds, in the pre-flowering stage, no significant differences were found (p < 0.05). At 39 dds, in the flowering stage, the ClO_2 treatment stood out compared to the control, with an 11 % increase over the control. However, it did not show a significant difference compared to nano Zn-Mo (p > 0.01). Finally, at 46 dds, when the pods were formed, no significant differences were found; however, the highest SPAD units were found in the nano Zn-Mo treatment, which was 17 % higher than the control.

Table 2. Effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on the chlorophyll index of Strike bean plants.

	SPAD units				
	18 das	25 das	32 das	39 das	46 das
CONTROL	38.78±1.70 a	41.64±0.95 b	42.75±4.20 ^{a}	42.98±2.10 b	40.46±3.49 ^{b}
ClO_2	38.84±0.72 ^{a}	42.74±2.14 ^{b}	44.02±0.81 ^{a}	47.51±4.44 a	46.76±6.31 ^{a}
NANO Zn-Mo	38.27±1.10 ^{a}	45.08±1.41 ^{a}	45.60±2.97 ^{a}	46.31±3.84 a^{b}	47.40±4.59 ^{a}
Significance level	NS	**	NS	**	NS

das= Days after sowing. Significance level: NS: No significance; ; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$
Different letters indicate statistically significant differences according to the LSD-Fisher test ($p \leq 0.05$).

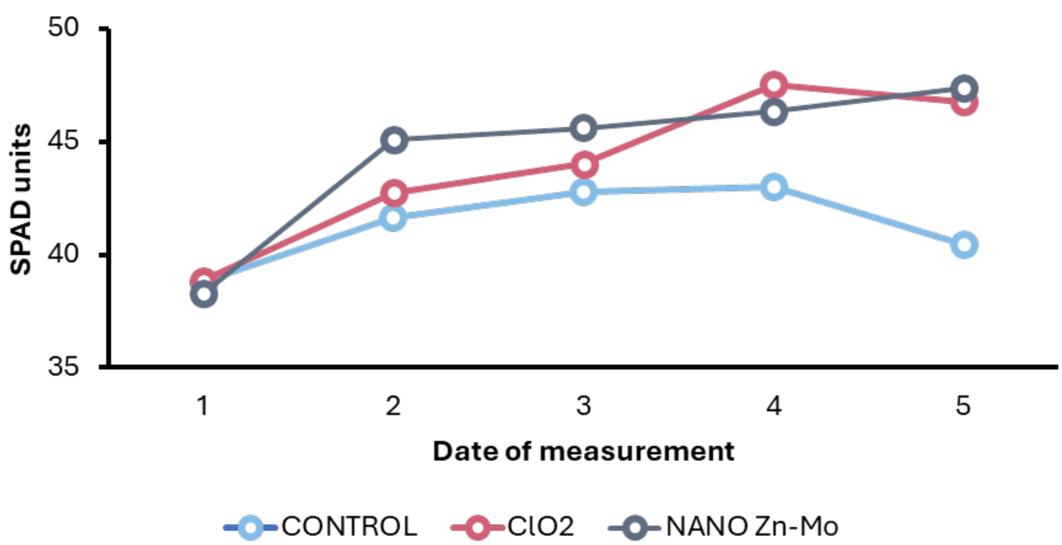

Figure 3. Effect of foliar application of chlorine dioxide (ClO2) and nano Zn-Mo on Strike bean plants on the evolution of units during the crop cycle.

Revista Bio Ciencias
ISSN 2007-3380

Nitrate reductase enzyme activity "*in vivo*" (E.C. 1.7.1.1)

In the present experiment, nitrate reductase enzyme activity measured in vivo was affected by the application of ClO_2 and nano Zn-Mo. The highest activity was observed in the control treatment, with a significant increase of 141 % compared to the application of nano Zn-Mo and 77 % compared to the application of ClO_2 ($p > 0.001$).

Soluble amino acids concentration

The highest concentration of soluble amino acids was found in the nano Zn-Mo treatment. This treatment increased significantly by 71 % compared to the control and by 49% compared to ClO_2 application ($p > 0.001$).

Total chlorophyll concentration

Similar to the concentration of soluble amino acids, total chlorophyll concentration increased significantly in the nano Zn-Mo treatment, by 29 % compared to the control and 38 % compared to ClO_2 application ($p > 0.001$).

Total nitrogen concentration

The nano Zn-Mo and ClO_2 treatments increased total foliar nitrogen concentration compared to the control. Furthermore, no significant difference was found between the two treatments, but there was a significant difference compared to the control ($p > 0.001$). ClO_2 treatment increased by 30 % compared to the control, while the nano Zn-Mo treatment increased by 33 % compared to the control. Finally, this demonstrates that both compounds are effective in increasing the nitrogen concentration in ejotero bean leaves.

Table 3. Effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on Strike bean plants on NR enzyme activity, soluble amino acid concentration, total chlorophyll concentration, and total nitrogen concentration.

	NR activity	Soluble aminoacids	Total chlorophyll	Total nitrogen
	(μM de NO_2^- formados · g FW · h^{-1})	(mg · g^{-1} FW)	(μg · cm^2 FW)	(%)
CONTROL	1.45±0.18 [a]	0.55±0.06 [b]	4.94±0.32 [b]	3.21±0.12 [b]
ClO_2	0.82±0.17 [b]	0.63±0.05 [b]	4.61±0.46 [b]	4.18±0.23 [a]

Revista Bio ciencias
ISSN 2007-3380

Continuation

Table 3. Effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on Strike bean plants on NR enzyme activity, soluble amino acid concentration, total chlorophyll concentration, and total nitrogen concentration.

	NR activity	Soluble aminoacids	Total chlorophyll	Total nitrogen
	(µM de NO_2^- formados · g FW · h^{-1})	(mg · g^{-1} FW)	(µg · cm^2 FW)	(%)
NANO Zn-Mo	0.60±0.17 [c]	0.94±0.1 [a]	6.37±0.49 [a]	4.28±0.27 [a]
Significance level	***	***	***	***

Identical letters indicate no statistically significant difference according to the LSD-Fisher test ($p \leq 0.05$). *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Photosynthetic rate

With regard to the photosynthetic rate, the application of chlorine dioxide obtained the best results. The highest photosynthetic rate was found in the ClO_2 treatment, with a significant increase of 141 % compared to the control and 70 % compared to the nano Zn-Mo treatment ($p > 0.001$).

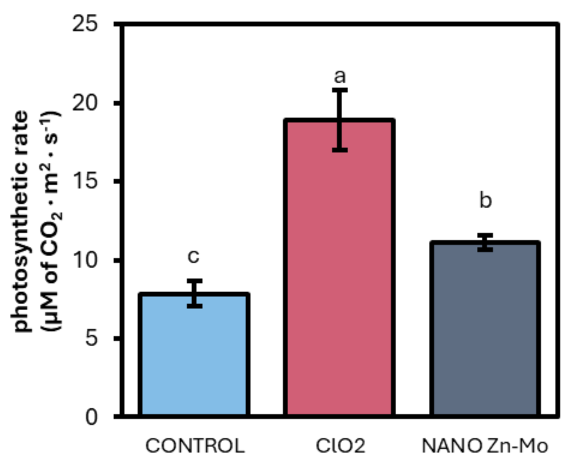

Figure 4. Effect of foliar application of chlorine dioxide (ClO2) and nano Zn-Mo on Strike bean plants on photosynthetic rate.

Revista Bio ciencias
ISSN 2007-3380

Internal CO_2 concentration

The internal CO_2 concentration was affected by the application of ClO_2 and nano Zn-Mo. The highest concentration was found in the control treatment. In this case, the nano Zn-Mo treatment was significantly reduced by 3 % compared to the control, and the ClO_2 treatment was significantly reduced by 7 % ($p > 0.001$).

Electron transfer rate

In terms of electron transfer rate, both chlorine dioxide and nano Zn-Mo increased the rate compared to the control. However, the ClO_2 treatment achieved the highest rate, with a significant increase of 76 % compared to the control and 34 % compared to nano Zn-Mo, making it the most effective treatment ($p > 0.001$).

Sucrose concentration

Regarding sucrose concentration, the treatment with the highest values was nano Zn-Mo application. This treatment showed a significant 14 % increase compared to the control and a non-significant 6 % increase compared to ClO_2. In this case, no significant differences were found between the control treatment and chlorine dioxide application ($p > 0.05$).

Carbon total concentration

Finally, total foliar carbon concentration was more strongly stimulated by chlorine dioxide. The ClO_2 treatment obtained a significant increase of 3 % compared to the control, however, no significant difference was found compared to the nano Zn-Mo treatment ($p > 0.05$).

Table 4. Effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on Strike bean plants on internal CO_2 concentration, electron transfer rate, sucrose concentration, and total carbon concentration.

	Internal CO_2 concentration	Electron transfer rate	Sucrose	Total carbon
	($\mu M\ CO_2 \cdot mol^{-1}$)	($\mu M\ m^{-2}\ s^{-1}$)	($mg \cdot g^{-1}$ FW)	(%)
CONTROL	395.19±5.80 ^{a}	79.39±17.13 ^{c}	16.22±1.71 ^{b}	40.19±0.47 ^{b}
ClO2	371.06±6.38 ^{c}	139.47±12.39 ^{a}	17.34±1.24 ^{ab}	41.45±0.31 ^{a}
NANO Zn-Mo	384.03±4.06 ^{b}	104.14±18.94 ^{b}	18.46±1.81 ^{a}	40.99±0.98 ^{ab}
Significance level	***	***	*	*

Identical letters indicate no statistically significant difference according to the LSD-Fisher test (p≤0.05). ***:** $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Discusion

Previous studies have documented the effectiveness of chlorine and nano-Zn-Mo applications on growth, nitrogen assimilation, and photosynthesis in various species, including rice, wheat, barley, and lettuce. In the present study, the total biomass of bean plants increased with the application of both chlorine dioxide and nano Zn-Mo (Figure 2A). This behavior has been previously reported by several authors, who indicate that because chlorine, present as Cl^- ions, is osmotically active and necessary for turgidity and osmoregulation, it is related to normal plant growth (Geilfus, 2018). For example, Franco-Navarro *et al*. (2016), reported a 50 % increase in dry biomass in tobacco plants treated with 5 mM Cl-, compared to untreated plants. The increase in dry biomass in green bean plants observed in this experiment may be due to chlorine acting as a beneficial micronutrient at higher concentrations. Some of the responses reported when supplying Cl as a macronutrient include leaf expansion, overall plant growth, osmoregulation, and improved water balance. In general, the effect on the growth of plants nourished with chlorine is associated with physiological and morphological alterations in leaf tissue (Franco-Navarro *et al*., 2016). On the other hand, zinc and molybdenum are cofactors in metabolic processes, so plant development depends on their absorption and assimilation (Manuel *et al*., 2018; Solanki, 2021). Mahdieh *et al*. (2018) reported that foliar application of zinc nanoparticles on bean plants increased dry biomass by 16 %. Similarly, Chen *et al*. (2023) indicate that the dry biomass of tobacco plants increased by 15 % with the application of molybdenum nanoparticles at 25 through irrigation µg mL^{-1}. It has previously been reported that zinc application improves the vigor and stability of plant membranes, thereby increasing growth (Mahdieh *et al*., 2018). In addition, other authors report that zinc nanoparticles promote growth by increasing auxin production (Du *et al*., 2019).

The efficiency of metabolic processes such as photosynthesis and nutrient accumulation, among other important processes that occur within plants, determines fruit production (Monreal *et al*., 2016). Regarding pod yield in the present experiment, the results are similar to those reported for other species. Prasad *et al*. (2012), indicate that the addition of Zn sulfate nanoparticles at a dose of 133 mg L increased peanut plant yield by 30 % compared to the control. Likewise, Ybañez *et al*. (2020) reported that the application of zinc oxide nanoparticles synthesized with $ZnCl_2$ at a dose of 32 mg per plant increased tomato yield by 134 % compared to the control and exceeded the yield of plants treated with commercial nano Zn and granulated Zn. Muñoz-Márquez *et al*. (2022b), Those who applied nano Mo to bean plants at a dose of 10 ppm achieved a 91 % increase in yield compared to the control. The increase in pod yield can be attributed to improvements in enzyme-related physiological processes, such as enhanced N assimilation, in which zinc and molybdenum serve as cofactors, leading to higher assimilate concentrations and, therefore, greater growth and production (Ghasemi *et al*., 2013).

The chlorophyll index measured with SPAD units quantifies leaf greenness by measuring light transmission, where light absorption by the chlorophyll molecule occurs (Cunha *et al*., 2015). The increase in SPAD values observed in the present study is similar to that reported by Silva *et al*. (2020), who found no difference in SPAD units in bean crops when Mo was added at six doses (0, 20, 40, 80, 120, and 160 g ha^{-1}). However, biomass and pod number increased. Previously,

Revista Bio ciencias
ISSN 2007-3380

micronutrients such as Zn and Mo have been linked to the maintenance of chlorophyll structure and to the acquisition and conversion of N, which in turn forms part of chlorophyll (Yeboah *et al*., 2021). Possibly, the application of Nano Zn-Mo allowed for better chlorophyll formation, which ultimately resulted in greater greenness in this evaluated treatment.

The enzyme nitrate reductase (NR) participates in nitrogen assimilation and is regulated by nitrate and molybdenum levels, as well as by the ratio of sugar to amino acid content, making it a good indicator of nitrogen and carbon assimilation in plants (Ponce-García *et al*., 2019). Authors as Imran *et al*. (2019), describe significant increases in NR and NiR activities in wheat plants treated with Mo. NR enzyme activity is activated by the presence of the Mo cofactor (MoCo), which, in turn, allows the conversion of NO_3^- to NO_2^- and subsequent depletion of the NO_3^- substrate. Once this is done, high-affinity transporters are induced to absorb more NO_3^- from the solution (Vigani *et al*., 2017; Imran *et al*., 2019). In this regard, the results obtained for total biomass and organic nitrogen content indicate that ejotero bean plants treated with nano Zn-Mo accumulated more total organic N and ultimately grew more, as expressed in total biomass. The higher enzyme activity in the control treatment could be due to the lack of fruiting and a greater need for vegetative growth.

Amino acids perform numerous metabolic functions, so measuring their concentrations can provide information about the plant's physiological state (Ramírez-Estrada *et al*., 2022). The trend observed in the treatments in this study is similar to that reported by Imran *et al*. (2019), who indicate that in two wheat cultivars, the application of Mo significantly increased the content of soluble proteins and amino acids, compared to Mo-deficient plants. As mentioned above, the activation of N metabolism driven by the presence of Mo as a cofactor of NR ultimately triggers the synthesis of amino acids, one of the end products of the N assimilation pathway (Liu *et al*., 2017). The increase in soluble amino acid concentration is likely due to the effect of exogenous Mo application.

Photosynthesis is the mechanism by which plants capture sunlight, which in turn is carried out by the chlorophyll contained in chloroplasts (Muñoz-Márquez *et al*., 2022a). The results obtained are consistent with Liu *et al*. (2019), who managed to significantly increase the total chlorophyll content by 19.23 % with the application of zinc and molybdenum in wheat plants. This effect could be attributed to the roles that both Zn and Mo play in chlorophyll formation. It has been shown that Mo deficiency can inhibit chlorophyll synthesis, especially in winter, while Zn promotes chlorophyll formation by regulating cytoplasmic nutrient concentrations (Bharti *et al*., 2014).

Quantifying organic nitrogen concentration is a good indicator of the physiological and nutritional status of plants, making it an important variable for observing mineral absorption in treated plants (Leghari *et al*., 2016). The data from this study are consistent with those reported by Khan *et al*. (2014), who observed significant increases of 111 % with the application of Mo at a dose of 0.5 kg ha^{-1} in bean crops. The positive effect on the total nitrogen concentration in the plant may be due to the relationship between these nutrients. Mo is an integral part of one of the key enzymes in the nitrogen assimilation process, NR (Kaiser *et al*., 2005). Furthermore, in legumes, the effect may be even more pronounced, as molybdenum promotes the integrity of the nitrogenase enzyme in nodules that fix atmospheric N, thereby enhancing N capture and absorption (Khan *et al*., 2014). Finally, the data are consistent with those obtained for total biomass, yield, photosynthetic pigments, NR activity, and soluble amino acid concentration, supporting the theory that nano Zn-Mo increases the entire N assimilation process in bean plants.

Measuring the photosynthetic rate is an indicator of the proper functioning of plant metabolism, as it is one of the most important processes in plants, whereby they convert solar energy into chemical energy so that the plant can grow and develop properly (Gutiérrez-Ruelas *et al*., 2021).

As Xu *et al*. (2013) indicate, the data obtained in this research study demonstrate the effects of chlorine application on the photosynthetic rate of plants. The aforementioned authors observed increases of 51 % and 48.7 % in the photosynthetic rate of Zoysia japonica plants when chlorine was added at 10 and 20 mM, respectively. This increase could be due to the signaling role of chlorine in plant metabolism. Electron transport within the photosynthetic apparatus in thylakoid membranes is highly sensitive to ion concentrations, such as Cl- (Herdean *et al*., 2016). Furthermore, it has been demonstrated that plants treated with chlorine have a greater capacity to prevent cell dehydration damage by balancing the osmotic force of the cytoplasm, improving water status, and therefore promoting photosynthesis compared to those without application (Ben Amor *et al*., 2010). Likewise, the data obtained on the electron transport rate and internal CO_2 rate correspond to the role of chlorine in the activation of enzymes involved in photosystem II. Cl- is an essential cofactor for oxygen evolution, although it has not yet been fully determined how oxygen is catalyzed (Pokhrel *et al*., 2011).

Watanabe *et al*. (2016), They determined that the application of potassium chloride at a dose of 0.75 g per pot promoted the accumulation of sucrose in sugarcane crops, compared to other sources of potassium, which could be attributed to fertilization with chlorine. However, Wu *et al*. (2015), They found that applying doses ranging from 0 to 150 mM of sodium chloride to sugar beets had no significant effect on sucrose accumulation. The response of soluble sugar production appears to depend on the species and to be more influenced by N metabolism than by C metabolism. The growth of vegetative shoots is subject to the translocation of assimilated N and C, which in turn depends on the correct assimilation of NO_3- and CO_2. N uptake by roots is subject to more adverse factors than obtaining CO_2 through photosynthesis, so the accumulation of photosynthetic assimilation products could be more dependent on a correct N assimilation cycle (Lawlor, 2002).

From an agronomic perspective, yield is the most important factor to consider in crop production (Prahdan *et al*., 2015). However, when discussing the functioning of primary metabolism for research purposes, it is necessary to take this into account since yield is a consequence of plant biochemistry. Authors have mentioned that, in summary, the available nitrogen content is related to the synthesis of amino acids and, therefore, of proteins and other cellular components (Hildebrandt *et al*., 2015). For accelerated CO_2 assimilation, well-formed chloroplasts and normal synthesis of the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco) are necessary, in addition to NADP+ reducing power. The first two components are dependent on an adequate

Revista Bio ciencias
ISSN 2007-3380

supply of N. Likewise, in order to capture and transform photosynthetic energy, a large amount of chlorophyll is required, which is why N also supports this process. Consequently, the proper supply of N also enables the assimilation of CO_2 into triose phosphate and, ultimately, into sucrose (Zhong *et al*., 2017; Lawlor, 2002).

The treatment evaluated with the highest yield was Nano Zn-Mo (Figure 2). Similarly, the highest accumulation of total N was observed in the same treatment, as well as the highest concentration of soluble amino acids, total chlorophyll, and SPAD units (Table 3, Figure 3). In contrast, the highest photosynthetic and electron transfer rates were observed with ClO_2 application (Table 4). The control treatment obtained higher NR activity and a higher internal CO_2 concentration (Table 3, Table 4). N assimilation was probably the factor that favored the Nano Zn-Mo treatment, as evidenced by higher yield, unlike the ClO_2 treatment, which only showed an improved effect on photosynthetic energy transformation (Figure 5). The control treatment showed higher NR activity and a higher internal CO_2 concentration, possibly to compensate for the low N accumulation. Enzyme synthesis increased, and similarly, this low N accumulation limited the assimilation of CO_2 into soluble sugars, which is why the internal concentration rose.

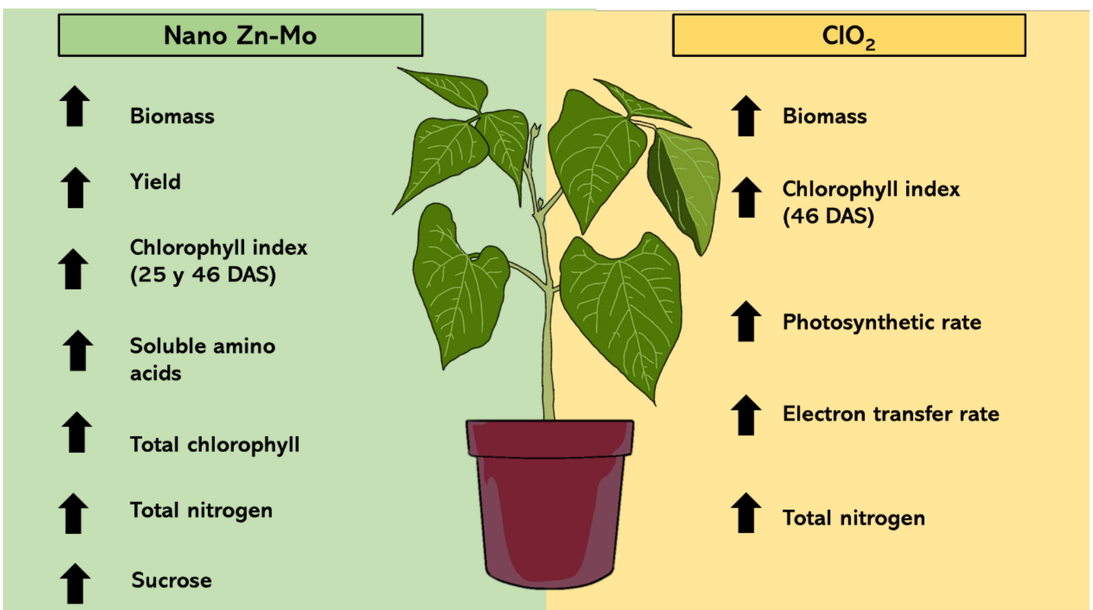

Figure 5. Graphical summary of the effect of foliar application of chlorine dioxide (ClO_2) and nano Zn-Mo on Strike variety green bean plants.

Revista Bio ciencias
ISSN 2007-3380

Conclusions

Based on the results obtained, it is concluded that foliar application of nano Zn-Mo at a dose of 4 ppm from the appearance of the first true leaf (V3, 15 days after sowing) is considered a viable option for increasing biomass, yield, soluble amino acid concentration, chlorophyll, and total nitrogen in Strike bean plants. In addition, foliar application of chlorine dioxide at a dose of 30 ppm increased biomass, photosynthetic rate, electron transfer rate, chlorophyll index, and total nitrogen. Finally, the results suggest that carbon assimilation is integrated with nitrogen assimilation in bean plants, with nitrogen supply as a cellular component being the crucial factor driving both metabolisms.

Authors' contribution

"Work conceptualization, Sánchez, E., Ramírez Estrada, C.A., Rábago Gonzalez, Y..; Methodology development, Sánchez, E., Ramírez Estrada, C.A., Palacio Márquez, A.; Software management, Ramírez Estrada, C.A.; Sánchez, E.; Results analysis, Palacio Márquez, A., Pérez Álvarez, S., Rivas Lucero, B. A..; Data managment, Ramírez Estrada, C.A., Palacio Márquez, A.; Writing and preparation of the manuscript, Ramírez Estrada, C.A., Rábago Gonzalez, Y., Sánchez, E., Palacio Márquez, A., Pérez Álvarez, S., Rivas Lucero, B. A..; Redaction, revision and editing, Ramírez Estrada, C.A., Palacio Márquez, A.; Writing, Ramírez Estrada, C.A., Rábago Gonzalez, Y., Sánchez, E., Palacio Márquez, A., Pérez Álvarez, S., Rivas Lucero, B. A.; Project manager, Sánchez, E.; Fund acquisition, Sánchez, E.

"All authors of this manuscript have read and accepted the published version."

Acknowledgements

This research was carried out thanks to the support of the National Council for Humanities, Science, and Technology (CONAHCYT, Mexico) through financial support granted by the national scholarship program for postgraduate studies under CVU No. 1011744 and the project of the Call for Attention to National Issues: Project #1529 "Biofortification of basic agricultural crops that are key to combating malnutrition and ensuring food security in Mexico".

Conflict of interest

"The authors declare that they have no conflict of interest".

Revista Bio ciencias
ISSN 2007-3380

References

Al-Mamun, M. R., Hasan, M. R., Ahommed, M. S., Bacchu M. S., Ali, M. R., & Khan, M. Z. H. (2021). Nanofertilizers towards sustainable agriculture and environment. *Environmental Technology & Innovation*, 23, 101658.https://doi.org/10.1016/j.eti.2021.101658

Alexander, P., Rounsevell, M. D., Dislich, C., Dodson, J. R., Engström, K., & Moran, D. (2015). Drivers for global agricultural land use change: The nexus of diet, population, yield and bioenergy. *Global Environmental Change*, 35, 138-147.https://doi.org/10.1016/j.gloenvcha.2015.08.011

Ben Amor, N., Megdiche, W., Jiménez, A., Sevilla, F., & Abdelly, C. (2010). The effect of calcium on the antioxidant systems in the halophyte Cakile maritima under salt stress. *Acta Physiologiae Plantarum*, 32, 453-461.https://doi.org/10.1007/s11738-009-0420-2

Bharti, K., Pandey, N., Shankhdhar, D., Srivastava, P. C., & Shankhdhar, S. C. (2014). Effect of exogenous zinc supply on photosynthetic rate, chlorophyll content and some growth parameters in different wheat genotypes. *Cereal Research Communications*, 42, 589-600. https://doi.org/10.1556/CRC.2014.0015

Bloom, A. J. (2015). Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. *Photosynthesis research*, 123(2), 117-128.https://doi.org/10.1007/s11120-014-0056-y

Chen, J., Yin, Y., Zhu, Y., Song, K., & Ding, W. (2023). Favorable physiological and morphological effects of molybdenum nanoparticles on tobacco (*Nicotiana tabacum* L.): root irrigation is superior to foliar spraying. *Frontiers in Plant Science*, 14, 1220109 https://doi.org/10.3389/fpls.2023.1220109

Cunha, A. R. D., Katz, I., Sousa, A. D. P., & Martinez Uribe, R. A. (2015). Índice SPAD en el crecimiento y desarrollo de plantas de *lisianthus* en función de diferentes dosis de nitrógeno en ambiente protegido. *Idesia (Arica),* 33(2), 97-105.http://dx.doi.org/10.4067/S0718-34292015000200012

Du, W., Yang, J., Peng, Q., Liang, X., & Mao, H. (2019). Comparison study of zinc nanoparticles and zinc sulphate on wheat growth: From toxicity and zinc biofortification. *Chemosphere*, 227, 109-116.https://doi.org/10.1016/j.chemosphere.2019.03.168

Franco-Navarro, J. D., Brumós, J., Rosales, M. A., Cubero-Font, P., Talón, M., & Colmenero-Flores, J. M. (2016). Chloride regulates leaf cell size and water relations in tobacco plants. *Journal of Experimental Botany*, 67(3), 873-891.https://doi.org/10.1093/jxb/erv502

Ghasemi, S., Khoshgoftarmanesh, A. H., Afyuni, M., & Hadadzadeh, H. (2013). The effectiveness of foliar applications of synthesized zinc-amino acid chelates in comparison with zinc sulfate to increase yield and grain nutritional quality of wheat. *European Journal of Agronomy*, 45, 68-74.https://doi.org/10.1016/j.eja.2012.10.012

Geilfus, C. M. (2018). Review on the significance of chlorine for crop yield and quality. *Plant Science*, 270, 114-122.https://doi.org/10.1016/j.plantsci.2018.02.014

Govindan, K. (2018). Sustainable consumption and production in the food supply chain: A conceptual framework. *International Journal of Production Economics*, 195, 419-431.https://doi.org/10.1016/j.ijpe.2017.03.003

Gutiérrez-Ruelas, N. J., Palacio-Márquez, A., Sanchez, E., Muñoz-Márquez, E., Chávez-

Mendoza, C., Ojeda-Barrios, D. L., & Flores-Cordova, M. A. (2021). Impact of the foliar application of nanoparticles, sulfate and iron chelate on the growth, yield and nitrogen assimilation in green beans. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49(3), 12437-12437.https://doi.org/10.15835/nbha49312437

Herdean, A., Teardo, E., Nilsson, A. K., Pfeil, B. E., Johansson, O. N., Ünnep, R., Nagy, G., Zsiros, O., Dana, S., Solymosi, K., Garab, G., Szabó, I., Cornelia Spetea & Lundin, B. (2016). A voltage-dependent chloride channel fine-tunes photosynthesis in plants. *Nature communications*, 7(1), 11654.https://doi.org/10.1038/ncomms11654

Hildebrandt, T. M., Nesi, A. N., Araújo, W. L., & Braun, H. P. (2015). Amino acid catabolism in plants. *Molecular plant*, 8(11), 1563-1579.https://doi.org/10.1016/j.molp.2015.09.005

Imran, M., Sun, X., Hussain, S., Ali, U., Rana, M. S., Rasul, F., Saleem, M. H., Mohamed, G. M., Parashuram, B., Javaria, A., Ali, M. E. & Hu, C. X. (2019). Molybdenum-induced effects on nitrogen metabolism enzymes and elemental profile of winter wheat (*Triticum aestivum* L.) under different nitrogen sources. *International Journal of Molecular Sciences*, 20(12), 3009. https://doi.org/10.3390/ijms20123009

Irigoyen, J. J., Einerich, D. W., & Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia plantarum*, 84(1), 55-60.https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

Kaiser, B. N., Gridley, K. L., Ngaire Brady, J., Phillips, T., & Tyerman, S. D. (2005). The role of molybdenum in agricultural plant production. *Annals of Botany*, 96(5), 745-754.https://doi.org/10.1093/aob/mci226

Khan, N., Tariq, M., Ullah, K., Muhammad, D., Khan, I., Rahatullah, K., & Ahmed, S. (2014). The effect of molybdenum and iron on nodulation, nitrogen fixation and yield of chickpea genotypes (*Cicer arietinum* L.). *IOSR Journal of Agriculture and Veterinary Science*, 7(1), 63-79.https://www.iosrjournals.org/iosr-javs/papers/vol7-issue1/Version-3/L07136379.pdf

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of experimental Botany*, 53(370), 773-787.https://doi.org/10.1093/jexbot/53.370.773

Leghari, S. J., Wahocho, N. A., Laghari, G. M., HafeezLaghari, A., MustafaBhabhan, G., HussainTalpur, K., & Lashari, A. A. (2016). Role of nitrogen for plant growth and development: A review. *Advances in Environmental Biology*, 10(9), 209-219.https://go.gale.com/ps

Liu, L., Xiao, W., Li, L., Li, D. M., Gao, D. S., Zhu, C. Y., & Fu, X. L. (2017). Effect of exogenously applied molybdenum on its absorption and nitrate metabolism in strawberry seedlings. *Plant Physiology and Biochemistry*, 115, 200-211. https://doi.org/10.1016/j.plaphy.2017.03.015

Liu, C., Hu, C., Tan, Q., Sun, X., Wu, S., & Zhao, X. (2019). Co-application of molybdenum and zinc increases grain yield and photosynthetic efficiency of wheat leaves. *Plant, Soil and Environment*, 65(10), 508-515. https://doi.org/10.17221/508/2019-PSE

Mahdieh, M., Sangi, M. R., Bamdad, F., & Ghanem, A. (2018). Effect of seed and foliar application of nano-zinc oxide, zinc chelate, and zinc sulphate rates on yield and growth of pinto bean (Phaseolus vulgaris) cultivars. *Journal of Plant Nutrition*, 41(18), 2401-2412. https://doi.org/10.1080/01904167.2018.1510517

Tejada-Jimenez, M., Chamizo-Ampudio, A., Llamas, A., Galván, A., & Fernandez, E. (2018). Roles of molybdenum in plants and improvement of its acquisition and use efficiency. In

Revista Bio ciencias
ISSN 2007-3380

Plant micronutrient use efficiency (pp. 137-159). *Academic Press*. https://doi.org/10.1016/B978-0-12-812104-7.00009-5

Monreal, C. M., DeRosa, M., Mallubhotla, S. C., Bindraban, P. S. & Dimkpa, C. 2016. Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biology and fertility of soils*, 52, 423-437. https://doi.org/10.1007/s00374-015-1073-5

Muñoz-Márquez, E., Soto-Parra, J. M., Noperi-Mosqueda, L. C., & Sánchez, E. (2022a). Application of molybdenum nanofertilizer on the nitrogen use efficiency, growth and yield in green beans. *Agronomy*, 12(12), 3163. https://doi.org/10.3390/agronomy12123163

Muñoz-Márquez, E., Soto-Parra, J. M., Pérez-Leal, R., Yánez-Muñoz, R. M., Noperi-Mosqueda, L. C., & Sánchez-Chávez, E. (2022b). Aplicación de nanomolibdeno en frijol y su impacto sobre la eficiencia del nitrógeno. *Revista Mexicana de Ciencias Agrícolas*, 13(SPE28), 319-329. https://doi.org/10.29312/remexca.v13i28.3286

Qaim, M. (2020). Role of new plant breeding technologies for food security and sustainable agricultural development. *Applied Economic Perspectives and Policy*, 42(2), 129-150. https://doi.org/10.1002/aepp.13044

Pardey, P. G., Beddow, J. M., Hurley, T. M., Beatty, T. K., & Eidman, V. R. (2014). A bounds analysis of world food futures: Global agriculture through to 2050. *Australian Journal of Agricultural and Resource Economics*, 58(4), 571-589. https://doi.org/10.1111/1467-8489.12072

Pokhrel, R., McConnell, I. L., & Brudvig, G. W. (2011). Chloride regulation of enzyme turnover: application to the role of chloride in photosystem II. *Biochemistry*, 50(14), 2725-2734. https://doi.org/10.1021/bi2000388

Ponce-García, C. O., Soto-Parra, J. M., Sánchez, E., Muñoz-Márquez, E., Piña-Ramírez, F. J., Flores-Córdova, M. A., ... & Yáñez Muñoz, R. M. (2019). Efficiency of nanoparticle, sulfate, and zinc-chelate use on biomass, yield, and nitrogen assimilation in green beans. *Agronomy*, 9(3), 128. https://doi.org/10.3390/agronomy9030128

Pradhan, P., Fischer, G., Van Velthuizen, H., Reusser, D. E., & Kropp, J. P. (2015). Closing yield gaps: how sustainable can we be?. *PloS one*, 10(6), e0129487. https://doi.org/10.1371/journal.pone.0129487

Prasad, T. N. V. K. V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K. R., Sreeprasad, T.S., Sajanlan, P.S. & Pradeep, T. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *Journal of plant nutrition*, 35(6), 905-927. https://doi.org/10.1080/01904167.2012.663443

Ramírez-Estrada, C. A., Sánchez, E., Flores-Cordova, M. A., Chávez-Mendoza, C., Muñoz-Márquez, E., Palacio-Márquez, A., & Hernández-Figueroa, K. I. (2022). Efficiency and assimilation of nitrogen in bean plants through foliar application of zinc and molybdenum nano fertilizer. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(2), 12719-12719. https://doi.org/10.15835/NBHA50212719

Sánchez, E., Rivero, R. M., Ruiz, J. M., & Romero, L. (2004). Changes in biomass, enzymatic activity and protein concentration in roots and leaves of green bean plants (*Phaseolus vulgaris* L. cv. Strike) under high NH4NO3 application rates. *Scientia horticulturae*, 99(3-4), 237-248. https://doi.org/10.1016/S0304-4238(03)00114-6

Schindler, F. V., & Knighton, R. E. (1999). Sample preparation for total nitrogen and 15N-ratio analysis by the automated Dumas combustion method. *Communications in soil science and plant analysis*, 30(9-10), 1315-1324. https://doi.org/10.1080/00103629909370287

Silva, M. S. D., Oliveira, G. R. F. D., Merloti, L. F., Bossolani, J. W., Camargos, L. S. D., de Sá, M. E., & Reis, A. R. D. (2020). New insights on molybdenum fertilization in common bean under no-tillage system: rates and application time to obtain high vigor seeds. *Journal of Plant Nutrition*, 44(10), 1420-1431. https://doi.org/10.1080/01904167.2020.1849290

Singh, M. D. (2017). Nano-fertilizers is a new way to increase nutrients use efficiency in crop production. *International Journal of Agriculture Sciences*, 9(7), 0975-3710. https://www.researchgate.net/profile/Hari-Meena/publication/344318260_Nanofertilizers_is_a_New_Way_to_Increase_Nutrients_Use_Efficiency_in_Crop_Production/links/5f670700299bf1b53ee440d9/Nano-Fertilizers-is-a-New-Way-to-Increase-Nutrients-Use-Efficiency-in-Crop-Production.pdf

Solanki, M. (2021). The Zn as a vital micronutrient in plants. *Journal of microbiology, biotechnology and food sciences*, 11(3), e4026-e4026. https://doi.org/10.15414/jmbfs.4026

Subramanian, K. S., Manikandan, A., Thirunavukkarasu, M., & Rahale, C. S. (2015). Nano-fertilizers for balanced crop nutrition. *Nanotechnologies in food and agriculture*, 69-80. https://doi.org/10.1007/978-3-319-14024-7_3

Sun, X., Baldwin, E., & Bai, J. (2019). Applications of gaseous chlorine dioxide on postharvest handling and storage of fruits and vegetables–a review. *Food Control*, 95, 18-26. https://doi.org/10.1016/j.foodcont.2018.07.044

Vigani, G., Di Silvestre, D., Agresta, A. M., Donnini, S., Mauri, P., Gehl, C., Bittner, F. & Murgia, I. (2017). Molybdenum and iron mutually impact their homeostasis in cucumber (Cucumis sativus) plants. *New Phytologist*, 213(3), 1222-1241. https://doi.org/10.1111/nph.14214

Watanabe, K., Fukuzawa, Y., Kawasaki, S. I., Ueno, M., & Kawamitsu, Y. (2016). Effects of potassium chloride and potassium sulfate on sucrose concentration in sugarcane juice under pot conditions. *Sugar Tech*, 18, 258-265. https://doi.org/10.1007/s12355-015-0392-z

Xu, C., Li, X., & Zhang, L. (2013). The effect of calcium chloride on growth, photosynthesis, and antioxidant responses of Zoysia japonica under drought conditions. *PloS One,* 8(7), e68214. https://doi.org/10.1371/journal.pone.0068214

Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of plant physiology*, 144(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

Wu, G. Q., Feng, R. J., Liang, N., Yuan, H. J., & Sun, W. B. (2015). Sodium chloride stimulates growth and alleviates sorbitol-induced osmotic stress in sugar beet seedlings. *Plant growth regulation*, 75, 307-316. https://doi.org/10.1007/s10725-014-9954-4

Ybañez, Q. E., Sanchez, P. B., Badayos, R. B., & Agravante, J. U. (2020). Synthesis and characterization of nano zinc oxide foliar fertilizer and its influence on yield and postharvest quality of tomato. *e Philippine Agricultural Scientist*, 103(1), 55-65. https://www.researchgate.net/profile/Quincy-Ybanez/publication/346658621_Synthesis_and_Characterization_of_Nano_Zinc_Oxide_Foliar_Fertilizer_and_its_Influence_on_Yield_and_Postharvest_Quality_of_Tomato/links/5fd6f3c3299bf140880a6524/Synthesis-and-Characterization-of-Nano-Zinc-Oxide-Foliar-Fertilizer-and-its-Influence-on-Yield-and-Postharvest-Quality-of-Tomato.pdf

Yeboah, S., Asibuo, J., Oteng-Darko, P., Asamoah Adjei, E., Lamptey, M., Owusu Danquah, E., Lamptey, M., Owusu-Danquah, E., Waswa, B. & Butare, L. (2021). Impact of foliar application of zinc and magnesium aminochelate on bean physiology and productivity in Ghana. *International Journal of Agronomy*, 2021, 1-9. https://doi.org/10.1155/2021/9766709

Yemm, E.W., Cocking, E.C., & Ricketts, R.E. (1955). The determination of amino-acids with

Revista Bio ciencias
ISSN 2007-3380

ninhydrin. *Analyst*, 80(948), 209-214. https://doi.org/10.1039/AN9558000209
Zhong, C., Cao, X., Hu, J., Zhu, L., Zhang, J., Huang, J., & Jin, Q. (2017). Nitrogen metabolism in adaptation of photosynthesis to water stress in rice grown under different nitrogen levels. *Frontiers in plant science*, 8, 1079. https://doi.org/10.3389/fpls.2017.01079