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Vegetation Indices for Predicting the Growth and Harvest Rate of Lettuce

Ana Luisa Alves Ribeiro [1], Gabriel Mascarenhas Maciel [2,*], Ana Carolina Silva Siquieroli [3], José Magno Queiroz Luz [4], Rodrigo Bezerra de Araujo Gallis [5], Pablo Henrique de Souza Assis [6], Hugo César Rodrigues Moreira Catão [4] and Rickey Yoshio Yada [7]

1 Postgraduate Program in Agronomy, Institute of Agrarian Sciences, Federal University of Uberlândia, Uberlândia 38410-337, Brazil; analuisaribeiro@ufu.br
2 Institute of Agrarian Sciences, Federal University of Uberlândia, Monte Carmelo 38500-000, Brazil
3 Institute of Biotechnology, Federal University of Uberlândia, Monte Carmelo 38500-000, Brazil; carol@ufu.br
4 Institute of Agrarian Sciences, Federal University of Uberlândia, Uberlândia 38410-337, Brazil; jmagno@ufu.br (J.M.Q.L.); hugo.catao@ufu.br (H.C.R.M.C.)
5 Institute of Geography, Federal University of Uberlândia, Monte Carmelo 38500-000, Brazil; rodrigogallis@ufu.br
6 Postgraduate Program in Agriculture and Geospatial Information, Institute of Agrarian Sciences, Federal University of Uberlândia, Monte Carmelo 38500-000, Brazil; pablohnrqsa@gmail.com
7 Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6T 1Z4, Canada; r.yada@ubc.c
* Correspondence: gabrielmaciel@ufu.br

Abstract: Urbanization has provided greater demand for food, and the search for strategies capable of reducing waste is essential to ensure food security. Lettuce (*Lactuca sativa* L.) culture has a short life cycle and its harvest point is determined visually, causing waste and important losses. Using vegetation indices could be an important alternative to reduce errors during harvest definition. The objective of this study was to evaluate different vegetation indices to predict the growth rate and harvest point of lettuce. Twenty-five genotypes of biofortified green lettuce were evaluated. The Green Leaf Index (GLI), Normalized Green Red Difference Index (NGRDI), Spectral Slope Saturation Index (SI), and Overall Hue Index (HUE) were calculated from images captured at 1, 8, 18, 24, and 36 days after transplanting (vegetative state). The diameter and average leaf area of plants were measured using QGIS software. Green mass, number of leaves, and plant and stem diameter were measured in the field. The means were compared using the Scott–Knott test ($p \leq 0.05$) and simple linear regression models were generated to monitor the growth rate, obtaining R^2 values ranging from 62% to 99%. Genetic dissimilarity was confirmed by the multivariate analysis presenting a cophenetic correlation coefficient of 88.49%. Furthermore, validation between data collected in the field versus data obtained by imaging was performed using Pearson's correlations and showed moderate to high values. Overall, the vegetation indices SI, GLI, and NGRDI were efficient for monitoring the growth rate and determining the harvest point of different green lettuce genotypes, in attempts to reduce waste and losses. It is suggested that the definition of the harvest point based on vegetation indices are specific for each genotype.

Keywords: food safety; image phenotyping; *Lactuca sativa* L.; vegetables

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1. Introduction

Every strategy to improve food security is of fundamental importance. The interest and importance of these actions have increased, including in the scientific area. It is now a consensus opinion to say that food waste reflects directly on the lack of food and consequently on the price, causing hunger. It is estimated that food production should increase by 30% by 2030 due to population growth [1].

Among the main vegetables, lettuce (*Lactuca sativa* L.) stands out and is present daily in food. Lettuce, which belongs to the Asteraceae family, is considered an annual and herbaceous plant and is among the most popular and consumed vegetables in Brazil and worldwide. More than 1.5 million tons of this crop are produced in Brazil, and its activity is concentrated near the large centers called "green belts" [2,3].

With the country facing the search for a healthier diet, especially post-COVID-19, lettuce cultivation in Brazil has increased considerably. In this context, producers have increased cultivation areas. Despite all the benefits, growing lettuce presents great difficulty in defining the harvesting point, causing significant losses and waste [4], mainly due to the plant presenting a short cycle and early bolting [5].

The main parameter that defines the development of the lettuce crop is the number of leaves [6], and in small and large areas of cultivation, the growth rate and harvest point are identified visually. However, in large plantation areas, producers face difficulties in performing this monitoring, causing significant waste and losses [7]. The use of digital images collected by UAVs can be useful and assist in decision-making. Thus, new strategies to define the harvest point in lettuce are needed.

Image phenotyping has been used to assist in the selection and characterization of quantitative and qualitative variables in specific individuals through non-destructive analyses [8]. Unmanned aerial vehicles (UAVs) with attached cameras and sensors perform analyses and follow the stages of crop development, from the visible electromagnetic spectrum to the infrared spectrum [9]. The cost, time, and labor to obtain information in the field and laboratory are reduced when remote sensing is employed.

Vegetation indices are based on reflectance, and their values vary according to the vegetation cover and its biophysical characteristics [10]. There have been reports of the potential use of images in several plant species [11–16]. In lettuce, vegetation indices are being used to differentiate pigment levels present in the leaves and to estimate leaf area indices using infrared images [17–19]. For eucalyptus cultures, remote sensing has proven efficient at monitoring plantations using vegetation indices. Overall, remote sensing is a low-cost technique and can be applied in large extensions [20]. Studies of the prediction of growth rate and harvest point of vegetables from images have been insufficient.

In this context, the objective of this study was to evaluate different vegetation indices to predict the growth rate and harvest point of lettuce.

2. Materials and Methods

2.1. Genetic Material and Place of Experiment

The experiment was conducted at the Experimental Vegetable Station (18°42′43.19″ S and 47°29′55.8″ O, 873-m altitude) of the Federal University of Uberlândia (UFU), Monte Carmelo campus, Minas Gerais, Brazil.

Twenty-five genotypes were evaluated (Figure 1), with two commercial controls (cv. Grand Rapids and Uberlândia 10,000) and 23 biofortified tropicalized green lettuce lines belonging to the UFU germplasm bank registered with BG α BIOFORT Software [21].

The genotypes employed in this study were derived from seven successive self-fertilizations between the cultivars PIRA 72 and Uberlândia 10,000 from 2013 to 2018. The seedlings were produced in expanded polyethylene trays with 200 cells filled with coconut fiber commercial substrate. Transplanting was performed when the lettuce plants presented four definitive leaves. The seedlings were transferred to 1.3-m beds fabricated with a rotary bed former.

The following physical and chemical characteristics of the soil were assessed: clayey texture (>50%); pH in $CaCl_2$ = 4.9; Ca = 3.3 $cmol_c$ dm^{-3}; Mg = 1.3 $cmol_c$ dm^{-3}; H + Al = 4.9 $cmol_c$ dm^{-3}; SB = 4.90 $cmol_c$ dm^{-3}; SOM = 3.9 dag kg^{-1}; P (rem) = 79.1 mg dm^{-3}; K = 0.29 $cmol_c$ dm^{-3}, CEC = 9.80 $cmol_c$ dm^{-3}; and BS% = 50. Cultivation was performed as recommended for lettuce culture [22]. Climate conditions were monitored daily during the experiment.

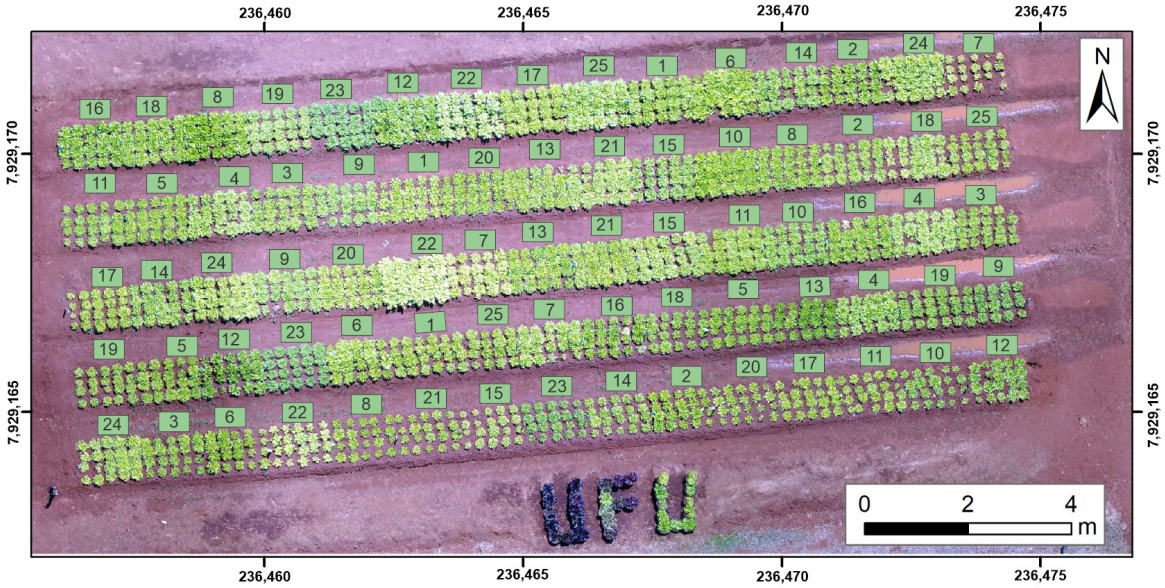

Figure 1. Distribution of green lettuce genotypes in the field. 1: UFU-206#1#6#1; 2: UFU BIOFORT189E8; 3: UFU-197#3#1#1; 4: UFU-125#1#1#1; 5: UFU-7#1#2#1; 6: UFU BIOFORT155E12; 7: UFU BIOFORT120E21; 8: UFU BIOFORT189E22; 9: UFU-197#2#1#1; 10: UFU-199#3#1#1; 11: UFU-206#1#1#1; 12: UFU BIOFORT206E32; 13: UFU BIOFORT197E34; 14: UFU-197#2#2#1; 15: UFU BIOFORT155E39; 16: UFU BIOFORT189E43; 17: UFU-206#1#4#1; 18: UFU-125#2#2#1; 19: UFU-206#1#2#1; 20: UFU BIOFORT189E48; 21: UFU-206#1#5#1; 22: UFU-040#5#5#1; 23: UFU MC BIOFORT; 24: Grand Rapids; and 25: Uberlândia 10,000.

The experiment was conducted in a randomized block design with three repetitions, totaling 75 plots. The plots consisted of 20 plants, with spacing of 0.25 × 0.25 m between plants.

2.2. Acquisition and Processing of Aerial Images

During the execution of the experiment, five flights were performed on different days after transplanting (DAT) (1, 8, 18, 24, and 36 DAT). The aerial images were captured using a Phantom 4 Advanced drone model, with a visible camera (RGB) that had a resolution of 20 megapixels.

Using DroneDeploy software, the flights were performed following the parameters of 20 m in height, 80% longitudinal overlap, and 75% lateral overlap. The orthoimage was generated using Pix4d software. The calculation of the vegetation indices (Table 1) and the image reclassification were performed using R software, version 3.6.3 [23], and the R package FieldImageR [24].

Table 1. Vegetation indices used in the experiment.

Vegetation Indices	Equation	Reference
SI—Spectral Slope Saturation Index	$\frac{R-B}{R+B}$	[25]
HUE—Overall Hue Index	$\text{atan}\left(2 \times \frac{(B-G-R)}{30.5 \times (G-R)}\right)$	[25]
GLI—Green Leaf Index	$\frac{(2 \times G - R - B)}{(2 \times G + R + B)}$	[26]
NGRDI—Normalized Green Red Difference Index	$\frac{G-R}{G+R}$	[27]

G = green band; R = red band; B = blue band

The Overall Hue Index (HUE) was calculated and used to form the mask layer and reclassify the RGB image, excluding the soil. After calculating the Green Leaf Index (GLI), Normalized Green Red Difference Index (NGRDI), and Spectral Slope Saturation Index (SI), the average index for each plot was obtained for all flights.

Growth rate monitoring was performed with non-destructive methods using imagery. In addition to vegetation indices, leaf area in software (LAS) and plant diameter in software (PDS) were extracted using QGIS software, version 3.0, for all flights. PDS was measured using the Raster Calculator tool with six central plants measured.

To obtain LAS, the pixel values in the green band were extracted from the RGB image. Using QGIS software, version 3.0, and the function r. recode, a classification from 1 to 0 could be assigned for the soil and plant, respectively. Thus, the contour of the plants was measured, enabling the calculation of the average leaf area in the respective plots.

2.3. Evaluation of Agronomic Data in the Field

At the commercial point (36 DAT), in addition to capturing images, the green mass (GM) was measured in the field by weighing the leaves, counting the number of leaves (NL), and determining plant diameter (PD) and stem diameter (SD). Six central plants from each plot were used for the evaluations.

2.4. Experimental Flowchart

The methodological steps, including image processing and data analysis, are presented in the experimental flowchart (Figure 2).

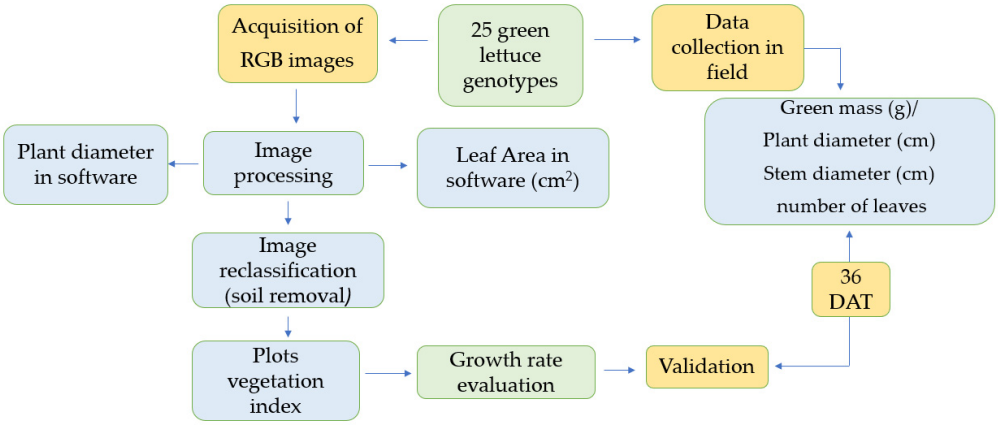

Figure 2. Flowchart of the experiment steps: data collection in the field and information obtained using images.

2.5. Statistical Analysis

The results measured in the field and the data from images extracted in the last flight (36 DAT) were subjected to analysis of variance using the F test ($p \leq 0.05$). Furthermore, means were compared using the Scott–Knott test ($p \leq 0.05$). A dendrogram was obtained through multivariate analysis using the Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA). This method was applied to prove the genetic diversity among the treatments of the experiment. To validate vegetation indices, experiments with demonstrably dissimilar treatments (greater number of branches of the dendrogram) were necessary, increasing the spectrum of efficiency of the indices evaluated [17].

Pearson's correlation between the variables collected in the field and from the images was determined individually between the genotypes (per se), and the significance of the coefficients was verified. Simple linear regression models were generated after the observation of the correlations to monitor the growth rate and the ideal harvest point for the lettuce culture. Graphs were generated for the response variables LAS and PDS and the vegetation indices GLI, NGRDI, and SI at different DAT. Statistical analyses were performed using R [23] and Genes version 1990.2019.91 [28] software.

3. Results

During the experiment, maximum temperatures ranged from 18.8 °C to 32 °C and minimum temperatures ranged from 5.7 °C to 20.9 °C (Figure 3).

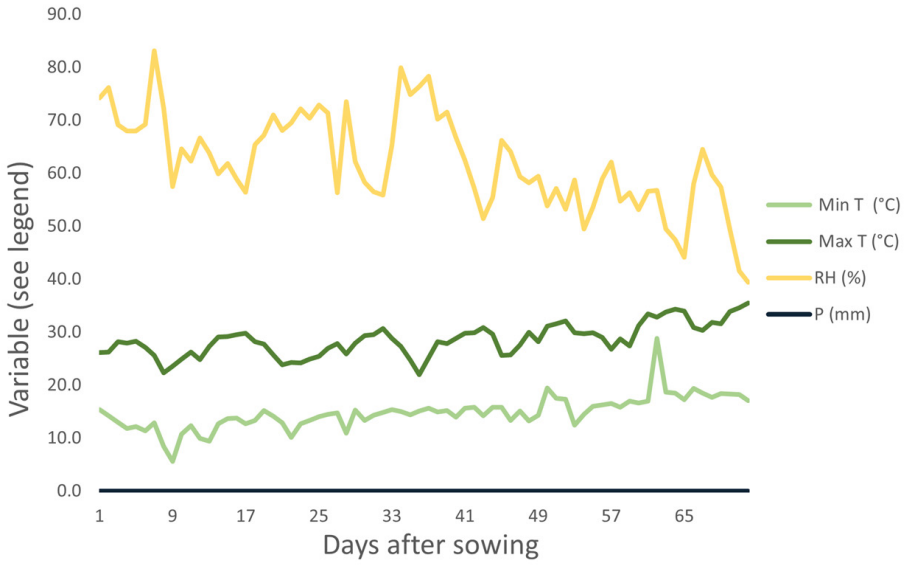

Figure 3. Climate conditions during the execution of the experiment (August and September 2019) in Monte Carmelo, Minas Gerais, Brazil. Humidity (RH), minimum temperature (Min Temp), maximum temperature (Max Temp), and precipitation (P).

3.1. Germplasm Evaluation

The lettuce genotypes differed from one another for the vegetation indices GLI, SI, and NGRDI and the variable NL (Table 2). There were no differences in the characteristics of GM, PD, and SD.

NL was highlighted for the genotypes UFU BIOFORT189E8, UFU-125#1#1#1, UFU-7#1#2#1, UFU BIOFORT155E12, UFU BIOFORT189E22, UFU-197#2#1#1, UFU-199#3#1#1, UFU-206#1#1#1, UFU BIOFORT206E32, UFU BIOFORT197E34, UFU BIOFORT189E43, UFU-125#2#2#1, UFU BIOFORT189E48, UFU-040#5#5#1, and Uberlândia 10,000, the values of which were superior to those of the other genotypes and the commercial control cv. Grand Rapids (Table 2). The increase in NL, compared with that in cv. Grand Rapids, was 45.4% for UFU BIOFORT189E22, 44.9% for UFU BIOFORT189E43, and 42.35% for UFUBIOFORT197E34.

The genotypes showed different behaviors ($p \leq 0.05$) among the vegetation indices SI, GLI, and NGRDI (Table 3). UFU-206#1#6#1, UFU-125#1#1#1, UFU BIOFORT120E21, UFU-197#2#1#1, UFU BIOFORT155E39, UFU BIOFORT189E48, UFU-206#1#5#1, and UFU-040#5#5#1 did not differ from genotype Uberlândia 10,000 or the commercial cultivar Grand Rapids in the vegetation index SI.

For GLI, the genotypes UFU BIOFORT189E8, UFU-197#3#1#1, UFU-7#1#2#1, UFU BIOFORT155E12, UFU BIOFORT189E22, UFU-199#3#1#1, UFU BIOFORT206E32, UFU BIOFORT197E34, and UFU BIOFORT189E43 were superior to the others. Regarding NGRDI, the genotypes UFU-7#1#2#1, UFU BIOFORT189E22, UFU-199#3#1#1, UFU BIOFORT206E32, UFU BIOFORT197E34, UFU-197#2#2#1, UFU BIOFORT189E43, and UFU MC BIOFORT1 showed average increments of 24%, 14.1%, 20%, 35.8%, 28.2%, 11.17%, 28.8%, and 26.47%, respectively, which were all higher than the readout for the commercial cv. Grand Rapids (Table 3).

Table 2. Means for the data collected in the field. Green mass (GM), plant diameter (PD), stem diameter (SD), and number of leaves (NL) in the green lettuce genotypes.

Genotype	MV (g)		DP (cm)		DH (cm)		NF
	$\bar{x}$	σ	$\bar{x}$	Ơ	$\bar{x}$	σ	$\bar{x}$
UFU-206#1#6#1	178.8	±84.0	25.3	±3.11	1.84	±0.22	23.3 b
UFU BIOFORT189E8	163.9	±31.8	24.5	±1.02	2.12	±0.18	26.5 a
UFU-197#3#1#1	197.1	±22.3	26.3	±0.86	1.96	±0.11	22.2 b
UFU-125#1#1#1	195.8	±8.17	28.3	±0.71	2.12	±0.08	27.7 a
UFU-7#1#2#1	154.3	±34.8	25.5	±1.42	2.12	±0.11	29.3 a
UFU BIOFORT155E12	154.3	±38.1	24.5	±2.14	2.00	±0.16	28.6 a
UFU BIOFORT120E21	157.5	±60.3	24.8	±4.60	1.86	±0.51	17.8 b
UFU BIOFORT189E22	181.8	±86.8	27.6	±3.33	2.00	±0.20	33.3 a
UFU-197#2#1#1	168.6	±32.2	25.1	±2.35	1.63	±0.34	29.5 a
UFU-199#3#1#1	132.2	±45.0	25.4	±1.68	1.94	±0.40	28.5 a
UFU-206#1#1#1	180.8	±76.4	24.6	±1.72	1.65	±0.27	27.7 a
UFU BIOFORT206E32	114.9	±51.5	24.5	±2.62	1.97	±0.38	29.4 a
UFU BIOFORT197E34	120.8	±53.8	25.3	±1.79	2.09	±0.25	32.6 a
UFU-197#2#2#1	167.3	±57.2	25.0	±3.80	1.99	±0.49	25.5 b
UFU BIOFORT155E39	141.2	±66.9	24.0	±3.68	1.50	±0.08	25.5 b
UFU BIOFORT189E43	140.5	±36.5	25.4	±1.96	1.87	±0.24	33.2 a
UFU-206#1#4#1	180.2	±60.0	27.0	±2.54	2.16	±0.22	23.0 b
UFU-125#2#2#1	191.1	±67.2	26.9	±3.43	2.09	±0.18	28.0 a
UFU-206#1#2#1	134.3	±45.5	25.3	±1.36	1.78	±0.08	22.0 b
UFU BIOFORT189E48	161.3	±57.2	28.7	±2.45	2.30	±0.39	27.2 a
UFU-206#1#5#1	160.9	±64.4	25.1	±3.70	1.78	±0.27	23.5 b
UFU-040#5#5#1	139.3	±41.1	26.4	±0.49	1.91	±0.12	27.0 a
UFU MC BIOFORT1	105.8	±32.8	22.7	±2.03	1.77	±0.32	20.0 b
Grand Rapids	189.7	±7.14	26.9	±1.16	1.95	±0.12	22.9 b
Uberlândia 10000	140.8	±59.8	25.1	±2.12	2.14	±0.04	29.6 a
Overall Average	160.9		25.3		1.967		27.25

$\bar{x}$: mean; σ: standard deviation. Averages followed by different letters in the column differ from one another by the Scott–Knott test ($p \leq 0.05$).

Table 3. Means for the data obtained from image analysis. Plant diameter in software (PDS), leaf area in software (LAS), Spectral Slope Saturation Index (SI), Normalized Green Red Difference Index (NGRDI), and Green Leaf Index (GLI) of the green lettuce strains.

Genotype	DPS (cm)		AFS (cm^2)		SI	GLI	NGRDI
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	$\bar{x}$	$\bar{x}$
UFU-206#1#6#1	22.93	±2.70	476.2	±63.52	147.9 a	0.262 b	0.162 b
UFU BIOFORT189E8	20.31	±2.73	433.2	±49.73	135.2 b	0.286 a	0.183 b
UFU-197#3#1#1	20.78	±0.31	432.9	±71.69	132.8 b	0.279 a	0.183 b
UFU-125#1#1#1	18.46	±4.55	487.3	±17.16	151.2 a	0.254 b	0.172 b
UFU-7#1#2#1	18.58	±5.83	462.1	±60.48	122.7 b	0.325 a	0.212 a
UFU BIOFORT155E12	22.81	±1.41	477.6	±88.73	138.5 b	0.286 a	0.175 b
UFU BIOFORT120E21	20.23	±4.12	391.1	±150.2	155.2 a	0.206 b	0.118 b
UFU BIOFORT189E22	20.10	±4.08	453.1	±131.6	134.2 b	0.307 a	0.194 a
UFU-197#2#1#1	20.53	±5.53	445.0	±58.51	146.7 a	0.243 b	0.181 b
UFU-199#3#1#1	20.97	±5.06	448.5	±136.2	132.9 b	0.302 a	0.204 a
UFU-206#1#1#1	20.47	±4.56	424.1	±100.3	134.9 b	0.270 b	0.169 b
UFU BIOFORT206E32	20.24	±4.88	485.3	±93.04	123.5 b	0.309 a	0.231 a
UFU BIOFORT197E34	20.91	±7.13	492.4	±62.31	135.5 b	0.319 a	0.218 a
UFU-197#2#2#1	20.47	±1.88	443.3	±90.66	138.6 b	0.272 b	0.189 a
UFU BIOFORT155E39	20.63	±2.99	431.9	±137.4	148.4 a	0.235 b	0.156 b
UFU BIOFORT189E43	22.33	±2.44	482.1	±69.73	131.3 b	0.317 a	0.219 a
UFU-206#1#4#1	20.35	±4.10	435.6	±108.9	138.7 b	0.263 b	0.156 b

Table 3. *Cont.*

Genotype	DPS (cm)		AFS (cm^2)		SI	GLI	NGRDI
UFU-125#2#2#1	18.88	±6.25	459.9	±98.53	139.5 b	0.272 b	0.177 b
UFU-206#1#2#1	18.49	±4.75	427.8	±78.82	132.4 b	0.257 b	0.180 b
UFU BIOFORT189E48	21.67	±4.26	439.0	±130.5	144.5 a	0.263 b	0.161 b
UFU-206#1#5#1	22.37	±3.10	458.5	±154.6	148.5 a	0.255 b	0.158 b
UFU-040#5#5#1	23.98	±1.73	510.8	±140.2	168.2 a	0.196 b	0.128 b
UFU MC BIOFORT1	20.90	±1.40	442.2	±77.55	136.2 b	0.245 b	0.215 a
Grand Rapids	24.61	±1.08	531.8	±39.36	145.0 a	0.267 b	0.170 b
Uberlândia 10000	23.41	±3.10	456.1	±118.0	144.4 a	0.256 b	0.162 b
Overall Average	21.82		477.56			0.267	0.177

$\bar{x}$: mean; σ: standard deviation. Averages followed by different letters in the column differed from each other by the Scott-Knott test ($p \leq 0.05$).

The indices GLI and NGRDI had approximately 67% similarity in the choice of genotypes with the largest estimates of vegetative development. Both indices selected the genotypes UFU-7#1#2#1, UFU BIOFORT189E22, UFU-199#3#1#1, UFU BIOFORT206E32, UFU BIOFORT197E34, and UFU BIOFORT189E43.

3.2. Genetic Dissimilarity

The dendrogram (UPGMA) obtained by the generalized Mahalanobis distance confirmed the existence of genetic dissimilarity among the genotypes evaluated. The cophenetic correlation coefficient was 88.49%. A cut-off line was drawn at 31.23% dissimilarity, and the formation of four groups was identified (Figure 4).

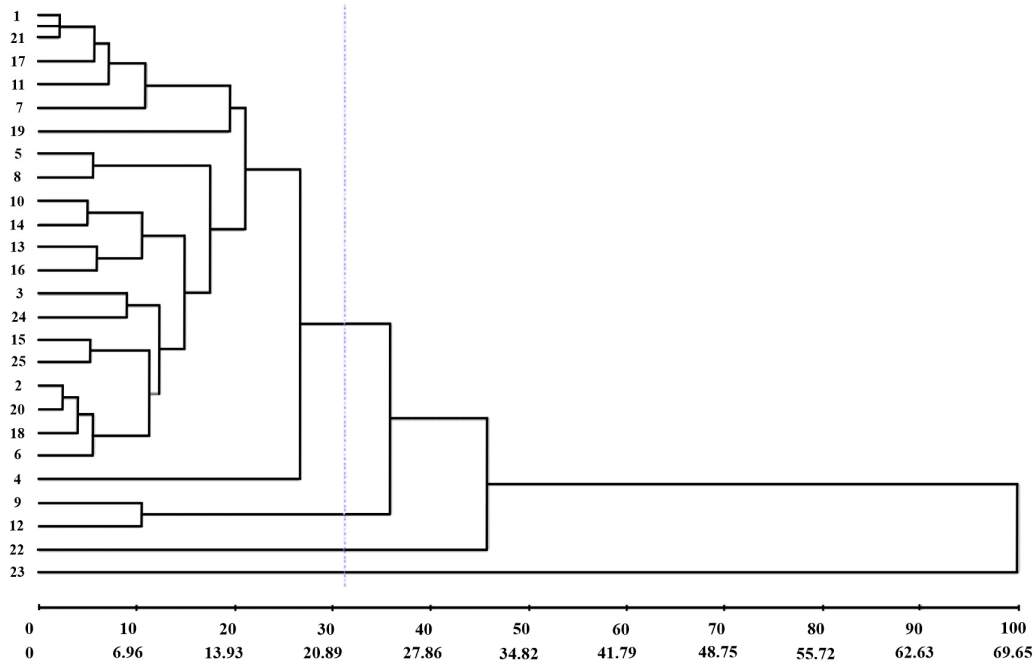

Figure 4. UPGMA dendrogram obtained by a generalized Mahalanobis distance of 25 green lettuce genotypes. 1: UFU-206#1#6#1; 2: UFU BIOFORT189E8; 3: UFU-197#3#1#1; 4: UFU-125#1#1#1; 5: UFU-7#1#2#1; 6: UFU BIOFORT155E12; 7: UFU BIOFORT120E21; 8: UFU BIOFORT189E22; 9: UFU-197#2#1#1; 10: UFU-199#3#1#1; 11: UFU-206#1#1#1; 12: UFU BIOFORT206E32; 13: UFU BIOFORT197E34; 14: UFU-197#2#2#1; 15: UFU BIOFORT155E39; 16: UFU BIOFORT189E43; 17: UFU-206#1#4#1; 18: UFU-125#2#2#1; 19: UFU-206#1#2#1; 20: UFU BIOFORT189E48; 21: UFU-206#1#5#1; 22: UFU-040#5#5#1; 23: UFU MC BIOFORT; 24: Grand Rapids; and 25: Uberlândia 10,000. Purple line: cut-off line at 31.23% dissimilarity.

Group I consisted of 84% of the genotypes analyzed; Group II comprised the genotypes UFU-197#2#1#1 and UFU BIOFORT206E32; and Groups III and IV comprised only one genotype each, UFU-040#5#5#1 and UFU MC BIOFORT1, respectively.

3.3. Monitoring Growth Rate

The acquisition of RGB images using UAVs enabled the monitoring of the growth rate in the lettuce crop over DAT (Figure 5).

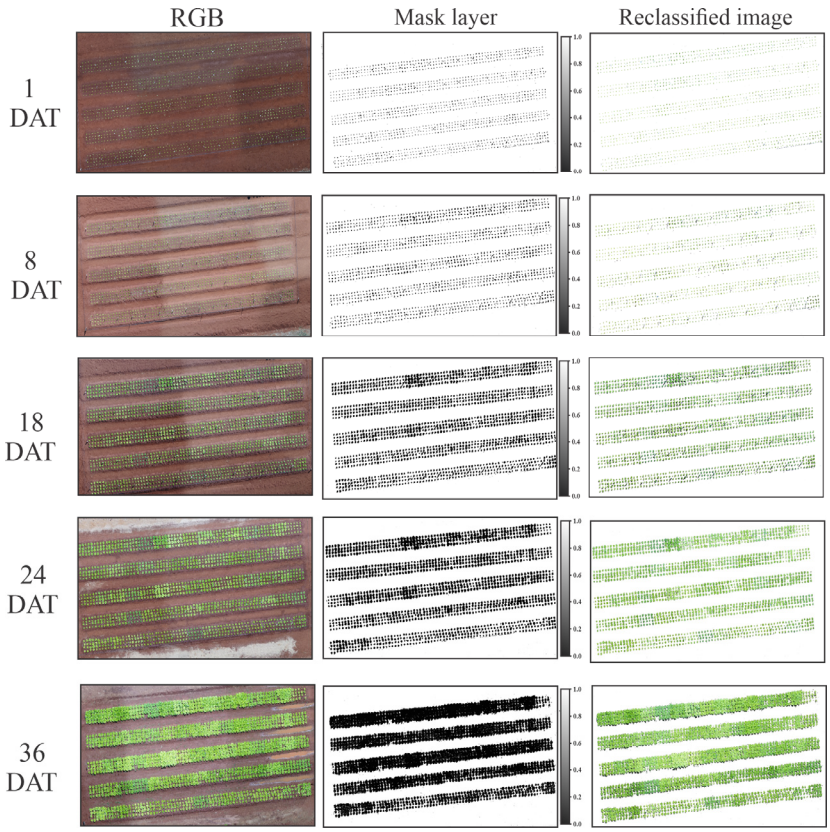

Figure 5. RGB image, mask layer, and reclassified image of the flights obtained by UAVs during the experiment on different days after transplanting (1, 8, 18, 24, and 36 DAT).

The images were reclassified, or the soil was removed in an automated manner using the vegetation indices and the RGB image. This step was performed to obtain better reproducibility and noise reduction of the image (Figure 6). After analysis of the pixel values in the histogram obtained by the vegetation indices, the HUE index enabled the discrimination of the plant and soil, using a cut-off value of 1.5 for the formation of the mask layer.

The evaluation of the PDS and LAS extracted using the images revealed growth over the DAT. The diameter obtained by the QGIS software showed a variation among the genotypes of 5.6 to 9.21 cm in the first flight (DAT 1); 7.86 to 13 cm in the second flight; and 10.6 to 18.56 cm, 11.1 to 21.5 cm, and 11 to 24.6 cm in the third, fourth, and fifth flights, respectively. For LAS, the lowest values were observed for the first flight (DAT 1) and the highest values were found for the fifth flight (DAT 5), which varied from 6.74 cm^2 to 531.8 cm^2 among the genotypes.

Obtaining the LAS and PDS values from the images was efficient. The regression equations presented values of the determination coefficient (R^2) between 78% and 99% for the genotypes evaluated. The vegetation indices SI, GLI, and NGRDI with the PDS and LAS values were coherent for lettuce growth rate, with an increase in the values observed over the flights for the genotypes (Figures 6–8).

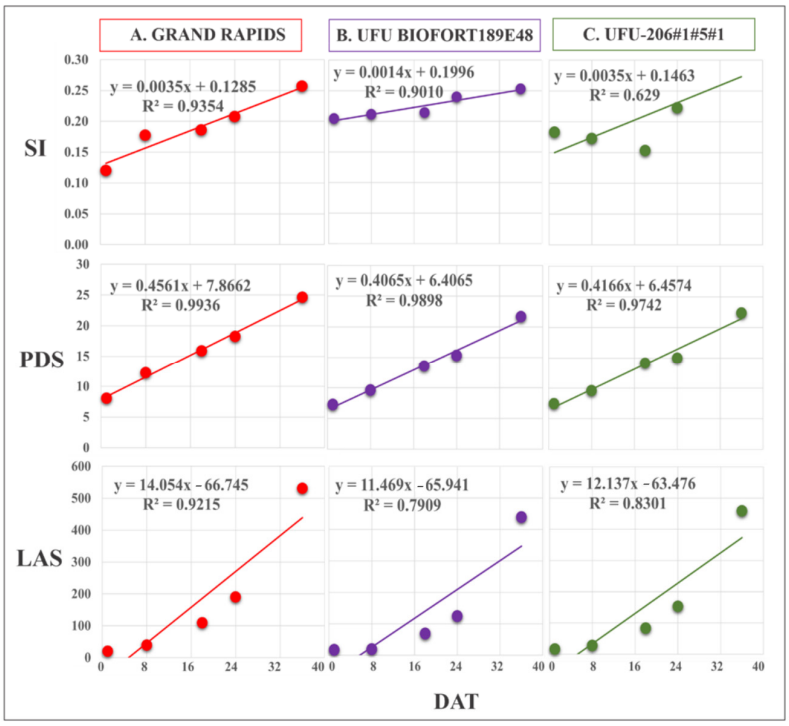

Figure 6. Regression equations of Spectral Slope Saturation Index (SI), leaf area in software (LAS) (cm^2), and plant diameter (PDS) obtained by imaging on different days after transplanting (DAT). The presented genotypes were selected based on their performance for growth rate monitoring using the vegetation index. (**A**) superior (Grand Rapids), (**B**) intermediate (UFU BIOFORT189E4), and (**C**) inferior (UFU-206#1#5#1).

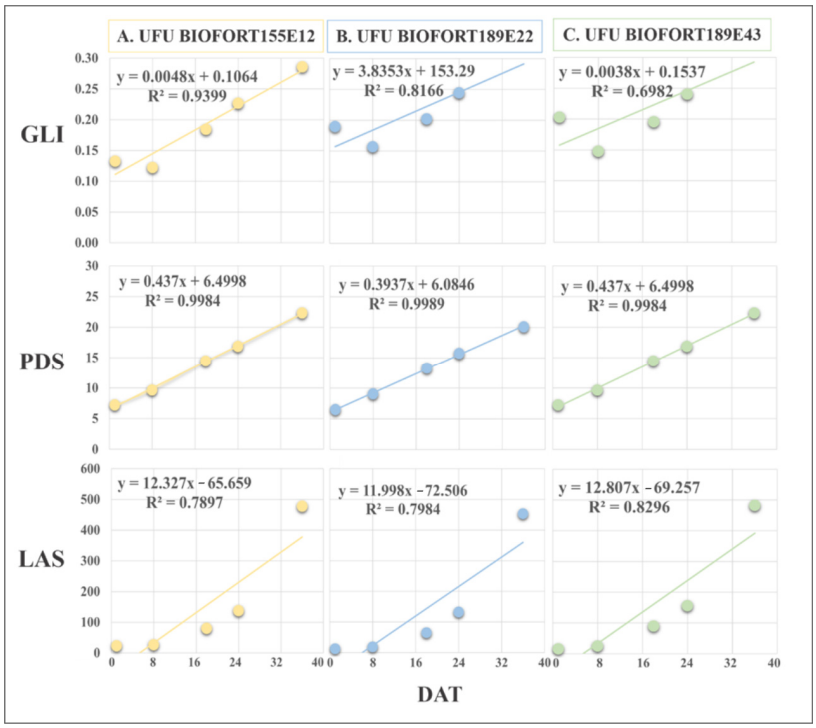

Figure 7. Regression equations of Green Leaf Index (GLI), leaf area in software (LAS) (cm^2), and plant diameter (PDS) obtained by imaging on different days after transplanting (DAT). The presented genotypes were selected based on their performance for growth rate monitoring using the vegetation index. (**A**) superior (UFU BIOFORT155E12), (**B**) intermediate (UFU BIOFORT189E22), and (**C**) inferior (UFU BIOFORT189E43).

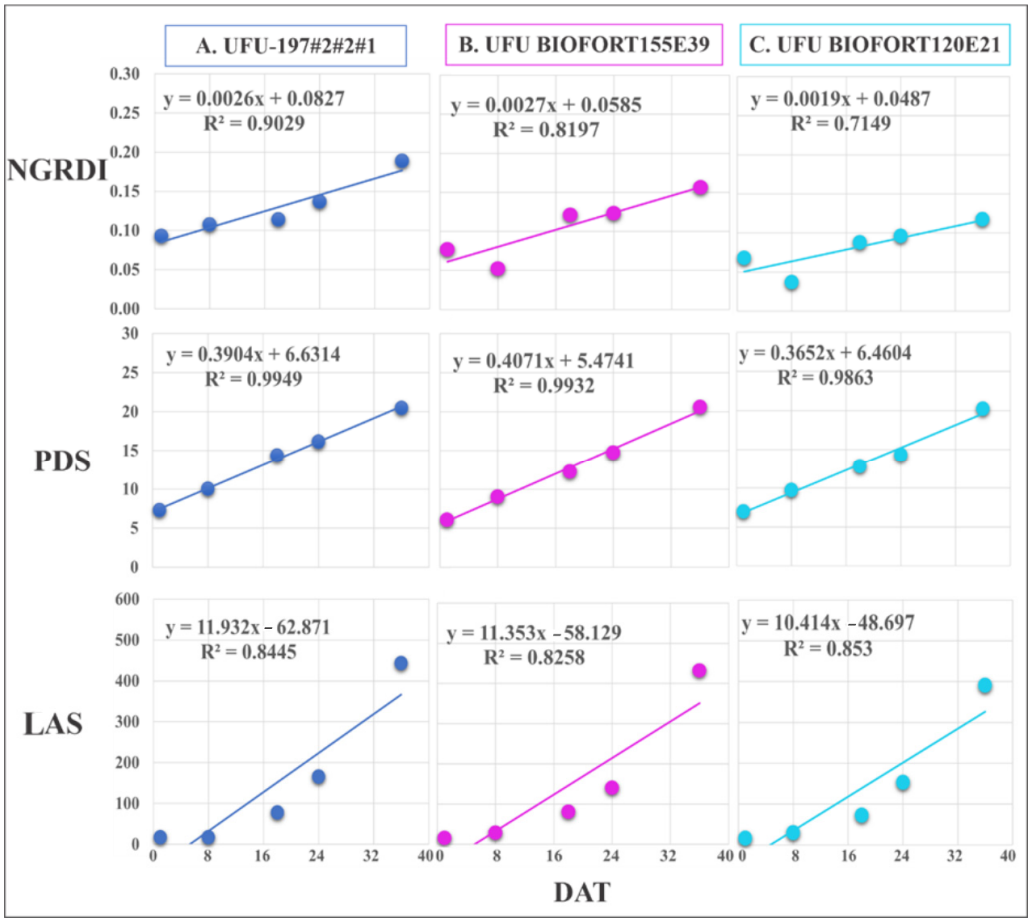

Figure 8. Regression equations of Normalized Green Red Difference Index (NGRDI), leaf area in software (LAS) (cm^2), and plant diameter (PDS) obtained by imaging on different days after transplanting (DAT). The presented genotypes were selected based on their performance for growth rate monitoring using the vegetation index. (**A**) superior (UFU-197#2#2#1), (**B**) intermediate (UFU BIOFORT155E39), and (**C**) inferior (UFU BIOFORT120E21).

Grand Rapids and the genotype UFUBIOFORT189E48 had good adjustments in the regression, with R^2 = 93.5% and 90%, respectively. The genotype UFU-206#1#5#1 had an R^2 = 62.9%, indicating that the linear regression did not explain the genotype behavior throughout its cycle (Figure 6).

GLI was efficient at determining the plant cover, along with the development and harvest point of the genotypes UFU BIOFORT155E12 (R^2 = 94%) and UFU BIOFORT189E22 (R^2 = 81%). The regression equation of genotype UFU BIOFORT189E43 referring to GLI had an R^2 = 69.8%.

NGRDI best estimated the development of genotypes UFU BIOFORT155E39 and UFU 197#2#2#1 (R^2 = 81.9% and 90.2%, respectively) in lettuce crop monitoring using RGB images. Compared with the genotypes above, UFU BIOFORT155E21 had a regression equation with an R^2 = 71.4%.

3.4. Validation of the Image Phenotyping Technique

The vegetation indices showed different behaviors for the genotypes under study and enabled the analysis of each genotype individually (Figure 9).

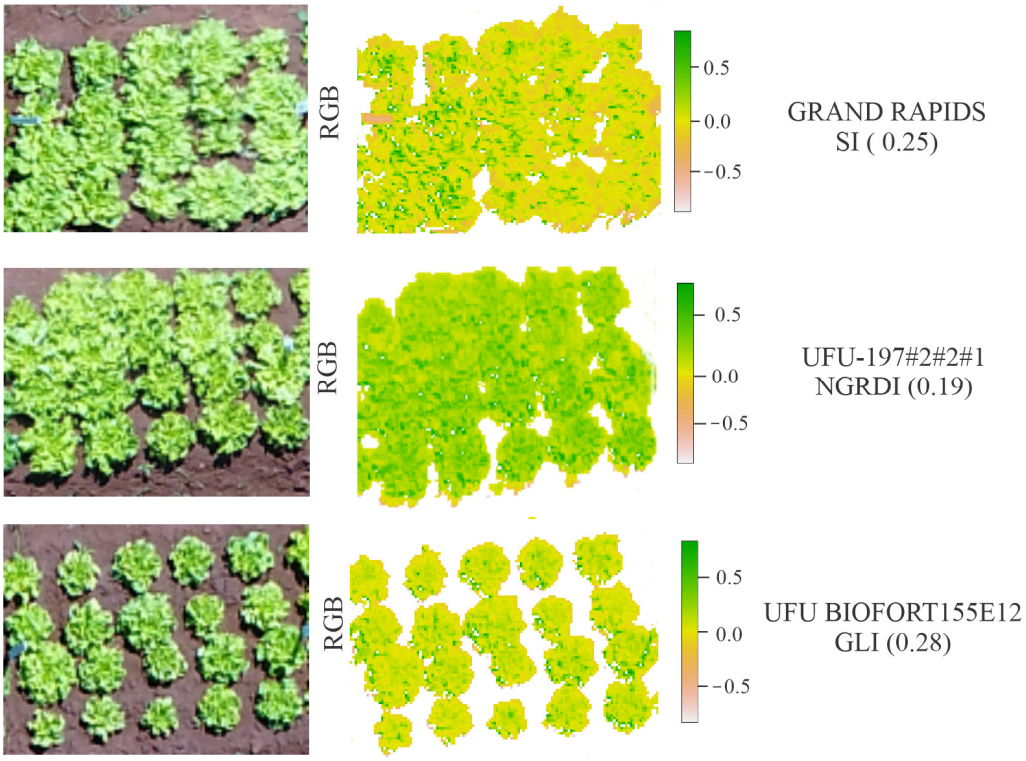

Figure 9. Representation of the behaviors of the vegetation indices: Spectral Slope Saturation Index (SI), Normalized Green Red Difference Index (NGRDI), and Green Leaf Index (GLI) for lettuce genotypes Grand Rapids, UFU-197 #2#2#1, and UFU BIOFORT 155E12.

Commercial cv. Grand Rapids and the genotypes UFU BIOFORT189E48, UFU BIOFORT189E43, UFU BIOFORT155E39, UFU BIOFORT155E12, UFU-206#1#5#1, and UFU BIOFORT189E22 had high correlations between GM values collected in the field and LAS from image analysis (0.78, 0.81, 0.82, 0.88, 0.88, 0.99, and 1.00, respectively). A high and positive correlation was found between PDS from image analysis and PD in the field. This behavior was observed for the genotypes UFU BIOFORT155E48, UFU-206#1#5#1, UFU BIOFORT120E21, UFU BIOFORT155E43, UFU BIOFORT155E39, and UFU BIOFORT189E22 (0.83, 0.85, 0.92, 0.98, 1, and 1, respectively) (Table 4).

A positive correlation was found of the vegetation indices SI, GLI, and NGRDI with the variables determined in the field: GM, PD, SD, and NL. The values presented a variation of 0.55–1.0 for the genotypes UFU BIOFORT155E12, UFU BIOFORT120E21, and UFU-206#1#5#1 in relation to SI. A positive correlation was verified of GLI and NGRDI with all the characteristics analyzed in the field for the genotypes UFU BIOFORT189E22 and UFU BIOFORT155E39. Furthermore, the highest correlation values were observed in the association of these indices with GM in UFU BIOFORT189E22 (0.99 and 1.00, respectively) and UFU BIOFORT155E39 (0.99 and 0.97, respectively). This second genotype also showed the highest correlation values with NL (0.97 for NGRDI and 0.99 for GLI) (Table 4).

PDS and LAS positively correlated with GM, PP, SD, and NL. This result was observed for the genotypes UFU BIOFORT155E12, UFU BIOFORT120E21, UFU BIOFORT189E22, UFU BIOFORT189E48, and UFU-206#1#5#1. In Grand Rapids, GLI and NGRDI positively correlated with PD and SD. In addition, PDS and LAS had high correlations with GM (0.97 and 0.78, respectively) and NL (0.94 and 1.0, respectively) (Table 4).

Table 4. Correlations per se between data collected in the field and data obtained by imaging at 36 days after transplanting (DAT) of nine green lettuce genotypes. Plant diameter in software (PDS), leaf area in software (LAS), Spectral Slope Saturation Index (SI), Normalized Green Red Difference Index (NGRDI), Green Leaf Index (GLI), green mass (GM), plant diameter (PD), stem diameter (SD), and number of leaves (NL).

		GM	PD	SD	NL
UFU BIOFORT155E12	SI	0.88 **	0.90 **	0.89 **	0.84 **
	GLI	0.64 *	0.60 *	0.62 *	0.69 *
	NGRDI	0.77 **	0.74 **	0.75 **	0.81 **
	PDS	0.48 *	0.52 *	0.50 *	0.42 *
	LAS	0.88 **	0.90 **	0.89 **	0.85 **
UFU BIOFORT120E21	SI	0.89 **	1.00 *	0.67 *	0.93 **
	GLI	1.00 *	0.88 **	0.96 **	0.98 **
	NGRDI	0.99 **	0.87 **	0.97 **	0.98 **
	PDS	0.68 *	0.92 **	0.37 *	0.75 **
	LAS	0.68 *	0.92 **	0.37 *	0.75 **
UFU BIOFORT189E22	SI	−0.35 ^{ns}	−0.43 ^{ns}	0.00 ^{ns}	−0.94 ^{ns}
	GLI	0.99 **	0.98 **	0.97 **	0.55 *
	NGRDI	1.00 *	0.99 **	0.95 **	0.61 *
	PDS	1.00 *	1.00 *	0.93 **	0.66 *
	LAS	1.00 *	1.00 *	0.90 **	0.71 **
UFU—197#2#1#1	SI	−0.94 ^{ns}	−0.93 ^{ns}	−0.74 ^{ns}	−0.92 ^{ns}
	GLI	−0.72 ^{ns}	−0.07 ^{ns}	−0.93 ^{ns}	−0.75 ^{ns}
	NGRDI	−0.70 ^{ns}	−0.04 ^{ns}	−0.92 ^{ns}	−0.74 ^{ns}
	PDS	0.71 **	0.04 *	0.92 **	0.74 **
	LAS	0.59 *	−0.11 ^{ns}	0.85 **	0.63 *
UFU BIOFORT155E39	SI	−0.56 ^{ns}	−0.64 ^{ns}	0.46 *	−0.56 ^{ns}
	GLI	0.99 **	0.97 **	0.60 *	0.99 **
	NGRDI	0.97 **	0.94 **	0.69 *	0.97 **
	PDS	0.99 **	1.00 *	0.39 *	0.99 **
	LAS	0.88 **	0.92 **	0.00 ^{ns}	0.88 **
UFU BIOFORT189E43	SI	−0.54 ^{ns}	−0.71 ^{ns}	0.82 **	0.53 *
	GLI	0.99 **	1.00 *	−0.97 ^{ns}	−0.99 ^{ns}
	NGRDI	0.98 **	0.91 **	−0.83 ^{ns}	−0.98 ^{ns}
	PDS	0.91 **	0.98 **	−1.00 ^{ns}	−0.90 ^{ns}
	LAS	0.82 **	0.92 **	−0.98 ^{ns}	−0.81 ^{ns}
UFU BIOFORT189E48	SI	0.98 **	1.00 *	1.00 *	0.89 **
	GLI	−0.22 ^{ns}	−0.09 ^{ns}	0.07 *	−0.45 ^{ns}
	NGRDI	−0.13 ^{ns}	−0.01 ^{ns}	0.16 *	−0.37 ^{ns}
	PDS	0.76 **	0.83 **	0.91 **	0.57 *
	LAS	0.81 **	0.87 **	0.94 **	0.64 *
UFU—206#1#5#1	SI	0.87 **	0.89 **	0.76 **	0.83 **
	GLI	0.59 *	0.55 *	0.74 **	0.65 *
	NGRDI	0.79 **	0.76 **	0.90 **	0.84 **
	PDS	0.88 **	0.85 **	0.95 **	0.91 **
	LAS	0.99 **	0.99 **	1.00 *	1.00 *
GRAND RAPIDS	SI	−0.33 ^{ns}	−0.37 ^{ns}	−0.79 ^{ns}	0.24 *
	GLI	−0.11 ^{ns}	0.73 **	0.98 **	−0.64 ^{ns}
	NGRDI	0.03 *	0.63 *	0.94 **	−0.53 ^{ns}
	PDS	0.97 **	−0.89 ^{ns}	−0.53 ^{ns}	0.94 **
	LAS	0.78 **	−1.00 ^{ns}	−0.84 ^{ns}	1.00 *

*: significant at 5% or less probability; **: significant at 1% or less probability, both by Student's *t* test; ^{ns}: not significant.

4. Discussion

In the present study, the climate was not very favorable, as the optimal temperature for the development of the lettuce culture is approximately 18 °C [29], but despite the irregular weather conditions, the plants did not suffer alterations.

The agronomic characteristics presented in the lettuce crop could be related to climate, genetic factors, and photoperiod [30,31]. These characteristics are defined and used as tools for product selection. Lettuce with greater GM is often selected by the consumer [31].

A greater NL on the lettuce plant positively impacts its commercialization. This characteristic can be used as a parameter to define climatic adaptations of the genotypes [32]. Other studies have revealed genotypes with better performance in NL compared to the commercial cv. Grand Rapids [33–35]. This result highlights the efficiency of genetic improvement in the lettuce crop, generating products of higher quality than those available on the market.

Remote sensing has become a tool with the potential to assist in monitoring and decision-making regarding crops. The evaluation of plant development by images is linked to records over time [36]. Therefore, the vegetation indices are an important tool in the evaluation of the vegetative development and identification of the harvest point in the lettuce crop.

Data analysis revealed the existence of genetic variability among the characterized genotypes. This information validates the use of phenotyping using images [17,18]. Furthermore, knowing the genetic variability among genotypes is essential for the selection of the best genotypes in breeding programs [37].

Variability was identified in vegetative development among the evaluated strains. The high correlation values highlight greater reliability in the clustering generated, and the closer these values are to one, the better the representativeness and quality of the cluster [38].

Similarity in the vegetative development of the strains was observed for the GLI and NGRDI. Similar behavior of the indices that use RGB (red, blue, and green) can be explained by their having similar detection characteristics for vegetation. GLI can be used to evaluate vegetation and has a good correlation with the chlorophyll content present in plants [39,40]. NGRDI has a strong relationship with chlorophyll content at different times of crop development, in addition to presenting strong potential to estimate the biomass of vegetation [41,42]. HUE has been used in different vegetation covers to differentiate between vegetation and non-vegetation pixels [43].

The vegetation indices analyzed by means of images were expressed differently among the lettuce genotypes. This difference occurred, for instance, when there were different levels of carotenoids [17,18]. When studying wheat crops, researchers found that the NDVI values among cultivars were influenced by the phenological stages of the crop and the amount of nitrogen present in the soil [44,45].

Plant phenotyping using images is more consistent than that using the conventional phenotyping method and can be useful in breeding programs [46,47]. In a study conducted with lettuce, researchers found a correlation of 0.68 between the anthocyanin contents quantified in the laboratory and the vegetation indices CIG, CIV, GNDVI, and NDVI [17].

Research with information extracted from images has revealed high correlations between the indices and different phenotypic characteristics of some crops. In brachiaria grass, a correlation of 0.92 was observed between control (%) and NDVI values extracted using images [48]. In corn, digital images were used to evaluate crop performance [46].

Studies have shown that the measurements of the leaf area of lettuce crops are performed via the traditional approach of counting leaves and electronic meters, which may or may not be destructible [49]. However, in large plantation areas, producers face difficulties in performing this monitoring, causing significant waste and losses [7]. In this context, the evaluation of leaf area and plant diameter obtained through images becomes a fast, effective, and low-cost tool for the use of plant phenotyping. The results presented in this work suggest that the methodology of collecting information through images adequately monitors the development of lettuce plants over time.

The monitoring of leaf area in different years in other crops, such as eucalyptus, using NDVI, SRI, and SAVI revealed equations with R^2 values ranging from 6.1% to 67.2% [20]. This study obtained R^2 values ranging from 78% to 92% for leaf area obtained through images. This result highlights the efficiency in phenotyping by imaging in the characterization of the development of lettuce plants. Regression models were generated for the respective vegetation indices and genotypes during winter. However, it is suggested to use the methodology during another season of the year and for other lettuce segments.

The information obtained in the present work indicates that phenotyping technology using RGB images to analyze and obtain information regarding vegetation indices, leaf area, and lettuce plant diameter has great potential. These results could facilitate the monitoring of the growth rate of lettuce plants and enable the determination of their harvest point. Image phenotyping is a low-cost technology and tool using RGB sensors, which can assist in decision-making and reduce the labor and costs associated with the existing crops. Image phenotyping is also a useful tool in genetic improvement, facilitating the characterization and selection of plants.

5. Conclusions

The vegetation indices SI, GLI, and NGRDI with the PDS and LAS values were coherent for lettuce growth rate, with an increase in the values observed over the flights for each genotype.

The correlations between data collected in the field and data obtained by imaging ranged from moderate to strong. Overall, the vegetation indices SI, GLI, and NGRDI were efficient for monitoring the growth rate and determining the harvest point of different green lettuce genotypes, in attempts to reduce waste and losses.

It is suggested that the definition of the harvest point based on vegetation indices be specific for each genotype.

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