Australian Journal of

Crop Science

AJCS 16(05):596-604 (2022) doi: 10.21475/ajcs.22.16.05.p3561



Hydroponic kale: effects of row spacing and number of plants per cell on yield and quality

Caio Salvador Noboa¹, Bianca Machado de Lima¹, Silvia Raquel Bettan², Dorin Gupta³, Marta Regina Verruma-Bernardi², Luis Felipe Villani Purquerio⁴, Fernando Cesar Sala^{1,*}

¹Departamento de Biotecnologia e Produção Vegetal e Animal, Universidade Federal de São Carlos, Centro de Ciências Agrárias, Rodovia Anhanguera, Km 174, Araras, SP, CEP: 13600-970 Brazil

²Departamento de Tecnologia Agroindustrial e Sócio-Economia Rural, Universidade Federal de São Carlos, Centro de Ciências Agrárias, Rodovia Anhanguera, Km 174, Araras, SP, CEP: 13600-970 Brazil

³School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Australia

⁴Instituto Agronômico, Avenida Barão de Itapura, 1.481, Campinas, SP, CEP: 13020-902 Brazil

*Corresponding author: fcsala@ufscar.br

Abstract

The consumption of kale is increasing, and it is sold in bunches of adult leaves. However, there is the possibility of growing in bunches of young plants in a hydroponic system with several advantages, but different densities were not tested. The aim was to generate information on the hydroponic cultivation in NFT (nutrient film technique) of curly kale in bunches of young plants, with a study of spacing (0.07, 0.11, 0.15 and 0.20 m) and several plants per cell (one, two, three and four), aiming to improve the productivity, quality and homogeneity of kale. A split-plot experiment was executed in randomized block design, with four repetitions. Agronomic characteristics were measured, and bromatological and nutrient analyses were performed. Plant height and productivity were higher with smaller spacing. With fewer plants per cell, the leaf dimensions were higher and more homogeneous were the different plants of the same cell. With four plants per cell, it was recorded, on average, 19.3 g of fresh weight of shoot in the largest plant and 6.4 g in the smallest one. The bromatological and nutrient content was consistent with literature reports. Thus, the desired product in cultivating hydroponic kale in bunches of young plants is decisive for choosing spacing and numbers of plants per cell. Larger leaves can be harvested with fewer plants per cell than more plants per cell and vice versa. The use of smaller spacing increases productivity. The hydroponic system is technically viable to produce curly kale, providing plants with good nutritional quality.

Keywords: *Brassica oleracea* L. var. *acephala*; bromatological analyses; hydroponics; leafy vegetable; nutrient analyses; nutrient film technique.

Introduction

Kale (Brassica oleracea L. var. acephala), a member of the Brassicaceae family, has become very popular in the last decade both due to the health benefits as well as due to good tolerance for temperature fluctuations (Šamec et al., 2019), serving as a good option in a climate change context. Curly kale, with crisp and dark green leaves, is not widespread in the Brazilian market. However, collards are common among consumers and producers as they have smooth and light green leaves (Novo et al., 2010). Both types of kale are a good source of nutrients, protein, and fibre; however, there are indications that curly kale outperforms collards by 31, 48 and 16%, respectively (UNICAMP, 2011; USDA, 2016). Despite being a perennial plant, it is an annual crop with multiple harvests, forming bunches of adult leaves. Although in the United States it is usual to take a single harvest in the commercial scope, which removes the entire plant (Kadam and Shinde, 1998).

Kale cultivation in the United States increased from 1,616 ha in 2007 to 6,202 ha in 2017, increasing 284% in the

harvested area (USDA, 2019, 2014). In Brazil's state of São Paulo alone, there was an expansion in the production of collards from 27 thousand tons in 2014 from an area of 1,300 ha to 69 thousand tons in 2019 from 3,170 ha area (IEA, 2020). This data shows a production compound annual growth rate of 20.5%, with a similar increase in planted area of 19.5%. Higher crop yields from less land are most beneficial and desired by growers, which could be due to concerns for environmental sustainability or economic benefits. Therefore, hydroponic farming has become increasingly attractive to maximise input use efficiency. Nutrient film technique (NFT) systems are widely used due to higher yields compared to conventional cultivation, earlier harvest (shorter cycle), greater efficiency in the use of water and fertilisers, no weed competition, fewer phytosanitary problems, better marketing due to cleaner plants and lower labour requirements (Purquerio et al., 2018).

Leafy green vegetables are produced and sold in different forms, either individually, like lettuce, or in bunches of

plants, like arugula. This distinction of selling the harvest in a particular way also affects and demands specific crop management. While in lettuce, one plant is raised per tray cell, more than one plant is raised per cell in arugula. For higher yields, changes in planting density are considered. The spacing between rows or plants is altered, and, for arugula (sold in bunches), the number of plants per cell can be altered. Commonly, kale is marketed as bunches of adult leaves; however, Noboa et al. (2019) proposed the concept of marketing bunches of young plants of kale produced through NFT. They suggested harvesting and market bunches of the entire young kale plants like arugula and watercress. Thus, there is an opportunity to explore various treatments for spacing and number of plants per cell to standardise production standards for marketing bunches of young kale plants, with a hydroponic structure already in use for vegetables. Such a production system can open new avenues into the growing market for smaller-sized vegetable growers and add value to the growing market for fresh yearround healthier foods. Therefore, this research aims to generate information about appropriate cultivation technologies, which includes the study of spacing and number of plants per cell to improve the yield and quality of kale plants.

Results

The interaction between spacing and number of plants per cell was significant for the agronomic characteristics such as the number of leaves, leaf length and width, fresh and dry mass of the shoot, productivity, and total chlorophyll index. However, plant height, petiole length and bromatological analysis did not show significant interaction, and among nutrient analysis, a significant interaction was observed only for calcium.

Agronomic characteristics

Increasing the spacing (Figure 1) resulted in: (a) linear decrease in plant height, leaf length and petiole length; (b) linear (two and three plants per cell) and quadratic (four plants per cell) increase in the number of leaves; (c) quadratic behaviour (one plant per cell) in leaf width; (d) linear (one, three and four plants per cell) and quadratic (two plants per cell) increase in total chlorophyll index. Increasing the number of plants per cell resulted in: (a) linear decrease in plant height; (b) linear (0.07, 0.15 and 0.20 m spacing) and quadratic (0.11 m) increase in number of leaves; (c) linear (0.07, 0.15 and 0.20 m) and quadratic decrease in leaf width; (d) quadratic decrease in leaf length.

Increasing the spacing (Figure 2) resulted in: (a) linear increase (one plant per cell) in fresh weight of the shoot; (b) linear (one plant per cell) and quadratic (three plants per cell) increase in dry weight of the shoot; (c) linear (one and two plants per cell) and quadratic (three and four plants per cell) decrease in productivity. Increasing the number of plants per cell resulted in: (a) linear (0.07 m) and quadratic (0.11 m) increase in fresh and dry weight of the shoot; (b) quadratic increase (0.07 and 0.11 m) in productivity.

With more plants per cell, each plant, individually, was discrepant among themselves and had inferior performance than those with fewer plants per cell (Figure 3). In cells with four plants per cell recorded the largest plant with an average height of 26.1 cm, 11 leaves, and 19.3 g of fresh weight, and the smallest plant recorded 20.1 cm height,

eight leaves, and 6.4 g fresh weight. However, for similar traits, one plant per cell treatment recorded an average height of 27.3 cm, 14 leaves and 43.1 g fresh weight.

Bromatological and nutrient analyses

Bromatological analysis (Table 1) for the leaf samples collected from different treatment levels for spacing had significantly higher moisture content at 0.07 and 0.11 m spacing, reaching values 1.3% higher than at spacing of 0.20 m, which recorded the lowest moisture content. Protein, fibre, and total carbohydrate contents were significantly higher at a spacing of 0.20 m, 11.4, 14.9 and 18.5% higher, respectively, compared to the lowest contents recorded at 0.07 and 0.11 m spacing (and 0.15 m in the case of fibre). Results for the treatment having a variable number of plants per cell showed significantly higher moisture content (0.47%) for four plants per cell. In contrast, the lowest moisture content was recorded for one plant per cell. The total carbohydrate content was significantly higher with one, two and three plants per cell, reaching 6.40% higher than with four plants per cell.

Results of nutrient analysis (Table 1) for different spacings revealed significantly higher contents of N, Mg and Cu at 0.20 m spacing, reaching values 14.3; 13.7 and 29.3%, respectively, higher than at spacings of 0.07 and 0.11 m (only at 0.07 m for Mg), which obtained the lowest values. Only Cu content was significantly higher with one plant per cell, reaching a value of 24.5% higher than with four plants per cell, which had the lowest value. For the calcium content (Table 2), the interaction between the spacing treatments and the number of plants per cell was significant. Thus, spacing within each level of the number of plants per cell, with one and four plants per cell, the highest calcium content was recorded with a spacing of 0.20 m. With the number of plants per cell within each level of spacing, at the 0.20 m spacing, the highest calcium content was recorded with one plant per cell.

The PCA explained 69.6% of the data variation (Figure 4). Among various characteristics, total chlorophyll index, Mg, N, protein, fibre, and total carbohydrates were positively correlated with each other and negatively correlated with productivity and moisture. Dry and fresh weights of the shoot were correlated positively with each other and negatively with plant height, whereas the number of leaves was correlated negatively with the width and length of the leaves.

Discussion

Plant competition

Two effects originating from the competition between plants were observed in the results due to (a) different row spacings, which led to shade avoidance responses and (b) the different number of plants per cell which led to dominance and suppression effect.

The plants perceive light intensity and quality through photoreceptors, which might initial phenotypic changes (Ma and Li, 2019). Phytochrome is one of the examples of a photoreceptor, a molecule that has one form for red light spectrum absorption and another one for far-red. However, both forms convert into each other depending on the rates of red and far-red light received by the plant, provoking responses such as changes that allow the plant to avoid shading (Iglesias et al., 2018). Shade avoidance responses **Table 1.** Mean values of bromatological and nutrient composition of kale cultivated in NFT hydroponic system in bunches of young plants as a function of different row spacings and number of plants per cell (NPC), 27 days after transplanting. UFSCar, Araras (SP), 2020.

Spacing (m)	Bromatological composition (g 100 g ⁻¹)							Nutrient composition (mg 100 g ⁻¹)						
	Moisture		Protein		Fibre		Total Carbohydrates		N		Mg		Cu	
0.07	90.49	а	2.64	с	3.34	b	4.43	с	506.10	с	50.47	b	0.022	b
0.11	90.21	а	2.71	с	3.69	b	4.63	с	528.84	С	51.53	ab	0.022	b
0.15	89.72	b	2.87	b	3.71	b	5.01	b	564.29	b	54.40	ab	0.026	ab
0.20	89.21	с	2.98	а	4.11	а	5.37	а	591.41	а	57.36	а	0.029	а
Number of plants per cell														
1	89.72	b	2.84	ns	3.79	ns	5.02	а	555.02	ns	53.96	ns	0.023	b
2	89.84	ab	2.81	ns	3.68	ns	4.90	а	550.65	ns	53.69	ns	0.024	ab
3	89.93	ab	2.78	ns	3.72	ns	4.88	а	543.66	ns	53.69	ns	0.025	ab
4	90.14	а	2.77	ns	3.66	ns	4.64	b	541.31	ns	52.42	ns	0.028	а
CV Main plot (%)	0.30		2.52		9.08		6.48		4.13		10.81		19.74	
CV Sub-plot (%)	0.39		4.73		9.48		4.80		4.74		5.75		20.67	

Means within a column with similar letter are not significantly different (p<0.05) by the Tukey's test; ns: non-significant; CV: coefficient of variation.



Figure 1. Mean values of plant height (A; B), number of leaves (C; D), total chlorophyll index, (E; F) leaf width (G; H) and length (I; J), and petiole length (K) of kale cultivated in NFT hydroponic system in bunches of young plants as a function of different row spacings and number of plants per cell, 27 days after transplanting. UFSCar, Araras (SP), 2020.

Table 2. Mean values of calcium of kale cultivated in NFT hydroponic system in bunches of young plants as a function of different row spacings and number of plants per cell (NPC), 27 days after transplanting. UFSCar, Araras (SP), 2020.

Ca (mg 100 g ⁻¹)											
Number of plants per cell	Spacing (m)										
	0.07		0.11		0.15		0.20				
1	256.87	bNS	256.54	bNS	248.97	bNS	303.48	aA			
2	245.12	nsNS	248.16	nsNS	288.33	nsNS	260.64	nsAB			
3	245.40	nsNS	270.97	nsNS	285.84	nsNS	283.03	nsAB			
4	233.40	bNS	247.96	abNS	257.29	abNS	284.83	aB			

Means followed by different capital letters do not differ statistically in columns and means followed by different lower-case letters do not differ statistically in rows by the Tukey's test (p<0.05); ns and NS: non-significant. CV (coefficient of variation) Main plot: 12.31%; CV Sub-plot: 7.86%.



Figure 2. Mean values of fresh (A; B) and dry (C; D) mass of shoot, and productivity (E; F) of kale cultivated in NFT hydroponic system in bunches of young plants as a function of different row spacings and number of plants per cell, 27 days after transplanting. UFSCar, Araras (SP), 2020.



Figure 3. Mean values (with standard error) of plant height (A), number of leaves (B) and fresh mass of shoot (C) of the different plants of the same cell, in descending order. UFSCar, Araras (SP), 2020.



Figure 4. Principal component analysis (PCA) of the plant height (PH), number of leaves (NL), leaf width (LW) and length (LL), petiole length (PL), fresh (FMS) and dry (DMS) weight of the shoot, productivity, total chlorophyll index (TCI), moisture, ashes, protein, fibre, lipids, total carbohydrates (T.Carb) and nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B) of kale cultivated in NFT hydroponic system in bunches of young plants as a function of different row spacings and number of plants per cell (NPC), 27 days after transplanting. PC1: principal component 1; PC2: principal component 2. It is possible to verify a positive correlation between the characteristics evaluated with parallel arrows, negative correlation with arrows in opposite directions and no correlation with perpendicular arrows. UFSCar, Araras (SP), 2020.

are reflected in the elongation of stems, petioles, internodes, more erect leaves and can reduce leaf thickness and branching (Iglesias et al., 2018; Ma and Li, 2019). Light, especially far-red, has been reported to induce shade avoidance responses in kale (Meng et al., 2019). It corresponds and explains the greater plant height, leaf and petiole length at smaller spacings found in the present study.

Studies of dominance and suppression in plants are common in other agricultural science areas, such as in forestry (Campoe et al., 2013; Soares et al., 2020) and weed science (Cho et al., 2015). However, the inequality between plants in the same cell is remarkable. It can be explained by the high competition among plants, which is reflected through dominance and suppression, where some plants outperformed (dominant) others (suppressed). Despite the differences being statistically significant for various traits due to a variable number of plants per cell, the height difference was not visually perceptible due to the inequality found in the plants within the same cell. The statistical difference occurred due to the average of all plants in the same cell. However, the higher number of plants, the greater was the difference between them, the greater was the inequality. Observing only the largest plant in each cell, it is noticeable that there did not seem to be any differences. Inequality, as a function of the number of plants per cell, the sum of the number of leaves and fresh weight of the shoot of the different plants in the same cell generated statistical differences in some cases and not in others.

According to Forrester (2019), size inequality is influenced by several factors, such as differences in light absorption, water and nutrient uptake, plant architectures, physiological processes and plant health. As there was only one kale cultivar without any competition from weeds and the plants were supplied with adequate nutrients in a hydroponic system, the most probable factor for the inequality could be the differences in light absorption. The homogeneity of the plants is a relevant characteristic because besides being an attraction to the consumer (Reghin et al., 2005; Sabio et al., 2013), it represents an optimization of the use of seeds. Seed is an essential input in the production cost of vegetables, representing about 10% of this value, varying by seed technology, species, and production system (Deleo et al., 2011; Souza and Garcia, 2013).

Agronomic characteristics

The obtained values of the evaluated characteristics were compatible with those found by Noboa et al. (2019) while studying three kale varieties (Darkibor, Starbor and Redbor) and one collard (Butter Green) in the same hydroponic system (a bunch of young plants with four plants per cell). They reported the averages of number (23 leaves), width and length of leaves (9.7 cm, 14.9 cm), length of petioles (14.8 cm), fresh and dry plant weights (93.7 g, 7.1 g) and productivity (4.5 kg m⁻² per month) of Darkibor hybrid. These values were relatively close to the present research. Sensorial analysis showed an equal preference and purchase intention for the produce, suggesting acceptance of the young kale plant by the Brazilian consumers. The authors also noticed size variation among plants from the same cell, and the present study showed this inequality.

Reghin et al. (2005) also observed visual inequality in arugula plants cultivated with four plants per cell. They explained that in higher densities, competition among plants leads to such inequalities. The authors further suggested that the type of product desired will define the spacing and number of plants per cell to be used. Such as, if arugula plants were marketed per unit, wider spacing with lower plant density would be a good option, as it promotes larger plant sizes. However, as arugula is commercialized in bunches of plants, the higher productivity generated in smaller spacing and higher density of plants becomes the preferred choice. Commercial bunches of arugula have between 10 and 12 plants per cell. The plants are similar, suggesting that many plants per cell force such competition that there is no dominance and suppression. If kale behaves similarly at high numbers of plants per cell, this will open another possibility for kale commercialization. However, it would require openpollinated varieties and raw seeds, as Darkibor seeds have value addition because they are pelleted and hybrid.

For spacing, differently than in the present study, Reghin et al. (2005) reported no statistical difference in plant height of arugula. However, they tested similar spacing to the present research, and the plants were grown conventionally, which may be the reason for the difference. As for the number of plants per cell and the present study, a decrease in the height of arugula plants was reported (Reghin et al., 2005, 2004).

For spacing and the present study, an increasing number of leaves in kale (Naik and Gupta, 2010) and arugula (Reghin et al., 2005) plants were reported. However, in arugula, Gonçalves-Trevisoli et al. (2017) reported no difference in summer, autumn, and winter seasons, but more leaves were observed in the wider spacing production system during spring. As for the number of plants per cell and the present study, Reghin et al. (2005, 2004) reported an increased number of leaves when considering all the plants in the cell; however, a decreased number when considering the individual plants.

The number of plants per cell had a higher impact on the number and dimension of leaves than spacing, which the PCA demonstrates. In contrast, a higher number of leaves led to a smaller width and length of the leaves, corroborating with the findings of Terfa et al. (2013). Although there are studies that have shown greater leaf area with wider spacing as in kale (Naik and Gupta, 2010) and arugula (Purquerio et al., 2007) plants, Gonçalves-Trevisoli et al. (2017) reported no difference in leaf area in arugula. Contrary to the present study, Naik and Gupta (2010) reported greater petiole length in kale in wider spacing; the most probable factor for this difference could be the higher competition among plants in the present study and the production systems.

Although with only one and three plants per cell, there was an increase in dry weight as a spacing function. Several sources reported an increase in the fresh and dry weight and wider spacing linked with lower productivity and the present research in kale (Naik and Gupta, 2010) and arugula plants (Purquerio et al., 2007; Reghin et al., 2005). However, in Gonçalves-Trevisoli et al. (2017) reported no difference in arugula's summer, fall, and winter cultivation, whereas spring cultivation led to higher fresh and dry weight in the wider spacing. As for the number of plants per cell, like this study, a decrease in fresh and dry weight per plant was reported for arugula (Reghin et al., 2005, 2004).

Similar to the present study, the increase of chlorophyll content as a function of spacing was also reported in kale (Naik and Gupta, 2010), groundnut (Meena et al., 2011), water spinach (Sarkar et al., 2014) and coriander (Sharma et al., 2016). The influence of light on chlorophyll content in

kale has been reported (Naznin et al., 2019), which can support the results of this study as the kale plants were grown in a hydroponic system, with adequate water and nutrients supply and sunlight could be a determinant factor for the variation in the results. The total chlorophyll index found was consistent with the values reported by Silva et al. (2021) to hydroponic curly kale. They also reported lower chlorophyll content in hydroponic kale than in the conventional system.

Bromatological and nutrient analyses

The moisture, protein and total carbohydrate values of hydroponic kale in the present study were different compared to those reported previously for kale, which ranges between 82.9 and 84.0; 4.2 and 4.3; 8.8 and 10.1 g 100 g⁻¹, respectively (Sikora and Bodziarczyk, 2012; USDA, 2016). However, they are similar to those reported for collards, which varied between 89.6 and 90.9; 2.9 and 3.0; 4.3 and 5.4 g 100 g⁻¹, respectively (Kawashima and Soares, 2003; UNICAMP, 2011; USDA, 2016). On the other hand, the ash, fibre and lipids values were similar to those reported for kale, which ranged between 2.0 and 2.1; 3.6 and 8.4; 0.7 and 0.9 g 100 g⁻¹, respectively (Sikora and Bodziarczyk, 2012; USDA, 2016). Although leaf thickness and sensory analyses were not performed, the leaves of hydroponic kale in bunches of young plants appear to be softer than conventionally grown kale; the presence of higher moisture may be an influencing factor. The apparent softness of the kale leaves achieved through this production system may be a positive differential for better acceptance of this product. However, validation of such results with sensory analysis should be considered.

The contents of P, K, Ca, Mg, Fe, Mn, Cu and Z of the hydroponic kale in the present study were consistent with those reported in the literature for kale, which varies between 49.2 and 105; 315.4 and 491; 106 and 384.8; 34.9 and 92; 0.5 and 1.47; 0.4 and 1.16; 0.04 and 1.5; 0.26 and 0.83 mg 100 g⁻¹, respectively (Metallo et al., 2018; Pathirana et al., 2017; Sikora and Bodziarczyk, 2012; Thavarajah et al., 2016; USDA, 2016). The contents of N, Mg, protein and chlorophyll, are positively correlated, as showed by PCA; this is explained by the chlorophyll molecule containing N and the protein molecules containing N and Mg (Resh, 2013; Taiz and Zeiger, 2013).

Waterland et al. (2017) studied kale at different developmental stages. Moisture decreased with plant development, around 95% in microgreens, 90% in baby leaf, similar to the present work, and 80% as adults. The content of total minerals on a fresh basis was statistically equal in microgreens and adults. However, the baby leaf stage was higher than the others; thus, there was an increase in mineral content and a subsequent decrease during the development of the plants. In the present work, kale was evaluated at 64 DAS, a period intermediate to baby leaf and adults mentioned by Waterland et al. (2017). According to Di Gioia et al. (2017), the baby leaf period goes until completing eight true leaves; for this reason, the present work treats kales produced as young plants and not as baby leaves. The mineral contents of the kales in the present study were similar to those reported for adult kale in the literature, thus agreeing with Waterland et al. (2017).

In general, the decision for spacing and number of plants per cell depends on the desired product. If the objective is large leaves, fewer shall be the number of plants per cell, whereas if the objective is small leaves and in high quantity, more plants per cell shall be used. As for the spacing, smaller spacing brings higher productivity. Kale is known and marketed as a vegetable for bunches of leaves rather than a bunch of young plants. The model of producing kales in bunches of young plants brings increased productivity and precocity, with plants as nutritious as the adults. Such innovation brings challenges and numerous possibilities, such as taking advantage of the growing market of smallsized vegetables (Purquerio et al., 2018). Also, taking advantage of hydroponic structures and management already used for other leafy vegetables, such as arugula, kale can be considered an alternative for winter cultivation, as kale consumption increases (Di Fabio et al., 2018). Something to be further investigated is sensory and purchase intention analyses between leaves of the smallest and largest spacings. Although leaf thickness was not measured in this study, there are impressions of less thickness in smaller spacings, which is possible to occur because it is one of the shade avoidance responses (Iglesias et al., 2018) and can also impact consumer preferences.

Materials and methods

Plant materials and conduction of study

The experiment was carried out from 30th June to 2nd September 2020, in NFT hydroponic system established in the greenhouse facility of the Department of Biotechnology and Plant and Animal Production, Center of Agricultural Sciences of the Federal University of São Carlos, Araras-SP (22°18'30.8" S, 47°22'53.5" W). Darkibor (Bejo®) kale hybrid cultivar seeds were used for this experiment, and the seedlings were produced in commercial trays with 128 cells (20 cm³ each cell) and transplanted to the hydroponic system with three true leaves (five weeks after sowing). The NFT system, Hidrogood[®] HPM (moving hydroponic channels) system, was used to grow kale hydroponically. The maximum and minimum temperature inside the greenhouse was collected daily and during the experimental period. The maximum and minimum temperature recorded ranged from 20.2 - 36.4 °C (average 31.0 °C) and 8.7 - 17.9 °C (average 13.4 °C), respectively, and the overall average was 22.2 °C. The greenhouse has a ceiling height of 3.5 m, 15 m long and 7 m wide, covered with diffuser plastic and closed sides with ChromatiNet[®] Leno (20% shading mesh). The hydroponic channels are polyethylene 61 mm wide and 40 mm high. The structural arrangement corresponded to two motor pumps Dancor[®] model Pratika CP-4R, 0.5 hp, and two reservoirs with a capacity of 1000 L.

The nutrient solution used was proposed by Furlani et al. (1999) for arugula: 120 g 1000 L⁻¹ MAP (monoammonium phosphate, N: 11% + P_2O_5 : 60%, Ominia[®]); 500 g 1000 L⁻¹ calcium nitrate (N: 15.5% + Ca: 19%, YaraLiva®); 650 g 1000 L⁻¹ potassium nitrate (N: 12% + K₂O: 45%, S: 1.2%, DripSol[®]); 350 g 1000 L⁻¹ Mg sulfate (Mg: 9% + S: 11.9%, Heringer®); 20 g 1000 L⁻¹ of micronutrient cocktail (B: 1.82% - Cu EDTA: 1.82% - Fe EDTA: 7.26% - Mn EDTA: 1.82% - Mo: 0.3%, Ni: 0.335% - Zn EDTA: 0.73%, Conplant®); and 30 g 1000 L⁻¹ of FeQ48 - iron chelate (Fe: 16%, DripSol®). During the experiments, daily measurements of electrical conductivity (CE) of the nutrient solution were taken, which was kept between 1,400 and 1,800 μ S cm⁻¹. The pH was maintained between 5.5 and 6.5. During the period from 06:00am to 19:15pm, the nutrient solution circulation scheme was alternated in 15 minutes intervals (on/off). During the night period, the solution was circulated for 30 min at 22:30pm and 03:00am.

Treatments and experimental design

The split-plot experiment was performed in randomized block design, with four repetitions. The main treatments were four-row spacings (0.07, 0.11, 0.15 and 0.20 m) and the secondary treatments corresponded to the number of plants per cell (one, two, three and four plants). The plots were composed of four subplots; each consisted of 30 cells, with six hydroponic channels and five openings each. The border was composed of the outermost cells (equivalent to 18 per subplot), and the data was recorded from the inner 12 cells. The spacing between plants was maintained at 0.15 m.

Traits measured

With two random cells in each subplot, the following measurements were recorded: (a) plant height; (b) number and dimensions of leaves of each plant in the cell (leaves larger than 1 cm; leaf width and length, and petiole length of the largest leaf of each plant in the cell); (c) fresh and dry weight of the shoot (for dry weight, the plants were dried in an oven with air circulation at a temperature of 60°C until reaching constant weight) and productivity (ratio between fresh weight and the product of fixed spacing between plants (0.15 m) by the equivalent row spacing); (d) total chlorophyll index by the SPAD-502-PLUS equipment (MINOLTA CORP, 2007).

Five cells per subplot comprised one sample for bromatological analysis and the other five cells for nutrient analysis, with a total of 64 samples for each analysis. Leaf samples in triplicate were used to analyze the following traits:

Moisture content, which was determined in an oven between 100 and 105 °C until constant weight (AOAC, 2012); Ash content, following burning method between 600 and 650°C (AOAC, 2012);

Protein content, following Kjeldahl method (AOAC, 2012); Fibre content (ANKOM, 2017);

Lipid content, made with ethyl ether, using the Soxhlet equipment (IUPAC, 1979);

Total carbohydrates, calculated from the difference between per cent water, protein, lipids, ash and fibre from 100;

The leaves from each of the 64 samples were rinsed and dried (oven with air circulation at a temperature of 60°C), and nutrient analyses were performed (Malavolta et al., 1997).

Statistical analysis

The data were analyzed by the analysis of variance and the F test. When the interaction between spacing and number of plants per cell was significant, spacing within each level of number of plants per cell and number of plants per cell within each level of spacing were studied. For the agronomic characteristics, regression analysis was performed, and in the treatments without significant difference, a trend line was drawn passing through the mean, keeping the original points. A principal component analysis (PCA) was performed to evaluate possible relationships among the measured variables. The Tukey's test (p≤0.05) was performed for bromatological and nutritional analysis. The R software (R CORE TEAM, 2020) was used for the analyses, and the Origin software (ORIGIN, 2020) was used to construct the graphs. It was considered necessary to study the homogeneity of the different plants in the same cell, as there was heterogeneity between plants in the same cell. Thus, the plants were arranged in decreasing order, in plant 1 (one plant per cell); plant 1 and 2 (two plants per cell); plant 1, 2 and 3 (three plants per cell); plants 1, 2, 3 and 4 (four plants per cell); being plant one the biggest and plant *n* the smallest.

Conclusion

The desired product of hydroponic kale cultivated in bunches of young plants is decisive for the choice of spacing and number of plants per cell. If the goal is to harvest larger leaves, fewer plants per cell should be considered. If the goal is smaller and more leaves, one could opt for more plants per cell, but this decision will bring inequality in the plants, which may impact the commercialization. The use of smaller spacing enhances the chances of higher productivity. The hydroponic system is technically feasible for kale production, providing plants of good nutritional quality.

Acknowledgements

The authors would like to thank the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), for the financial support, code 001.

References

- ANKOM (2017) Neutral detergent fiber in feed: Filter bag technique (for A2000 and A2000I).
- AOAC ASSOCIATION OF ANALITICAL CHEMISTS (2012) Official methods of analysis of AOAC International, 19th edn. AOAC International, Washington.
- Campoe OC, Stape JL, Nouvellon Y, Laclau J-P, Bauerle WL, Binkley D, Le Maire G (2013) Stem production, light absorption and light use efficiency between dominant and non-dominant trees of *Eucalyptus grandis* across a productivity gradient in Brazil. For Ecol Manag. 288: 14–20.
- Cho AH, Chase CA, Treadwell DD, Koenig RL, Morris JB, Morales-Payan JP (2015) Apical dominance and planting density effects on weed suppression by sunn hemp (*Crotalaria juncea* L.). HortScience. 50: 263–267.
- Deleo JPB, Menegazzo TM, Tapetti R (2011) Gestão sustentável hortaliças. Hortifruti Bras. 10: 10–29.
- Di Fabio E, Nascimento DF, Oliveira EAC, Costa EA, Feiden A (2018) Sazonalidades na comercialização de hortaliças da agricultura familiar na fronteira Brasil-Bolívia. Cad Agroecol. 13: 1–10.
- Di Gioia F, Renna M, Santamaria P (2017) Sprouts, microgreens and "baby leaf" vegetables. In: Yildiz F, Wiley RC (eds.) Minimally processed refrigerated fruits and vegetables, Food Engineering Series. Springer US, Boston.
- Forrester DI (2019) Linking forest growth with stand structure: Tree size inequality, tree growth or resource partitioning and the asymmetry of competition. For Ecol Manag. 447: 139– 157.
- Furlani PR, Silveira LCP, Bolonhezi D, Faquin V (1999) Cultivo hidropônico de plantas. Instituto Agronômico, Campinas.
- Gonçalves-Trevisoli EDV, Mendonça HFC, Dangelo O, Dildey F, Dartora J, Rissato BB, Coltro-Roncato S, Silvério É, Tsutsumi CY, Echer MM (2017) Ambiência e desempenho produtivo de rúcula cultivada em diferentes espaçamentos. Sci Agrar Parana. 16: 230–236.

- IEA INSTITUTO DE ECONOMIA AGRÍCOLA (2020) Estatística de produção paulista.
- http://ciagri.iea.sp.gov.br/nia1/subjetiva.aspx?cod_. Iglesias MJ, Sellaro R, Zurbriggen MD, Casal JJ (2018) Multiple
- links between shade avoidance and auxin networks. J Exp Bot. 69: 213–228.
- IUPAC INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY (1979) Standard methods for the analysis of oils, fats and derivatives, 6th edn. Oxford, Pergamon Press.
- Kadam SS, Shinde KG (1998) Other crucifers. In: Handbook of vegetable science and technology: Production, composition, storage and processing. Marcel Dekker Inc, New York.
- Kawashima LM, Soares LMV (2003) Mineral profile of raw and cooked leafy vegetables consumed in Southern Brazil. J Food Compos Anal. 16: 605–611.
- Ma L, Li G (2019) Auxin-dependent cell elongation during the shade avoidance response. Front Plant Sci. 10: 1–8.
- Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do estado nutricional das plantas: Princípios e aplicações, 2nd edn. Associação Brasileira de Potassa e do Fósforo, Piracicaba.
- Meena BP, Kumawat SM, Yadav RS (2011) Effect of planting geometry and nitrogen management on groundnut (*Arachis hypogaea*) in loamy sand soil of Rajasthan. Indian J Agric Sci. 81: 86–88.
- Meng Q, Kelly N, Runkle ES (2019) Substituting green or farred radiation for blue radiation induces shade avoidance and promotes growth in lettuce and kale. Environ Exp Bot. 162: 383–391.
- Metallo RM, Kopsell DA, Sams CE, Bumgarner NR (2018) Influence of blue/red vs. white LED light treatments on biomass, shoot morphology, and quality parameters of hydroponically grown kale. Sci Hortic. 235: 189–197.
- MINOLTA CORP (2007) Precise color communication: Color control from feeling to instrumentation. MINOLTA Corp Ltda, Osaka.
- Naik IA, Gupta AJ (2010) Effect of plant density and integrated nutrient management on growth, yield, quality and economics of kale (*Brassica oleracea* var. *acephala*) in temperate region. Indian J Agric Sci. 80-84.
- Naznin M, Lefsrud M, Gravel V, Azad M (2019) Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. Plants. 8: 1–12.
- Noboa CS, Ravagnani CA, Santos CP, Oliveira BC, Fernandes N, Verruma-Bernardi MR, Sala FC (2019) Produção hidropônica e análise sensorial de couve-de-folhas na forma de maço de plantas jovens. Rev Cienc Tecnol Ambiente. 9: 1–9.
- Novo M do C de S, Prela-Pantano A, Trani PE, Blat SF (2010) Desenvolvimento e produção de genótipos de couve manteiga. Hortic Bras. 28: 321–325.

ORIGIN (2020) OriginLab, Massachusetts.

- Pathirana I, Thavarajah P, Siva N, Wickramasinghe ANK, Smith P, Thavarajah D (2017) Moisture deficit effects on kale (*Brassica oleracea* L. var. *acephala*) biomass, mineral, and low molecular weight carbohydrate concentrations. Sci Hortic. 226: 216–222.
- Purquerio LFV, Demant LAR, Goto R, Villas Boas RL (2007) Efeito da adubação nitrogenada de cobertura e do espaçamento sobre a produção de rúcula. Hortic Bras. 25: 464–470.
- Purquerio LFV, de Moraes CC, Factor TL, Calori AH (2018) Bioeconomia: Promoção da horticultura urbana do século XXI. O Agronômico. 70: 6–19.

- R CORE TEAM (2020) R Foundation for Statistical Computing, Austria.
- Reghin MY, Otto RF, Olinik JR, Jacoby CFS (2005) Efeito do espaçamento e do número de mudas por cova na produção de rúcula nas estações de outono e inverno. Cienc Agrotec. 29: 953–959.
- Reghin MY, Otto RF, Vinne JVD (2004) Efeito da densidade de mudas por célula e do volume da célula na produção de mudas e cultivo da rúcula. Cienc Agrotec. 28: 287–295.
- Resh HM (2013) Hydroponic food production: A definitive guidebook for the advanced home gardener and the commercial hydroponic grower, 7th edn. CRC Press, Boca Raton.
- Sabio RP, Ventura MB, Campoli SS (2013) Mini e "baby" frutas e hortaliças. Hortifruti Bras. 11: 8–20.
- Šamec D, Urlić B, Salopek-Sondi B (2019) Kale (*Brassica* oleracea var. acephala) as a superfood: Review of the scientific evidence behind the statement. Crit Rev Food Sci Nutr. 59: 2411–2422.
- Sarkar RK, Jana JC, Datta S (2014) Effect of different sowing times and spacings on growth, yield and quality of water spinach (*Ipomoea reptans* Poir.) under terai region of West Bengal. J Appl Nat Sci. 6: 489–494.
- Sharma A, Naruka IS, Shaktawat RPS (2016) Effect of row spacing and nitrogen on growth and yield of coriander (*Coriandrum sativum* L.). J Krishi Vigyan. 5: 49–53.
- Sikora E, Bodziarczyk I (2012) Composition and antioxidant activity of kale (*Brassica oleracea* L. var. *acephala*) raw and cooked. Acta Sci Pol Technol Aliment. 11: 239–248.
- Silva LC, Pimenta DM, Forti VA, Sala FC, Medeiros SDS, Verruma-Bernardi MR (2021) Sensory analysis of curly kale produced under conventional and hydroponic systems. Braz J Food Technol. 24: 1–8.
- Soares AAV, Scolforo HF, Forrester DI, Carneiro RL, Campoe OC (2020) Exploring the relationship between stand growth, structure and growth dominance in *Eucalyptus* monoclonal plantations across a continent-wide environmental gradient in Brazil. For Ecol Manag. 474: 1–9.
- Souza JL, Garcia RDC (2013) Custos e rentabilidades na produção de hortaliças orgânicas e convencionais no estado do Espírito Santo. Rev Bras Agropecuária Sustentável. 3: 11–24.
- Taiz L, Zeiger E (2013) Plant physiology, 5th ed. Sinauer Associates, Sunderland.
- Terfa MT, Solhaug KA, Gislerød HR, Olsen JE, Torre S (2013) A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of *Rosa* × *hybrida* but does not affect time to flower opening. Physiol Plantarum. 148: 146–159.
- Thavarajah D, Thavarajah P, Abare A, Basnagala S, Lacher C, Smith P, Combs GF (2016) Mineral micronutrient and prebiotic carbohydrate profiles of USA-grown kale (*Brassica oleracea* L. var. *acephala*). J Food Compos Anal. 52: 9–15.
- UNICAMP UNIVERSIDADE ESTADUAL DE CAMPINAS (2011) Tabela brasileira de composição de alimentos. UNICAMP, Campinas.
- USDA UNITED STATES DEPARTMENT OF AGRICULTURE (2014) 2012 Census of agriculture.
- https://www.nass.usda.gov/Publications/AgCensus/2012/. USDA - UNITED STATES DEPARTMENT OF AGRICULTURE (2016) USDA National nutrient database for standard reference, release 28 (slightly revised). http://www.ars.usda.gov/nea/bhnrc/mafcl.
- USDA UNITED STATES DEPARTMENT OF AGRICULTURE (2019) 2017 Census of agriculture.

https://www.nass.usda.gov/Publications/AgCensus/2017/in dex.php.

Waterland NL, Moon Y, Tou JC, Kim MJ, Pena-Yewtukhiw EM, Park S (2017) Mineral content differs among microgreen, baby leaf, and adult stages in three cultivars of kale. HortScience. 52: 566–571.