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WHAT IS THE OPTIMAL FERTIGATION START TIME AND FREQUENCY IN LETTUCE SEEDLINGS?

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Abstract
Although lettuce is one of the most important vegetable crops cultivated in Brazil, producers conduct seedling production empirically, as there are no published reports on the optimal start time and management strategy for seedling fertigation. The present aimed to assess the influence of fertigation management on the growth, physiological aspects and nutritional status of lettuce seedlings and to determine the optimal fertigation start time and frequency. Two experiments were conducted, each with a randomized block design and six repetitions. The first consisted of six treatments, namely six fertigation start times at 0, 3, 6, 9, 12, and 15 d after emergence (DAE), and the second consisted of five treatments, representing different application frequencies at 3, 4, 5, 6, and 7 d intervals. The assessment of nutrient accumulation levels and biometric and physiological characteristics of the seedlings were performed after transplanting. Fertigation start times significantly affected 14 of the 18 variables assessed, particularly the number of leaves, shoot dry weight, leaf area, initial chlorophyll fluorescence, and P, K, Ca, Mg, and S accumulation. The best results for ten variables were obtained when fertigation began at emergence, with values 17.77 - 35.63% higher than those at fertigation onset at 15 DAE. Application frequency only influenced chlorophyll content and N, P, K, and S accumulation, with optimal results obtained at 3 - 6 d intervals. Beginning fertigation at plant emergence favors dry weight production, nutrition and photosynthesis and shortens the production time of lettuce seedlings. The optimal start time for lettuce seedling fertigation is at emergence, with application performed every 6 d.

Keywords: Fertigation management. *Lactuca sativa* L. Lettuce fertilization. Nutrient solution. Seedling production.

1. Introduction

Widely grown and consumed worldwide, lettuce (*Lactuca sativa* L.) is one of the most popular leafy vegetables in Brazil (Gnoatto et al. 2018; Gusatti et al. 2019).

Seedling production is one of the most critical phases in the lettuce crop cycle and directly affects the final plant performance from the dual perspective of nutritional quality available to consumers and productivity perspective (Carmona et al. 2012; Silva et al. 2018; Chiomento et al. 2019; Gusatti et al. 2019; Lima et al. 2019).

Successful crop cultivation is closely associated with seedling quality. Quality seedlings are pathogen-free, uniform, and well-developed, and, after transplanting, are less vulnerable to biotic and abiotic stresses (Fávaris et al. 2016).

Lettuce seedlings were grown in multicell plug trays. Seedling development in trays depends on factors such as cell volume, the substrate used, the microclimate in the nursery, and the availability of water and nutrients to plants (Gnoatto et al. 2018).

Most research on vegetable seedling production focuses on substrate assessment and proposes new substrate formulations based on residue usage to lower seedling production costs (Tejada and Benítez 2015; Alvarez et al. 2019).

However, the main challenge in seedling production is ensuring adequate plant development in low-volume containers that allow only a limited amount of water and nutrients (Carmona et al. 2012; Chiomento et al. 2019). Additionally, as the substrates used are mainly inert, a supplementary nutrient supply is needed in organic form or mineral fertilizers mixed with the substrate or applied via irrigation, i.e., fertigation.

As such, fertigation with specific nutrient solutions is a decisive factor in high-quality seedling production, resulting in relatively more productive plants that are less susceptible to pests and diseases. Although in practice, the application of these nutrient solutions is empirical and not based on technical or scientific evidence regarding the optimal start time and frequency of fertigation.

Fertigation typically begins 8-10 d after emergence (DAE), with 5 d intervals between applications. However, research on the optimal start time and frequency of fertigation are limited.

The present study hypothesized that adequate nutrient supply via fertigation combined with correct management of the remaining production factors improves seedling quality, lowers costs, and shortens production time. Thus, the research question is to ascertain the optimal time for beginning fertigation begin and its application frequency in seedling production.

In light of the above, this study aimed to assess the influence of fertigation management on the growth, physiological aspects, and nutritional status of lettuce seedlings and to determine the optimal fertigation start time and application interval.

2. Material and Methods

The study consisted of two independent experiments: fertigation start times and application intervals, both conducted in an arched roof greenhouse covered in 150 micra thick light-diffusing plastic film with 50% shade cloth on the sides, located at 19°45'26" S and 47°55'27" W, in the municipality of Uberaba, Minas Gerais state (MG), Brazil. The experiments were performed from December 8-28, 2019, with sowing performed on the first day of the study and assessment and harvesting on the last day.

The climate in the region is characterized as warm tropical (Aw) based on Köppen's classification, with cold and dry winters and warm and wet summers (Beck et al. 2018). Temperatures and relative humidity inside the greenhouse were measured daily using a thermohygrometer installed 1.20 m above the ground, with average values of 27.51°C and 67.16%, respectively.

Six start times were assessed in the fertigation time experiment (T_1 = 0, T_2 = 3, T_3 = 6, T_4 = 9, T_5 = 12, and T_6 = 15 DAE), and five application intervals in the second experiment: T_1 = every 3 d, T_2 = every 4 d, T_3 = every 5 d, T_4 = every 6 d and T_5 = every 7 d. A randomized block design (RBD) was used in both experiments, with 6 repetitions. Each experimental unit consisted of 140 plants, with 40 central plants in each plot assessed (study area).

The nutrient solution used in this study was proposed by Furlani et al. (1999) and is presented in Table 1.

The "Vanda"® lettuce cultivar was obtained from SAKATA. According to the information provided by the company, plants of this cultivar are large, with high resistance to tip burn (calcium deficiency), long leaves and thick stems, a vigorous root system, an average cycle length of 55 d (early), and high resistance to lettuce mosaic virus strain II (LMV-II*)* (Sakata 2022).

Sowing was performed in polyethylene trays with 12.5 cm³ cells, filled with Bioplant Plus® substrate. According to the information available on the packaging, by the information available on the packaging, the substrate contained *Sphagnum* moss, coconut powder, rice husk, and vermiculite in proportions of 50, 20, 20 and 10%, respectively, with limestone PG mix fertilizer (14-16-18 + micros), and monoamonium phosphate (11-52-00) at concentrations of 3, 1.5, and 1.5 Kg m^{-3}, respectively. Physical and chemical analyses of the substrate were performed (1:1.5 v:v) according to de Boodt and Verdonck (1972). Results showed the following characteristics: 55% moisture content, 100% water retention capacity (WRC), a density of 160 kg m^{-3}, a pH of 6.5, and electrical conductivity (EC) of 0.8 mS cm^{-1}. The experiment was conducted on 0.9 m-high benches made of wires stretched across metal supports.

Table 1. Chemical composition of the nutrients in the nutrient solution used in the present study.

Nutrient	Amount of the nutrient*
	-------------------- g $1000L^{-1}$ of nutrient solution--------------------
N	198.0
P	39.0
K	183.0
Ca	142.0
Mg	38.0
S-SO_4	52.0
	----------------- mg $1000L^{-1}$ of nutrient solution--------------------
B	300
Cu	20
Fe	2000
Mn	400
Mo	60
Zn	60

The seedlings were watered four times a day (8:00 a.m., 12:00 p.m., 2:00 p.m., and 4:30 p.m.) throughout the experiment using an automated sprinkler irrigation system to maintain the substrate at the capacity of the container without drainage. From sowing to emergence (3 d after sowing), all treatments were irrigated using only water.

Fertigation began after plantlet emergence in at least 90% of cells sown in each experiment and was performed once a day (8:00 a.m.) in both experiments. Fertigation at the start of the experiment was initiated based on the different treatments. Following the first application for each treatment, fertigation was repeated at 5 d intervals. Thus, as a function of the start time, the number of applications differed between the treatments, with four applications in T_1, three in T_2 and T_3, two in T_4 and T_5, and one in T_6.

For the frequency experiment, fertigation began 3 DAE and was repeated in line with the treatments, indicating that the number of applications differed between the treatments based on the intervals stipulated, with five applications in T_1, four in T_2, three in T_3 and T_4, and three in T_5.

All treatments received the same amount of water per day. As a function of the treatment, when fertigation was applied, the water in the first irrigation (8:00 a.m.) was substituted with the exact volume of nutrient solution. Fertigation was performed by a backpack sprayer. Plants were sprayed with a small amount of water to prevent the nutrient solution from accumulating on the leaves after fertigation. The remaining daily irrigation, at 12:00 p.m., 2:00 p.m., and 4:30 p.m., was performed using only water for all treatments.

The number of leaves (NL) was assessed at 15 DAE by counting all fully developed leaves on ten plants from the study area of each plot.

The physiological parameters of the seedlings, i.e., chlorophyll-*a* fluorescence (OJIP) transient and chlorophyll-*a* content, were determined at 17 DAE. The OJIP transient was measured using a fluorometer (FP100) from Photon Systems Instruments company, between 12:00 and 3:00 a.m. to ensure that the plants were adapted to the dark. The initial (F_o), variable (F_v), and maximum fluorescence (F_m), as well as the maximum quantum efficiency of PS II (F_v/F_m), absorption flux per reaction center (ABS/RC), and trapped energy flux per reaction center (TR_o/RC), were assessed using five readings per plot on the second true leaf of five plants from the study area of each plot. Chlorophyll-*a* content was measured indirectly from 11:00 a.m. to 3:00 p.m. based on the Falker chlorophyll index, with five readings per plot on the second true leaf, using a chlorophyll content meter (Falker Clorofilog CFL1030).

At 21 d after sowing, that is, 18 d after treatment application began, when the seedlings had reached the commercial stage for transplanting, the following were assessed: leaf area (LA) (cm^2 $plant^{-1}$); shoot dry weight (SDW) (g $plant^{-1}$); root dry weight (RDW) (g $plant^{-1}$); shoot root ratio (SRR); and N, P, K, Ca, Mg, and S content (g kg^{-1}), following EMBRAPA (2009). The accumulation of N, P, K, Ca, Mg, and S (mg $plant^{-1}$) in the shoots was calculated by multiplying the nutrient contents by SDW.

The data were subjected to an analysis of variance using the F-test. On identifying a significant difference, polynomial regression was performed for fertigation start times, with first and second-order models selected as functions of the highest R^2 adjusted value. For fertigation frequency, the treatment means were grouped using the Scott-Knott clustering algorithm. A 5% significance level was used for all analyses. Data were analyzed using the R Core Team program (2019), and graphics were created using SigmaPlot 14.0.

3. Results

Fertigation start time significantly influenced the biometric variables NL, SDW, and LA, as well as all physiological variables and nutrient content accumulation, except for N (Figures 1 and 2).

The highest values were obtained for the following variables when fertigation began at plant emergence: SDW (0.0841 g $plant^{-1}$, Figure 1A), NL (3.77 leaves $plant^{-1}$, Figure 1B), F_o (7564.80, Figure 1D), ABS/RC (2422.16, Figure 2A), TR_o/RC (1959.12, Figure 2A), P (0.5967 mg $plant^{-1}$, Figure 2C), K (2.8363 mg $plant^{-1}$, Figure 2B), Ca (0.8664 mg $plant^{-1}$, Figure 2C), Mg (0.2056 mg $plant^{-1}$, Figure 2D), and S (0.1118 mg $plant^{-1}$, Figure 2D). The leaf area (31.18 cm^2 $plant^{-1}$, Figure 1C) and Fm (39778.66, Figure 1E) were the highest for fertigation onset at 5 DAE, and F_v (32542.53) and F_v/F_m (0.82) when fertigation started at 6 and 9 DAE, respectively (Figures 1E and 1D).

Fertigation frequency only significantly influenced chlorophyll-*a* content (Figure 3C) and N, P, K, and S accumulation (Figures 4B, 4C, and 4D) in lettuce seedlings. The lowest values for these variables were observed for an application interval of 7 d and the highest for 3, 4, 5, and 6 d intervals between fertigations (Figures 3C, 4B, 4C, and 4D). In general, the application intervals ad little effect on lettuce seedling production.

Fertigation frequency had no significant effect on the remaining biometric and nutritional variables or any of the physiological variables assessed (Figures 3 and 4).

Summarizing the results of the start time and frequency experiments indicated that early fertigation is more meaningful than fertigation at shorter intervals.

4. Discussion

The fertigation start time influenced approximately 78% of the variables studied. The results demonstrated that the earlier fertigation begins, the better the development of lettuce seedlings (Figures 1 and 2).

The supply of nutrients immediately after emergence likely met the nutritional needs of the plants during the initial stage of seedling development. Although the amount of nutrients required during this stage is not significant, the nutrient content in the seeds, particularly the substrate, may be insufficient to ensure optimal growth (Frugeri et al. 2017). Additionally, when fertigation begins, plants are supplied with higher nutrient doses until transplanting.

The availability of essential plant elements affects organic compound production (e.g., amino acids, proteins, enzymes, and nucleic acids), speed and intensity of enzymatic processes, osmotic potential of cells, membrane permeability and electrochemical potential (Kirkby 2012). Therefore, nutrient supply via fertigation immediately after seedling emergence may have provided better conditions for growth, thereby increasing NL and SDW (Figures 1A and 1B). Similarly, Souza et al. (2015) studied lettuce production under fertigation with N doses and foliar K application and found that the larger the dose of these nutrients (171 kg ha^{-1} of N, and foliar fertilizer with 21.85 kg ha^{-1} of K_2O), the higher the shoot dry weight in these plants. These findings are important because dry matter production has been identified as one of the best parameters for assessing seedling quality (Beninni et al. 2005; Souza et al. 2015; Ferreira et al. 2019).

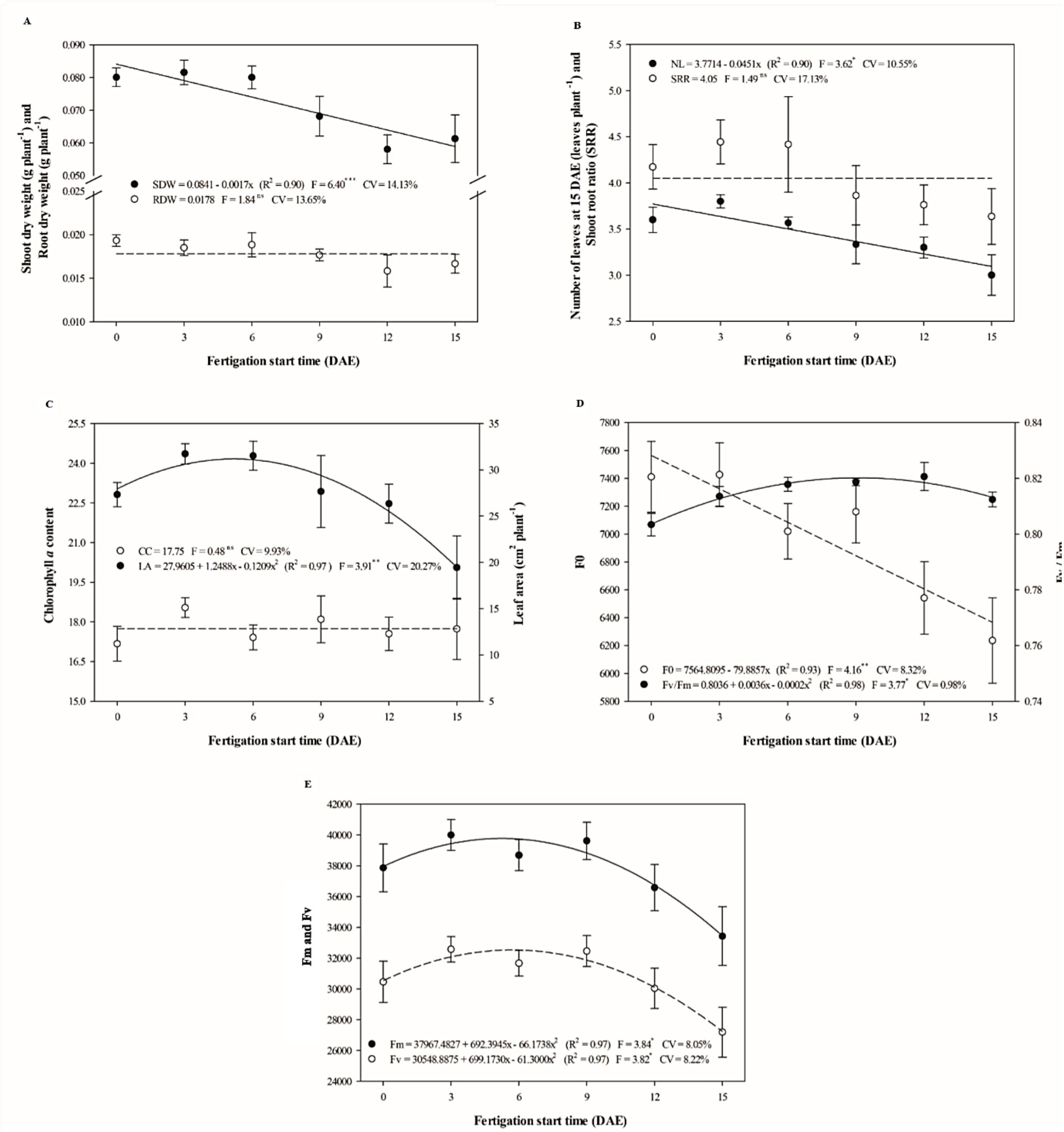

Figure 1. A - Shoot and root dry weight; B - number of leaves at 15 DAE and shoot root ratio; C - chlorophyll-*a* content and leaf area; D - initial fluorescence (F_o) and maximum quantum efficiency of PS II (F_v/F_m); E - maximum fluorescence (F_m), and variable fluorescence (F_v) as a function of fertigation start time in lettuce seedlings. Bars indicate the standard error of the mean (n = 6). ns = not significant (p>0,05); "*" significant (p≤0,05); "**" very significant (p≤0,01); "***" highly significant (p≤0,001) according to the F-test; C.V. = coefficient of variation.

In general, a similar effect was observed for the physiological and biometric variables assessed, demonstrating that nutrient supply via early fertigation (6 DAE) contributed to improving the plant photosynthesis system, since the F_o, F_v, F_m, F_v/F_m, ABS/RC, and TR_o/RC values obtained in the present study indicated normal development and no stress in the plants analyzed (Figures 1 and 2).

The nutritional status of the plant also improved under early fertigation, with higher nutrient accumulation for start times closer to plant emergence (Figure 2). This is related to the responses observed in the biometric and physiological variables evaluated, since according to Souza et al. (2015), a high nutrient level is needed to maintain optimal metabolic processes and physiological activity, resulting in greater nutrient absorption and accumulation, as observed in the present study (Figure 2).

Beninni et al. (2005) and Souza et al. (2015) evaluated lettuce grown under hydroponic and conventional systems and found that nutrient accumulation is directly related to dry matter production, with both these variables behaving similarly, as observed in the present study.

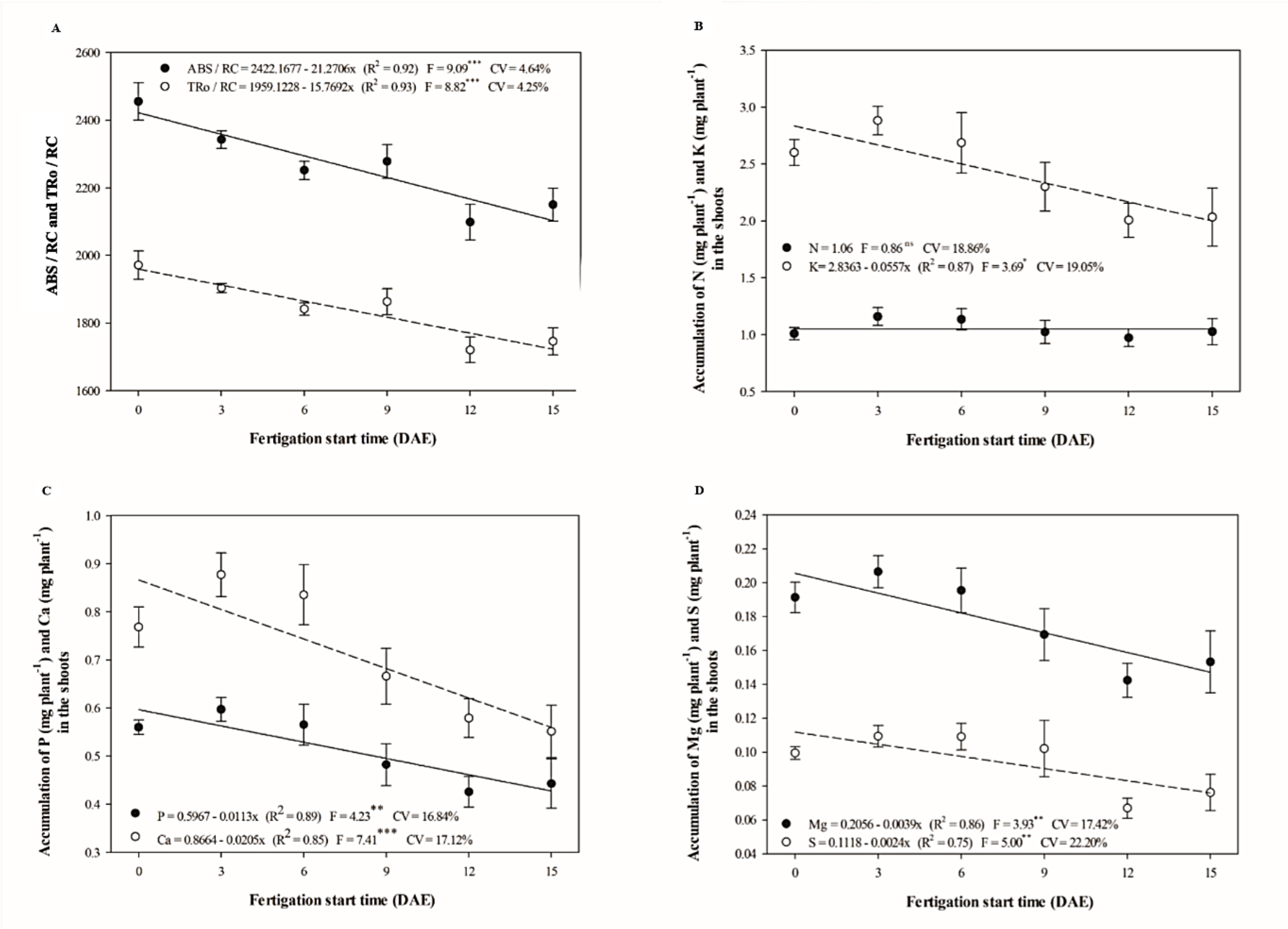

Figure 2. A - Absorption flux per reaction center (ABS/RC) and trapped energy flux per reaction center (TR_o/RC); B - accumulation of nitrogen and potassium in the shoots; C – accumulation of phosphorus and calcium in the shoots; D – accumulation of magnesium, and sulfur in lettuce shoots as a function of fertigation start times. Bars indicate the standard error of the mean (n = 6). ns = not significant (p>0,05); "*" significant (p≤0,05); "**" very significant (p≤0,01); "***" highly significant (p≤0,001) according to the F-test; C.V. = coefficient of variation.

Seedlings were transplanted to the field when they reached the desired commercial standard, which was determined based on plant development and visually assessed by the growers considering biometric parameters such as NL, SDW, and LA. As such, fertigation start time that ensure optimal results for these variables are preferred.

Considering the variables influenced by start time, fertigation that began close to plant emergence promoted higher values than those recorded for late-onset fertigation. The percentage increase in the highest values recorded for these variables when compared to those obtained for late-onset fertigation were: NL15 (NL at 15 DAE) (17.77%); SDW (30.32%); LA (37.49%); F_o (15.84%); F_v (16.28%); F_m (15.87%); F_v/F_m (0.90%); ABS/RC (13.17%); TR_o/RC (12.07%); P (28.33%); K (29.58%); Ca (35.63%); Mg (0.57%) and S (27.27%) (Figures 1 and 2).

Fertigation frequency had little impact on lettuce seedling production, as a statistically significant effect was only observed for chlorophyll-*a* content and N, P, K, and S accumulation (Figures 3 and 4). Based on the results of the fertigation start time experiment, it can be inferred that the small effect of the application intervals was likely because fertigation was first performed at 3 DAE, considered early concerning current practices, further confirming that early application is more important than frequency.

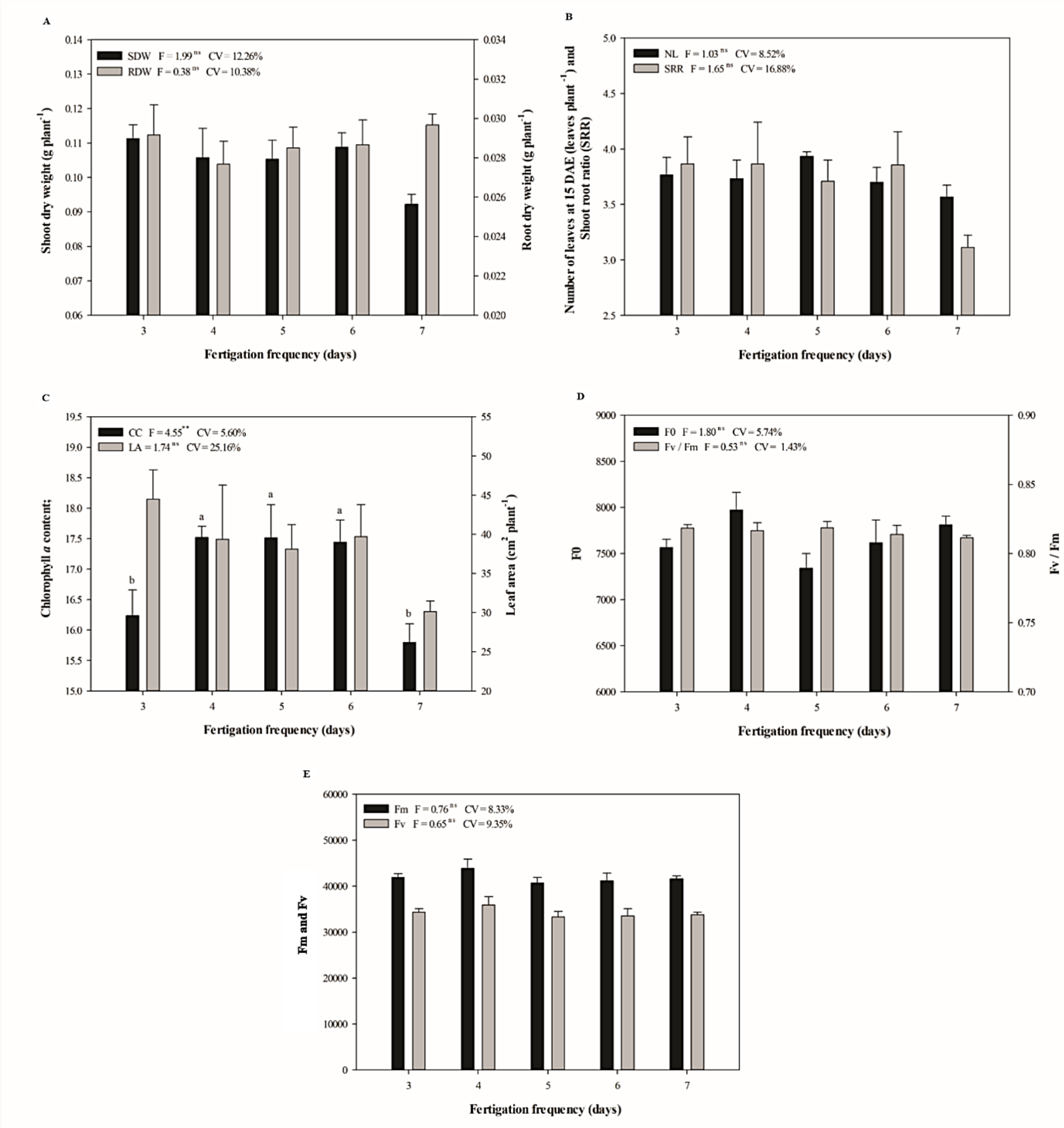

Figure 3. A - Shoot and root dry weight; B - number of leaves and shoot root ratio; C - chlorophyll-*a* content and leaf area; D - initial fluorescence (F_o) and maximum quantum efficiency of PS II (F_v/F_m); E - maximum fluorescence (F_m) and variable fluorescence (F_v) as a function of fertigation frequency in lettuce seedlings. Only application intervals with different letters in lower case are significantly different. Significant ($p \leq 0,05$) according to the Scott-Knott clustering algorithm. Bars indicate the standard error of the mean (n = 6).

Plants exhibited similar development, regardless of the application interval (Figure 3). This demonstrates that for plants subjected to early fertigation, an application frequency of 3-7 d can provide sufficient nutrients to ensure good lettuce seedling development (Figure 3).

Dalastra et al. (2020) assessed the periodicity of hydroponic lettuce exposed to a nutrient solution and found that iceberg lettuce grown under continuous flow exhibited high shoot and root dry and fresh weights. In general, biomass production tended to decline with longer intervals of no exposure to the nutrient solution.

The values obtained for the physiological variables indicate that any fertigation frequency between 3 and 7 d promotes adequate photosystem II function and contributes to the prevention of stress in plants (Figures 3 and 4). The physiological parameters evaluated in both experiments have been widely used as indicators of stress in plants in several studies (Bussotti et al. 2011; Desotgiu et al. 2012; Goltsev et al. 2012; Oukarroum et al. 2012; Krasteva et al. 2013; Oukarroum et al. 2013; Salvatori et al. 2013; Gottardini et al. 2014; Kalaji et al. 2014a; Kalaji et al. 2014b; Araújo et al. 2020).

Despite the variation in ABS/RC and TR_o/RC as a function of the treatments, potentially indicating stress, the results obtained for the physiological parameters in both experiments, especially F_v/F_m, demonstrated no stress in the plants studied because F_v/F_m did not reach the lower limit of 0.75. According to Reis and Campostrini (2011) and Cintra et al. (2020), F_v/F_m between 0.75 and 0.85 electrons quantum^{-1} indicates an intact photosynthetic apparatus, whereas values below 0.75 electrons quantum^{-1} reduce the photosynthetic potential of plants.

Nutrient accumulation was influenced by fertigation frequency, with the highest values recorded at shorter intervals, particularly at 3 d. Accumulation of N, P, and S was higher at 3-6-d application intervals, whereas K accumulation was greater for the 3-d interval than other frequencies (Figure 4). Greater nutrient accumulation at shorter fertigation intervals was due to the more frequent nutrient supply in these treatments. Providing more nutrients means that they tend to be better absorbed and accumulated by plants, corroborating the behavior observed by Beninni et al. (2005) and Souza et al. (2015).

Chlorophyll-*a* content was higher at application intervals of 4, 5, and 6 d. This may be associated with greater nutrient accumulation, especially N (Figures 3 and 4), an important enzymatic constituent of chloroplasts and a component of chlorophyll molecules, which may have increased chlorophyll-*a* content (Taiz et al. 2017).

The data analyzed demonstrated the benefits of early fertigation in lettuce seedlings, with 0 DAE being the most recommended start time. In addition to ensuring optimal plant development, early fertigation shortens this phase of the production cycle, as seedlings reach the transplanting stage faster than usual.

Concerning fertigation frequency, the best results were observed at intervals of 3-6 d. However, given that plant development was similar between these intervals, application every 6 d is recommended because it significantly reduces the number of fertigations performed and the amount of fertilizer used, thereby lowering production costs.

It is important to note that a 5-d interval was adopted in the fertigation start time experiment, and the best results were obtained when the first application occurred at 0 DAE. For the fertigation frequency experiment, the start time was 3 DAE, with the best results observed for a 6-d interval. A joint assessment of the two experiments demonstrated that initiating fertigation close to plant emergence (0 DAE) at a frequency of 6 d promotes better plant development and shortened seedling production time.

From an economic perspective, lettuce seedlings produced in both experiments reached the transplanting stage (commercial standard) 20 d after sowing (DAS). Commercial lettuce seedling production currently takes an average of 30 d from sowing to transplanting. The 10-d reduction observed here would ensure lower seedling production costs, thereby reducing the total production costs. The seedlings produced were ready for transplantation at 33.33% less time than the commercial standard. As a result, several production factors also declined, including the time seedlings spent in the nursery, costs involved in seedling fertilization, irrigation, and pest and disease control.

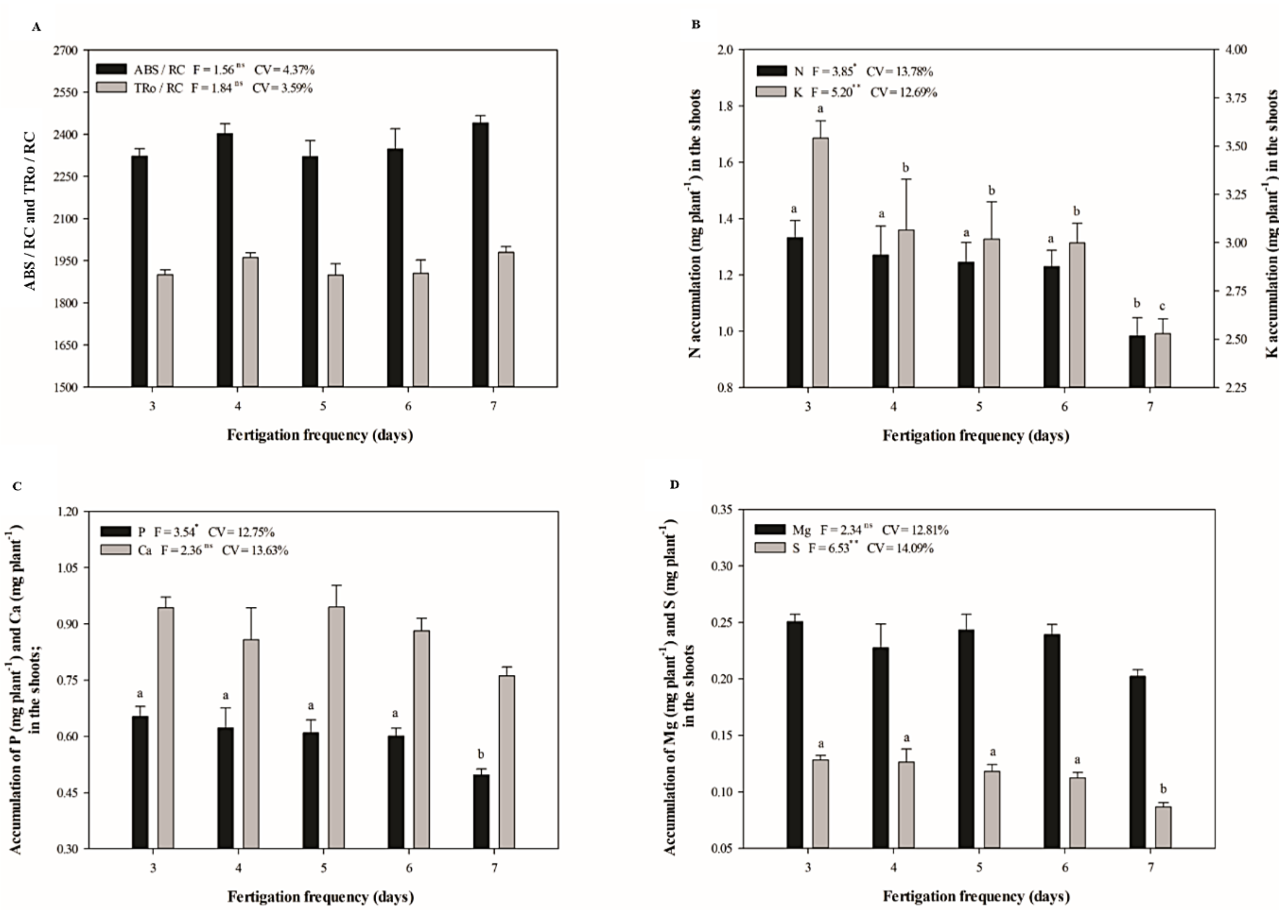

Figure 4. A - Absorption flux per reaction center (ABS/RC) and trapped energy flux per reaction center (TR_o/RC); B - accumulation of nitrogen and potassium in the shoots; C- accumulation of phosphorus and calcium in the shoots; D – accumulation of magnesium and sulfur in lettuce shoots as a function of fertigation frequency. Only application intervals with different letters in lower case are significantly different. Significant ($p \leq 0,05$) according to the Scott-Knott clustering algorithm. Bars indicate the standard error of the mean (n = 6).

Most growers begin fertigation at 10 DAE and adopt an average frequency of 5 d, with the seedling production stage lasting approximately 30 d, requiring six applications during this period. In contrast, in the present study, the most recommended start time for lettuce seedlings (0 DAE) and an application frequency of 6 d shortened seedling production by 10 d, indicating that only three applications were needed. This adjustment in start time and application interval not only reduces the previously mentioned factors, but also improves seedling quality.

As such, the fertigation start time has a greater influence on lettuce seedling production than the application intervals adopted.

5. Conclusions

In both experiments, the physiological parameters analyzed indicated no plant stress regardless of treatment. However, early fertigation improved the physiological performance of the lettuce seedlings.

Starting fertigation at plant emergence favors dry weight production, nutrient accumulation, and photosynthesis and shortens the production time of lettuce seedlings.

For optimal lettuce seedling production, fertigation should begin at emergence (0 DAE) and be applied every 6 d.

Authors' Contributions: DA SILVA NETO, O. F.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MOREIRA, E. F.: conception and design, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; ORIOLI JÚNIOR, V.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; CASTOLDI, R.: conception and design, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; TORRES, J. L. R.:

acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; CHARLO, H. C. O.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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Ethics Approval: Not applicable.

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