# Growth and production of crisp lettuce (*Lactuca sativa* L.) using different doses of nitrogen and magnesium

Crecimiento y producción de lechuga crespa (*Lactuca sativa* L.) sometida a diferentes dosis de nitrógeno y magnesio



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Crisp lettuce var. Vera. Photo: A.J. Reyes-Medina

# ABSTRACT

Lettuce is part of the group of leafy vegetables that sees high consumption worldwide. However, population increases have forced excessive increases in fertilization, raising N levels both in the water and the soil and leading to serious environmental problems. As a result, appropriate fertilization management must be carried out; therefore, this study aimed to determine the effect of nitrogen and magnesium on the growth and yield of crisp lettuce. A randomized complete block design was carried out with 10 treatments: a control plus nine treatments with a central compound and different combinations of nitrogen and magnesium doses. The variables leaf area ratio, SPAD chlorophyll index and relative growth rate decreased throughout the crop cycle. The application of 150 kg ha<sup>-1</sup> of N + 18 kg ha<sup>-1</sup> of Mg generated the highest production values and net assimilation rate. The response surface graph yielded a model with a maximum yield of 21 t ha<sup>-1</sup> using fertilization with 93.8 kg ha<sup>-1</sup> of nitrogen and 12.4 kg ha<sup>-1</sup> of magnesium. The lower doses of both nitrogenous and magnesium fertilization showed the highest values of agronomic efficiency and partial productivity.

Additional key words: dry mass; leaf area; yield; agronomic efficiency; response surface.

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#### RESUMEN

La lechuga hace parte del grupo de hortalizas de hoja que tiene un alto consumo a nivel mundial. Sin embargo, el aumento de la población ha obligado a aumentar excesivamente la fertilización, elevando los niveles de N tanto en el agua como en el suelo y provocando graves problemas ambientales. En consecuencia, se debe llevar un manejo adecuado de la fertilización, para lo tanto, este estudio tuvo como objetivo determinar el efecto del nitrógeno y el magnesio en el crecimiento y rendimiento de lechuga crespa. Se realizó un diseño en bloques completos al azar con diez tratamientos, un testigo más un arreglo de nueve tratamientos en forma de compuesto central, con diferentes combinaciones de dosis de nitrógeno y magnesio. Las variables relación de área foliar, el índice de clorofila SPAD y la tasa de crecimiento relativo disminuyeron a lo largo del ciclo de cultivo. La aplicación de 150 kg ha<sup>-1</sup> N + 18 kg ha<sup>-1</sup> Mg generó los mayores valores de producción y tasa de asimilación neta. El gráfico de superficie de respuesta arrojó un modelo con un rendimiento máximo de 21 t ha<sup>-1</sup>, con una fertilización de 93,8 kg ha<sup>-1</sup> N y 12,4 kg ha<sup>-1</sup> Mg. Las dosis más bajas de fertilización tanto nitrogenada como magnésica mostraron los valores más altos de eficiencia agronómica y productividad parcial.

Palabras clave adicionales: masa seca; área foliar; rendimiento; eficiencia agronómica; superficie de respuesta.

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## INTRODUCTION

Lettuce is a widely consumed vegetable worldwide, with great commercial availability because of its short growth time and nutraceutical quality given its content of ascorbic acid, carotenoids, flavonoids, and tocopherols, favoring human health (Hong *et al.*, 2022). In 2021, approximately 27.7 million t of lettuce were produced worldwide, with China having the highest production (51.8%), followed by the United States with 15.9% and India with 4.1% (FAO, 2021). Production in Colombia was 97,240 t in 2016, with a yield of 21.95 t ha<sup>-1</sup>, where production is concentrated in the departments of Cundinamarca, Antioquia, and Nariño given their large planting areas (Agronet, 2023).

It is estimated that the global population will increase by between two and three billion by 2050 and that half of the population will be fed with crops grown with nitrogen fertilizer (Zhang *et al.*, 2015). Likewise, according to the UNEP (2019), excess nitrogen fertilization has increased N levels both in the soil and water, altering life. N contributes greatly to climate change and constitutes a threat to human health, leading to the need for careful management of nitrogen and fertilizer elements, such as applying adequate magnesium to satisfy crop requirements and nourish a growing population, while minimizing environmental effects.

Crop yield and quality are directly related to the supply of mineral nutrients during the growth stages, with nitrogen (N) and magnesium (Mg) being essential for lettuce cultivation (Mahlangu et al., 2016). N plays an important role in chlorophyll content and photosynthetic capacity; therefore, a N deficiency reduces productivity (Gong et al., 2020; Hong et al., 2022) by decreasing the development of the leaf area, as well as photosynthetic assimilation, which decreases the source of carbon skeletons, important characteristics in the commercial production of leafy vegetables such as lettuce (Mahlangu et al., 2016). According to Coulombier et al. (2020), the adequate availability of N increases the contents of B1 vitamins and carotenes such as lutein, beta-carotenes, neoxanthin, and zeaxanthins, in addition to regulating the biosynthesis of secondary metabolites such as phenols.

Likewise, Mg is a constituent of chlorophyll; therefore, it is important to the processes of photosynthesis, enzymatic activation, production, and transport of photoassimilates. In addition, it serves as a link between substrates and, in combination with ATP, stabilizes ribosomal particles, acting as an indicator of enzymes involved in protein synthesis (Ishfaq *et al.*, 2022). Hence, the growth and development of plants can be seriously inhibited by its deficiency, which



generates detrimental effects on the productivity and quality of crops (Chen *et al.*, 2018).

Therefore, the objective of this work was to evaluate the growth and production of lettuce cv. Vera crops subjected to different doses of nitrogen and magnesium to provide producers with optimal fertilization management with these nutrients.

# **MATERIALS AND METHODS**

#### Location and plant material

This study was carried out under a plastic roof in the San Francisco zone, municipality of Mosquera-Cundinamarca, at 4°41′27″ N and 74°12 09″ W, with an altitude of 2,542 m. Laboratory analyses were carried out in the Plant Physiology Laboratory of the National University of Colombia. The plastic greenhouse had a chapel-type structure, with an area of 160 m<sup>2</sup> and a maximum height of 6 m, and 80% white polyshade was used to control light intensity. Crispy lettuce Vera variety seedlings from a Rioplant certified propagator were sown in three 1.2 m wide by 12 m

long beds, with a planting distance of 0.3 m between plants and 0.3 m between rows.

The physical and chemical properties of the soil are in Tab. 1. Fertilization was carried out according to the nutritional requirements of the crop in an edaphic form divided into two parts. The first fertilizer application was made seven days after transplanting (dat), and the second was done at 20 dat, incorporated into the crown. The nitrogen and magnesium were applied according to Tab. 1. In addition, 70 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 200 kg ha<sup>-1</sup> of K<sub>2</sub>O, 51 kg ha<sup>-1</sup> of CaO, and 10 kg ha<sup>-1</sup> of S were added, using as source potassium sulfate, potassium chloride, dicalcium phosphate, triple superphosphate, magnesium nitrate and urea. Agronomic management for pests, diseases and weeds was carried out opportunely according to the monitoring.

## Experiment design

A randomized complete block design was developed with ten treatments: a control plus nine treatments with a central compound (Tab. 2) according to Martínez (1988). Each block was equivalent to a replicate,

Table 1. Physical and chemical analysis of the soil from the municipality of Mosquera, Cundinamarca.										
pН	EC	0	C	Ν	Са	K	Mg	Na	EAI	ECEC
	(dS m <sup>-1</sup> )	n <sup>-1</sup> ) %		meq 100 g <sup>-1</sup>						
5.50	-	3.33		0.29	19.60	0.18	3.46	3.29	0.00	26.50
Particle size	Clay	Silt	Sand	Р	S	Cu	Fe	Mn	Zn	В
	%			mg kg <sup>-1</sup>						
Clay loam	35	43	22	55.5	149	0.78	76.3	19.7	31.8	0.41

EC: electric conductivity; OC: organic carbon; EAI: exchangeable aluminum; ECEC: effective cation exchange capacity.

Treatment structure	Number of reatments	X <sub>1</sub>	X <sub>2</sub>	Doses of N (kg ha <sup>-1</sup> )	Doses of Mg (kg ha⁻¹)
Control	1	-	-	0	0
	2	-1	-1	50	6
Desis festarial	3	-1	+1	50	18
Basic factorial	4	+1	-1	150	6
-	5	+1	+1	150	18
	6	-α	0	0	12
Avial agint	7	$+\alpha$	0	200	12
Axial point	8	0	-α	100	0
	9	0	$+\alpha$	100	24
Central point	10	0	0	100	12

 Table 2.
 Treatment combination codes for two factors in a central composite design.

for a total of 30 experiment units (EU), and each EU contained 16 plants, for a total of 480 plants.

#### **Response variables**

Samplings were carried out every 8 d for the relative chlorophyll content and every 15 d for the other variables, taking three random plants per treatment and per block, for a total of 360 plants evaluated in four samplings at 21, 35, 49, and 60 dat. The leaf area (LA) was measured by taking photos of the fully extended leaves, which were analyzed according to the methodology recommended by Schneider et al. (2012) using ImageJ version 1.45. The relative chlorophyll content was determined with a SPAD-502 Plus (Konica Minolta, Inc., Tokyo, Japan). The fresh and dry mass were measured with an Adam PGW-2502e electronic balance (Adam Equipment Co. Ltd., Milton Keynes, UK) with a precision of 0.01 g and a Memmert drying oven, model UNB500 (Memmert GmbH + Co. KG, Schwabach, Germany), at a temperature of 75°C for 72 h.

The following growth indices were calculated: leaf area ratio (LAR), relative growth rate (RGR), net assimilation rate (NAR), agronomic efficiency, and partial productivity factor, according to Tab. 3.

Tabla 3.   Growth and efficiency rates.					
Index	Equation				
Leaf area ratio (LAR)	$LAR = \frac{\left(\frac{LA_1}{W_1}\right) + \left(\frac{LA_2}{W_2}\right)}{2}$				
Relative growth rate (RGR)	$\mathrm{RGR} = \frac{\ln(W_2) - \ln(W_1)}{T_2 - T_1}$				
Net assimilation rate (NAR)	NAR= $\frac{\frac{W_2 - W_1}{T_2 - T_1} * \ln(LA_2) - \ln(LA_1)}{LA_2 - LA_1}$				
Agronomic efficiency (AE)	$AE = \frac{Y - Y_0}{F}$				
Partial factor productivity (PFP)	$PFP = \frac{(Y_0 + \Delta Y)}{F}$				

W: dry mass; T: time; LA = leaf area; In: natural logarithm; Y: yield with applied fertilizer;  $Y_0$ : yield without fertilizer input; F: amount of fertilizer applied;  $\Delta Y$ : incremental yield that results from de fertilizer application.

## Data analysis

The data were subjected to a normality test, analysis of variance (Anova), and Tukey test ( $P \le 0.05$ ) to establish differences between treatments and classify them. For the performance variable, a response surface analysis was performed to establish the optimal combination of N and Mg that generated the maximum yield values. This model was fitted to a quadratic function for the factorial design with SAS version 9.4 (SAS Institute Inc., Cary, NC).

# **RESULTS AND DISCUSSION**

#### Leaf area (LA) and Leaf area ratio (LAR)

There were only significant differences for LA between treatments at 21 and 35 dat (Fig. 1A). However, a significant and progressive increase was observed over time, reaching a maximum of 1,753  $cm^2$  with 150 kg ha<sup>-1</sup> N + 6 kg ha<sup>-1</sup> Mg at 60 dat, and the treatments in general had a significant average value of 1,533 cm<sup>2</sup>, which is higher than the 902.3 cm<sup>2</sup> reported by Cho et al. (2018) in 'Cheongchima' lettuce subjected to a light intensity of 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. It should be noted that one of the main functions of leaves is the interception of solar radiation to carry out the photosynthesis process, where a greater LA allows a greater absorption of radiation and thus achieve maximum physiological development (Fraszczak and Kula-Maximenko, 2021). These authors found that lettuce with different colors and thicknesses intercepted different spectra of light.

On the other hand, the LAR decreased over time (Fig. 1B), going from a maximum value at 35 dat of 296.07  $\text{cm}^2 \text{g}^{-1}$  to 165  $\text{cm}^2 \text{g}^{-1}$  at 60 dat, at which time the control treatment presented the highest LAR value with 222  $\text{cm}^2 \text{g}^{-1}$ , which indicated greater efficiency in the generation of LA with a lower dry mass requirement. This response can be related to the production of photoassimilates, required in greater quantities during the early stages of development in photosynthetically active parts, which increases energy expenditure; consequently, a lower mass was obtained, which is consistent with Simkin *et al.* (2020) who found that crop yield is determined by the cumulative rate of photosynthesis during the growing season.

#### Relative chlorophyll content

There were significant differences between treatments and between the different measurement times, however, the interaction did not have significant differences (Fig, 1C). The SPAD values varied



over time and decreased from 19.7 at 14 dat to 16.26 at 56 dat, which were lower than the values of 26 to 35 obtained by Liu *et al.* (2014) and from the 24.1 to 30.1 values observed by Cho *et al.* (2018) by varying the light intensity from 100 to 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively, and higher than those reported by Frąszczak and Kula-Maximenko (2021), with oscillated between 2 and 12. Likewise, these authors stated that the application of blue light in lettuce reduced the SPAD content.

Higher chlorophyll contents were found in SPAD units at 14 dat, while lower values occurred at 49 and 60 dat, which coincided with the lowest applications of magnesium fertilization (Figure 1C). Mg is the central atom of the chlorophyll molecule, is fundamental in photosynthesis, is also involved in protein synthesis and has an important role in energy transfer (Peng *et al.*, 2019). Therefore, the decrease in the concentration of chlorophylls measured in the SPAD

units could be due to the fact that the contents of N and Mg decreased with the age of the plants because, during the growth and development of crops, the size and number of leaves increases and, therefore, the requirements of these elements are increased by photosynthetic activity, where N applications increase the photosynthetic rate (Leghari *et al.*, 2016).

#### Net assimilation rate (NAR)

The NAR showed significant differences between treatments only at 50 dat, where the application of 150 kg ha<sup>-1</sup> N + 18 kg ha<sup>-1</sup> of Mg showed higher values (Fig. 2A), 77.4% higher than the control treatment, which had the lowest NAR. Despite the fact that the measurements and the interaction did not present significant differences over time for the NAR, it increased until 49 dat, subsequently decreasing slightly until the end of the cycle at 60 dat. This

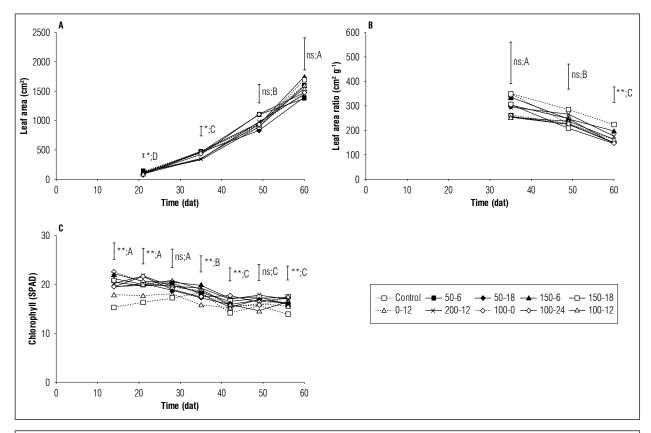


Figure 1. A) Leaf area, B) leaf area ratio, and C) chlorophyll (SPAD) of the 'Vera' lettuce crop subjected to different doses of nitrogen and magnesium. ns: not significant, \* and \*\* indicate significant effect according to the Anova ( $P \le 0.05$  and  $P \le 0.01$ , respectively) between the treatments before the semicolon, and different uppercase letters after the semicolon indicate significant differences between measurements over time according to Tukey ( $P \le 0.05$ ). Vertical bars indicate the minimum significant difference between treatments for the Tukey test.

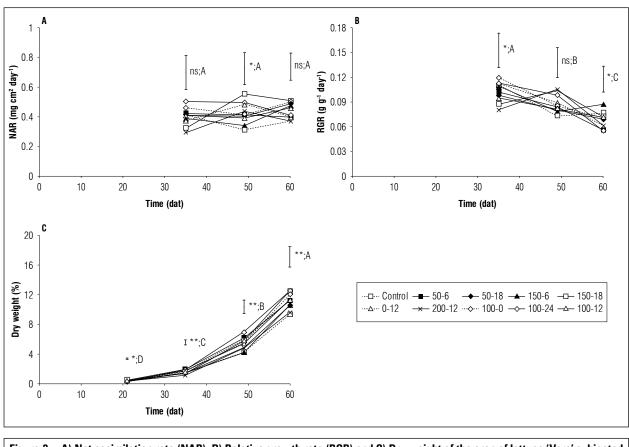


Figure 2. A) Net assimilation rate (NAR), B) Relative growth rate (RGR) and C) Dry weight of the crop of lettuce 'Vera' subjected to different doses of nutrition with Nitrogen and Magnesium. ns: not significant, \* and \*\* indicate significant effect according to the Anova (P≤0.05 and P≤0.01, respectively) between treatments before the semicolon, and different uppercase letters after the semicolon indicate differences significant between measurements over time according to Tukey (P≤0.05). Vertical bars indicate the minimum significant difference between treatments of the Tukey test.

reduction NAR is related to the behavior of LA because leaves are more exposed to radiation and are more efficient in the uptake and assimilation of  $CO_2$ during the early stages of development; therefore, an adequate supply of nitrogen fertilization in the first stages will be reflected in a greater LA, greater photosynthetic capacity and greater chlorophyll content (Mahlangu *et al.*, 2016). Likewise, as plants develop, external leaves increase shading on internal leaves, which translates into a reduction in the capture of photosynthetic rates according to Chapepa *et al.* (2020), who reported that attenuation in light capture is affected by canopy architecture.

The constant increase or increase following a decrease in the NAR in some treatments was explained by the fact that it is possible that the concentration of assimilates, and the generation of dry biomass were higher because of a higher production and better distribution of leaves and, as a consequence, because a reduction of photosynthetic activity was not seen (Frąszczak and Kula-Maximenko, 2021). It is also probable that having a greater availability of nutrients resulted in optimal distribution of photoassimilates in the different plant organs (Leghari *et al.*, 2016).

#### Relative growth rate (RGR)

There were significant differences between interaction at 35 and 60 dat for RGR (Fig. 2B). At 35 dat, the control treatment showed the best results, while at 60 dat, the application of 150 kg ha<sup>-1</sup> of N + 6 kg ha<sup>-1</sup> of Mg reached the highest RGR data. In the same way, the measurements had significant differences, and the RGR decreased during the entire crop cycle; at 35 dat, it was 51.26% higher than at 60 dat, going from 0.102 to 0.067 g g<sup>-1</sup> d<sup>-1</sup>. These results are similar



to those obtained by Martínez and Garcés (2010) in 'Romana' variety lettuce, who, at 40 dat, found values of 0.117 g g<sup>-1</sup> d<sup>-1</sup> in plants subjected to 180 mg L<sup>-1</sup> of potassium. The RGR is important because it refers to the growth speed of the plant in relation to its current biomass; likewise, it determines the time it takes for the plant to reach harvest. Generally, low RGR rates are indicators of the presence of some type of stress (El-Nakhel *et al.*, 2019).

#### Dry biomass

There were significant differences between interaction and between the different measurement times for the accumulation of dry biomass (Fig. 2C). The highest values of dry mass were obtained in the treatments with applications of 100 kg ha<sup>-1</sup> N + 24 kg ha<sup>-1</sup> Mg and 150 kg ha<sup>-1</sup> N + 18 kg ha<sup>-1</sup> Mg with 12.52 and 12.50%. In this regard, Liu *et al.* (2014) stated that the application of 200 kg ha<sup>-1</sup> N (0.39 g/plant) in lettuce saw a higher value than the dose of 400 kg ha<sup>-1</sup> N (0.12 to 0.31 g/plant) and the control (0.14 g/plant) in terms of accumulated dry biomass at 50 dat. Similarly, Cho *et al.* (2018) reported that the dry mass at 20 dat reached values of 3.03 g/plant under light intensities of 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>.

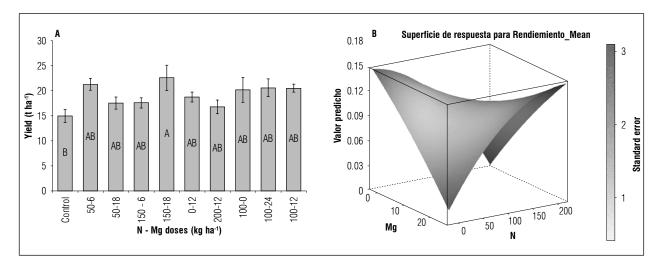
In this regard, it is estimated that a plant requires 1.5 to 3.5 g kg<sup>-1</sup> of dry mass of Mg, which is used in numerous physiological and biochemical processes (Chen *et al.*, 2018), of which, between 15 and 35% of the plant's Mg binds to chloroplasts, mainly as a

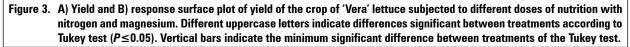
component of chlorophyll where it is key in the energy transfer process (Chen *et al.*, 2018). Furthermore, a Mg deficiency decreases the photosynthetic rate and blocks carbohydrate allocation to sink organs, which inhibits growth and ultimately leads to low productivity in many plant species (Yang *et al.*, 2012).

#### Yield

There were significant differences between interactions for crop yield. The highest yield was obtained with the application of 150 kg ha<sup>-1</sup> of N + 18 kg ha<sup>-1</sup> of Mg with 22.59 t ha-1, which was 51.15% higher than the yield of the control, which, in turn, was the treatment with the lowest value (Fig. 3A). It should be noted that an adequate supply of N positively increases its absorption and, consequently, the production of leaves and their size, as reported by Hong et al. (2022) who found increases in production between 13% and 22% when they increased the application of N to 315 kg ha<sup>-1</sup>. Likewise, cell division and expansion increase the growth rate of leaves; therefore, a nutritional deficit directly affects the production of new leaves and leaf growth (Martínez and Garcés, 2010). Regarding the contribution of Mg to production, Peng et al. (2019) reported that this element is relatively mobile since it translocates from old to young tissues in plants under limited conditions, being stored in greater quantity in the latter.

Multiple linear regression was plotted as a response surface (Fig. 3B), which had a degree of fit of 0.942





with a coefficient of variation of 3.69%. The adjusted regression model was the quadratic one, being significant for the treatments (P=0.0479), the interaction (P=0.0145), and for each of the independent factors, N and Mg (P =0 0.0322 and P=0.0492, respectively). The optimum application level was estimated with the regression equation, which showed that fertilization should be carried out with 93.8 kg ha<sup>-1</sup> N and 12.4 kg ha<sup>-1</sup> Mg to obtain the maximum yield (21 t ha<sup>-1</sup>). These N doses were relatively low because Liu *et al.* (2014) applied 315 kg ha<sup>-1</sup> N and obtained a production of 15 t ha<sup>-1</sup>, which was lower because the present trial also used Mg fertilization, which may have increased production.

## Agronomic efficiency (AE) and Partial Factor Productivity (PFP)

The highest AE for N occurred with the application of 50 kg ha<sup>-1</sup> of N + 6 kg ha<sup>-1</sup> of Mg since it managed to increase yield by 126 kg when compared to the control treatment (Tab. 4). In the case of Mg, the highest AE was obtained with the dose of 50 kg ha<sup>-1</sup> of N + 6 kg ha<sup>-1</sup> of Mg with a 1051 kg increase in yield compared to the control, per kg of Mg applied, which coincided with the second highest yield of 21.2 t ha<sup>-1</sup>, which indicated that, by only applying 6 kg of Mg, high production efficiency was already achieved. These values were higher than those of Guelfi-Silva *et al.* (2013), who reported AE values that ranged between 12 and 19 kg kg  $^{\rm -1}$  of potassium with 200 kg  $ha^{\rm -1}$  in a lettuce crop.

On the other hand, when analyzing the PFP for the two elements, the application of 50 kg ha<sup>-1</sup> of N and 6 kg ha<sup>-1</sup> of Mg obtained higher values with a PFP of 425.1 and 3,542.6 kg kg<sup>-1</sup> for N and Mg, respectively (Tab. 4). When comparing this treatment with the one that registered the highest production (150 kg ha<sup>-1</sup> of N and 18 kg ha<sup>-1</sup> of Mg), it can be seen that the PFP was 182% higher, which implied that add-ing more fertilization did not improve performance linearly, in accordance with the law of diminishing returns or Mitscherlich's law (Hasnain *et al.*, 2020). Similarly, the values were similar to the PFP reported for lettuce, which ranged from 78 to 252 kg kg<sup>-1</sup>, 292 to 885 kg kg<sup>-1</sup> and 82 to 190 kg kg<sup>-1</sup>, for N, P, and K, respectively (Sangare *et al.*, 2012).

# CONCLUSION

The leaf area ratio, SPAD chlorophyll index, and relative growth rate decreased over time during the crop cycle. The application of 150 kg ha<sup>-1</sup> of N + 18 kg ha<sup>-1</sup> of Mg generated the highest production and NAR values. According to the response surface graph, fertilization with 93.8 kg ha<sup>-1</sup> of N and 12.4 kg ha<sup>-1</sup> of Mg should be carried out to obtain maximum yield in 'Vera' lettuce. The treatments with lower applications of both nitrogenous and magnesium

Table 4. Yield, dry mass, agronomic efficiency (AE) and partial factor productivity (PFP) of the crop of 'Vera' variety of crisp lettuce.

Ν	Mg	Yield	Dry mass	Relative yield	AE (kg increment/kg element)		PFP (kg FW/kg element)	
(kg ha⁻¹)		(%)		N	Mg	N	Mg	
0	0	14,944.4	66	6.42	-	-	-	-
50	6	21,255.6	94	5.58	126.2	1051.9	425.1	3,542.6
50	18	18,855.6	83	5.90	78.2	217.3	377.1	1,047.5
150	6	17,577.8	78	6.07	17.6	438.9	117.2	2,929.6
150	18	22,588.9	100	5.95	51.0	424.7	150.6	1,254.9
0	12	18,711.1	83	6.10	-	313.9	-	1,559.3
200	12	16,755.6	74	5.81	9.1	150.9	83.8	1,396.3
100	0	20,166.7	89	6.37	52.2	-	201.7	-
100	24	20,577.8	91	6.33	56.3	234.7	205.8	857.4
100	12	20,500.0	91	5.82	55.6	463.0	205.0	1,708.3

FW: fresh weight.



fertilization showed higher values of agronomic efficiency and partial productivity factor.

**Conflict of interests:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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