

Article

Phenotyping in Green Lettuce Populations Through Multispectral Imaging

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Abstract: Lettuce (*Lactuca sativa*) is the most consumed leafy vegetable in the world, with great economic and social importance in Brazil. In breeding programs, selecting genotypes with high agronomic potential is essential to meet market demands and cultivation conditions. In this context, plant phenotyping by means of multispectral imaging emerges as a modern, efficient and non-destructive tool, which enhances the analysis of phenotypic characteristics quickly and accurately. Therefore, the aim of the present study was to group different lettuce situations according to their group using image-based phenotyping, in addition to morphological descriptors and agronomic evaluations. The experiment was carried out in an experimental area of the Federal University of Uberlândia, Campus of Monte Carmelo, MG, Brazil, in randomized blocks with three replicates and 17 treatments (lettuce populations of the F₂ generation, resulting from the cross between different lettuce cultivars and/or lines). Morphological descriptors and agronomic characteristics were obtained in the field. The vegetation indices GLI, NDVI, GNDVI, NGRDI and NDRE were calculated from images acquired at 49 days after transplanting. Means were compared using the Scott–Knott test ($p \leq 0.05$), and the results were presented in box plots. Genetic dissimilarity was confirmed by multivariate analysis, which resulted in a cophenetic correlation coefficient of 96.11%. In addition, validation between field-collected data and image-obtained data was performed using heat maps and Pearson's correlation. Populations UFU 003, UFU 006, UFU 009, UFU 011, UFU 012, UFU 013, UFU 014, UFU 016 and UFU 017 stood out, with high agronomic potential. Image-based phenotyping was correlated with agronomic traits and, therefore, can be considered an alternative to grouping different lettuce populations.

Keywords: *Lactuca sativa*; genotypes; image-based phenotyping



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1. Introduction

Lettuce (*Lactuca sativa*), belonging to the Asteraceae family, is the most consumed leafy vegetable in the world, with great relevance in the Brazilian economy [1]. According to data from the Brazilian Association of Seed and Seedling Trade, lettuce production was approximately 671.5 thousand tons in 2023, and this crop stood out in third place among the

vegetables with the highest production volume [2]. The preference for this leafy vegetable is due to the benefits to human health, since it is a source of vitamins and fibers, in addition to having medicinal properties capable of promoting the proper functioning of the body [3].

In Brazil, the lettuces sold are classified into five groups, according to their phenotypic characteristics, and those in the curly and butterhead groups are preferred by consumers [4]. In this context, phenotypic characterization of lettuce plants is of paramount importance in the differentiation of genotypes, in order to provide information for breeding programs, in addition to being a necessary step for the registration of cultivars.

The techniques used in the phenotyping of lettuce genotypes are simple and involve evaluations based on empirical and visual methods. However, such evaluations are usually destructive and require specialized labor, a long time and financial resources, which can compromise the accuracy of the results and the efficiency in decision-making [5,6].

Traditional phenotyping, based on destructive methods, limits the evaluation of genotypes by causing irreversible damage to plants, which is especially problematic in hybrids or new mutants with few available progenies. In addition, the time required for these analyses compromises efficiency in fast-growing leafy species with short phenological stages. These limitations reduce the number of genotypes evaluated in breeding programs, restricting genetic progress. Thus, the need for non-destructive and more agile methods that allow obtaining maximum information without compromising the plant material is highlighted. According to Coelho et al. [7] and Cortes et al. [8], among the challenges encountered in lettuce cultivation is the need to advance in the phenotyping of populations on a large scale, in a precise, fast and consistent way, in order to enable the exploration of new agronomic and genetic characteristics.

Large-scale plant phenotyping, also known as phenomics, allows the generation of a large amount of data in a non-destructive and fast way, with reductions in costs, time and labor, compared to the use of conventional methods [9]. Phenomics works in an interdisciplinary way, including spectroscopy, which generates images that are captured in different regions of the electromagnetic spectrum [6].

Image phenotyping, using multispectral sensors on board remotely piloted aircraft (RPA) or automated platforms, has emerged as an innovative and promising strategy for the analysis of agricultural crops, allowing high-resolution, fast and non-destructive evaluations. This technology allows the capture of images in different regions of the electromagnetic spectrum, enabling the generation of vegetation indices, such as NDVI (Normalized Difference Vegetation Index), GNDVI (Green Normalized Difference Vegetation Index) and GLI (Green Leaf Index), which help in the quantification of physiological parameters of plants, such as chlorophyll content, biomass and water stress [10]. Studies involving lettuce crops have used vegetation indices to monitor growth rate and distinguish foliar pigments, reinforcing the feasibility of using vegetation indices for image-based phenotyping. Multispectral sensors coupled to RPA offer a platform for capturing images with excellent resolution, allowing the development of vegetation indices that are correlated with various physiological properties of vegetation, as well as environmental, phenological and anthropogenic factors that influence them [11,12]. Large-scale application of these techniques has the potential to increase the efficiency of breeding programs, allowing a more precise selection of genotypes adapted to different environmental conditions.

Given the high spatial and temporal resolutions offered by this technology, its application in lettuce phenotyping becomes essential [13]. Studies have proven that the use of image-based phenotyping in the lettuce crop is feasible [13,14]. However, there are still a few studies involving the use of phenotyping techniques to assist in the indirect selection of lettuce genotypes. In this context, the present study aims to group different lettuce situations according to their group using image-based phenotyping, in addition to

morphological descriptors and agronomic evaluations. The validation of these techniques can significantly contribute to the optimization of cultivar selection processes, providing the basis for the advancement of sustainable and high-yielding lettuce crop production.

2. Materials and Methods

2.1. Characterization of Experimental Area and Treatments

The experiment was carried out from 5 February to 20 May 2024, in the experimental area of the Federal University of Uberlândia, Campus of Monte Carmelo, MG, Brazil, whose geographic coordinates are 18°52′70.93 South latitude, 47°52′31.44 West longitude and altitude of 903 m (Figure 1).

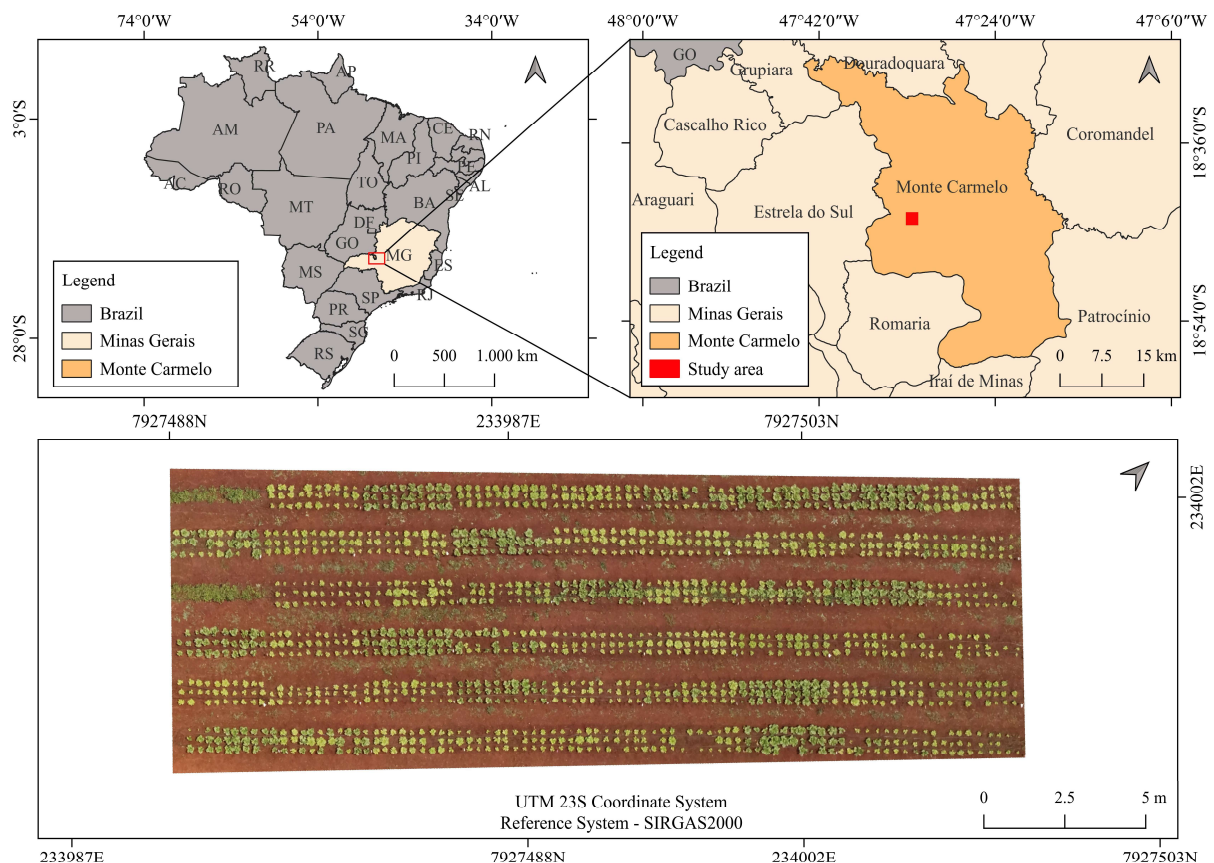


Figure 1. Location map of the study area.

The soil of the experimental area was classified as *Latossolo vermelho* (Oxisol), with clayey texture and flat relief, according to the criteria of the Brazilian Soil Classification System [15]. The climate of the region is tropical with a dry winter, classified as Aw (Köppen), with an average annual rainfall of 1370 mm and an average annual temperature of 24 °C [16].

The experimental design was randomized blocks with three replicates. Treatments consisted of 17 lettuce populations of the F₂ generation, obtained from crosses between different lettuce cultivars and/or lines (Table 1), developed by the Lettuce Breeding Program of the Federal University of Uberlândia, Campus of Monte Carmelo, through the genealogical method.

Table 1. Identification of the populations used in the experiment and their respective parents.

Lines	Parents
UFU 001	Coral × BS AC0055
UFU 002#2	BS AC0055 × Coral
UFU 003#1	BS AC0055 × Coral
UFU 004	BS AC0055 × Aliot
UFU 005	Luiza × L3
UFU 006	Vanda × L1
UFU 007	Vanda × L1
UFU 008	Vanda × Coral
UFU 009#2	Vanda × Coral
UFU 010	Vanda × Coral
UFU 011	Lectrice × Lais
UFU 012	Lectrice × Lais
UFU 013	Lectrice × Lais
UFU 014	Coral × L1
UFU 015	Coral × L7
UFU 016	Vanda × L7
UFU 017	Vanda × L7

L1, L3 and L7 = curly lettuce lines with resistance factors R18 and R38 to *Bremia lactucae*: L1—Vanda cultivar × JAB 4-13-17 line (resistance factor R18, male parent); L3—Veneranda cultivar × JAB 4-13-17 line (resistance factor R18, male parent); L7—Argelis line (resistance factor R38, female parent) × JAB 4-13-17 line (resistance factor R18, male parent).

Each experimental plot consisted of 30 plants arranged in three 3.5-meter-long planting rows, with spacing of 0.35 m between plants and 0.30 m between rows, with eight central plants being considered as usable area. Sowing was carried out in 200-cell expanded polyethylene trays, filled with substrate based on coconut fiber. The seedlings were kept in a greenhouse measuring (7 m × 4 m), covered with 150 micron anti-UV transparent plastic, until the transplanting phase, carried out in seedbeds 25 days after sowing, on 1 March 2024. The environmental conditions during the crop cycle were as follows: average temperature of 23.5 °C, average maximum temperature of 29.84 °C, average minimum temperature of 18.86 °C and relative humidity of 77.47%. The accumulated rainfall during the period was 572.6 mm (Figure 2).

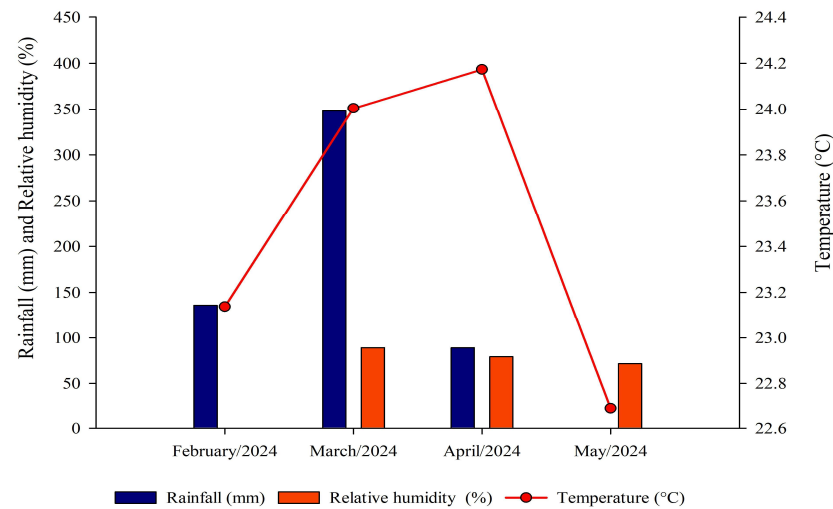


Figure 2. Monthly averages of air temperature, accumulated rainfall and relative humidity, recorded in the experimental area during the experiment. Source: Cooxupé, 2024 [17].

Prior to transplanting the seedlings, chemical analysis of the soil in the 0–20 cm layer was performed to determine its chemical properties (Table 2).

Table 2. Chemical characterization of the soil in the experimental area at 0–0.20 m depth, in the year 2023.

Characteristic	Values
pH (H ₂ O) (1:2.5)	6.4
Phosphorus (P) Mehlich-1—mg dm ^{−3}	9.9
Potassium (K)—mg dm ^{−3}	0.53
Calcium (Ca ²⁺)—cmol _c dm ^{−3}	5.79
Magnesium (Mg ²⁺)—cmol _c dm ^{−3}	1.3
Aluminum (Al ³⁺)—cmol _c dm ^{−3}	0.0
H+Al (SMP Extractant)—cmol _c dm ^{−3}	1.8
Sum of exchangeable bases (SB)—cmol _c dm ^{−3}	7.58
CEC (t)—cmol _c dm ^{−3}	7.58
CEC at pH 7.0 (T)—cmol _c dm ^{−3}	9.38
Base saturation index (V)—%	81.0
Aluminum saturation index (m)—%	0.0
Boron (B)—mg dm ^{−3}	0.24
Copper (Cu)—mg dm ^{−3}	1.9
Iron (Fe)—mg dm ^{−3}	57.0
Manganese (Mg)—mg dm ^{−3}	21.3
Zinc (Zn)—mg dm ^{−3}	6.8
Organic matter (OM)—%	2.4

SB: sum of bases; V: base saturation; m: aluminum saturation; t: effective CEC; T: potential CEC; OM: organic matter. Extraction methods: P, K, Na = Mehlich-1; S-SO₄^{2−} = [0.01 mol L^{−1} monobasic calcium phosphate]; Ca, Mg, Al = [1 mol L^{−1} KCl]; H+Al = [SMP Buffer Solution pH 7.5]; B = [0.125% BaCl₂·2H₂O, hot]; Cu, Fe, Mn, Zn = DTPA. dm^{−3} = cubic decimeter. Analyses carried out at Laboratório Brasileiro de Análises Ambientais e Agrícolas L.T.D.A. (LABRAS), Monte Carmelo, MG, Brazil.

In view of the results, the practice of liming was not recommended, since the soil had a base saturation (V%) of 81% [18]. Planting and top-dressing fertilization in the experimental area was carried out according to the recommendations for the crop [18]. In planting fertilization, the main sources of fertilizer used were the formulation 06-27-06 (N–P₂O₅–K₂O) at a dose of 20 g m^{−1}, urea (45% of N) at a dose of 4 g m^{−1} and single superphosphate at a dose of 25.5 g m^{−1}. Top-dressing fertilization was divided into three portions and applied at 15, 30 and 40 days after transplanting, using the formulation 06-27-06 (N–P₂O₅–K₂O) at a dose of 5.6 g m^{−1} and urea (45% of N) at a dose of 9.0 g m^{−1}.

2.2. Morphological and Agronomic Characterization of Lettuce Populations

Morphological characterization of the plants and agronomic evaluations were carried out at 49 days after transplanting. Morphological characterization of the plants was performed according to the guidelines for conducting tests on distinguishability, uniformity and stability of lettuce cultivars recommended by the Ministry of Agriculture, Livestock and Food Supply [19], using thirteen morphological descriptors (Table 3).

The agronomic characteristics evaluated were as follows: plant height and diameter (cm), number of leaves and tolerance to bolting. Tolerance to bolting was evaluated considering the number of days after transplantation until the bolting stage, marked by stem elongation and the beginning of the formation of floral structures [20].

Table 3. Morphological descriptors used for lettuce crop, according to the guidelines for performing distinguishability, uniformity and stability tests.

Characteristics ¹	Descriptive Scale
Degree of overlap of the upper leaves (DOV)	1 = very weak, 3 = weak, 5 = medium, 7 = strong and 9 = very strong
Degree of base closure (DBC)	3 = weak, 5 = medium and 7 = strong
Longitudinal section shape (LSS)	1 = elliptical, 2 = enlarged elliptical, 3 = circular and 4 = transverse elliptic
Growth habit (GH)	3 = erect, 5 = semi-erect and 7 = nearly horizontal
Leaf shape (LS)	1 = narrowed elliptical, 2 = elliptical, 3 = enlarged elliptical, 4 = circular, 5 = enlarged transverse elliptical, 6 = transverse elliptical, 7 = oval, 8 = enlarged transverse rhomboidal, 9 = triangular
Leaf thickness (LT)	3 = thin, 5 = medium, 7 = thick
Leaf color intensity (CI)	1 = very light, 3 = light, 5 = medium, 7 = dark and 9 = very dark
Leaf top side brightness (LB)	1 = very weak, 3 = weak, 5 = medium, 7 = strong and 9 = very strong
Profile of outer leaves (POL)	3 = concave, 5 = flat, 7 = convex
Bumpiness (BMP)	1 = absent or very weak, 3 = weak, 5 = medium, 7 = strong and 9 = very strong
Degree of margin undulation (UND)	1 = absent or very weak, 3 = weak, 5 = medium, 7 = strong and 9 = very strong
Head compactness (CO)	1 = very loose, 3 = loose, 5 = medium, 7 = compact and 9 = very compact
Bump size (BS)	3 = small, 5 = medium and 7 = large

¹ MAPA—Ministry of Agriculture, Livestock and Food Supply [19]. Guidelines for conducting distinguishability, uniformity and stability tests of lettuce cultivars (*Lactuca sativa* L.).

2.3. Acquisition and Processing of Aerial Images

To enable high-resolution phenotyping of lettuce populations, an aerial survey was conducted at 49 days after transplanting (DAT), a period coinciding with the harvest stage when morphological and agronomic traits are fully expressed. A DJI Phantom 4 Multispectral drone was utilized, equipped with a six-sensor imaging system: one RGB sensor (visible range) and five narrow-band multispectral sensors with the following spectral characteristics—blue (B: 450 ± 16 nm), green (G: 560 ± 16 nm), red (R: 650 ± 16 nm), red edge (RE: 730 ± 16 nm) and near infrared (NIR: 840 ± 26 nm). Each multispectral sensor captures images at a resolution of 1600×1300 pixels, with radiometric calibration performed using the drone's integrated sunshine sensor and calibration reflectance panel prior to the flight, ensuring consistency in reflectance values across the scene. Flight planning was conducted using the DJI GS Pro application, which allows precise control over flight paths and acquisition parameters. The mission was flown at solar noon (~12:00 p.m. local time) under clear sky conditions to maximize sunlight intensity and reduce shadow artifacts. The flight altitude was set to 30 m above ground level (AGL), and image overlap was configured with 80% front (longitudinal) and 75% side (lateral) overlap. These parameters were selected to guarantee high-quality structure-from-motion (SfM) processing and to maintain spatial resolution below 3 cm/pixel. The resulting dataset included over 250 images with high spatial redundancy and spectral integrity.

Image preprocessing and orthomosaic generation were performed using Agisoft Metashape Professional (version 2.2.1). The standard photogrammetric workflow included the following steps: (i) photo alignment, which determined camera positions and extracted tie points between overlapping images; (ii) dense point cloud reconstruction, using depth maps; (iii) digital surface model (DSM) and 3D mesh generation based on triangulated surface geometry; and (iv) true orthomosaic generation, with band-to-band registration of the multispectral layers. A scale-invariant feature transform (SIFT) algorithm was applied during alignment to improve the tie point detection accuracy. The final orthomosaic was georeferenced and exported in GeoTIFF format for subsequent spatial analysis.

Subsequently, the radiometric values of each plant were extracted to calculate the vegetation indices (Table 4), using the Envi Classic program. In addition, the average vegetation index was extracted for each experimental plot.

Table 4. Vegetation indices applicable to the monitoring of agricultural variables and of interest in this study.

Vegetation Indices	Formula	Application	Reference
NDVI	$\frac{(NIR-R)}{(NIR+R)}$	Plant biomass.	[21]
GNDVI	$\frac{(NIR-G)}{(NIR+G)}$	Chlorophyll concentration.	[22]
GLI	$\frac{2 \times G - R - B}{2 \times G + R + B}$	Chlorophyll indicator created to classify the presence of live plants, dead plants and exposed soil.	[23]
NGRDI	$\frac{(G-R)}{(G+R)}$	Biomass.	[24]
NDRE	$\frac{(NIR-RE)}{(NIR+RE)}$	Chlorophyll content in plants, as well as their nitrogen uptake and possible fertilizer demands.	[25]

NDVI (Normalized Difference Vegetation Index); GNDVI (Green Normalized Difference Vegetation Index); GLI (Green Leaf Index); NGRDI (Normalized Green–Red Difference Index); NDRE (Normalized Difference Red Edge Index). R (red band); G (green band), B (blue band), RE (red edge); NIR (near infrared).

2.4. Statistical Analysis

The results of the agronomic evaluations and the images obtained at 49 days after transplanting were subjected to analysis of variance by the F test ($p \leq 0.05$) and comparison of means by the Scott–Knott test ($p \leq 0.05$), presented in box plots.

The data were also subjected to multivariate analysis, aiming to detail the dissimilarity between the genotypes through the heat map dendrogram. For the dissimilarity matrix, the Euclidean distance was used with the genetic dissimilarity represented by the hierarchical unweighted pair-group method using arithmetic averages (UPGMA). The cut-off line was established at the point where an abrupt change in the branches present in the dendrogram was observed [26].

The validation of the UPGMA clustering was confirmed by the calculation of the cophenetic correlation coefficient (CCC), obtained through the Mantel test [27]. The relative contribution of traits in relation to dissimilarity between genotypes was calculated according to Singh’s criterion [28]. Pearson’s correlation matrix was calculated between agronomic parameters, morphological parameters and vegetation indices ($p > 0.05$).

All statistical analyses were performed using GENES software and R software, version 3.6 [29,30].

3. Results and Discussion

Variation is observed among the genotypes for most descriptors, which indicates phenotypic variability (Table 5). This variability is fundamental in breeding programs, as it facilitates the selection of plants with desirable characteristics [31].

When analyzing the degree of overlap of the upper leaves (DOV), it was observed that the populations UFU 011, UFU 012, UFU 013 and UFU 016 showed weak overlap of the leaves, which suggests a more open growth structure. This arrangement can be advantageous, as it avoids the accumulation of water in the plants, consequently reducing the appearance of bacterial problems and leaf burn. The leaf overlapping characteristic is directly related to the arrangement of the leaves on the plant, which can influence both its growth and the quality of production. In addition, the spacing between plants plays an important role in this characteristic, because the higher the density, the greater the competition for light due to the overlapping of leaves, which can reduce the efficiency of photosynthesis and, consequently, reduce plant size [32,33].

Table 5. Morphological descriptors and the respective descriptive scales used to characterize the lettuce germplasm bank.

Populations	DOV	CO	DBC	LSS	GH	LS	LT	LCI	LB	POL	BMP	BS	UND
UFU 001	1	1	7	1	5	8	5	5	3	3	7	5	7
UFU 002#2	1	1	3	4	5	8	5	3	5	3	7	3	7
UFU 003#1	1	1	3	1	5	8	7	7	3	3	7	5	7
UFU 004	1	1	7	1	5	8	7	5	3	3	5	5	7
UFU 005	1	1	7	1	5	8	5	5	5	3	7	5	7
UFU 006	1	1	3	1	5	8	5	3	3	3	7	5	7
UFU 007	1	1	3	1	5	8	5	3	3	3	7	5	5
UFU 008	1	1	7	1	5	8	5	5	5	3	7	5	7
UFU 009#2	1	1	7	1	5	2	3	5	3	3	7	5	7
UFU 010	1	1	5	2	3	8	7	5	7	3	7	3	5
UFU 011	3	5	7	2	5	3	7	7	5	3	3	5	3
UFU 012	3	9	7	2	3	3	7	7	7	5	5	5	3
UFU 013	3	9	7	2	3	7	5	7	5	5	3	7	5
UFU 014	1	1	3	1	5	8	7	5	3	3	7	5	7
UFU 015	1	1	7	1	5	8	7	5	3	3	7	7	7
UFU 016	3	3	7	2	3	4	5	7	7	5	3	7	3
UFU 017	1	1	5	1	5	8	5	5	5	3	9	5	7

DOV (degree of overlap of the upper leaves): 1 = absence, 3 = weak; CO (head compactness): 1 = very loose, 3 = loose, 5 = medium, 9 = very compact; DBC (degree of base closure): 3 = weak, 5 = medium, 7 = strong; LSS (longitudinal section shape): 1 = elliptical, 2 = enlarged elliptical, 4 = transverse elliptical; GH (growth habit): 3 = erect, 5 = semi-erect; LS (leaf shape): 2 = elliptical, 3 = enlarged elliptical, 4 = circular, 7 = oval, 8 = enlarged transverse rhomboidal; LT (leaf thickness): 3 = thin; 5 = medium, 7 = thick; LCI (leaf color intensity): 3 = light, 5 = medium, 7 = dark; LB (leaf top side brightness): 3 = weak, 5 = medium, 7 = strong; POL (profile of the outer leaves): 3 = concave, 5 = flat; BMP (bumpiness): 3 = weak, 5 = medium, 7 = strong, 9 = very strong; BS (bump size): 3 = small, 5 = medium, 7 = large; UND (degree of margin undulation): 3 = weak, 5 = medium, 7 = strong.

Populations UFU 012 and UFU 013 showed high compactness (CO), so they are interesting for iceberg lettuce breeding programs, since this is a fundamental characteristic of this lettuce group, because plants whose leaves are neatly imbricated are more compact and consequently have greater fresh mass and better visual quality. In addition, the higher degree of compactness indicates that the cultivar is adapted to the growing conditions [34].

Regarding the characteristics degree of base closure (DBC) and the longitudinal section shape (LSS), it was observed that 58.82% of the populations had a strong degree of base closure, while 64.70% of the populations had an elliptical shape of the longitudinal section. These attributes are closely related, as they both indicate how compact and well-structured the lettuce head is. In addition, a good base closure is favorable to making lettuce plants less sensitive to soil-borne diseases [35].

Regarding growth habit (GH), 76.47% of the population showed semi-erect growth, while 23.53% of the population had an erect growth habit. Jacinto et al. [36], when analyzing conventional morphological descriptors in the characterization of biofortified lettuce genotypes, found that 50% of the genotypes had a semi-erect growth habit, corroborating the results found here.

For the morphological descriptors leaf shape (LS) and leaf thickness (LT), it was observed that 70.58% and 52.94% of the populations had leaves with an enlarged transverse rhomboidal shape and thick thickness, respectively. Alves et al. [37] and Costa et al. [38] state that thick leaves are more adapted to withstand the attack of pests, diseases and a high incidence of sun rays. Thus, a large part of these populations could be selected for generation advancement, since they could be more resistant to biotic and abiotic factors.

Leaf color intensity (LCI) in lettuce populations varied from light to dark, with no observations for very light or very dark intensity, corroborating the results found by [36]. Leaf brightness (LB) varied from weak to medium among the populations. According to Santos et al. [39], leaf color in lettuce is an important qualitative characteristic and is related to chlorophyll content; the higher the content of this pigment in the leaf, the greater the photosynthetic activity. In addition, highly pigmented leaves are often associated with very ripe products, being rejected by consumers, since they prefer lettuce cultivars with greater brightness and light green color [36,40].

Regarding the morphological characteristic profile of the outer leaves (POL), it was observed that 82.35% of the populations had a concave profile. As for bumpiness (BMP), it was verified that 64.71% of the populations showed strong bumpiness, with 70.59% of the genotypes having medium bump size (BS), 17.65% having large bumps and 11.76% having small bumps. According to Jacinto et al. [36], the type of leaf margin is a very important attribute to discriminate lettuce of the butterhead or curly group. In this context, in the present experiment, it can be seen that 70.59% of the population can fit into the curly group, since they exhibited a strong undulation at the margin of the leaf.

Concomitantly with the morphological characterization of the populations in the lettuce crop, evaluations of the agronomic characteristics were carried out. Figure 3 represents the box plot defined by the means comparison test of the attributes: plant diameter (PD), plant height (PH), number of leaves (NL) and days after transplanting (DAT), obtained by the Scott–Knott test at a 5% significance level.

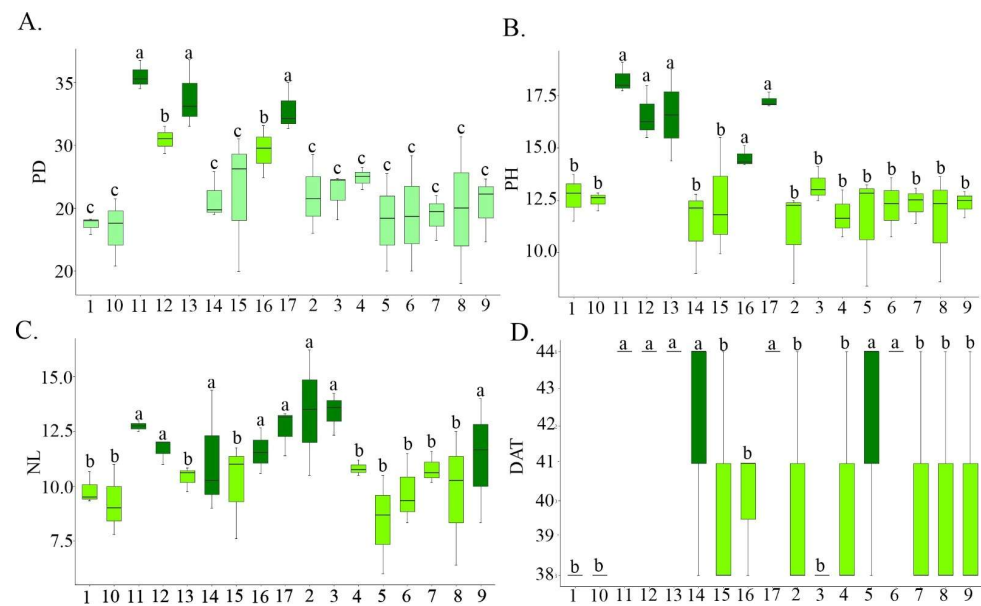


Figure 3. Comparison of lettuce populations regarding plant diameter (PD) (A), plant height (PH) (B), number of leaves (NL) (C) and days after transplanting (DAT) (D) of 17 lettuce populations. 1—UFU 001, 2—UFU 002#2, 3—UFU 003#1, 4—UFU 004, 5—UFU 005, 6—UFU 006, 7—UFU 007, 8—UFU 008, 9—UFU 009#2, 10—UFU 010, 11—UFU 011, 12—UFU 012, 13—UFU 013, 14—UFU 014, 15—UFU 015, 16—UFU 016, 17—UFU 017. Means followed by the same letter do not differ statically by the *Scott–Knott* test at the 5% probability level.

Plant diameter is an important characteristic for lettuce commercialization in Brazil, and the consumer has a greater preference for plants with a larger diameter and more attractive [41]. In addition, it is an attribute strongly influenced by environmental factors such as solar radiation, temperature and planting spacing [34,42]. In this study, the populations UFU 011, UFU 012, UFU 013, UFU 016 and UFU 017, classified as curly and iceberg, had a larger plant diameter than the others, with means of 35.5 cm, 30.46 cm, 33.79 cm, 29.58 cm and 32.81 cm, respectively (Figure 3A). These values are similar to those found by Cardoso et al. [43] when evaluating morphological aspects in the lettuce crop; these authors found that the curly and iceberg cultivars had an average plant diameter of 32 cm.

Plant height and diameter are characteristics related to stature and provide important information about the packaging of plants in plastic boxes for transport, in addition to being attributes that interfere with yield [44]. It is known that the greater the height of the lettuce plant, the more elongated the stem tends to be. This is because the plant may be in the process of flowering, which results in higher latex production and, consequently, lower final quality of the product [45,46]. Thus, lettuce populations UFU 001, UFU 002#2, UFU 003#1, UFU 004, UFU 005, UFU 006, UFU 007, UFU 008, UFU 009#2, UFU 010, UFU 014 and UFU 015 are the most promising in the breeding program, as they are shorter than the others (Figure 3B).

Vialle et al. [47], when evaluating the performance of curly and iceberg lettuce cultivars, found that the ‘Vera’ cultivar reached a higher average height (26.09 cm) compared to the ‘Delícia Americana’ cultivar, a value higher than that observed in the present study. Shorter lettuce plants, with a higher number of leaves and a larger diameter, are generally better because these factors indicate vigorous development and greater resistance to bolting.

A higher number of leaves suggests a greater capacity for photosynthesis, which favors plant growth and promotes greater production of photoassimilates [47]. In addition, a larger diameter indicates more robust plants, with more attractive visual characteristics for the consumer, which increases the chance of commercialization [48]. Shorter plants also

tend to be less prone to excessive stem elongation and early flowering, which helps prevent excessive latex production and loss of quality [45].

For the characteristic number of leaves, populations UFU 002, UFU 003, UFU 009, UFU 011, UFU 012, UFU 014, UFU 016 and UFU 017 obtained the highest means, ranging from 11.22 to 13.42 leaves (Figure 3C). Pereira et al. [46], when evaluating the performance of different cultivars of butterhead and curly lettuce, found the highest number of leaves in the cultivar of the butterhead group, 'Baba-de-verão'. Volpato et al. [49], when analyzing different lettuce cultivars as a function of different types of soil cover in two cropping systems, found that the cultivar 'Ariel', of the curly group, had a higher number of leaves, which is associated with its significant volume. Therefore, plants with a higher number of leaves promote a greater increase in biomass and are, therefore, more desirable in the market [50]. In addition, by measuring this variable, it becomes possible to find out whether or not the cultivar is adapted to the environment in which it will be cultivated [51,52].

In addition to the morphological attributes, what is sought in lettuce breeding programs are tropicalized cultivars, that is, plants resistant to early bolting and, consequently, indicated for cultivation in warmer regions, such as in the Brazilian territory [52]. In this context, populations UFU 005, UFU 006, UFU 011, UFU 012, UFU 013, UFU 014 and UFU 017 are the most resistant to bolting, as they had higher means of days after transplantation for bolting (42 to 44 days after transplantation for the beginning of bolting) (Figure 3D).

Although most cultivars develop adequately in regions with air temperatures ranging from 20 to 25 °C, it can be verified that the lettuce populations mentioned above have greater tolerance to heat, since the maximum temperatures throughout the cultivation ranged from 21.3 to 34.3 °C and the minimum temperatures ranged from 13.8 to 21.9 °C. In general, most cultivars develop well in regions with air temperatures ranging from 20 to 25 °C, especially in the vegetative growth phase of the crop. Thus, although the other populations are more susceptible to early bolting, this does not prevent them from being cultivated in Brazil. However, they should be cultivated in places where temperatures do not exceed the average temperatures tolerable for the crop, because high temperatures, as well as long photoperiod, can cause greater production of latex, making the flavor of the plants astringent and making their commercialization more difficult [53,54].

In order to monitor vegetation cover in lettuce populations, the following vegetation indices were calculated: GLI, NGRDI, NDVI, GNDVI and NDRE (Figure 4).

When analyzing the GLI and NGRDI indices, a similar behavior was observed among the lettuce populations, i.e., lettuce populations UFU 003, UFU 011, UFU 012, UFU 013 and UFU 017 showed higher means for these indices compared to the other genotypes. These indices may be able to discriminate genotypes based on their growth rate, according to Ribeiro et al. [55].

Regarding GNDVI, it is known that it is a normalized index that considers the near-infrared and green regions of the spectrum. In the present experiment, the GNDVI index was able to discriminate the populations UFU 011, UFU 012, UFU 013, UFU 016 and UFU 017 from the others, which can indicate that these populations may have darker leaf pigments than the others. This can be confirmed in the study conducted by Clemente et al. [56], who evaluated lettuce genotypes through high-throughput phenotyping and found significant differences between them in terms of anthocyanin and chlorophyll contents.

It can be seen that the populations UFU 011, UFU 012 and UFU 017 had higher means of NDVI. Likewise, these populations are among those that had the highest number of leaves, plant height and number of days for bolting (Figure 3C), which corroborates Sousa et al. [6], who state that NDVI is one of the most used indices to monitor vegetative vigor quickly and non-destructively.

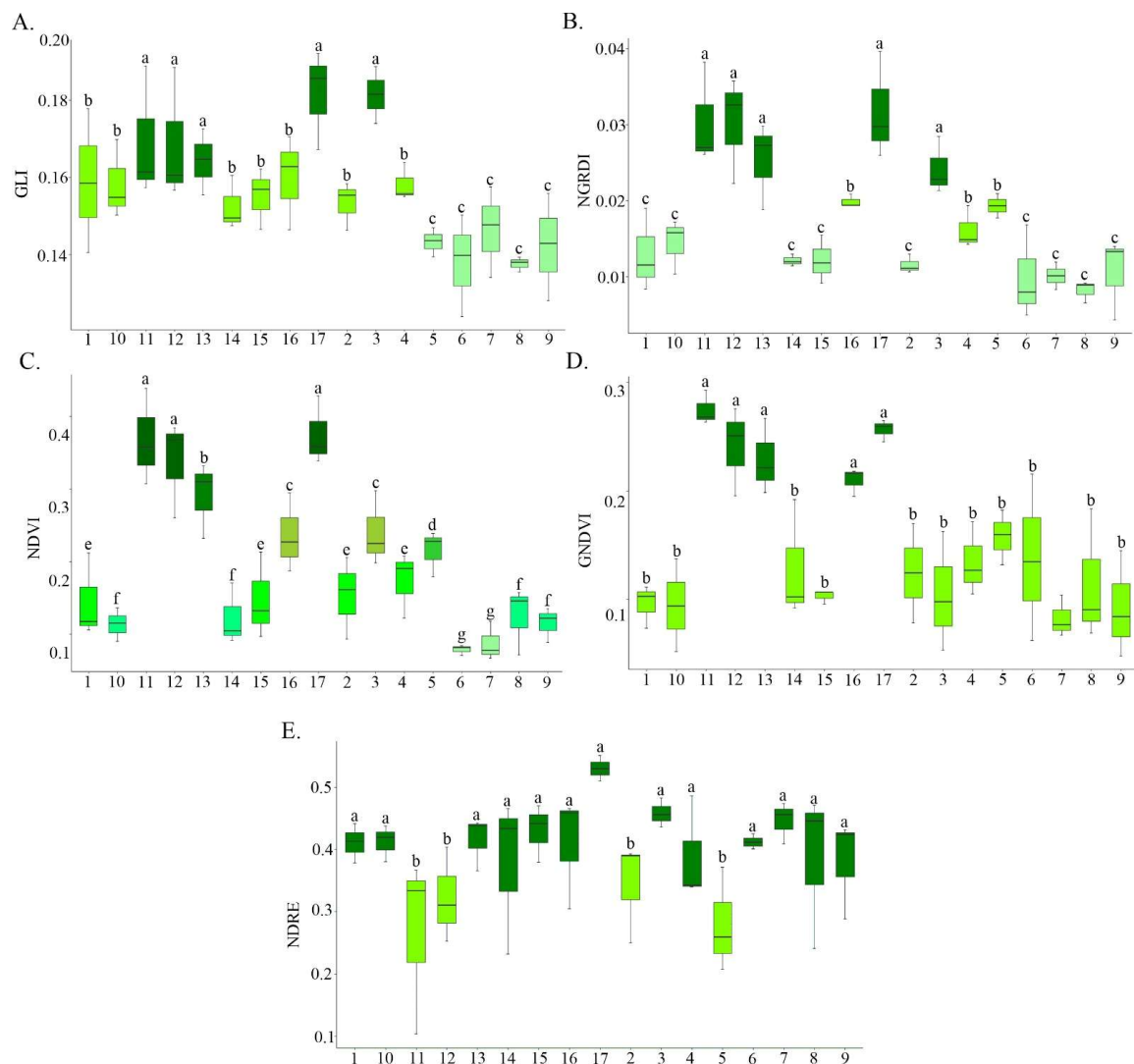


Figure 4. Comparison of the vegetation indices: GLI (Green Leaf Index) (A); NGRDI (Normalized Green-Red Difference Index) (B); NDVI (Normalized Difference Vegetation Index) (C); GNDVI (Green Normalized Difference Vegetation Index) (D) and NDRE (Normalized Difference Red Edge Index) (E) in lettuce populations. 1—UFU 001, 2—UFU 002#2, 3—UFU 003#1, 4—UFU 004, 5—UFU 005, 6—UFU 006, 7—UFU 007, 8—UFU 008, 9—UFU 009#2, 10—UFU 010, 11—UFU 011, 12—UFU 012, 13—UFU 013, 14—UFU 014, 15—UFU 015, 16—UFU 016, 17—UFU 017. Means followed by the same letter do not differ statically by the *Scott-Knott* test at the 5% probability level.

Higher means of the NDRE vegetation index were observed for the populations UFU 01, UFU 003, UFU 004, UFU 006, UFU 007, UFU 008, UFU 009, UFU 010, UFU 013, UFU 014, UFU 015, UFU 016 and UFU 017. Like NDVI, this index uses the near-infrared band, but it has greater sensitivity in representing photosynthetic activity in the entire biomass of the crop, especially in more advanced phenological stages [57].

In general, the populations that had higher means for vegetation indices also showed better performance in the agronomic characteristics determined in the field (UFU 003, UFU 006, UFU 009, UFU 011, UFU 012, UFU 013, UFU 014, UFU 016, UFU 017) (Figure 3), which corroborates several authors [55,58,59], who state that vegetation indices are important tools in the evaluation of vegetative development in lettuce, providing accurate data, which will assist in monitoring and decision-making.

Based on the results referring to the vegetation indices GLI, NGRDI, GNDVI, NDVI and NDRE, it is possible to notice the variation of their values in five categories (Figure 5).

This categorization uses the color scheme, which signals the change in the intensity and vigor of the vegetation in the study area. Red tone indicates the absence of vegetation and low vegetative vigor, whereas blue and green tones point to greater vegetative vigor.

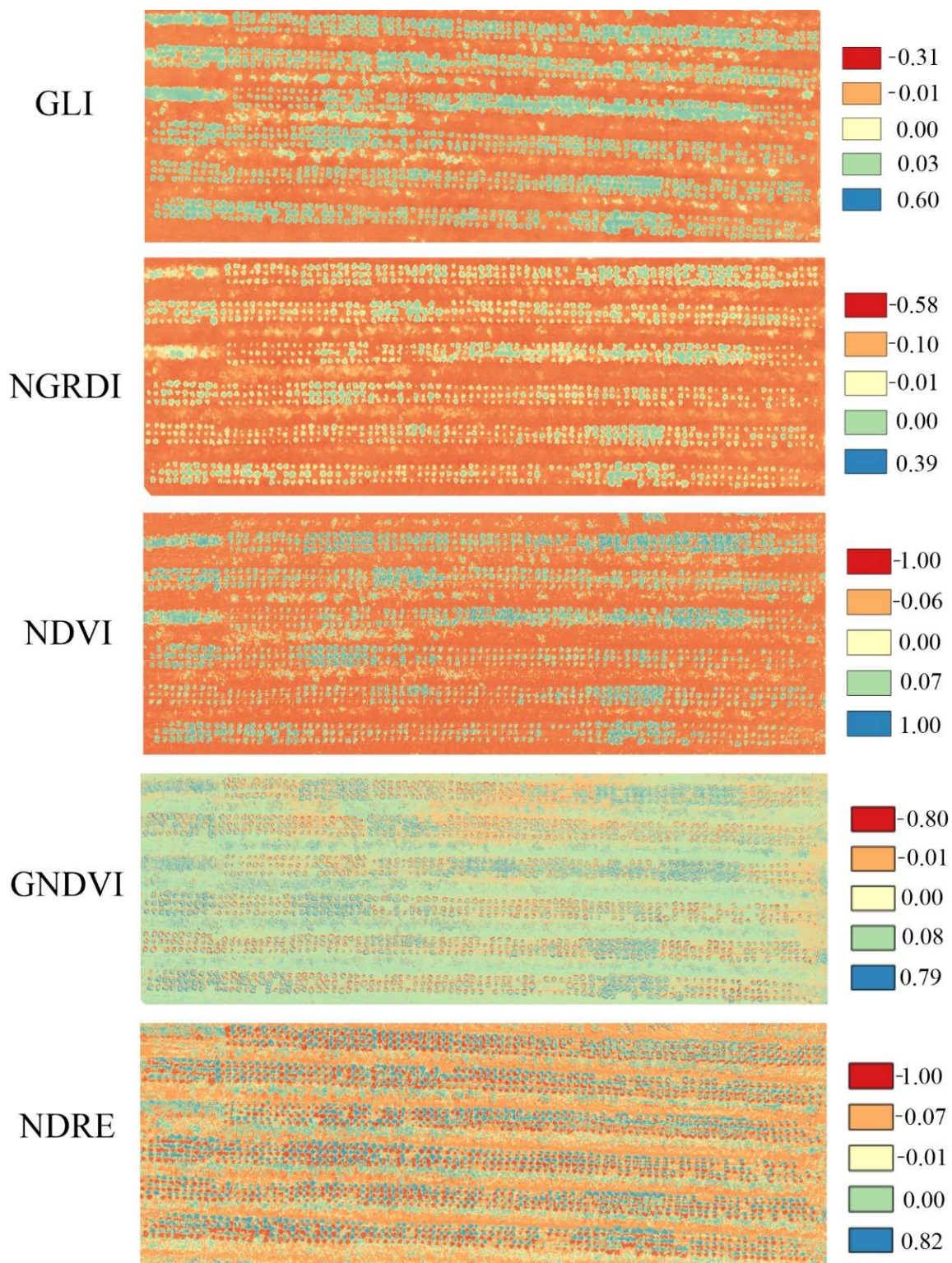


Figure 5. Representation of vegetation indices: NDVI (Normalized Difference Vegetation Index); GNDVI (Green Normalized Difference Vegetation Index); GLI (Green Leaf Index); NGRDI (Normalized Green–Red Difference Index); NDRE (Normalized Difference Red Edge Index).

Aiming at validating the use of image-based phenotyping, it becomes necessary to prove the existence of variability between the lettuce populations analyzed [56], which can be confirmed by the dendrogram, as the cophenetic correlation coefficient was 96.11% (Figure 6), a value that suggests high reliability between the original distance matrix and the matrix produced by the UPGMA clustering.

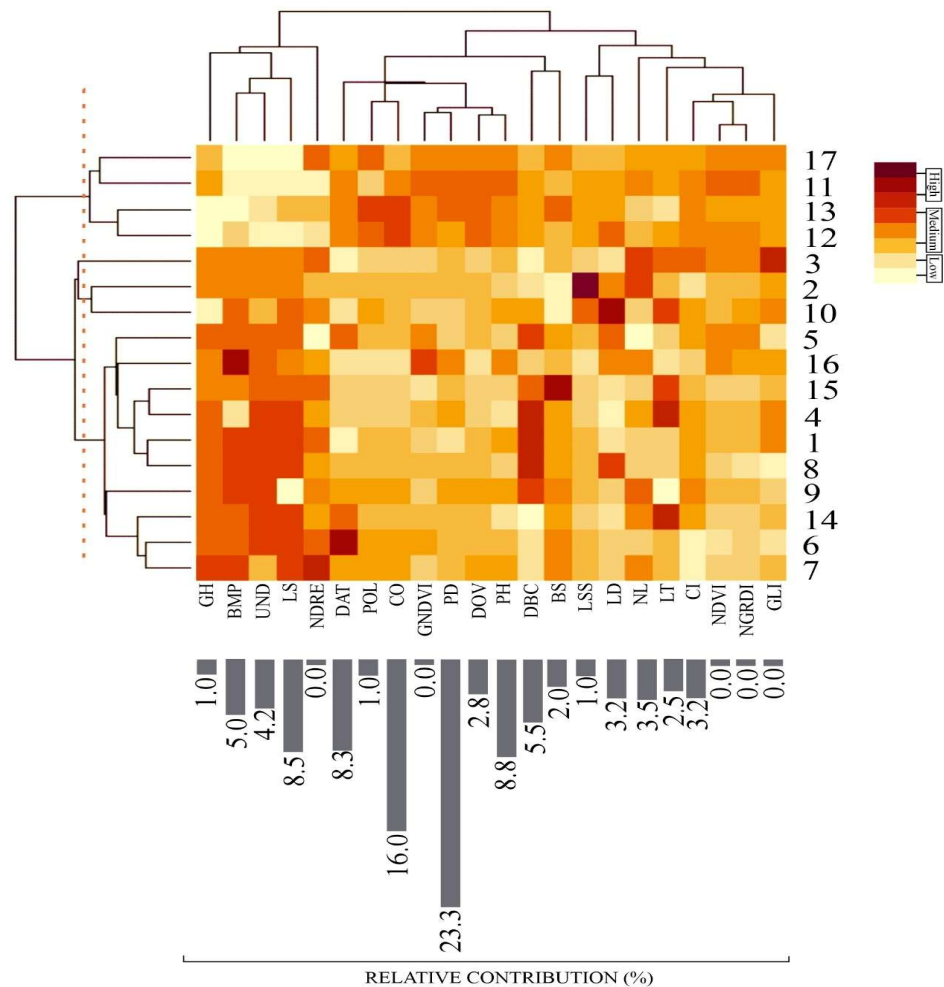


Figure 6. Dendrogram of genetic dissimilarity among 17 populations of lettuce, using the Euclidean distance, UPGMA Method. CCC = 96.11%.

The cut-off line delineated at 10.24% divergence, defined at a point of abrupt change in the dendrogram branching [26], allowed the formation of four distinct groups of lettuce populations. The first group was composed of the populations UFU 011, UFU 012, UFU 013 and UFU 017. The second group was composed of the populations UFU 002, UFU 003 and UFU 010. The third group was formed by the populations UFU 001, UFU 004, UFU 005, UFU 008, UFU 015 and UFU 016. The fourth group comprised the populations UFU 06, UFU 07, UFU 09 and UFU 014. In view of this, the formation of these groups suggests that the experiment generated varied responses, creating a favorable environment for validating the vegetation indices analyzed [60].

The results found in the formation of the first group suggest a strong alignment between the agronomic potential and the image-based phenotyping, since there is coherence of the results, because indeed these populations showed the best performance for all the morphological characteristics evaluated and vegetation indices (Figures 4 and 5). Thus, it can be proven that image-based phenotyping is a fast, accurate and non-destructive

tool, besides assisting in the indirect selection of lettuce genotypes, as already reported by several authors [55,56,61].

The internal portion of the dendrogram was visualized as a heat map, where warm (dark) colors indicate a greater response of the genotype in relation to the analyzed trait, and the same reasoning is valid for cold colors. Among the attributes studied, the ones that most contributed to the dissimilarity between the genotypes were plant diameter (23.3%) and compactness (16%).

To evaluate the relevance of these variables identified in the study, a correlation matrix (Pearson's r) was created (Figure 7). According to Coelho et al. [7], a coefficient of 0.30 is considered weak, 0.30 to 0.59 is considered moderate, 0.60 to 0.89 is considered strong, and 0.90 to 1.00 is considered very strong. Thus, understanding the correlation is relevant, because strongly correlated characteristics make indirect selection easier [62].

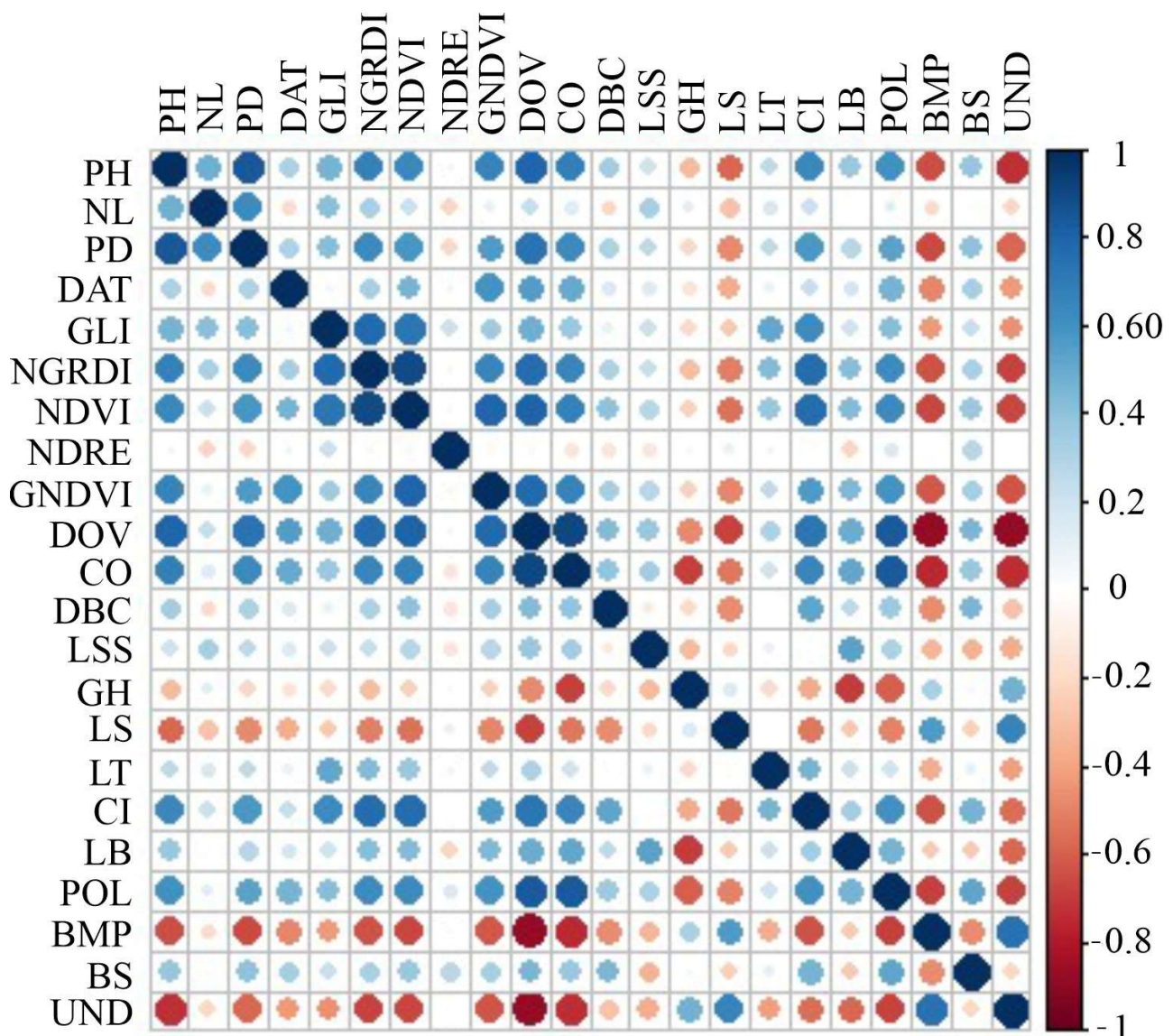


Figure 7. Correlation matrix (Pearson's r) for morphological descriptors, agronomic parameters and vegetation indices in lettuce populations. Significant correlations by the test are presented in shades of blue (positive) and red (negative). NL = number of leaves, PD = plant diameter, DAT = number of days for bolting, DOV = degree of overlap of the upper leaves, DBC = degree of base closure, LSS = longitudinal section shape, GH = growth habit, LS = leaf shape, LT = leaf thickness, CI = color intensity, LB = leaf brightness, POL = profile of the outer leaves.

When analyzing the morphological descriptors with the agronomic parameters and vegetation indices, it was observed that the correlation, in general, ranged from -1 (negative correlation) to 0.8 . Plant height was strongly correlated (0.80) with plant diameter and moderately correlated with number of leaves (0.50) and days after transplanting (0.40). On the other hand, the number of days after transplanting, which expresses crop resistance to bolting, showed a negative correlation with the number of leaves and a moderate correlation with plant diameter (0.4). The number of leaves, in turn, showed a strong correlation with plant diameter (0.8). Souza et al. [63] observed a negative correlation (-0.0337) between the number of leaves and the number of days for bolting, indicating that, although there is a weak relationship between these characteristics, genetic and environmental factors influence both, possibly due to different physiological mechanisms.

It was possible to observe the existence of correlation between the vegetation indices GLI, NGRDI, NVDI and GNDVI and the agronomic parameters, ranging from 0.2 (weak correlation) to 0.6 (moderate correlation), which indicates that the lettuce genotypes can be analyzed indirectly using remote sensing techniques. NDRE did not correlate with plant height and number of days after transplanting and showed a negative correlation with number of leaves and plant diameter (-0.2). GLI showed a strong correlation with plant height (0.6) and a moderate correlation with number of leaves and plant diameter (0.4), not correlating with the number of days after transplanting. NGRDI and NVDI behaved similarly in relation to agronomic characteristics, since they are directly associated with photosynthetic activity. Plant height and diameter showed a strong correlation (0.8), and the number of leaves showed a moderate correlation (0.4) [58]. GNDVI showed a low correlation with the number of leaves (0.2), a strong correlation with plant height and number of days after transplanting (0.8), and a moderate correlation with plant diameter (0.4).

The GNDVI and GLI indices are recommended for future research involving image-based phenotyping in lettuce cultivation, as they exhibited strong correlations with plant height. Recent studies on several vegetables have demonstrated the application of vegetation indices for crop monitoring. Clemente et al. [14], aiming to detect photosynthetic pigments in lettuce crop using image-based phenotyping, found that the GLI vegetation index had high potential to discriminate the anthocyanin content in purple lettuce genotypes. Assis et al. [60], when analyzing the vegetation indices NDVI, GLI and NGRDI in order to monitor agronomic performance in potato cultivation under mineral and organic fertilization, found a strong correlation when relating them to agronomic criteria.

In this context, the results of this study highlight the great potential of image-based phenotyping with multispectral sensors in the characterization of lettuce genotypes, through agronomic parameters and vegetation indices. This approach proves to be effective as it reduces labor costs and provides fast analysis and accurate data. In addition, the technique has a high correlation with anatomical, physiological and biochemical characteristics of plants, consolidating itself as a strategic tool in breeding programs, since it facilitates the characterization and selection of plants [55,64].

4. Conclusions

The results of this study indicate that image-based phenotyping has potential as a complementary and non-destructive tool for the characterization of lettuce populations. Analysis of the morphological and agronomic descriptors, combined with the vegetation indices derived from the multispectral images, allowed the identification of populations with high agronomic potential, particularly UFU 003, UFU 006, UFU 009, UFU 011, UFU 012, UFU 013, UFU 014, UFU 016 and UFU 017. These genotypes showed superior performance in traits such as plant diameter, number of leaves and tolerance to bolting, which reinforces their potential for advancement in breeding programs.

The strong correlation observed between image-based phenotyping data and field agronomic evaluations confirms the feasibility of using this approach for the indirect selection of promising genotypes, contributing to more efficient decision-making based on objective data. In addition, the application of vegetation indices, such as NDVI, GNDVI and GLI, proved to be effective in discriminating populations and identifying genetic variability, being a promising alternative for monitoring the crop at different phenological stages.

However, despite the advantages highlighted, the study reinforces the need for additional research to improve and validate this technique under different growing conditions and for different types of lettuce. Integration of low-cost sensors, combined with artificial intelligence algorithms, can further expand the use of image-based phenotyping, making it accessible to different producer profiles. In addition, conducting long-term studies can contribute to the definition of standardized protocols for data acquisition and analysis, allowing better comparability between experiments and different production regions.

Thus, image-based phenotyping emerges as an innovative and strategic approach for breeding programs, allowing not only a more accurate phenotypic characterization of lettuce populations, but also the optimization of agronomic selection and management processes, resulting in productivity and sustainability gains for the vegetable production chain.

Limitations and Future Prospects

Image-based phenotyping of lettuce genotypes has several advantages that make it a promising tool in breeding programs and agricultural management. Among the main benefits, precision and objectivity stand out for reducing subjectivity in phenotypic evaluations, allowing standardized and reproducible measurements, as well as better discrimination of genotypes based on detailed morphological and physiological parameters.

The speed and efficiency of the technique are also remarkable, since it allows large-scale data collection in a reduced time, optimizing the analysis of large plant populations and enabling continuous monitoring of the crop cycle. In addition, image-based phenotyping promotes a significant reduction in operating costs by reducing the need for specialized labor and field evaluation time, minimizing the use of inputs due to more accurate monitoring of plant pathology.

Another positive point is that the technique is non-destructive, allowing successive evaluations of the same plants without compromising their development, in addition to its multidisciplinary application, which integrates other precision agriculture tools, such as nutritional monitoring and water management. Finally, the capacity to detect water, nutritional and biotic stresses early stands out, allowing for rapid and effective interventions.

However, despite the advantages, image-based phenotyping also has some disadvantages that need to be considered. The high initial cost represents a significant barrier, especially for small producers, due to the investment required in equipment such as drones and multispectral sensors. In addition, operation and data interpretation require technical training, as specialized knowledge on remote sensing and image processing is required. Environmental conditions, such as lighting, humidity and wind, can influence the quality of the images acquired, limiting the accuracy of the analyses.

Another limitation is the spatial resolution of some sensors, which may not be sufficient to capture specific phenotypic details. Generating large volumes of data also poses challenges, as it requires storage infrastructure and computational capacity for complex analyses. Finally, the lack of standardization in data acquisition and processing methods can hamper comparison between different studies, compromising large-scale applicability.

In view of these aspects, there are several possibilities for future studies that can contribute to the evolution and improvement of image-based phenotyping in the lettuce crop. One such possibility is the development of predictive models based on artificial

intelligence, applying machine learning algorithms to predict agronomic performance based on multispectral data. Integration with climate and soil data also represents a valuable opportunity, as the combination of all this information can provide a more complete analysis of crop response to environmental conditions. Studies that validate this technology under different growing conditions are essential to evaluate its applicability on a large scale.

In addition, real-time monitoring, through the development of automated systems for continuous data capture and analysis, can help in making more agile decisions. Comparing multispectral and hyperspectral sensors can also yield important insights into which spectral ranges are most relevant to lettuce phenotyping.

Another promising aspect is the use of vegetation indices to predict post-harvest quality, contributing to improving the durability and nutritional value of lettuce after harvest. Reduction of costs with the use of low-cost sensors, such as RGB cameras, can expand access to this technology for small producers.

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