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## Abstract

Lettuce is the most important leafy vegetable in Brazil. Hydroponic lettuce cultivation has grown due to the viability of harvesting throughout the year. In this context, this study aimed to evaluate the agronomic characteristics, color, and preference of curly lettuce in the NFT hydroponic system. Six lineages (6601-1A, 6601-2L, 7016-6A, 7119-1B, 7223-1A, and 7224-4A) and two commercial cultivars (Brida and Vanda) of lettuce were used, in a completely randomized blocks design with four replications. Shoot length, root length, stem length, number of leaves, stem diameter, plant diameter, shoot fresh mass, root fresh mass, chlorophyll content, instrumental color, and sensory characteristics were evaluated. As for agronomic evaluation, the bolting of 6601-2L lettuce was relevant in different attributes. The Vanda lettuce and the lineage 7016-6A presented best performances for shoot fresh mass (399.44 and 378.63 g, respectively), while the lineages 7119-1B and 6601-2L present the worst performance (279.50 and 273.13 g, respectively). There was variation in chlorophyll content and luminosity, however, the evaluators did not notice differences between lettuces for brightness or green color, as well as for crunchy texture. Lettuces 6601-2L, 7224-4A, 6601-1A, Brida, 7223-1A, and 7119-1B were preferred. The variation among plants may be due to different situations, such as harvesting times and bolting, and a direct relationship between agronomic properties and preference among lettuces has not been established yet.

**Keywords:** Color. Hydroponics. *Lactuca sativa* L. sensory evaluation.

## 1. Introduction

Lettuce is the most important leafy vegetable in Brazil (Sala and Costa 2012), preferably used in salads, with high acceptance and ease preparation. According to Filgueira (2003), the plant grows all year round but adapts better in mild temperatures.

Lettuce has changed in terms of the varietal types preferred by consumers and cultivation technologies. Until the 1980s, Brazil had a pattern of consumption of smooth lettuce. One of the big changes was the adoption of curly lettuce, both by the farmer and the consumer. This type tends to be more resistant in the summer, showing an advantage concerning the smooth type since its leaves do not form heads, which leads to less loss by the producer (Sala and Costa 2012).

In the agronomic evaluation of new lineages of lettuce, several characteristics must be considered, such as plant structure, productivity, number of leaves, leaf shape, and color of plants. A coloring that best pleases and attracts the attention of the final consumer, as well as longer shelf life, is of great importance to always be meeting the needs of an increasingly dynamic and demanding market.

The type of cultivation must be viable. Hydroponic lettuce has been preferred due to the possibility of cultivation in all seasons of the year, in a limited space, and with excellent quality in the final product (Menegaes et al. 2015; Silva et al. 2015).

Hydroponics offers advantages in reducing the effects of seasonality, rain particles, and soil movement. Production conditions are close to ideal, even in places where there is no fertile ground since growth is stimulated by nutritious solutions (Luz et al. 2006).

Similar studies, using other varieties and lineages, can be found in the literature. Vicentini-Polette et al. (2018) evaluated the characteristics and acceptance of Crocantela, Crespa Crocante (SVR-2005), and Vanda lettuces, grown in a hydroponic system. According to the authors, Vanda lettuce showed high acceptance due to its flavor (high content of soluble solids), and for its light color and aroma, in comparison to the others. Longatti et al. (2014) evaluated the agronomic performance of thirteen different curly lettuces, both commercial and new strains, under cultivation conditions in an NFT hydroponic system under a red screen (30%). Santos et al. (2010) evaluated the use of brackish groundwater in the hydroponic cultivation of two varieties of lettuce (Vera and AF-1743), concluding that the increase in salinity led to a linear and equivalent reduction in the growth of lettuce plants, regardless of the variety evaluated. Sanches et al. (2005) evaluated two varieties of lettuce (Lucy-Brown and Sandy), grown in NFT hydroponic system with or without cooling the nutrient solution. According to the authors, a larger number of leaves and a larger head diameter were observed in plants grown with the nutrient solution at room temperature. Vital et al. (2002) evaluated the influence of three nutritious hydroponic solutions on four lettuce varieties (Cinderella, Elizabeth, Mônica, and Princesa) regarding the shoot and root mass. According to the authors, the cultivars showed different behavior according to the solution employed.

This study aimed to evaluate the agronomic characteristics, color, and preference of curly lettuce (six lineages and two commercial cultivars) in hydroponic cultivation system.

## **2. Material and Methods**

### **Description of experimental location, design, and structure**

This study was carried out under hydroponic conditions in a protected environment under a red screen (35%), from December 2016 to February 2017 in the experimental area of the horticulture sector at the Center for Agricultural Sciences (CCA) in Federal University of São Carlos (UFSCar) in the city of Araras, São Paulo State, Brazil (latitude of 22°18'00", longitude of 47°23'03" and altitude of approximately 611 m above mean sea level). Six lineages of F8 lettuce from the UFSCar breeding program were used: 6601-1A, 6601-2L, 7016-6A, 7119-1B, 7223-1A, 7224-4A, and two commercial cultivars: Brida (Hortec<sup>®</sup>, Brazil) and Vanda (Sakata<sup>®</sup>, Brazil).

The eight types of curly lettuce (6601-1A, 6601-2L, 7016-6A, 7119-1B, 7223-1A, 7224-4A, Brida, and Vanda) were grown in an NFT (Nutrient Film Technique) hydroponic system, in a completely randomized blocks design with four replications, each consisting of 35 plants.

Four benches (each one with 12 m long x 2.1 m wide) were used. Seven polyethylene profiles (75 mm in diameter) were used per bench, installed with a 3% slope, using 0.30 m x 0.30 m spacing between profiles and plants.

A plastic tank (4,000 L capacity) to storage the nutrient solution and a pump (0.5 horsepower) to inject the solution into of all hydroponic profiles simultaneously were used. The nutrient solution was circulated every 15 min from 6:00 am to 6:00 pm, and 15 min every 4 h from 6:00 pm to 6:00 am. The flow rate of the solution was adjusted to 1.8 L min<sup>-1</sup> per profile.

## Crop conduction and nutrient solution management

On 12/30th/2016, seeds of lettuce were sown in 200-cell trays, using coconut fiber substrate (Amafibra®, Brazil). In the first 23 days after sowing (DAS), the seedlings were manually irrigated using just water. After this period, the seedlings were transferred to the intermediate cultivation system (NFT hydroponic system), where they remained for 10 days, and were transplanted to the final cultivation system, remaining for 23 days.

Furlani et al. (1999) proposed both in the intermediate and final cultivation system, the nutrient solution used for leafy vegetables. The control of both the pH and the electrical conductivity of the nutrient solution were carried out and maintained daily from 5.5 to 6.5 and 1.5 dS m<sup>-1</sup>, respectively.

## Variables evaluated

The evaluations of agronomic characteristics, instrumental, and sensory color were performed at 23 days after transplanting (53 DAS).

## Growth and production of the lettuce

For the evaluations, five plants were randomly collected from the center of each plot, to determine: shoot length (ShL, in cm), root length (RL, in cm), stem length (StL, in cm), number of leaves (NL, units), stem diameter (StD, in mm), plant diameter (PD, in cm), shoot fresh mass (SFM) (g plant<sup>-1</sup>) and root fresh mass (RFM) (g plant<sup>-1</sup>). NL was counted, detaching the leaves of the plant stem, and counting the ones with a size equal to or greater than 5 cm. StD was performed with the aid of a caliper. PD was performed with the aid of a measuring tape allocated in the middle part of the shoot. The SFM and RFM were performed with the aid of precision scale (Milesimal Model, Bel Engineering, Italy).

## Instrumental color analysis

The leaves chlorophyll content was analyzed using the Chlorophyll Meter SPAD-502 equipment (Konica Minolta Sensing, Tokyo, Japan). Three plants per plot were evaluated, analyzing two leaves from the median region of the plant.

The instrumental color was evaluated using the Hunterlab colorimeter. The values of luminosity (L), hue, and chroma were recorded. The L value varies from black (L = 0) to white (L = 100). The hue or color angle shows the location of the color in the diagram, which ranges from 0 to 360°, with the 0° and 360° angles corresponding to the red color parameter; the 90° angle where the yellow is located; 180° corresponding to green; and 270° where the blue is located. The intermediate angles refer to the combinations of these colors. The chroma indicates the intensity of the color and is the distance from the center to the end of the diagram, which varies from 0 to 60. The results were expressed in luminosity (L), hue, or color angle (°h), and the chroma (C) (Minolta 2007). One plant of each lettuce per plot was analyzed, where a leaf in the apical region, a leaf in the median region, and a leaf in the basal region of each plant were evaluated.

## Sensory evaluation

Sensory tests were performed in individual booths using white light, with 26 untrained evaluators, consumers of leafy vegetables. Lettuces were served on coded plastic plates. The evaluators received two whole leaves of each treatment at room temperature.

For the sensory evaluation, the difference and preference ordination test was used (ascending order: color, brightness, crunchiness, and preference) (ABNT 1994).

## Statistical analysis

The data (growth, production, and instrumental color) were subjected to analysis of variance (ANOVA) by F test, and the means were compared by the Scott-Knott test at 0.05 probability level using the AgroEstat software (Barbosa and Maldonado Junior 2015). The interpretation of the results obtained in the sensory

ordering tests was carried out according to the Friedman test (Newell and MacFarlane 1987), with the sum differences equal to or greater than 54 considered significant.

Principal component analysis (PCA) and hierarchical cluster analysis also were performed using the Statistical Analysis Software (SAS 2005).

### 3. Results and Discussion

#### Growth and production

Except for the root fresh mass, the other characteristics (shoot length, root length, stem length, number of leaves, plant diameter, stem diameter, shoot fresh mass and root fresh mass) were significantly affected ( $p \leq 0.05$ ) by different types of curly lettuce (Table 1).

**Table 1.** Mean values of shoot length (ShL, cm), root length (RL, cm), stem length (StL, cm), number of leaves (NL, units), stem diameter (StD, mm), plant diameter (PD, cm), shoot fresh mass (SFM, g plant<sup>-1</sup>) and root fresh mass (RFM, g plant<sup>-1</sup>) of curly lettuce in the hydroponic cultivation.

Parameters Lettuce	ShL	RL	StL	NL	StD	PD	SFM	RFM
6601-1A	22.16 <sup>b</sup>	35.13 <sup>b</sup>	6.41 <sup>d</sup>	30.56 <sup>a</sup>	25.34 <sup>b</sup>	25.94 <sup>b</sup>	334.75 <sup>b</sup>	61.75 <sup>a</sup>
7119-1B	16.31 <sup>d</sup>	35.63 <sup>b</sup>	6.90 <sup>d</sup>	24.00 <sup>b</sup>	25.87 <sup>a</sup>	26.44 <sup>b</sup>	279.50 <sup>c</sup>	57.63 <sup>a</sup>
7223-1A	20.00 <sup>c</sup>	47.63 <sup>a</sup>	9.44 <sup>c</sup>	23.87 <sup>b</sup>	26.22 <sup>a</sup>	28.11 <sup>a</sup>	338.25 <sup>b</sup>	67.63 <sup>a</sup>
6601-2L	29.38 <sup>a</sup>	32.19 <sup>c</sup>	26.78 <sup>a</sup>	26.81 <sup>b</sup>	24.76 <sup>b</sup>	24.97 <sup>b</sup>	273.13 <sup>c</sup>	49.38 <sup>a</sup>
7224-4A	22.53 <sup>b</sup>	39.00 <sup>b</sup>	13.88 <sup>b</sup>	26.31 <sup>b</sup>	24.80 <sup>b</sup>	26.70 <sup>b</sup>	331.50 <sup>b</sup>	64.13 <sup>a</sup>
7016-6A	23.97 <sup>b</sup>	26.78 <sup>c</sup>	10.70 <sup>c</sup>	27.06 <sup>b</sup>	24.19 <sup>b</sup>	28.19 <sup>a</sup>	378.63 <sup>a</sup>	65.13 <sup>a</sup>
Brida	20.13 <sup>c</sup>	30.38 <sup>c</sup>	7.19 <sup>d</sup>	26.56 <sup>b</sup>	24.43 <sup>b</sup>	27.21 <sup>b</sup>	317.63 <sup>b</sup>	66.63 <sup>a</sup>
Vanda	21.31 <sup>c</sup>	23.94 <sup>c</sup>	9.84 <sup>c</sup>	28.56 <sup>a</sup>	27.39 <sup>a</sup>	29.32 <sup>a</sup>	399.44 <sup>a</sup>	68.63 <sup>a</sup>
CV (%)	6.09	13.00	14.16	7.97	4.5	4.26	8.41	14.61
SD	1.34	4.40	1.61	2.13	1.14	1.15	27.89	9.15

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 0.05 probability level; CV - coefficient of variation; SD - standard deviation.

For the shoot length, lineage 6601-2L obtained a significantly higher value (29.38 cm) than the other lineages and commercial cultivars Brida and Vanda (Table 1). This result may be due to its bolting. Early bolting is an undesirable characteristic in lettuce since the plants lose their commercial value. According to Luz et al. (2009), when the plant is early bolting the vegetable becomes unfit for consumption, as there is latex production that gives the leaves a bitter taste. In the present study, among lettuces that did not bolt, the lineages 7016-6A, 7224-4A, 6601-1A, and cultivar Vanda showed the best performance for shoot length (Table 1).

The root length is an important characteristic to be evaluated in lettuce since it can influence the efficiency in the supply of water and nutrients to the plant. The lineage 7223-1A obtained higher root length (47.63 cm) compared to the other lineages and commercial cultivars Brida and Vanda (Table 1).

Plants with shorter stem length are more adapted to climatic conditions in Brazil, which is a characteristic highly valued by breeders in the selection of lettuce, and this is a vitally important attribute in the selection of a material (lineage or cultivars) since larger stems are undesirable due to the possibility of bolting (Oliveira et al. 2004). It was found that lineage 6601-1A showed the best result for stem length (6.41 cm), not significantly differing from lineage 7119-1B (6.90 cm) and cultivar Brida (7.19 cm). The lettuce with the longest stem (26.74 cm) was the lineage 6601-2L, being the only one that presented early bolting, significantly differing from the other lettuce types (Table 1). In a study with six commercial lettuce cultivars grown in a hydroponic system in summer and winter, Sediya (2009) obtained stem length values in the summer ranging between 6.4 and 14.6 cm and in the winter between 4.8 and 7.6 cm, showing a correlation between plant temperature and bolting.

For the number of leaves, the higher values (30.56 and 28.56 leaves) were obtained for lineage 6601-1A and cultivar Vanda, respectively (Table 1). For the production of lettuce in the curly segment, the number of leaves is an important characteristic, and it is associated with the temperature of the growing

environment and the photoperiod, and as the flower tassel emits, there is a reduction in the number of leaves (Oliveira et al. 2004; Longatti et al. 2014).

Regarding the stem diameter of lettuce, where plants with a larger diameter are sought to facilitate the detachment of the plants stem leaves (Blind and Silva Filho 015), the lineages 7119-1B and 7223-1A, and cultivar Vanda presented highest stem diameter, without significant difference between them ( $p>0.05$ ), in the order of 25.87, 26.22 and 27.39 cm, respectively (Table 1).

The largest diameters of the plants were found for lineages 7223-1A (28.11 cm) and 7016-6A (28.19 cm) and cultivar Vanda (29.32 cm). Usually, the diameter is related to the fresh mass content, when also were obtained larger production of shoot fresh mass for cultivar Vanda (399.44 g plant<sup>-1</sup>) and lineage 7016-6A (378.63 g plant<sup>-1</sup>) (Table 1). These results show the superiority of the cultivar Vanda, this also because of the larger number of leaves. Vanda is the best-selling curly lettuce in the country.

Differently of results of the present study, when was registered plant diameter of 27.23 cm for cultivar Brida, under hydroponic cultivation Longatti et al. (2014) obtained a plant diameter of 38.70 cm for the same lettuce cultivar used in the present study. This may be due to the evaluation of lettuces with the greatest number of days after sowing, once the mentioned authors carried out the evaluation with 67 DAS and the present study evaluated with 56 DAS.

Regarding the production of shoot fresh mass obtained for cultivar Vanda (399.44 g plant<sup>-1</sup>), a larger value of shoot fresh mass was found by Lima et al. (2018), who reported production of 439.50 g plant<sup>-1</sup> for the same cultivar at harvest at 29 days after transplanting (DAT), presenting an increase of approximately 10% in relation to the present study at 23 DAT. This is due both to the greater number of days after transplantation and the greater volume of substrate used by Lima (2018) for the production of seedlings, where the seedlings developed in cells of 50 cm<sup>3</sup> and obtained greater development of the root system, against the available 10 cm<sup>3</sup> for the seedlings that were used in this study.

For the root fresh mass, the values obtained ranged from 49.30 to 68.63 g, regardless of lettuce types. Root fresh mass in the same magnitude (68.70 g) was obtained by Longatti et al. (2014) for cultivar Vanda.

### Instrumental color analysis and sensory evaluation

For the results of total chlorophyll in the leaves (SPAD units), there was a significant difference ( $p\leq 0.05$ ) between the lettuce types. A larger value of chlorophyll (32.77 SPAD units) was obtained for lineage 6601-1A, while the lower values of 19.51, 18.57, and 18.98 SPAD units were obtained for lineage 7016-6A and cultivars Vanda and Brida, respectively (Table 2).

The SPAD measures the amount of chlorophyll in the plant, a pigment that gives them a green color. The greater the amount of chlorophyll present in the tissues, the greater the green shade presented in the plant (Minolta 2009). Plants that have a more intense green color during the shelf period will increase their attractiveness and, consequently, prolong their time of acceptance by the consumer, making the color attribute one of the decisive factors that will make the consumer choose to purchase a plant or not.

**Table 2.** Mean values of chlorophyll (SPAD units), luminosity, hue, and chroma of curly lettuce in the hydroponic cultivation.

Lettuce Parameters	6601-1A	7119-1B	7223-1A	6601-2L	7224-4A	7016-6A	Brida	Vanda	CV (%)	SD
Chlorophyll	32.77 <sup>a</sup>	28.17 <sup>b</sup>	25.88 <sup>c</sup>	24.64 <sup>c</sup>	21.74 <sup>d</sup>	19.51 <sup>e</sup>	18.98 <sup>e</sup>	18.57 <sup>e</sup>	4.97	1.18
Luminosity	47.63 <sup>a</sup>	50.88 <sup>a</sup>	52.62 <sup>a</sup>	50.63 <sup>a</sup>	48.22 <sup>a</sup>	52.68 <sup>a</sup>	51.83 <sup>a</sup>	56.70 <sup>a</sup>	3.86	1.47
Hue angle	109.43 <sup>b</sup>	108.87 <sup>b</sup>	108.76 <sup>b</sup>	108.70 <sup>b</sup>	108.81 <sup>b</sup>	108.03 <sup>b</sup>	111.00 <sup>a</sup>	107.85 <sup>b</sup>	1.02	0.97
Chroma	38.73 <sup>a</sup>	37.80 <sup>a</sup>	39.14 <sup>a</sup>	40.08 <sup>a</sup>	39.13 <sup>a</sup>	39.93 <sup>a</sup>	37.65 <sup>a</sup>	39.00 <sup>a</sup>	5.26	0.55

Means followed by the same letter in the line do not differ statistically by the Scott-Knott test at 0.05 probability level; CV - coefficient of variation; SD - standard deviation.

For luminosity and chroma, there was no significant difference ( $p>0.05$ ) between lettuce types (Table 2).

The hue angle shows us the color of the material. According to Minolta (2007), the green color is detected at angles greater than 90 and lesser than 180, ranging from green-yellow to blue-green. Although

there is a significant difference ( $p \leq 0.05$ ) between Brida lettuce and the others, the angles observed in this study are in the light green range of the scale, as expected.

The color of lettuces can vary for different reasons. McWatters et al. (2002) found in iceberg lettuces a range of 110 to 116 °hue in one system replicate, and 122 and 126 °hue in another. In curly lettuce, Fontana et al. (2018) found between 114.7 and 115.7 °hue, values very close to those obtained in this study.

Sensory evaluation showed that the evaluators indicated the lineages 6601-1A, 7016-6A and 7119-1B, and commercial cultivars Vanda and Brida as the lettuces with darker green color; while the lineages 6601-2L, 7223-1A, and 7224-4A were indicated with lighter green color (Table 3).

**Table 3.** Sum results for sensory attributes evaluated in the difference and preference ordering sensory test of lettuce lineages and cultivars.

Lettuce Parameters	6601-1A	7119-1B	7223-1A	6601-2L	7224-4A	7016-6A	Brida	Vanda
Green color	130 <sup>b</sup>	142 <sup>b</sup>	76 <sup>a</sup>	71 <sup>a</sup>	95 <sup>a</sup>	130 <sup>b</sup>	138 <sup>b</sup>	154 <sup>b</sup>
Brightness	129 <sup>a</sup>	104 <sup>a</sup>	117 <sup>a</sup>	126 <sup>a</sup>	119 <sup>a</sup>	112 <sup>a</sup>	121 <sup>a</sup>	108 <sup>a</sup>
Crunchiness	145 <sup>a</sup>	111 <sup>a</sup>	119 <sup>a</sup>	123 <sup>a</sup>	114 <sup>a</sup>	100 <sup>a</sup>	127 <sup>a</sup>	97 <sup>a</sup>
Preference	119 <sup>a</sup>	115 <sup>a</sup>	149 <sup>a</sup>	116 <sup>a</sup>	130 <sup>a</sup>	104 <sup>ab</sup>	117 <sup>a</sup>	86 <sup>b</sup>

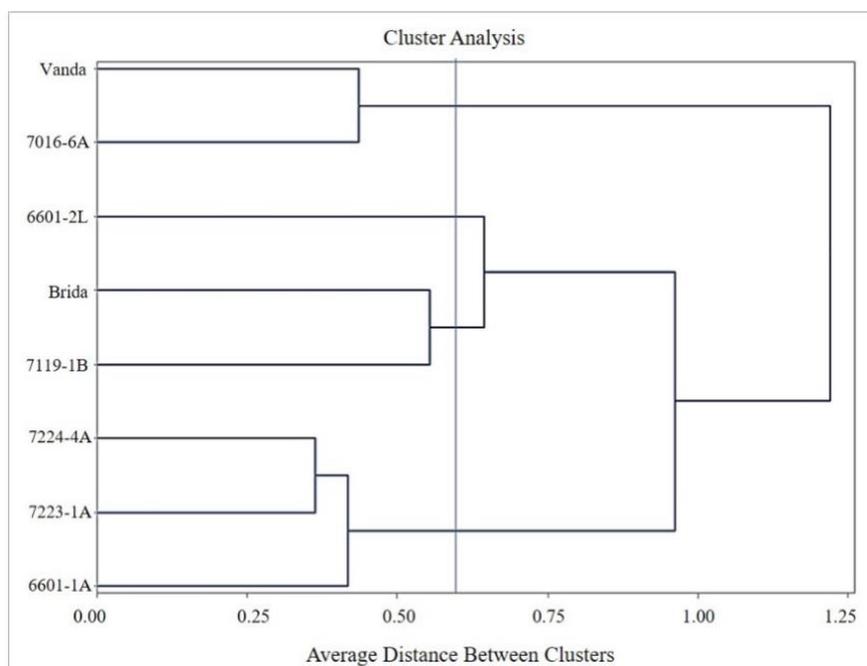
Values followed by the same letters in the line do not differ significantly ( $p \geq 0.05$ ). Minimum difference  $\geq 54$ .

The brightness and crunchiness attributes of the lettuces did not differ between treatments ( $p > 0.05$ ). Vicentini-Polette et al. (2018) reported that lettuces with a sweet taste are usually preferred, which is directly related to the chemical composition of the leaves, especially the content of soluble solids. In addition, the light green color may be related to the perception of a sweeter taste.

Cultivar Vanda and lineage 7016-6A were the least preferred, and the others showed no significant difference ( $p > 0.05$ ) among those evaluated in this study (Table 3).

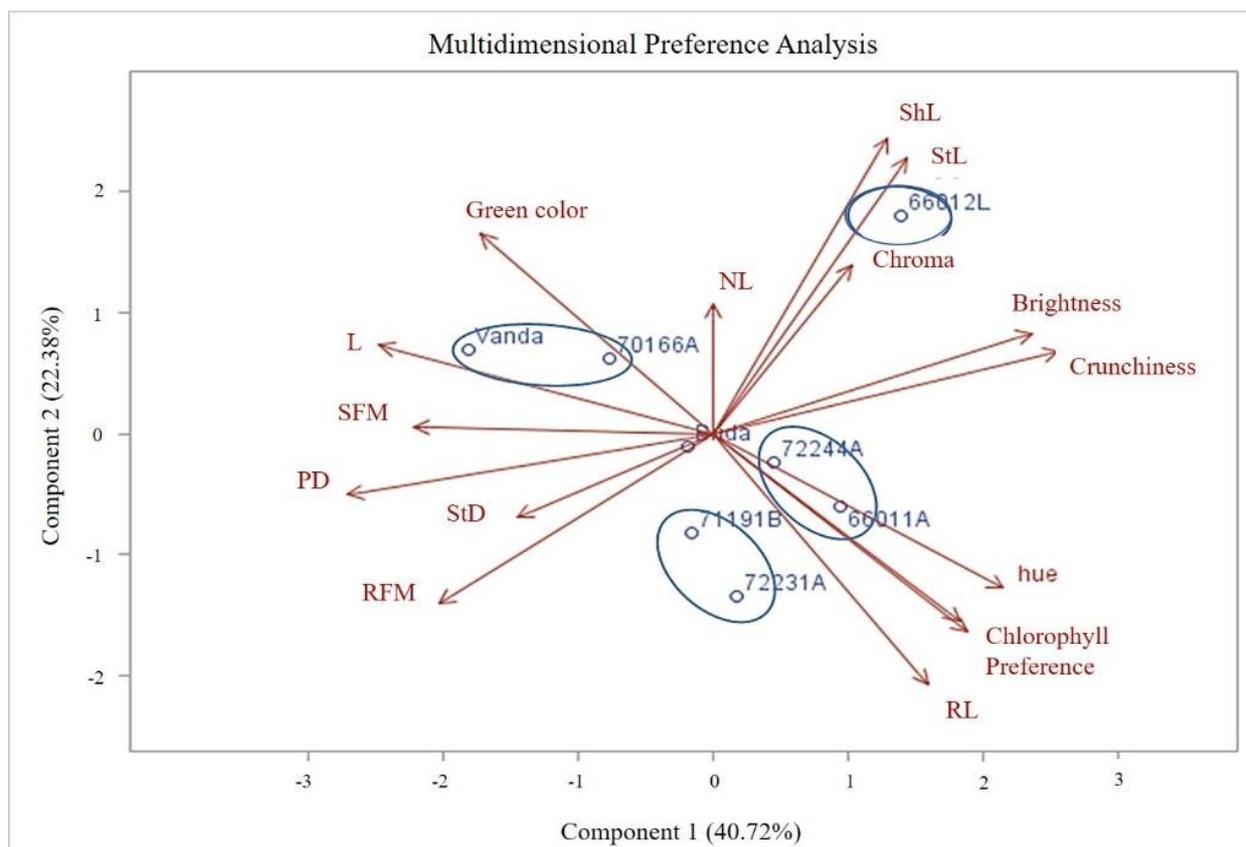
### Multivariate analysis

To assess the interdependence between agronomic, physicochemical, and sensory variables, the correlation matrix was calculated from self-scaled data. From this matrix, the cluster analysis was obtained which divides the set of lettuces (lineages and cultivars) into four groups: the first represented by the cultivar Vanda and lineage 7016-6A; the second represented by lineage 6601-2L; the third group by lineage 7119-1B and cultivar Brida; and the fourth by lineages 7224-4A, 7223-1A, and 6601-1A (Figure 1).



**Figure 1.** Cluster analysis of treatment groups according to agronomic, physicochemical, and sensory characteristics.

The clusters mentioned in Figure 1 can be explained by the variables that characterize them, that is, the results of the cultivar Vanda and lineage 7016-6A (Group 1) are directed to the variables luminosity and sensory green color; lineage 6601-2L (Group 2), is strongly characterized by the variables StL, ShL and chroma; the grouping of lineages 7224-4A, 7223-1A, 6601-1A and 7119-1B (Group 3) can be explained by the variables preference, chlorophyll (Cl), RL and hue. The variables RL and FSM also influence the characterization of lineages 7119-1B and 7223-1A (Group 4). The other variables, brightness, crispness, PD, and SFM, although they are parameters of high score in the graph, do not present expressive characterizations representative of the studied samples. The cultivar Brida, with a low value in its vector, suffered a less important contribution from the studied variables (Figure 2).



**Figure 2.** Principal Component Analysis of agronomic, physicochemical, and sensory parameters for the characterization of lettuce of different lineages and cultivars. ShL - shoot length; RL - root length; StL - stem length; PD - plant diameter; StD - stem diameter; SFM - shoot fresh mass; RFM – root fresh mass; NL - number of leaves; Cl = chlorophyll.

#### 4. Conclusions

There was a great variation in the agronomic and sensory characteristics of the lettuces studied. Such variation may be due to different situations, such as harvest time and bolting. The lettuce with early bolting also showed the lowest performance among the evaluated lettuces, in relation to shoot fresh mass.

Although it was not possible to establish a direct relationship between agronomic properties and preference among lettuces when evaluating the interdependence between agronomic, physicochemical, and sensory variables, the formation of four distinct groups was observed, and the cultivar Brida presented the lowest value in its vector, with the lowest contribution of the studied variables.

This study collaborates with the characterization of different lettuces that are part of the daily human diet. It is suggested that, in future studies, such results should be compared to the physicochemical characteristics of lettuces, seeking to identify the attributes with the greatest impact for the preference of the vegetable consumer.

**Authors' Contributions:** de SÁ, J.J.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; VICENTINI-POLETTE, C.M.: analysis and interpretation of data, drafting the article; SPOTO, M.H.F.: acquisition of data, analysis and interpretation of data; VERRUMA-BERNARDI, M.R.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; SALA, F.C.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article. All authors have read and approved the final version of the manuscript.

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**Ethics Approval:** Not applicable.

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