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ESTIMATION OF GENETIC GAINS BY DIFFERENT SELECTION CRITERIA IN BIOFORTIFIED MINI LETTUCE GENOTYPES

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ABSTRACT

The development of mini lettuce genotypes with good agronomic characteristics are fundamental to launch new cultivars. Thus, the objective was to compare the variability of biofortified mini lettuce genotypes by analyzing their principal components and hierarchical clusters, and evaluating different selection indices to estimate selection gain. The experiment was carried out at the Federal University of Uberlândia in a randomized block design implemented with 11 treatments (10 genotypes of mini lettuce generation F5:6 and one mini lettuce commercial cultivar) and four repetitions. The following variables were evaluated: chlorophyll content (SPAD índex), stem diameter and length, plant diameter and height, number of leaves per plant, fresh mass of the aerial part, and bolting tolerance. The data were submitted to analysis of variance and genotypes were compared using the Scott-Knott test ($P \leq 0.05$). Additionally, principal components, hierarchical clusters, and their correlations were evaluated ($P \leq 0.05$). The best genotypes were chosen by appropriate direct and indirect selection and the main indices: William's index, selection index from Smith and Hazel, and Mulamba y Mock index. There were five groups and significant negative correlations of all agronomic characteristics evaluated with resistance to bolting, except stem length. William's index provided a balanced distribution of genetic gains by selecting the genotypes UFU 66#8, UFU 215#2, UFU 215#7 and UFU 215#13.
Additional keywords: Bolting, direct selection, *Lactuca sativa*, selection gain

RESUMEN

Estimación de ganancias genéticas mediante diferentes criterios de selección en genotipos de minilechugas biofortificadas

El desarrollo de genotipos de mini lechugas con buenas características agronómicas es fundamental para el lanzamiento de nuevos cultivares. Por lo tanto, el objetivo fue comparar la variabilidad de genotipos de minilechugas biofortificadas mediante el análisis de sus componentes principales y grupos jerárquicos, y evaluar diferentes índices de selección para estimar la ganancia de selección. El experimento se realizó en la Universidad Federal de Uberlândia mediante un ensayo en bloques al azar, implementado con 11 tratamientos (10 genotipos de mini lechuga generación F5:6 y una mini lechuga cultivar comercial) y cuatro repeticiones. Se evaluó el contenido de clorofila (índice SPAD), diámetro y longitud del tallo, diámetro y altura de la planta, número de hojas por planta, masa fresca de la parte aérea y resistencia al espigado o floración prematura. Los datos se sometieron a análisis de varianza y se compararon los genotipos mediante la prueba de Scott-Knott ($P \leq 0,05$). Además, se evaluaron los componentes principales, los conglomerados jerárquicos y su correlación ($P \leq 0,05$). Los mejores genotipos fueron elegidos por selección directa e indirecta y por los principales índices: índice base de William, índice de selección de Smith y Hazel, y de Mulamba y Mock. Hubo cinco grupos y correlaciones negativas significativas de todas las características agronómicas evaluadas con la resistencia a la floración prematura, excepto para la longitud del tallo. El índice base de William proporcionó una distribución equilibrada de ganancias genéticas al seleccionar los genotipos UFU 66#8, UFU 215#2, UFU 215#7 y UFU 215#13.
Palabras clave adicionales: Floración prematura, ganancia de selección, *Lactuca sativa*, selección directa

INTRODUCCIÓN

Lettuce crops are being increasingly grown in small and medium farms located in the outskirts of big cities and commercialization centers, mainly owing to low production costs, not needing extensive areas for high economic

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profitability, and the search for healthier eating habits by the Brazilian population (Carvalho et al., 2016).

The growing demand for higher yields and differentiated and better-quality products has inspired genetic improvement programs to develop new cultivars with diversified standards of consistency, leaf color, plant size, and chemical composition. Miniature vegetables and biofortified foods stand out among these products.

There are specific mini lettuce cultivars, which have peculiar characteristics besides their small size, such as longer leaves, a narrower leaf limb, thicker leaves, and a thicker central rib than full-size lettuce (Martínez-Sánchez et al., 2012). However, specific mini lettuce cultivars are not being used by Brazilian producers.

Biofortified foods have a higher mineral and/or vitamin content than natural foods, there by complementing the nutritional requirements of the population. Several studies have been conducted on sweet potato and lettuce genotype biofortification with high beta-carotene content, which is a precursor of vitamin A (Cassetari et al., 2015; Jacinto et al., 2019; Silveira et al., 2019).

Nevertheless, there are still obstacles to lettuce cultivation, one of them related to the lack of cultivars adapted to tropical regions, i.e., excessive rainfall and heat, which impair leaf quality and, consequently, productivity and profitability. Additionally, as lettuce cultivars must have characteristics that meet the expectations of consumers and producers, genotype selection tools are necessary.

Multivariate analysis techniques can be useful to select the best genitors in breeding programs, as they allow the quantification of the genetic dissimilarity among genotypes (Lee et al., 2010; Shi et al., 2010). Some multivariate methods are used to predict genetic divergence, with principal component analysis by canonical variables and clustering methods being the most used techniques (Cruz et al., 2012).

Selection indices associated with multivariate analysis techniques allow genotype selection by considering several simultaneous characteristics (Cruz et al., 2012). Several studies highlight the use of selection indices in vegetables, such as potato (Silva et al., 2019), beans (Gomes et al., 2018) and sweet corn (Candido et al., 2020); however, this type of study has not been conducted in mini lettuce.

The hypothesis of this study was that the Vegetable Germplasm Bank of the Federal University of Uberlândia (UFU) contains promising lineages of biofortified mini lettuce genotypes to be launched as cultivars and one of the proposed selection indices is the most suitable to select mini lettuce genotypes based on agronomic characteristics. Therefore, the questions were: What are the most promising mini lettuce lineages in the UFU Germplasm Bank and what is the most appropriate selection index to estimate selection gain in mini lettuce?

Thus, the objective of this study was to compare the variability of biofortified mini lettuce genotypes by analyzing principal components and hierarchical clusters and evaluate different selection indices to estimate selection gain.

MATERIALS AND METHODS

The experiment was carried out in 2019 the UFU Vegetable Experimental Station, Monte Carmelo campus (18°43’36” South, 47°31’29” West, and 903 m altitude), which is part of the UFUs Breeding Program for Biofortified and Tropicalized Lettuce.

The soil is characterized as red-yellow Latosol with medium texture, and wavy to flat relief. According to the Koppen classification, the area is characterized as Aw (tropical, humid area, hot summers, with cold and dry winters). The temperature and rainfall data obtained from the Cooxupé meteorological station is shown in Figure 1.

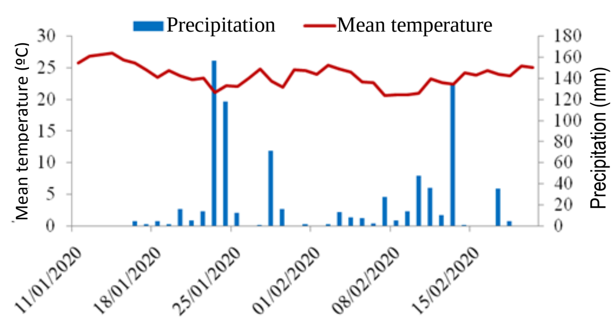


Figure 1. Variation of mean temperature of the air and pluviometric precipitation during the experiment

The study evaluated ten inbred lines of lettuce that were hybridized from the Belíssima cultivar and the carotenoid-rich Uberlândia 10,000 cultivar (Sousa et al., 2019) and five successive self-fertilizations carried out between 2013 and 2019.

A randomized block design was implemented, with 11 treatments and four repetitions. The treatments consisted of 10 $F_{5:6}$ generation mini lettuce genotypes from the cross between the Uberlândia 10,000 lineage (UDI 10.000) and Pira 72 (Belíssima) cultivar: 1 (UFU 66#3), 2 (UFU 66#4), 3 (UFU 66#7), 4 (UFU 66#8), 5 (UFU 215#1), 6 (UFU 215#2), 7 (UFU 215#6), 8 (UFU 215#7), 9 (UFU 215#13), and 10 (UFU MC MINIBIOFORT2). Additionally, a mini lettuce commercial cultivar (11-Purpurita) was used. The genotypes belong to the UFU Vegetable Germplasm Bank, Monte Carmelo campus, previously selected for high carotenoid levels in leaves.

Each experimental plot consisted of four 1.05 m long planting rows arranged with 0.15 m spacing between plants and 0.15 m between rows, which is recommended for mini lettuce cultivars (Castoldi et al., 2012). Ten plants per plot from the two central rows were evaluated.

The seeds were sown in 200-cell expanded polystyrene trays filled with Maxfertil coconut fiber-based substrate. The seedlings were maintained in a greenhouse (7 m × 4 m), covered with 150 µm transparent anti-UV plastic, until they reached the transplant stage.

When the seedlings had three to five permanent leaves, they were transplanted to beds. Previously, the soil in the experimental area was limed to increase base saturation to 70 % and fertilized with 30 $kg{\cdot}ha^{-1}$N, 300 $kg{\cdot}ha^{-1}P_2O_5$, and 18 $kg{\cdot}ha^{-1}K_2O$, based on the soil analysis results and the recommendations of Fontes (1999). The cover fertilization consisted of 30 $kg{\cdot}ha^{-1}$ N and 18 $kg{\cdot}ha^{-1}$ K_2O 15 days after planting, and 45 $kg{\cdot}ha^{-1}$ N and 27 $kg{\cdot}ha^{-1}$ K_2O 30 and 45 days after transplantation (Fontes, 1999).

The following variables were evaluated 40 days after transplantation, when the plants reached maximum vegetative development: stem diameter (SD), expressed in mm; plant diameter (PD), stem length (SL), and plant height (PH), expressed in cm; fresh mass of the aerial part (FM), expressed in $g{\cdot}plant^{-1}$; and number of leaves per plant (NL). The rest of the plot remained in the field to determine bolting tolerance (Bolt), i.e., the number of days to grow a floral bolt from transplant. This evaluation was carried out as follows: the plants that remained in the field were monitored daily. When the floral bolt was emitted, the number of plants and the number of days from transplant it took for this event to occur was noted. After making this note, these plants were marked so that they would not be included in the accounting again. When all the plants in the plot emitted the floral bolt, the weighted average of each plot of each block was made, thus obtaining the average number of days to tasseling.

At the morning time, one day before harvest, chlorophyll content was measured in four freshly ripened leaves from the middle third of each of the 10 plants using a SPAD Minolta model 502 CFL1030 chlorophyll meter.

The data obtained were submitted for the analysis of homogeneity of residual variance, residual normality and additivity. Once the assumptions were met, variance analysis was performed and, when significant effects were observed, the genotypes were compared using Scott-Knott clustering means. Additionally, multivariate analyses of principal components, hierarchical clusters (using the mean of the groups as a measure of dissimilarity), and correlation (using the t-test for coefficient significance) were performed. All analyses considered a 5 % probability significance level. The R Core Team software (2019) was used for data analyses and Sigma Plot 14.0 was used for the graphic presentation of the means.

The data were also analyzed to estimate genetic and phenotypic parameters using analysis of variance with mean square of genotypes (MSg) of the evaluated characteristics, as well as the environmental coefficient of variation (CVe), broad-sense heritability (h^2), genetic coefficient of variation (CVg), and relationship CVg/CVe of each variable.

The h^2 was calculated using the formula:

$$h^2 = \sigma_g^2/\sigma_f^2$$

where h^2= estimated coefficient of broad-sense heritability, σ_g^2 = estimated genotypic variance, and σ_f^2 = estimated phenotypic variance.

The calculation of the genetic coefficient of variation was obtained using the formula:

$$CV_g = 100 \times \sigma_g / m$$

where σg is the genetic standard deviation and m the experimental mean

Selection gain and best genotypes were selected using direct and indirect selection, William's base index (1962), the Smith (1936) and Hazel (1943) selection index, and the Mulamba and Mock (1978) rank-sum index, with 31 % selection intensity.

The CVg value was used for all selection index methods, as it provides a better distribution of gains and tends to increase gain (Cruz, 2013). All genetic analyses were processed using Genes software.

RESULTS

There were no significant differences among the genotypes evaluated regarding SD (F=1.43 ns, CV=15.71 %, mean= 7.53 mm) and SL (F=1.73 ns, CV=15.67 %, mean= 1.81 cm). The other characteristics evaluated presented significant differences among the genotypes evaluated (Figure 2).

PD varied from 9.01 (5-UFU 215#1) to 16.22 cm (4-UFU 66#8) and genotypes 1, 3, 4, 6, 8, and 9 presented higher PD than the other genotypes evaluated (Figure 2A). Six genotypes presented a superior PD to the commercial Purpurita cultivar (Figure 2A).

The genotypes were divided into four groups regarding PH, with the highest plant in genotype 10 and the lowest in genotype 2 and the Purpurita cultivar (Figure 2B).

The lowest NL was observed in the Purpurita cultivar, followed by genotype 5, which differed significantly from the other genotypes (Figure 2C).

In FM, the genotypes were divided into two groups, and the lowest means for this important characteristic were observed in Purpurita cultivar and genotypes 2, 5, and 7. However, the other genotypes had a higher FM than the commercial cultivar (Figure 2D).

The SPAD index, which has a positive correlation with carotenoid concentration (Cassetari et al., 2015), was significantly higher in genotypes 5 and 6 than in all other genotypes (Figure 2E). Genotypes 8, 9, and 10 had a SPAD index value higher than the standard commercial cultivar, indicating a higher carotenoid concentration. All genotypes presented a mean ≥ 40 days to bolting (Figure 2F). However, genotypes 2, 5, and 8 and the Purpurita cultivar presented more days to bolting than the other genotypes evaluated (Figure 2F).

The multivariate analysis of principal components showed that 77.3 % of the total variance was explained by components 1 and 2, with component 1 representing 53.21 % of the total variance (Figure 3A). Genotypes 5, 6 and 10 had the highest SPAD index values (Figure 2E), indicating that they are the richest in carotenoids (Cassetari et al., 2015), and genotypes 2, 5, and 11 (Purpurita) were the most tolerant to bolting, as they presented the highest number of days before bolt (Figure 2F); however, they exhibited the least increase of fresh mass of aerial part (Figure 2D).

Multivariate cluster analysis separated the tested genotypes into five groups. Two individual groups were formed by genotypes 5 and 7 (Figure 3B). A third group was formed by genotype 2 and the commercial cultivar Purpurita, a fourth group was formed by genotypes 9 and 10, and the fifth group was formed by two subgroups: one subgroup formed by genotypes 1, 3 and 4; and the other subgroup formed by genotypes 6 and 8 (Figure 3B).

The correlation analysis between the evaluated characteristics showed significant correlations; both positive and negative (Figure 4). SD showed a significant positive correlation with PD, NL, and FM and a significant negative correlation with flowering (Bolt) (Figure 4). PD presented a significant positive correlation with NL and FM. Stem length had no correlation with any characteristic evaluated (Figure 4).

PH a significant positive correlation with NL, FM, and the SPAD index and a negative correlation with Bolt. PH was the only variable correlated with the SPAD index. NL had a significant positive correlation with FM and negative correlation with bolt flowering. Similar to the other characteristics, FM had a significant negative correlation with flowering (Figure 4).

MSg showed genetic variability among genotypes, for all analyzed characteristics, except SD and SL (Table 1).

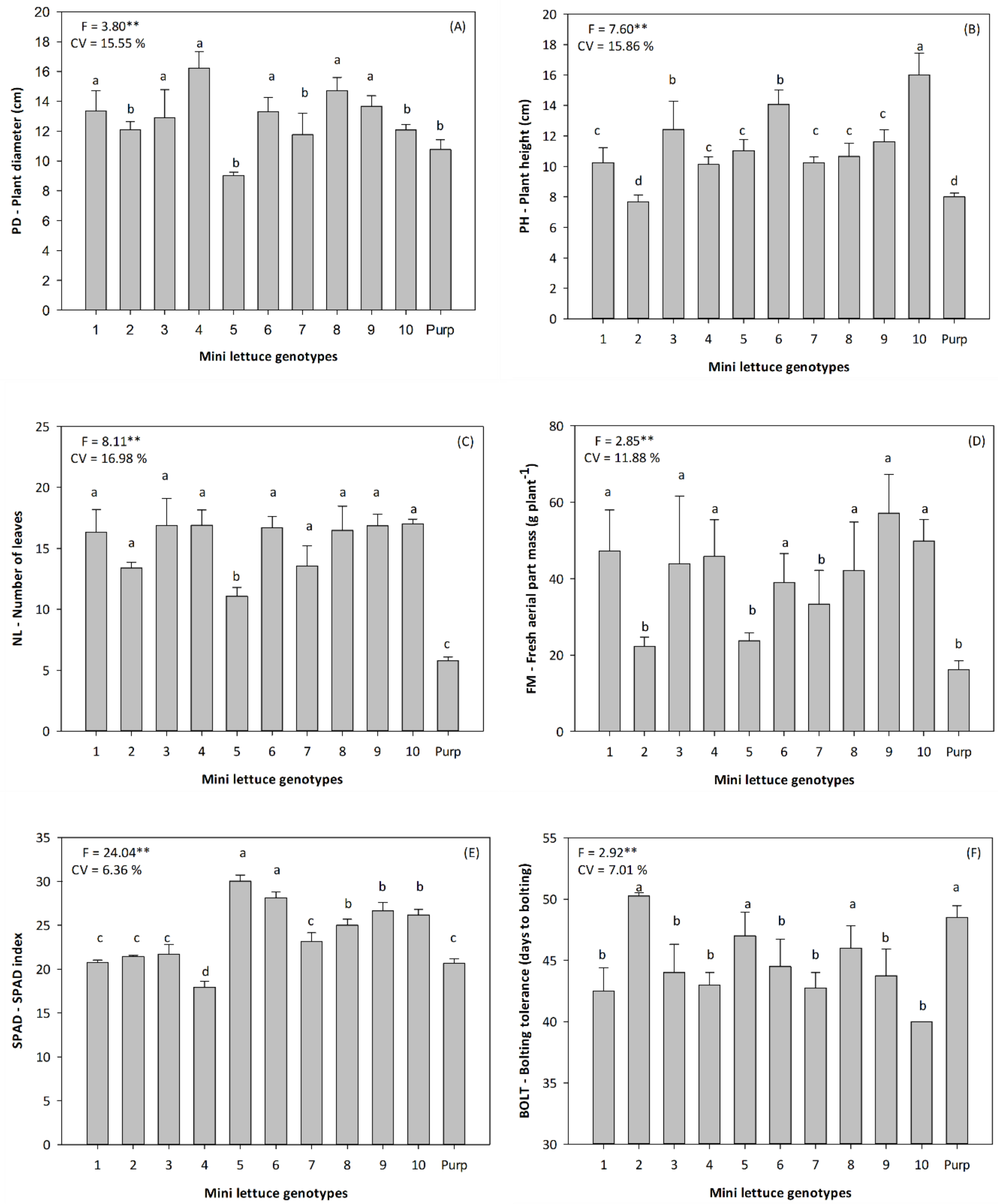
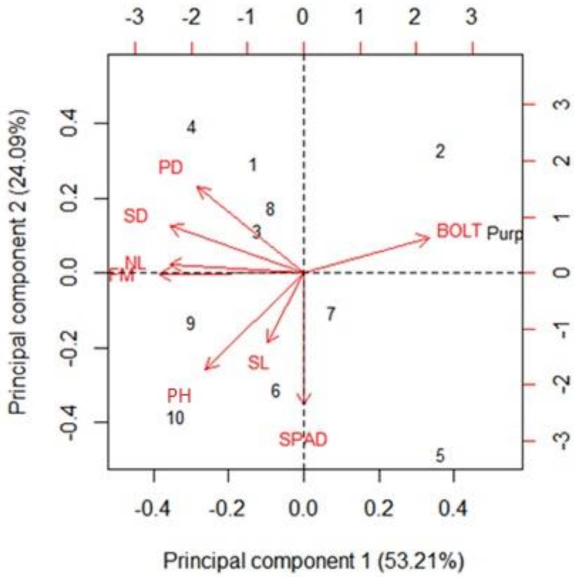
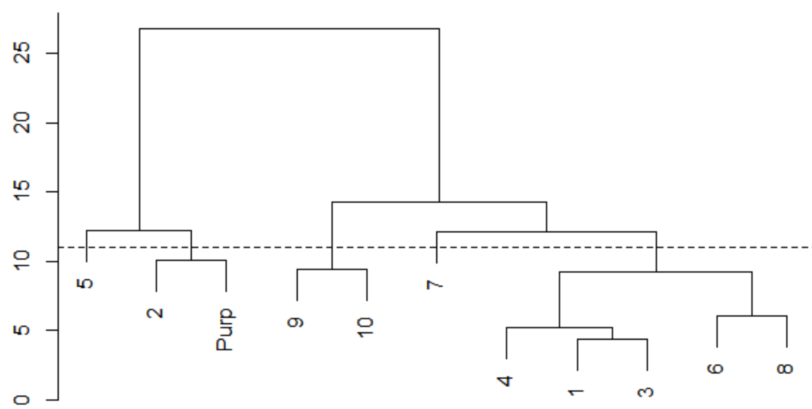


Figure 2. Mean agronomic characteristics, SPAD index, and bolting tolerance in biofortified mini lettuce genotypes selected for tropical conditions. Genotypes: 1-UFU 66#3, 2-UFU 66#4, 3-UFU 66#7, 4-UFU 66#8, 5-UFU 215#1, 6-UFU 215#2, 7-UFU 215#6, 8-UFU 215#7, 9-UFU 215#13, 10-UFU MC MINIBIOFORT2, and 11-Purpurita cultivar



(A)



(B)

Figure 3. Multivariate principal component (A) and cluster (B) analysis of biofortified mini lettuce genotypes selected for tropical conditions. Genotypes: 1-UFU 66#3, 2-UFU 66#4, 3-UFU 66#7, 4-UFU 66#8, 5-UFU 215#1, 6-UFU 215#2, 7-UFU 215#6, 8-UFU 215#7, 9-UFU 215#13, 10-UFU MC MINIBIOFORT2, and 11-Purpurita cultivar (Purp). SD: stem diameter, PD: plant diameter, SL: stem length, PH: plant height, FM: fresh aerial part mass, NL: number of leaves per plant, BOLT: bolting tolerance

The estimated h^2 ranged from 30.53 % (SD) to 95.84 % (SPAD) (Table 1), indicating genetic gain with selection (Luz et al., 2018).

CVg varied from 5.21 % (SD) to 22.65 % (NL) (Table 1) and is an extremely relevant parameter to infer the dimension of genetic variability in the population (Leite et al., 2016).

The superior genotypes could be identified through the following characteristics: PH (1.29), NL (1.33), and SPAD (2.40), as they had CVg/CVe values of greater than one (Table 1).

A selection intensity of 31 % selected four genotypes for each analyzed characteristic (Table 2).

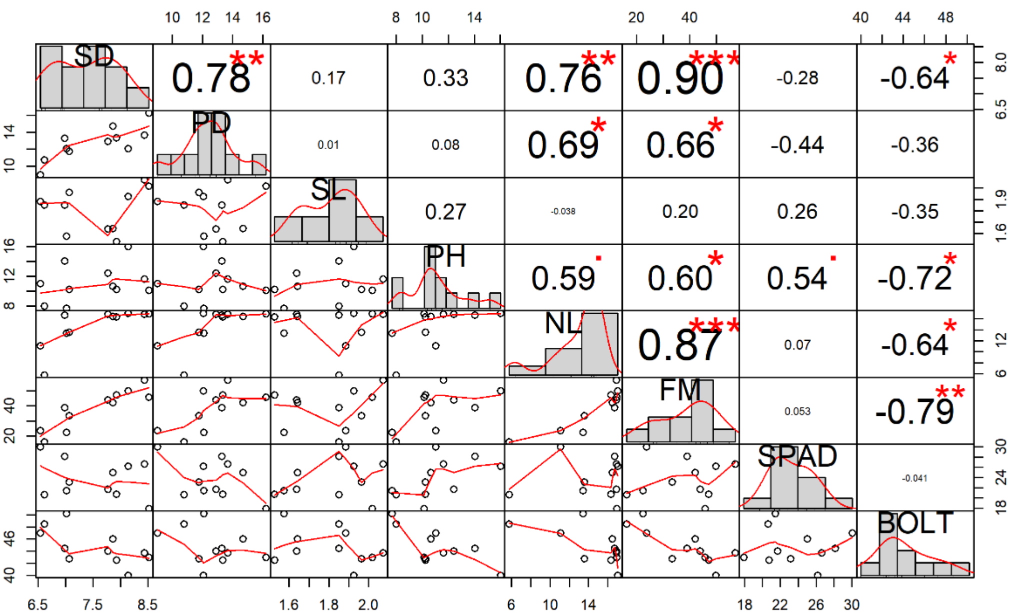


Figure 4. Pearson's correlation matrix of agronomic characteristics in the biofortified mini lettuce genotypes selected for tropical conditions. SD: stem diameter, PD: plant diameter, SL: stem length, PH: plant height, FM: fresh aerial part mass, NL: number of leaves per plant, BOLT: bolting tolerance

Table 1. Mean square of genotypes and estimation of agronomic characteristics genetic parameters of ten biofortified mini lettuce genotypes and one mini lettuce cultivar, Monte Carmelo, MG, Brazil, 2020.

Variables	MSg	h^2 (%)	CVg (%)	CVg/CVe	CV (%)
SD	2.02 ns	30.53	5.21	0.33	15.71
PD	14.88**	73.72	13.03	0.84	15.55
SL	0.14 ns	42.30	6.71	0.43	15.67
PH	23.55**	86.85	20.38	1.29	15.86
NL	50.12**	87.68	22.65	1.33	16.98
FM	0.56**	69.26	8.92	0.75	11.88
SPAD	54.93**	95.84	15.26	2.40	6.36
BOLT	34.90**	71.81	5.59	0.80	7.01

SD: stem diameter (mm); PD: plant diameter (cm); SL: stem length (cm); PH: plant height (cm); NL: number of leaves; FM: fresh aerial part mass (g); BOLT: bolting tolerance; MSg: mean square of the genotypes; h^2: coefficient of heritability; CVg: genetic coefficient of variation; CVg/CVe: genetic and environmental coefficient of variation ratio; *: Significant at 5 % probability, **: Significant at 1 % probability, ns: non-significant by the F test. Genotype 1: UFU 66#3; Genotype 2: UFU 66#4; Genotype 3: UFU 66#7; Genotype 4: UFU 66#8; Genotype 5: UFU 215#1; Genotype 6: UFU 215#2; Genotype 7: UFU 215#6; Genotype 8: UFU 215#7; Genotype 9: UFU 215#13; Genotype 10: UFU MC MINIBIOFORT2; Genotype 11: Purpurita

Table 2. Genetic gains estimate percentages by direct (red diagonal values) and indirect selection methods for six characteristics in ten biofortified mini lettuce genotypes and one mini lettuce cultivar, Monte Carmelo, MG, Brazil, 2020.

	PD	PH	NL	FM	SPAD	BOLT
PD	**10.28**	-0.02	5.82	6.48	-4.06	-6.21
PH	-3.43	**-16.34**	11.31	7.06	16.28	-13.77
NL	12.03	-13.34	**13.67**	12.85	4.67	-17.69
FM	5.32	-5.12	6.02	**6.75**	3.09	-7.04
SPAD	-4.84	-12.06	-2.71	-3.65	**16.01**	2.01
BOLT	-1.5	2.21	-3.31	-3.91	-1.5	**5.11**
Total	17.86	-44.67	30.8	25.58	34.49	-37.59
Selected genotypes	4; 8; 9; 1	2; 11; 4; 7	10; 4; 3; 9	9; 10; 4; 1	5; 6; 9; 10	2; 11; 5; 8

PD: plant diameter (cm); PH: plant height (cm); NL: number of leaves; FM: fresh aerial part mass (g); BOLT: bolting tolerance. Genotype 1: UFU 66#3; Genotype 2: UFU 66#4; Genotype 3: UFU 66#7; Genotype 4: UFU 66#8; Genotype 5: UFU 215#1; Genotype 6: UFU 215#2; Genotype 7: UFU 215#6; Genotype 8: UFU 215#7; Genotype 9: UFU 215#13; Genotype 10: UFU MC MINIBIOFORT2; Genotype 11: Purpurita

The greatest total positive genetic gains were obtained for SPAD, NL, FM, and PD, with 34.49 %, 30.80 %, 25.58 %, and 17.86 % respectively. Similarly, the greatest direct positive genetic gains were observed for SPAD (16.01 %), NL (13.67 %), and PD (10.28 %) (Table 2).

Table 3 shows genetic gain estimate results obtained with different selection indices. All indices provided negative bolt gain and positive gains for the other characteristics evaluated.

Table 3. Percentage of estimated genetic gain by index selection method in ten genotypes and one mini lettuce cultivar, Monte Carmelo, MG, Brazil, 2020.

Variables	Selection index		
	William's (1962)	Smith (1936) and Hazel (1943)	Mulamba and Mock (1978)
PD	4.20	3.07	10.21
PH	15.55	17.14	4.03
NL	12.78	12.81	12.59
FM	5.27	3.24	4.64
SPAD	10.92	5.91	2.59
BOLT	-1.91	-1.81	-0.7
Total	46.81	40.36	33.36
Selected genotypes	6; 8; 9; 10	3; 6; 8; 10	4; 6; 8; 9

PD: plant diameter (cm); PH: plant height (cm); NL: number of leaves; FM: fresh aerial part mass (g); BOLT: bolting tolerance. Selected genotypes are Genotype 3: UFU 66#7; Genotype 4: UFU 66#8; Genotype 6: UFU 215#2; Genotype 8: UFU 215#7; Genotype 9: UFU 215#13; Genotype 10: UFU MC MINIBIOFORT2

All indices selected genotypes 6 and 8; however, it was the Mulamba and Mock (1978) index that selected the genotypes with lower PH gain, lower bolt loss, and satisfactory values for the other characteristics.

The Mulamba and Mock (1978) rank-sum index had the lowest total gain value (33.36 %), followed by the Smith (1936) and Hazel tests (1943) (40.36 %) and Williams' index (1962) (46.81 %).

DISCUSSION

The genotypes used in this study have already been suggested, in the UFU Lettuce Improvement Program, for future launch as cultivars. These genotypes were selected for tropical conditions (average annual temperature of 24-25°C and average rainfall of 1500 mm) and, therefore, showed no SL differences, as they are bolting tolerance. There were no correlations between SL and Bolt (Figure 4). SL is related to early bolting tolerance, as the longer the stem the lower the tolerance (Resende et al., 2017). This effect was observed in the present study, as all genotypes required 40 days or more for flowering (Figure 2F), which is longer than the mini lettuce cycle, which generally lasts 35 days. Although all genotypes presented adequate performance in terms of bolting, it is worth highlighting the high value of genotypes 2, 5, and 8, which presented similar resistance to bolting as Purpurita cultivar (Figure 2F).

From the agronomic point of view, an ideal mini lettuce cultivar must present, besides bolting tolerance, other characteristics that are important to the consumer. Thus, ideal mini lettuce plants should present a high NL and an appearance that resembles a compact, but completely developed, common lettuce plant. Diamante et al. (2013) highlighted that NL is important both for the producer and the market, as it indicates the adaptation of the plant to the environment and is a relevant characteristic at the time of purchase. In this study, all genotypes presented superior performance to the commercial cultivar in terms of NL (Figure 2C), indicating that appropriate genotypes were selected to meet market demands. Genotype 5 presented the lowest NL; however, it had 52 % more leaves than the commercial cultivar (Figure 2C).

As all genotypes presented adequate NL when PD and FM were analyzed together, important characteristics in a superior plant, genotypes 1, 3, 4, 6, 8, 9, and 10 had the highest mean FM (Figure 2D), with dimensions suitable for mini lettuce (Figure 2A). It is also worth mentioning that these genotypes presented an agronomic performance equal or superior to commercial cultivars (Castoldi et al., 2012; Takahashi and Cardoso, 2014), indicating their potential to be launched as cultivars.

However, the consumer market is increasingly demanding regarding the appearance and nutritional quality of vegetables. In this sense, in addition to selecting plants with adequate agronomic and commercial performance, the selection of genotypes with special nutritional characteristics, such as carotenoids, is relevant. In this study, associating agronomic performance with high carotenoid concentrations highlights genotype 6, followed by genotypes 8, 9, and 10, which presented high SPAD index values (Figure 2E), indicating a high carotenoid concentration (Cassetari et al., 2015).

It is worth mentioning that these genotypes have UDI 10,000 as parental cultivar, which has been used as a reference in several studies as a high carotenoid level genotype (Jacinto et al., 2019; Sousa et al., 2019; Maciel et al., 2020). Additionally, genotype 10 has been registered in the Ministry of Agriculture and Livestock (Process number 35967) as a mini lettuce cultivar biofortified in carotenoids.

Principal component analysis indicated that there was minimal loss of information, as 77 % of the total variance was explained by the first and second principal components.

A significant MSg indicates the existence of genetic variability among genotypes for PD, PH, NL, FM, SPAD index, and BOLT (Table 1). According to Baldissera et al. (2014), most of the estimated h^2 values in the present study can be considered adequate to assume a phenotypic selection success for genetic gain. However, according to Ramalho et al. (2012), as h^2 values for SD and SL were less than 70 %, they cannot be considered high enough to successfully select superior genotypes.

One of the most important parameters in plant breeding is h^2 because it is directly related to genetic gain.

A low h^2 for SD and SL was probably observed because these were advanced genotypes for SD selection, i.e., tolerant to bolting. Cruz et al. (2012) reported that the use of secondary characteristics with a high h^2 and high correlation with the characteristic of interest may increase genetic gain.

Direct selection is based on maximum genetic gains for only one characteristic, indirectly resulting in favorable or unfavorable genetic gains for other variables (Cruz et al., 2012).

The CVg/CVe ratio can be used to indicate the degree of genotype selection for each character. When the estimated quotient is greater than or equal to one, genetic variation is responsible for the estimated variation in experimental data (Leite et al., 2016). The CVg was higher than the CVe in 38 % of the characteristics evaluated (PH, NL, and SPAD), which was verified by the high CVg/CVe ratio (≥1), indicating that genetic factors have a greater influence on expressing these characteristics than environmental factors (Leite et al., 2016).

Knowledge about direct selection and the indirect effects of principal characteristics on the secondary ones can optimize the selection of superior genotypes, as characteristics of little relevance can be rejected early (Cruz et al., 2012).

Total genetic gains were positive for 67 % of the evaluated characteristics (PD, NL, FM, and SPAD). The most important factors that directly or indirectly interfere with selection gains are selection intensity, genetic characteristics of the population, and environmental conditions. The gain is directly related to the difference between the mean of the selected group and that of the original population (Hamawaki et al., 2012).

The indirect response of SPAD, NL, and PD characteristics compared with the others present unsatisfactory values of these characteristics for bolting because the direct selection of these characteristics decreases the sudden growth of a flower stalk in the plants (Table 2). Therefore, if the selection was based on SPAD, NL, and PD characteristics, plants with a lower bolt value, i.e., a reduced number of days before flowering, would be selected, which is not desired in tropicalized lettuce cultivars.

For mini lettuce cultivars, the intention is to select more compact plants; thus, the PH value should be negative. However, selection based on this characteristic resulted in unsatisfactory indirect gains.

In this study, no direct selection resulted in genetic gains for all characteristics evaluated, which corroborates the results by Finzi et al. (2020) for tomatoes. William’s index showed the largest total gain, whereas Mulamba and Mock had the smallest gain, diverging from the results reported by Silva et al. (2020a) and Silva et al. (2020b), who showed the largest total gain for beans and sweet corn, respectively, using the Mulamba and Mock index.

Although the rank-sum index showed the smallest total gain, it provided a balanced distribution of the selection gains for the evaluated characteristics. Similarly, Candido et al. (2017) verified that the Mulamba and Mock index results in good gains for the evaluated characteristics when working with selection indices for curly lettuce genotypes and, as it is strongly correlated with the other studied indices owing to is easy construction, they recommended its use to select curly lettuce genotypes.

CONCLUSIONS

The multivariate analysis of principal components showed that 77.3 % of the total variance was explained by components 1 and 2.

Multivariate cluster analysis separated the evaluated genotypes into five groups (group i: UFU 215#1; group ii: UFU 215#6; group iii: UFU 66#4 and Purpurita commercial cultivar; group iv: UFU 251#13 and UFU MC minibiofort2; and group v: UFU 66#3, UFU 66#7, UFU 66#8, UFU 215#2, and UFU 215#7). All agronomic characteristics evaluated showed a significant negative correlation with Bolt, except SL, and a significant positive correlation was observed between PH and the SPAD index.

The genetic parameters for all characteristics were medium to high and these conditions were satisfactory to obtain significant genetic gains with superior genotype selection. The Mulamba and Mock Index was the most appropriate index to select genotypes based on the agronomic characteristics of biofortified mini lettuce. The genotypes UFU 66#8, UFU 215#2, UFU 215#7, and UFU 215#13 were selected for future improvement programs based on the analyzed variables and chosen index.

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