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Research Article

Agronomic and qualitative characteristics of baby leaf lettuce as a function of planting density in a floating hydroponic system[1]

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ABSTRACT

Planting density should be a priority in the improvement of the baby leaf cultivation technique, as it influences morphophysiological characteristics. This study aimed to evaluate seven planting densities (408-2,856 plants m^{-2}) on the growth, yield and quality of 'Vanda' baby leaf lettuce under floating hydroponic cultivation. Two experiments were carried out in a randomized block design, with four replicates. The leaf length had an initial increase followed by a decrease at the highest planting densities, whereas the fresh and dry masses decreased. The yield, in turn, increased 215 and 372 % between the lowest and highest planting density in the experiments 1 and 2, respectively. Regarding the baby leaf quality, the calcium and magnesium contents did not show a response pattern in the two growing seasons, whereas the potassium and zinc contents increased and the iron content decreased with the increase in planting density in both seasons. The vitamin C content increased, whereas the chlorophyll and carotenoid contents decreased with the increase in planting density.

KEYWORDS: *Lactuca sativa*, floating hydroponic system, protected cultivation, soilless cultivation.

RESUMO

Características agronômicas e qualitativas de alface *baby leaf* em função da densidade de plantio em sistema hidropônico flutuante

A densidade de plantio deve ser prioridade no aprimoramento da técnica de cultivo de hortaliças *baby leaf*, pois influencia nas características morfofisiológicas. Objetivou-se avaliar sete densidades de plantio (408-2.856 plantas m^{-2}) no crescimento, produtividade e qualidade de alface 'Vanda' *baby leaf*, em cultivo hidropônico *floating*. Realizaram-se dois experimentos, em delineamento de blocos ao acaso, com quatro repetições. O comprimento das folhas teve incremento inicial seguido de redução nas maiores densidades de plantio, enquanto as massas fresca e seca diminuíram. A produtividade, por sua vez, aumentou 215 e 372 % entre a menor e maior densidade de plantio nos experimentos 1 e 2, respectivamente. Com relação à qualidade da *baby leaf*, os teores de cálcio e magnésio não apresentaram padrão de resposta nas duas épocas de cultivo, enquanto os teores de potássio e zinco aumentaram e o teor de ferro diminuiu com o incremento na densidade de plantio nas duas épocas. O teor de vitamina C aumentou, enquanto os de clorofila e carotenoides diminuíram com o aumento da densidade de plantio.

PALAVRAS-CHAVE: *Lactuca sativa*, sistema hidropônico de flutuação, cultivo protegido, cultivo sem solo.

INTRODUCTION

The increase in vegetable consumption has occurred not only due to population growth, but also due to changes in eating habits, a scenario which requires a greater production (Pessoa et al. 2023). Lettuce (*Lactuca sativa*) is the most important leafy vegetable in the world, being eaten fresh, mainly in salads and sandwiches (Medina-Lozano et al. 2021). In terms of nutraceutical value, the highlights are its contents of fiber, carotenoids, phenolic compounds, antioxidants, vitamins A, C and minerals, as well as folate and lutein, which confer organoleptic properties and are beneficial to human health (Fortis-Hernández et al. 2024).

Baby-sized vegetables have aroused the interest of consumers by bringing with them different colors, textures, shapes and flavors (Moraes et al. 2016, Giménez et al. 2020).

Baby leaves have stood out in the baby-sized segment, being harvested early, long before the harvest of their traditionally harvested forms, still in

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the initial phase of development. According to Gil & Garrido (2020), they have leaf length and width of 8-10 cm and 7-8 cm, respectively. In Brazil, they are present mainly in larger consumer centers and have high market potential in the country's inland.

Baby leaves have been produced in the soil and also in hydroponic systems. According to Capanema & Costa (2022), the floating hydroponic system has operational simplicity from planting the seedlings to harvest. The plants are placed on floating plates, promoting uniformity in plant development and growth, and therefore in harvest time, resulting in a reduction in production costs (Gonnella et al. 2003). This hydroponic system has been widely used to produce baby leaf vegetables, especially lettuce (Puccinelli et al. 2021).

Agronomic management plays a crucial role in improving yield and the commercial presentation of the product. As an important agronomic technique, planting density determines, among other things, the vegetation area of cultivated plants (Nkansah et al. 2021). By defining the plant population per unit area, the objective is to maximize the biofactors in the cultivation area and achieve the yield potential of the genotype (Haque & Sakimin 2022). Thus, the production of baby leaf lettuce in a floating system requires the definition of planting density, since no results were found to guide the producer in decision making. Under a high planting density, plants respond to competition for light. Changes in the quantity and quality of light perceived by the plant, such as a reduction in photoactive radiation and decrease in the proportion of red/far red light, determine reactions externalized in the plasticity of the plant through adaptive actions such as stem elongation, reduced branching, leaf hyponastic orientation, early flowering and accelerated senescence, which is called shade avoidance syndrome (Liu et al. 2021).

This study aimed to evaluate the effect of planting densities on the growth, yield and quality of baby leaf lettuce under floating hydroponic cultivation.

MATERIAL AND METHODS

The experiments were carried out in a greenhouse, from Oct. 18 to Nov. 09 and from Nov. 19 to Dec. 10, 2024, at the Universidade Estadual Paulista, in Jaboticabal, São Paulo state, Brazil (21º14'33"S, 48º17'10"W and altitude of 565 m). The climate of the region is classified as subtropical, with a rainy summer and relatively dry winter, average annual rainfall of 1,425 mm and average temperature of 22 ºC (Alvares et al. 2013). Figure 1 shows the climatic parameters of the experimental periods.

Seven planting densities (408; 816; 1,224; 1,632; 2,040; 2,448; and 2,856 plants m^{-2}) were evaluated in a randomized block design, with four replicates. Each experimental unit contained a polyethylene box with capacity of 20 liters, but with 17 liters of nutrient solution, which received a tray with 63 cells, with each cell being 3.8 x 3.8 x 6.0 cm long, wide and deep, respectively. Phenolic foam with the number of plants to provide the density established in the treatment was placed in each cell of the tray. For this, 1, 2, 3 or 4 seeds were sown in each unit of phenolic foam, so that the combination of these foams provided the desired planting densities in the treatments (Figure 2).

'Vanda' lettuce seeds (Sakata Seed Sudamerica®) were sown in phenolic foam (Green up®) in 1.9 x 1.9 x 1.9 cm cells. After 2 days in a dark environment for germination, the trays were taken to the nursery for initial growth of the seedlings, receiving only water. After five days, when the seedlings had expanded cotyledons, the phenolic foam cells were individualized and transferred to the nursery, which consisted of polypropylene channels, 5 cm wide, in a Nutrient Film Technique hydroponic system, with 5 % slope. The used nutrient solution was that proposed for lettuce by Furlani et al. (1999), whose concentrations were: 24, 174, 39, 183, 142, 38, 52, 0.3, 0.02, 2.0, 0.40, 0.06 and 0.06 mg L^{-1} of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Mo and Zn, respectively. The spacing between plants was 0.05 x 0.05 m. After six days of permanence in the nursery, the plants were transferred to polyethylene trays with 63 cells of 3.8 x 3.8 cm, in a floating hydroponic system. Each cell received a phenolic foam piece of 1.9 x 1.9 x 1.9 cm with the number of plants to serve each treatment.

Daily, the nutrient solutions were aerated and the initial volume of each reservoir was completed with the addition of water corrected with pH between 6.0 and 6.5. After 10 days in the floating system, the plants were harvested by cutting the petiole close to the surface of the phenolic foam.

After harvest, the following agronomic traits were evaluated: a) shoot fresh mass (g $plant^{-1}$),

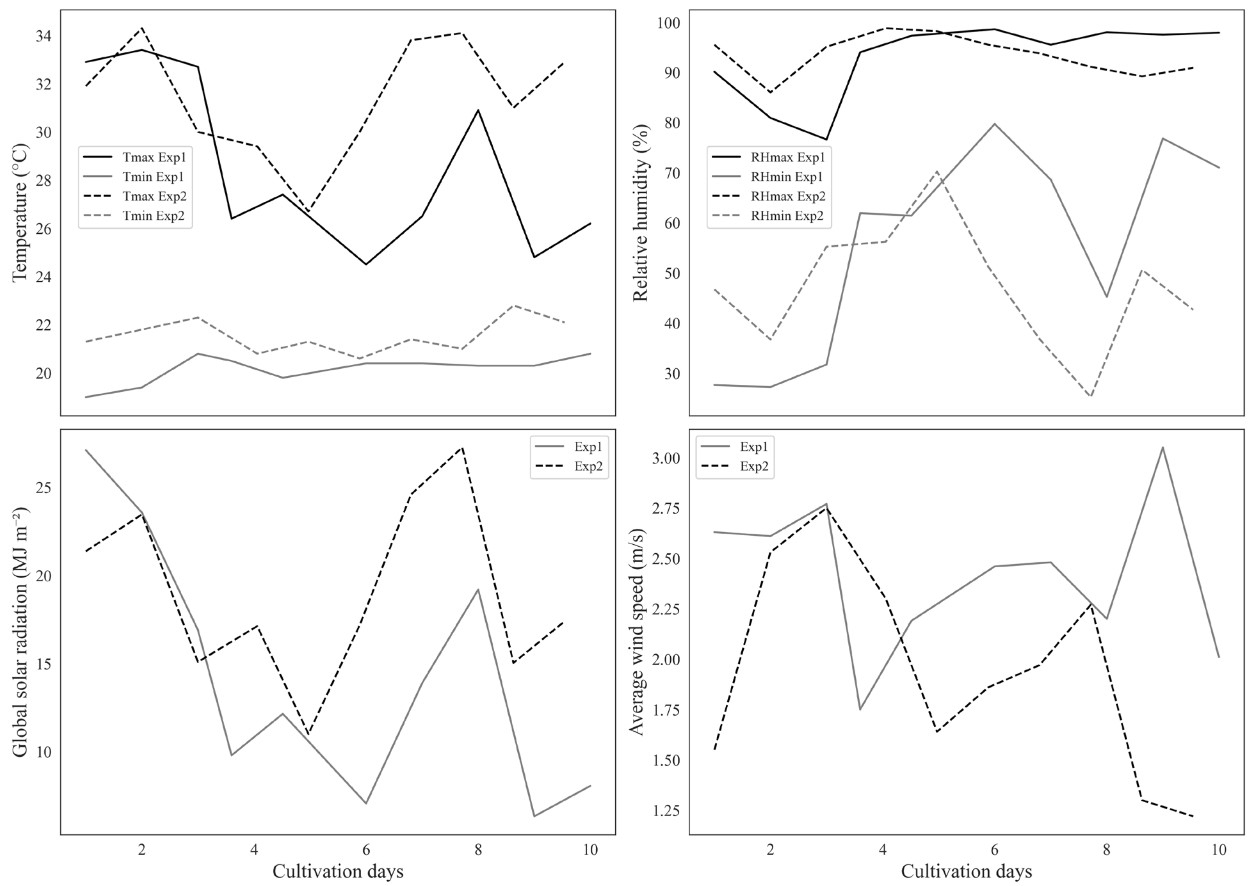


Figure 1. Temperature, relative humidity, global solar radiation and average wind speed for the experimental period (days) of the experiment 1 (Oct. 18 to Nov. 09, 2024) and experiment 2 (Nov. 19 to Dec. 10, 2024), in Jaboticabal, São Paulo state, Brazil.

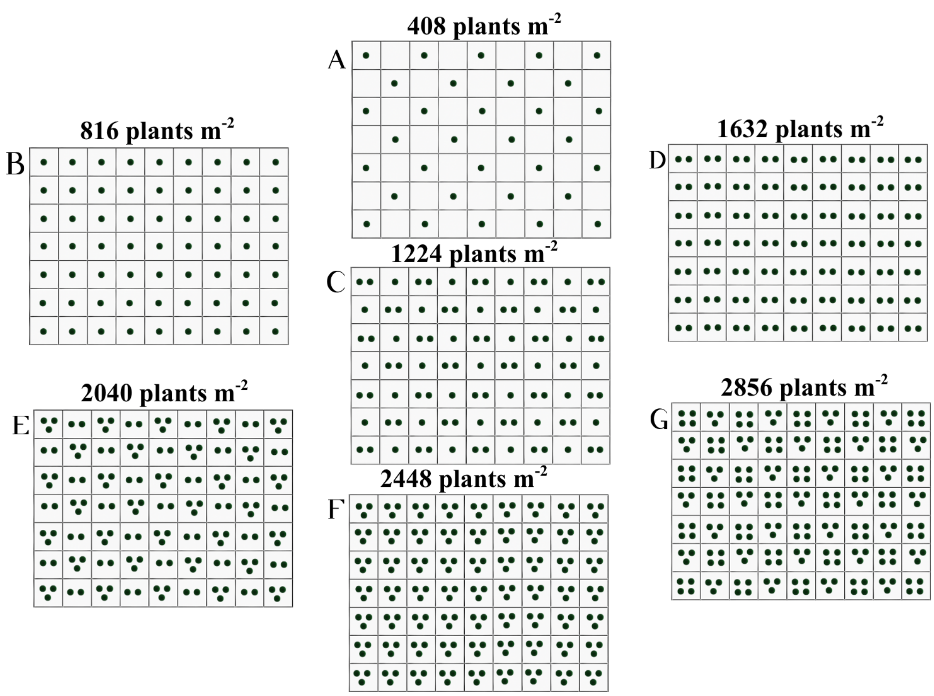


Figure 2. Illustration of planting densities of baby leaf lettuces in a floating system. Each dot in the cell represents a baby leaf lettuce.

obtained by harvesting the plants of each plot and immediately weighing their leaves; b) length of the largest leaf (cm), measured with a caliper; c) leaf area (cm^2 plant^{-1}), obtained using the LI-COR 3100 electronic area integrator, by quantifying the area of all leaves of the plants; d) shoot dry mass (mg plant^{-1}), determined by drying the baby leaves in an oven with forced air circulation at 65 ºC, for 72 hours, and weighing them on a precision scale; e) yield (kg m^{-2}), estimated from shoot fresh mass.

Regarding the qualitative traits of the baby leaf, the following were evaluated: a) chlorophyll *a*, chlorophyll *b* and carotenoid contents in the fresh mass (FM) (mg g^{-1} FM), determined according to the methodology proposed by Lichtenthaler & Wellburn (1983), with spectrophotometer readings at 663 nm (chlorophyll *a*), 647 nm (chlorophyll *b*) and 470 nm (carotenoids); b) ascorbic acid (mg 100 g^{-1} FM), determined as described by Strohecker & Henning (1967), where 1 mL of the filtered extract and 4 mL of 0.5 % oxalic acid were transferred to an Erlenmeyer® flask, mixed and titrated with 2,6-dichlorophenol indophenol (DCPIP) (0.02 %) up to the turning point; c) leaf contents of cationic micronutrients (mg kg^{-1}), determined by following a methodology in which the leaves were washed in deionized water, dried in an oven with forced air circulation at 65 ºC, ground and subjected to digestion to determine the contents of cationic micronutrients, according to Miyazawa et al. (2009).

Analysis of variance of the data (F-test, at 5 % of probability) was performed for each experiment, using the AgroEstat software (Barbosa & Maldonado Júnior 2015). For each experiment, a polynomial regression study was performed for the evaluated traits, and the equations with significant fit ($p < 0.05$) and the highest coefficient of determination were chosen. The Python 3.8 programming language, along with the Matplotlib, Pandas and Seaborn libraries, were used to construct and visualize the graphs.

RESULTS AND DISCUSSION

When the planting density (408-2,856 plants m^{-2}) was modified, effects were observed on the growth, yield and quality of the baby leaf lettuce (Figure 3).

Regarding the length of the largest leaf, in both experiments, larger leaves were observed as the planting density increased, but with decreasing increments characteristic of the quadratic model fitted (Figure 4). The maximum leaf lengths (13.9 and 10.9 cm in the experiments 1 and 2, respectively) observed with 2,856 plants m^{-2} were 31 and 49 % longer than those observed with 408 plants m^{-2}. Unlike leaf length, leaf area decreased as planting density increased. Between the lowest and highest planting density, the leaf area decreased linearly from 53.8 to 23.3 cm^2 in the experiment 1 and from 31.8 to 20.0 cm^2 in the experiment 2 (Figure 4).

The increase in leaf length as the planting density increased (Figure 3) indicates that the plants are activating shade avoidance, which is a response to

Photos: Gildeon Santos Brito

Figure 3. Baby leaf lettuces representative of the planting densities.

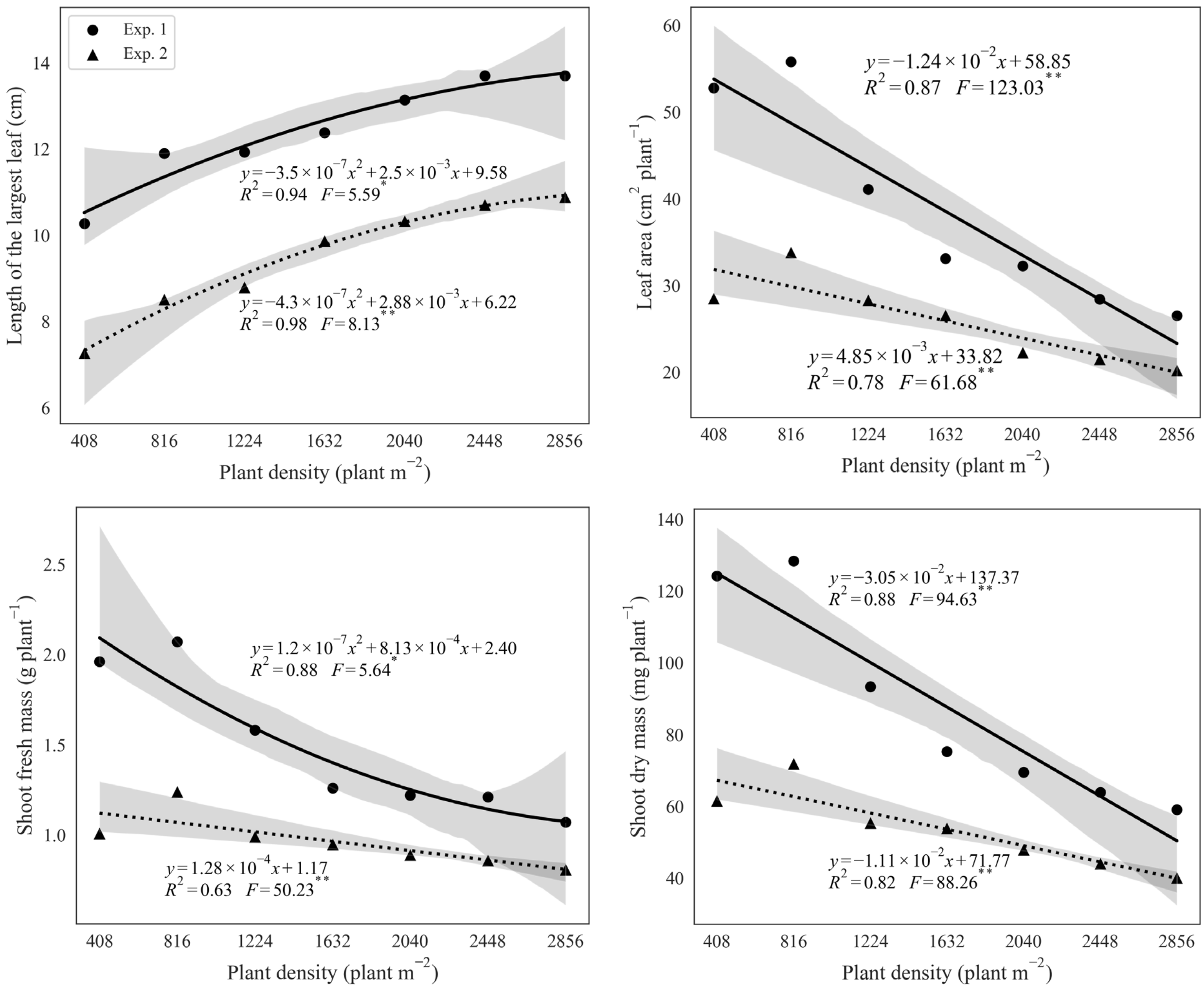


Figure 4. Length of the largest leaf, leaf area, shoot fresh mass and shoot dry mass of baby leaf lettuce as a function of planting density. * Significant by the F test ($p < 0.05$); ** significant by the F test ($p < 0.01$).

competition for light among neighboring individuals, resulting in elongation of the stem to increase its access to light (Han et al. 2024). Liu et al. (2025) highlight that light plays a dual role in plants, providing the energy necessary for photosynthesis and also acting as a signal that regulates various plant responses.

This competition for light also had an impact on the linear reduction of leaf area in both experiments with the increase in planting density (Figure 4), reflecting in a reorganization of vegetative growth in response to the smaller space available. According to Pôrto et al. (2012), population density increases intraspecific competition, which leads to a reduction in the size of organs, but not necessarily a decrease in their number, which was observed in the present study, since all baby leaves had five leaves. In lettuce plants, Vysotskaya et al. (2018) found that the concentration of cytokinins, a phytohormone that promotes cell division and expansion, decreased both in the shoots and roots due to the increase in planting density.

In addition to the observed morphological changes, such as leaf elongation and leaf area reduction, the higher planting density also negatively impacted the biomass production of the baby leaf lettuce. The plant fresh mass decreased with increasing planting density, being described by quadratic and linear models in the experiments 1 and 2, respectively (Figure 4). The lowest values were observed at the highest density, with reductions of approximately 45 and 20 %, when compared to the lowest density, respectively in the experiments 1 and 2. Plants under higher densities also showed less dry mass accumulation, with reductions of 52 % in the experiment 1 and 35 % in the experiment 2. Biomass production depends on the availability of solar radiation that reaches the surface of the vegetation cover and on the fraction of this radiation that is absorbed by the canopy to perform photosynthesis (Dellai et al. 2008). The reductions in the fresh and dry masses of baby leaf lettuce with the increase in planting density (Figure 4) can be explained, according to Zhang et al. (2018), by the lower photosynthetic capacity of the plant, resulting from insufficient amount of light absorbed due to the greater proximity of the plants. In addition, Vysotskaya et al. (2018) related the decline in shoot mass of lettuce plants resulting from the increase in planting density to the increase in the concentration of abscisic acid, since this phytohormone, according to Kishor et al. (2022), at high concentrations, impairs photosynthesis, growth and, ultimately, yield. Although the shading effects may vary widely among species, Yang et al. (2020) found similar results regarding the decrease in chlorophyll contents with increased shading, besides highlighting that shading reduces leaf thickness, altering the morphology of the chloroplast and leaf tissue, and reducing the photosynthetic rate.

These morphophysiological responses are directly linked to the final yield, since the yield of a crop is determined by the interaction among the plant, management and the production environment (Mauad et al. 2010). The relationship between planting density and yield was better described by a quadratic model and maximized, in both experiments, at the highest planting density, reaching approximately 2.9 kg m^{-2} in the experiment 1 and 2.4 kg m^{-2} in the experiment 2 (Figure 5). The magnitude of the increment was greater in the experiment 1, with a difference of almost 2 kg m^{-2} between the minimum and maximum densities. As observed for the biometric characteristics, with the increase in planting density, there was an increase in the length of the largest leaf and reductions in leaf area, shoot fresh and dry mass. As the yield of baby leaf lettuce is fundamentally represented by shoot fresh mass, the growing number of plants in the area compensated for the reduction in biomass and was responsible for the successive increase in yield (Figure 5), in agreement with Çekin et al. (2025). Kumaratenna & Cho (2025) also observed that, in a plant factory, the increase in the spacing between rows of lettuce promoted an increase in shoot fresh and dry mass, whereas yield decreased.

In both experiments, the electrical conductivity of the nutrient solution decreased linearly with the increase in planting density due to the greater extraction of nutrients from the solution motivated by the significant increase in yield. However, even with the higher density, due to the short growth period and characteristic size of the baby leaf, the reductions in the electrical conductivity of the nutrient solution were small, on the order of 10 and 6 % in the experiments 1 and 2, respectively (Figure 5).

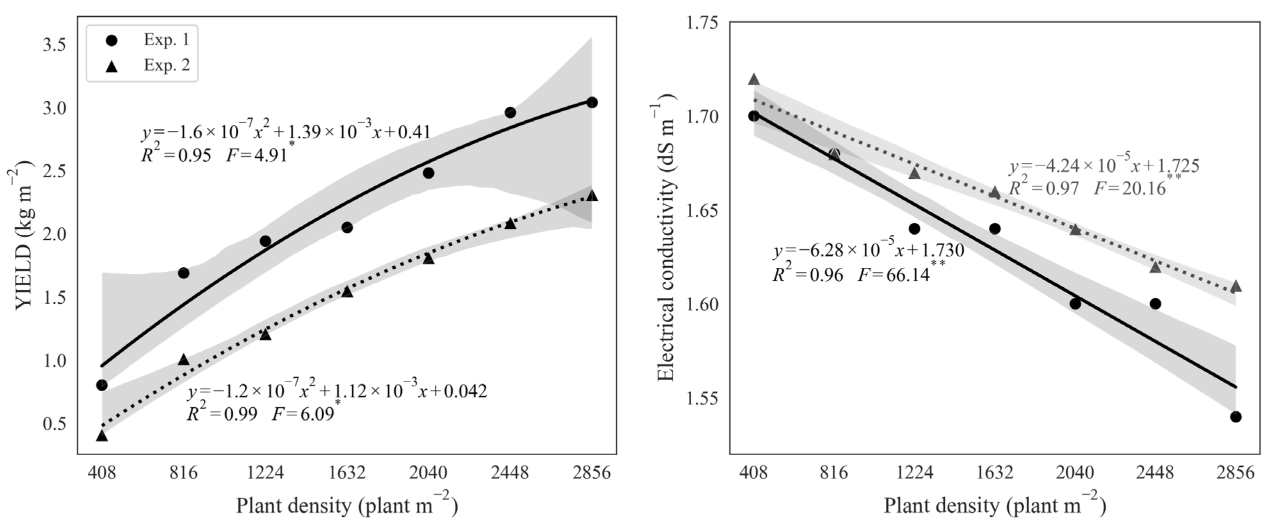


Figure 5. Yield of baby leaf lettuce and electrical conductivity of the nutrient solution as a function of planting density. * Significant by the F test ($p < 0.05$); ** significant by F test ($p < 0.01$).

The chlorophyll *a* content decreased linearly with the increase in planting density in both experiments, and the highest values were recorded at the lowest densities, with more accentuated reductions in the experiment 1. The reductions in the chlorophyll *a* and *b* contents in the experiment 1 were approximately 38 and 24 %, whereas, in the experiment 2, they were approximately 16 and 30 %, respectively (Figure 6). For carotenoids, their contents also showed a marked linear reduction with the increase in planting density, in both experiments. According to the results, baby leaves cultivated with lower planting density, hence greater availability of resources, including light, also showed higher contents of chlorophyll *a* and *b*, and carotenoids (Figure 6), corroborating the results reported by Givens et al. (2023), who observed an increase in these pigments in lettuce as light intensity increased. Carotenoids play a crucial role in plant growth and response to abiotic stress (Qian et al. 2025), acting as photoprotectors of the photosynthetic apparatus against excessive light (Llorente et al. 2015). As the greater density reduces the absorption of light by the plant, there was a decrease in the production of the photoprotector. In this context, in the second experiment, in which there were conditions of greater luminosity and higher global solar radiation, at all evaluated planting densities, baby leaf lettuces had higher carotenoid contents than in the first experiment, agreeing with the results reported by Martins et al. (2009), who observed that African basil plants grown in full sun produced higher amounts of carotenoids, if compared to those in shaded environments.

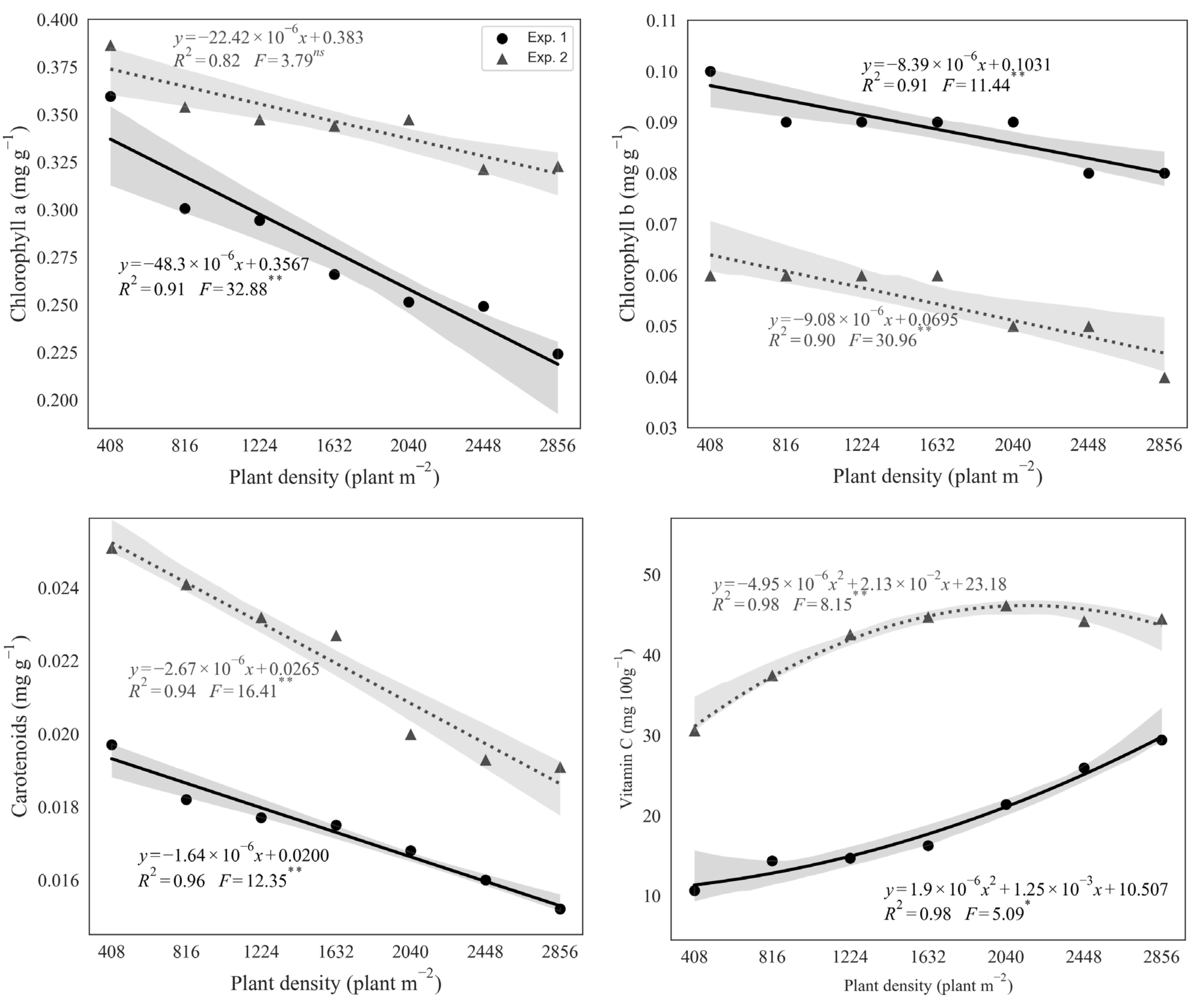


Figure 6. Chlorophyll *a*, chlorophyll *b*, carotenoids and vitamin C contents in baby leaf lettuce leaves as a function of planting density. * Significant by the F test ($p < 0.05$); ** significant by F test ($p < 0.01$); ^{ns} not significant.

On the other hand, differing from the effects observed on chlorophylls and carotenoids, the concentration of vitamin C increased with the increase in planting density in both experiments, and, in the experiment 1, this increase intensified from 1,612 plants m^{-2}.

The recommended daily dose of vitamin C is 75 mg day^{-1} for women and 90 mg day^{-1} for men, being sufficient to meet cellular needs and reduce human health risks, such as cardiovascular and neurodegenerative diseases, cancer and stroke (Granger & Eck 2018). It is considered by Sakamoto & Suzuki (2015) as the most important vitamin found in vegetables for human health. There was an increase in vitamin C content with the increase in planting density (Figure 6). Vitamin C synthesis depends on light quality, intensity and photoperiod (Paciolla et al. 2019, Liu et al. 2022). If light absorption is impaired in any way, the expression of genes involved in vitamin C biosynthesis is compromised (Yabuta et al. 2007, Bukh et al. 2012). Comparatively, Silva et al. (2011) observed that leaf lettuces grown in a hydroponic system had, on average, 26.4 mg 100 g^{-1} of fresh mass, whereas the contents in the present study exceeded this value at the highest planting densities, especially in the experiment 2, in which the content exceeded 45 mg 100 g^{-1}. This increase may be associated with the intensification of stress due to higher global solar radiation, which demanded a greater antioxidant activity, including ascorbic acid.

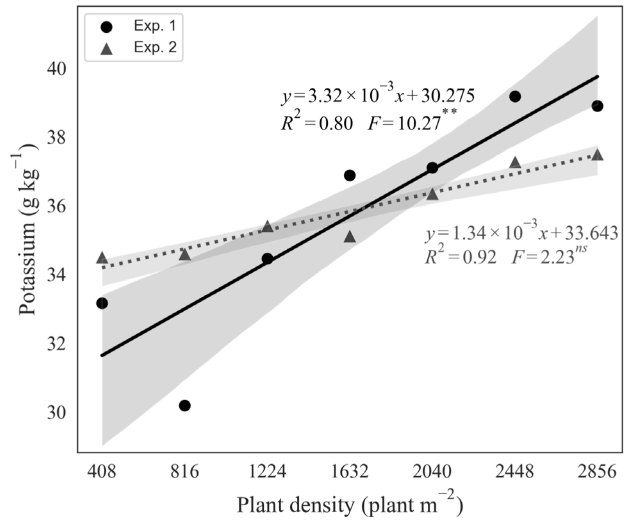
The results of the nutritional analysis on baby leaf lettuce leaves for concentrations of K, Ca, Mg, Fe and Zn are presented in Figure 7. Regarding K contents, an increase proportional to the increase in planting density was observed in both experiments, ranging from 30.2 to 39.2 g kg^{-1} in the experiment 1 and from 34.5 to 37.5 g kg^{-1} in the experiment 2. As for leaf Ca contents, a variation between 10.0 and 11.3 g kg^{-1} was observed in the experiment 1 as a function of planting density. In experiment 2, the values were higher, ranging from 11.7 to 13.7 g kg^{-1}, with a decreasing response as the plant population increased.

Similarly, the Mg contents also showed contrasting responses between the experiments. While in the experiment 1 the contents were increasing and ranged between 2.6 and 2.3 g kg^{-1}, in the experiment 2 the contents were higher and varied from 3.2 to 2.7 g kg^{-1}, with a clear reduction associated with the increase in planting density.

For the leaf Fe contents, a variation of 162.75 to 146.4 mg kg^{-1} was observed in the experiment 1, whereas, in the experiment 2, the values ranged between 141.22 and 123.72 mg kg^{-1}, indicating a slight reduction in the Fe contents with the increase in planting density, thus having a gradual and insignificant effect of competition among plants on the accumulation of this micronutrient. For Zn, the contents showed a similar behavior, ranging from 48.95 to 40.7 mg kg^{-1} in the experiment 1 and from 59.12 to 53.92 mg kg^{-1} in the experiment 2. While in the second crop the variations were not very significant for Zn content, in the first, the contents were significantly higher in plants grown under high planting density.

Few studies have been found regarding the influence of planting density on nutrient content. However, knowing the effect of the cultivation environment on the nutrient contents in the plant is of great importance, because these contents, in addition to directly influencing the food nutritional value, indicate the amount of nutrients exported at harvest (Aquino et al. 2007). Planting density had contrasting effects on Ca and Mg contents in the leaves, in the experiments carried out here. With the increase in planting density, leaf Ca and Mg contents were higher in the experiment 1, but decreased in the experiment 2 (Figure 7). This divergence may be associated with the different environmental conditions between the experiments, especially air temperature and global solar radiation. Grzebisz et al. (2022) stated that Ca concentration is highly sensitive to abiotic stimuli and, among the environmental variables, temperature is one of the most influential. As can be seen in Figure 1, the second experiment was characterized by higher temperatures and higher incidence of radiation, which increase transpiration and, consequently, the absorption of water and nutrients, in addition to increasing the plant metabolic activity, as observed by Zhou et al. (2019).

For K, unlike the macronutrients Ca and Mg, a positive response to population density was observed in both experiments (Figure 7). Similar results were noted by Singh et al. (2023), who reported that the use of shading material can increase the leaf K concentration in lettuce grown in a hydroponic



system. Fe contents decreased with the increase in population density. Therefore, these results suggest that increasing planting density can limit the absorption and accumulation of Fe in baby leaf lettuce, whereas, for Zn, the content can be favored by an increased density. The increase in planting density results in greater shading, which tends to increase the humidity of the microenvironment and reduce the incidence of direct solar radiation (Zhang et al. 2023). Similar results were observed by Kabir et al. (2022), when working with tomatoes and peppers, in which higher levels of shading increased Zn contents in plant tissues, even with the reduction in the transpiration rate.

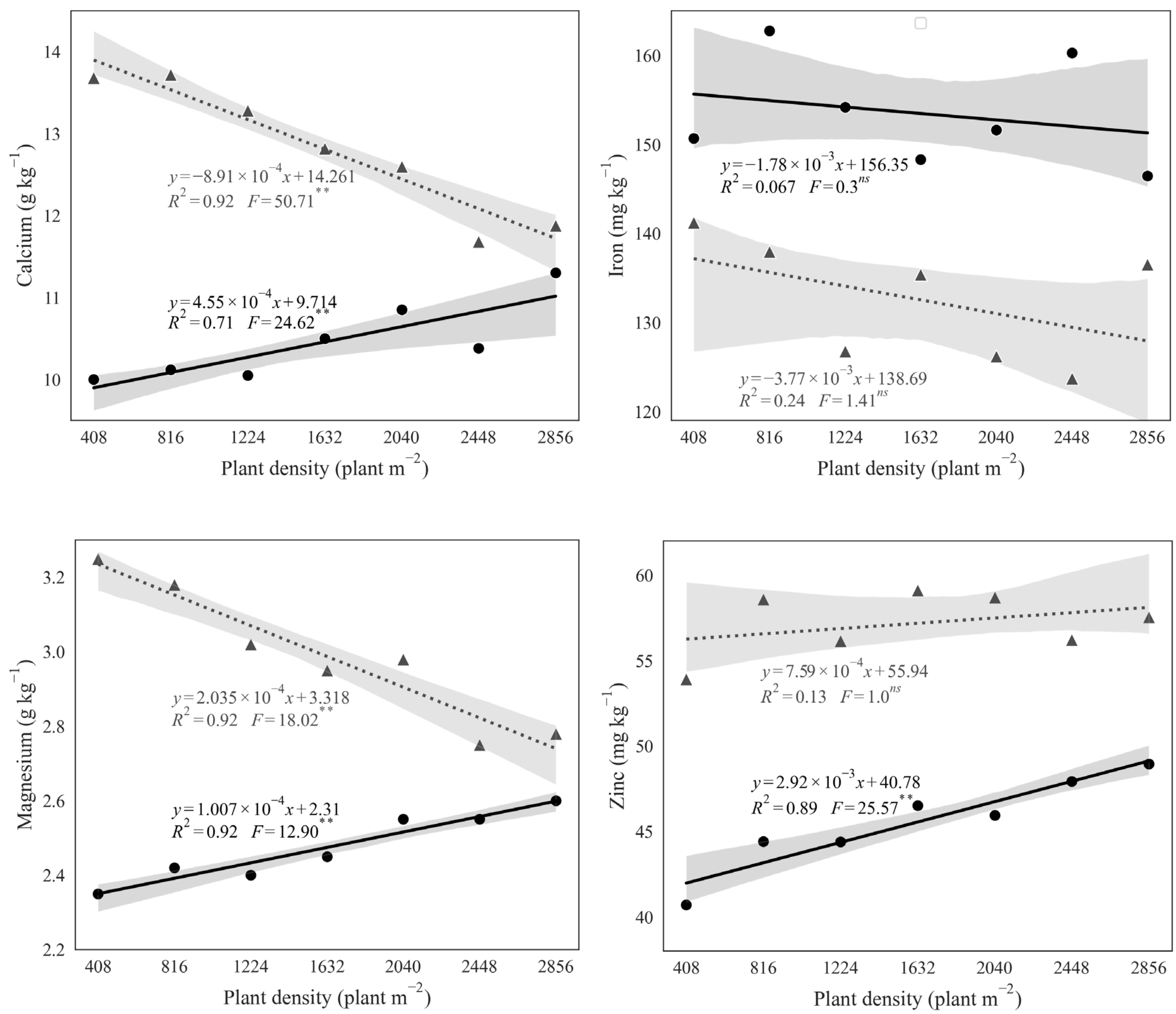


Figure 7. Leaf contents of potassium, calcium, magnesium, iron and zinc in baby leaf lettuce as a function of planting density. * Significant by the F test ($p < 0.01$); ^{ns} not significant.

CONCLUSIONS

1. Population density influences the morphological, physiological, productive and qualitative characteristics of baby leaf lettuce. Its increase reduces the leaf area and shoot fresh mass per plant, but the increase in the number of plants compensates for the reductions and promotes a higher yield;
2. The dry mass per plant and the contents of the pigments chlorophyll *a*, chlorophyll *b* and carotenoids reduce proportionally to the increase in planting density, indicating limitations in the synthesis of photosynthetic pigments, potentially affecting the efficiency of photosynthesis and reducing the visual and functional quality of baby leaf lettuce;
3. The vitamin C content in baby leaf lettuce leaves responds positively to increased planting density, favoring their nutritional quality;
4. The Ca, Mg, Fe and Zn contents varied as a function of the experimental season, whereas the K content increased as a function of planting density, regardless of the season. Therefore, planting season and density have implications on nutritional quality;
5. A density of 2,448 plants m^{-2} is recommended for the production of baby leaf lettuce, as it represents the best relationship between yield and quality of leaf attributes, such as vitamin C, K, Fe and Zn contents.

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