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Genetic parameters and selection of biofortified lettuce genotypes based on selection indices[1]

Parâmetros genéticos e seleção de genótipos de alface biofortificadas com base em índices de seleção

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HIGHLIGHTS:
There is genetic variability in the germplasm of biofortified lettuce.
There is a positive genotypic correlation between agronomic traits in biofortified lettuce.
Smith-Hazel, Williams, and Mulamba & Mock indices showed good direct gains in biofortified lettuce genotypes.

ABSTRACT: Obtaining biofortified vegetables with an emphasis on lettuce is a tool to improve the nutritional status of the population. Selection indices can maximize the simultaneous selection of good agronomic traits and high carotenoid levels. The objective of this study was to estimate the genetic parameters and efficiency of different indices in selecting genotypes of biofortified lettuce with high concentrations of carotenoids and favorable agronomic traits. Statistical analyses were performed on 91 genotypes belonging to the vegetable germplasm bank of the Federal University of Uberlândia, Brazil. The variables analyzed were the chlorophyll index of the leaves, plant diameter, stem diameter, and number of leaves per plant. The values found for narrow sense heritability (h^2) ranged from 89.63% (stem diameter) to 96.05% (chlorophyll), showing a high magnitude. The Smith-Hazel index, sum of ranks by the Mulamba & Mock index, direct and indirect selection, and Williams base index were used to predict the selection gains. A total of 17 individuals were selected using the selection methodologies. The Smith-Hazel, Williams, and Mulamba & Mock indices were efficient in showing good direct gains for the evaluated traits. Thirteen genotypes were selected for all indices presenting suitable agronomic traits, which show promise for advancing generations within the breeding program to obtain biofortified lettuce strains.

Key words: *Lactuca sativa* L., biofortification, correlation, selection gain

RESUMO: A obtenção de vegetais biofortificados, com destaque para a alface, é uma ferramenta para melhorar o estado nutricional da população. Os índices de seleção podem maximizar a seleção simultânea de boas características agronômicas e altos níveis de carotenoides. O objetivo deste estudo foi estimar os parâmetros genéticos e a eficiência de diferentes índices para a seleção de genótipos de alface biofortificados combinados com características agronômicas adequadas. As análises estatísticas foram realizadas com 91 genótipos pertencentes ao banco de germoplasma de alface biofortificada da Universidade Federal de Uberlândia. As variáveis analisadas foram: índice de clorofila das folhas, diâmetro da planta, diâmetro do caule e número de folhas por planta. Os valores encontrados para herdabilidade no sentido restrito (h^2) variaram de 89,63% (diâmetro do caule) a 96,05% (clorofila), apresentando alta magnitude. O índice de Smith-Hazel, a soma de "ranks" de Mulamba & Mock, a seleção direta e indireta e o índice base de Williams foram usados para prever os ganhos de seleção. No total, 17 indivíduos foram selecionados usando as metodologias de seleção. Os índices de Smith-Hazel, Williams e Mulamba & Mock são eficientes em mostrar bons ganhos diretos para as características avaliadas. Treze genótipos foram selecionados por todos os índices apresentando características agronômicas favoráveis, sendo promissores para o avanço de geração no programa de melhoramento para obter linhagens de alface biofortificadas.

Palavras-chave: *Lactuca sativa* L., biofortificação, correlação, ganho de seleção

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Introduction

Lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in the world; consequently, there is a market demand for high-quality lettuce (Candido et al., 2017). Additionally, the consumption of biofortified vegetables has become essential for improving the nutritional status of those with nutritional deficiencies in the population.

Among the various constituents that characterize biofortification, carotenoids are of great relevance in several species (Machada Junior et al., 2017; Oliveira et al., 2018); however, studies on the biofortification of lettuce are limited. In addition, a positive correlation was observed between carotenoid and chlorophyll concentrations measured indirectly using the soil plant analysis development (SPAD) index (Silva et al., 2014; Cassetari et al., 2015). In this way, the indirect selection of superior individuals with higher carotenoid concentrations is possible by measuring the chlorophyll index. Currently, there are no commercial lettuce cultivars available that have desirable agronomic traits along with a high carotenoid concentration. This may be due to the limited knowledge of the variables that govern the genetic inheritance of chlorophyll production and the main agronomic traits of interest in lettuce (Oliveira et al., 2019).

Estimates of the parameters of variability, heritability, and genetic gain are reliable indicators for the improvement of the characteristics of a particular genetic material through selection (Kumar et al., 2015). In addition, knowledge of the correlation between complex traits, such as the yield and its component characteristics, is of considerable importance for a rational approach to yield improvement (Samnotra et al., 2012).

Selection indices allow several characteristics to be improved simultaneously, regardless of the existence of a correlation among them (Cruz et al., 2014), but there have been limited studies on lettuce strains. Thus, the objective of this study was to estimate the genetic parameters and efficiency of different indices in selecting genotypes of biofortified lettuce with high concentrations of carotenoids and favorable agronomic traits.

Material and Methods

The experiment was carried out under field conditions between February and April 2016 in the city of Monte Carmelo, Brazil (18° 42' 43.19" S, 47° 29' 55.8" W; 873 m above sea level). The mean, minimum, and maximum temperatures during the experimental period were 23.54, 18.72, and 30.31 °C, respectively, with a mean relative air humidity of 77.85% and a total precipitation of 426.8 mm (Figures 1A and B).

A total of 91 genotypes were evaluated, with 86 lettuce genotypes from the hybridization between Pira 72 and Uberlândia 10000, which is rich in carotenoids (Sousa et al., 2007), followed by three successive self-fertilizations carried out between 2013 and 2016. A pedigree breeding method was used.

These genotypes are registered at the Federal University of Uberlândia's Biofortified Lettuce Genetic Improvement

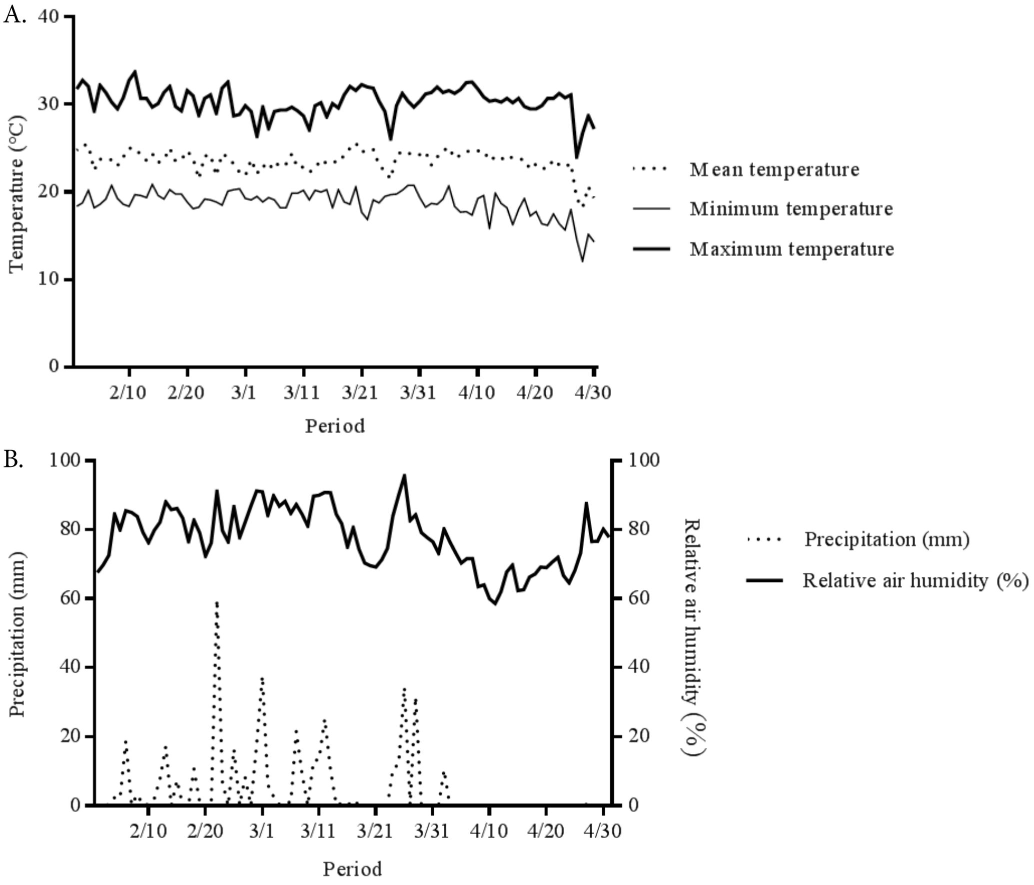

Figure 1. (A) Maximum, minimum, and mean temperature, and (B) precipitation and relative air humidity during the experimental period

Program, and the entire genealogy is stored in the "BG α BIOFORT" software registered at INPI BR512019002403-6 (Maciel et al., 2019). Five controls were used: the commercial cultivars Grand Rapids, Pira 72, and Robusta, with low carotenoid concentrations; cv. UFU-Biofort and genotype Uberlândia 10000 (Sousa et al., 2007), with high carotenoid concentrations.

Sowing was carried out in expanded polystyrene trays of 200 cells filled with the commercial substrate (70% sphagnum peat + 30% vermiculite + limestone; electrical conductivity = 0.7 mS cm^{-1}, pH = 5.5 ± 0.25, water holding capacity = 350 mm; dry density = 130 kg m^{-3}). After sowing, the trays were maintained in an arch-type greenhouse, with dimensions of 5 × 6 m and a 3.5 m high ceiling, covered with 150-micron transparent polyethylene film, which was protective against ultraviolet rays, and side curtains made of white anti-aphid screens.

Twenty-five days after sowing, the seedlings were transplanted to the field in 1.25 m seedbeds, which were previously prepared by a rotary harvester. These were fertilized according to recommendations required by the crop(Filgueira, 2013), which consisted of the application of 643 kg ha^{-1} of the N-P-K formula (4-14-8) applied at the time of the transplant, and weekly fertilization with the N-P-K formula of 20-5-20 (5 g per plant), and 50 g ha^{-1} of the mineral calcium borate Calbor®. The soil, classified as Oxisol, presented the following characteristics: clay texture, containing more than 50% clay in its composition; pH in $CaCl_2$ = 4.9; organic matter = 3.9 dag kg^{-1}; P Mehlich-1 = 79.1 mg dm^{-3}; K = 0.29 $cmol_c$ dm^{-3}; Ca = 3.3 $cmol_c$ dm^{-3}; Mg = 1.3 $cmol_c$ dm^{-3}; H + Al = 4.9 $cmol_c$ dm^{-3}; sum of bases = 4.90 $cmol_c$ dm^{-3}; T = 9.80 $cmol_c$ dm^{-3}; V% = 50. Each plot consisted of 20 plants with a spacing of 25 × 25 cm. Six central plants were evaluated.

Forty-five days after transplantation, the following evaluations were performed: (1) chlorophyll index, measured using the chlorophyll meter SPAD (Minolta SPAD-502 CFL1030 model), with an accuracy of ± 1.0 SPAD unit, for values between 0.0 and 50.0 at normal temperature or humidity on the median leaf of the plant in the morning; (2) plant diameter (cm) using a graduated ruler; (3) stem diameter (mm) using digital calipers; and (4) the number of leaves per plant.

The statistical design used was randomized blocks with 91 treatments and three replicates. To assess the existence of genetic variability for the quantitative traits, an analysis of variance (ANOVA) was performed using the following statistical model:

$$y_{ij} = \mu + b_j + g_i + e_{ij} \tag{1}$$

where:

y_{ij} - observation of the i-th genotype in the j-th block;
μ - fixed effect of the general mean;
b_j - effect of the j-th block;
g_i - effect of the i-th genotype; and,
e_{ij} - experimental error.

The data obtained were analyzed to estimate the selection gain. In addition, the F test ($p \leq 0.01$) was performed to obtain the mean square (MS) and coefficient of variation (CV). The coefficient of genetic variation (CVg), the ratio between the CVg and the coefficient of experimental variation (CVe): CVg/CVe, and the narrow sense heritability (h^2) for each variable were also assessed. The h^2 was calculated using the following equations:

$$h^2 = \frac{\sigma_p^2}{\frac{GMS}{r}} \tag{2}$$

$$\sigma_p^2 = \frac{GMS - EMS}{r} \tag{3}$$

where:

h^2 - narrow sense heritability;
$\sigma_p{}^2$ - variance component;
GMS - genotype mean square;
EMS - error mean square; and,
R - number of replicates.

The CVg and (CVe) were calculated using Eqs. 4 and 5:

$$CV_g\,\% = \frac{\sigma_p^{0.5}}{M} 100 \tag{4}$$

$$CV_e = \frac{\sigma_E}{M} 100 \tag{5}$$

where:

σ_E - standard deviation of the experimental residue, equal to $EMS^{0.5}$; and,
M - experimental average.

The genotypic correlation estimator was:

$$r_g = \frac{\sigma_{gxy}}{\sqrt{\sigma_{gx}^2 \sigma_{gy}^2}} \tag{6}$$

where:

r_g - genotypic correlation estimator;
X and Y - traits analyzed;
σ_{gxy} - genotypic covariance between X and Y; and,
$\sigma_{gx}{}^2$ and $\sigma_{gy}{}^2$ - genotypic variance estimators of X and Y, respectively (Cruz et al., 2014).

The estimates of the selection gains were obtained through methodologies using different indices, such as direct and indirect selection (Cruz et al., 2014), the classic index proposed by Smith (1936) and Hazel (1943), Mulamba & Mock rank index (1978), and Williams base index (1962). The selection intensity was set at 20%. The selection gain was obtained through the product of the heritability value by the selection differential value, and the results are presented as percentage values (Eq. 7):

$$SG(\%) = \left(X_{si} - X_{oi}\right) h_i^2 100 = DS_i h^2 100 \tag{7}$$

where:

SG(%) - selection gain in percentage;
X_{si} - average of the strains selected for character i;
X_{oi} - original average of the population;
DS_i - selection differential practiced in the population;
h^2i - heritability of character i; and,
h^2 - narrow sense heritability.

The Smith (1936) and Hazel (1943) indices were estimated using the selection index (I) and the genotypic aggregate (H) (Eqs. 8 and 9):

$$I = b_1y_1 + b_2y_2 + \ldots + b_ny_n = \sum_{i-1}^{n} b_iy_i = y'b \qquad (8)$$

$$H = a_1g_1 + a_2g_2 + \ldots + a_ng_n = \sum_{i-1}^{n} a_ig_i = g'a \qquad (9)$$

where:

n - number of characters evaluated;
b - vector of dimension 1 × n of the weighting coefficients of the selection index to be estimated;
y - dimension matrix n × p (plants) of phenotypic values of the characters;
a - vector of dimension 1 × n of previously established economic weights; and,
g - n × p dimension matrix of unknown genetic values of the n characters considered.

The vector used was:

$$b = P^{-1}Ga$$

where:

P^{-1} - inverse of the matrix, dimension n × n, of phenotypic variances, and covariance between the characters; and,
Ga - matrix, dimension n × n, of the genetic variances, and covariance between the characters.

The expected gain for character j was expressed by Eq. 10:

$$\Delta g_{i(i)} = DS_{i(i)}h^2j \qquad (10)$$

where:

$\Delta g_{j(i)}$ - expected gain for character j, with selection based on index I;
$DS_{j(i)}$ - selection differential for character j, with selection based on index I; and,
h^2j - heritability of character j.

In the Mulamba & Mock index, the order of each genotype was added, resulting in the selection index, as described in Eq. 11:

$$I = r_1 + r_2 + \ldots + r_n \qquad (11)$$

where:

I - value of the index for a given individual or family;
r_j - classification (or "rank") of an individual in relation to the j-th character; and,
n - number of characters considered in the index.

Weights were given by:

$$I = p_1r_1 + p_2r_2 + \ldots + p_nr_n \qquad (12)$$

where:

p_j - economic weight attributed to the j-th character.

For the Williams index, the following index was used as the selection criterion (Eq. 13):

$$I = a_1y_1 + a_2y_2 + \ldots + a_ny_n = \sum_{i-1}^{n} a_iy_i = y'a \qquad (13)$$

where:

y - averages; and,
a - economic weights of the characters studied.

The selection criterion used was increased for all traits for direct and indirect selection (Mulamba & Mock, 1978; Smith, 1936; Hazel, 1943; Williams, 1962) indices. The economic weight adopted was the coefficient of genetic variation for each variable, as recommended by Cruz (2012). The data were analyzed using the GENES software (version 2015.5.0) (Cruz, 2013).

RESULTS AND DISCUSSION

Genetic variability was observed in the following characteristics: chlorophyll index, plant diameter, stem diameter, and number of leaves (Table 1). Therefore, it is possible to select agronomically superior accessions for these traits (Cruz, 2012).

The CVe ranged from 13.20% for plant diameter to 19.19% for stem diameter (Table1). In this study, the CVe were considered to be medium, demonstrating the good precision of the experiment. According to Pimentel-Gomes (2009), in field conditions, CV values below 10% are considered low, medium if between 10 and 20%, high if between 20 and 30%, and very high if above 30%.

Cândido et al. (2017) found CV values of 7.79% for the number of leaves in lettuce. Peixoto et al. (2020), in their study

Table 1. Mean squares, coefficients of variance, and genetic parameters of variables analyzed in 91 lettuce genotypes

Parameters	Variables			
	Chlorophyll	Plant diameter	Stem diameter	Number of leaves
h^2 (%)	96.05	94.15	89.63	92.23
CVg (%)	23.33	16.76	17.82	20.13
CVg/CVe	1.56	1.26	0.93	1.09
CVe (%)	14.95	13.20	19.16	18.46
GMS	439.52**	181.74**	164.06**	521.77**
EMS	17.33	10.62	17.00	40.51

h^2 - Narrow sense heritability; CVg - Coefficient of genetic variation; CVe - Coefficient of experimental variation; GMS - Genotype mean square; EMS - Error mean square; ** - Significant at $p \leq 0.01$ by F test on biofortified curly lettuce, found CV values similar to those found in the present study for stem diameter (11.99%), and lower values for the number of leaves (9.77%), plant diameter (7.99%), and SPAD index (6.24%).

The CVg is an important parameter that makes it possible to determine the magnitude of genetic variability in the population for all the characters being analyzed (Vencovsky & Barriga, 1992). The CVg had values between 16.76% for the plant diameter and 23.33% for the chlorophyll index (Table 1), indicating that these traits had large amounts of genetic variability.

Souza et al. (2008) characterized heat-tolerant lettuce progenies and observed CVg values of 6.39% for plant diameter and 6.97% for the number of leaves. However, there were differences between the genotypes, which could account for the disparity in the results.

The variables for h^2 in the present study, with values in ascending order, were as follows: stem diameter (89.63%), number of leaves (92.23%), plant diameter (94.15%), and chlorophyll index (96.05%) (Table1). These values show that the genotype has a greater effect on the evaluated trait, and the h^2 is considered high for values greater than 0.7 (Baldissera et al., 2014). Characters with a high h^2 indicate a high component of the inheritable fraction of the variation (genetic component) in their phenotypic expressions, demonstrating that gains can be achieved through visual selection (Silva et al., 2019), as performed in the present study

Peixoto et al. (2020) estimated the genetic parameters of eight variables analyzed in 25 lettuce genotypes from the same breeding program (generation F7) and also found high h^2 values for the number of leaves (91.42%), SPAD index (95.96%), and plant diameter (72.77%), confirming the high magnitude of h^2 for these traits. In contrast to this study, Oliveira et al. (2019) found low heritability estimates of 6.94% for the chlorophyll index, which may be related to the fact that although it was carried out in the same experimental area with the same cultural practices, different parents were used. Thakur et al. (2016) found a heritability value of 58.10% for β-carotene in lettuce, which may have been influenced by the fact that the experiment was conducted under protected conditions.

The values obtained for the CVg/CVe ratio were close to or greater than one for all the evaluated traits, which corroborates that genetic variation is the main factor responsible for the estimated variation of the character, as when the CVg/CVe ratio approaches a value of one or greater, the selection gain is increased (Vencovsky & Barriga, 1992). Similar results were also observed by Souza et al. (2008) and Peixoto et al. (2020), who evaluated lettuce genotypes and found CVg/CVe ratios close to or greater than one for the variables analyzed.

It is possible to determine the association between the characters using genotypic correlations (Table 2).

Positive correlations were found between most of the agronomic traits, which is favorable for the improvement of lettuce (Azevedo et al., 2014). The stem diameter showed a significant positive correlation with the number of leaves (Table 2).

Peixoto et al. (2020) correlated six variables of biofortified lettuce and observed that the stem diameter was positively correlated with the number of leaves and plant diameter. In addition, the plant diameter was negatively correlated with the SPAD index, similar to these results.

Table 2. Genotypic correlations between the variables evaluated in 91 lettuce genotypes

	Variables			
	Chlorophyll	Plant diameter	Stem diameter	Number of leaves
Chlorophyll	-	-0.05	0.08	0.16
Plant diameter	-0.05	-	0.25*	0.28*
Stem diameter	0.08	0.25*	-	0.63**
Number of leaves	0.16	0.28*	0.63**	-

**, * - Significant at $p \leq 0.01$ and $p \leq 0.05$ by t test, respectively

The direct selection gains obtained for the variables evaluated were, in decreasing order: 32.17% for the chlorophyll index, 27.20% for the number of leaves, 23.73% for the stem diameter, and 22.89% for the plant diameter (Table 3). In this study, direct selection provided individual gains greater than those of indirect selection, similar to the results found by Peixoto et al. (2020).

Direct selection of the number of leaves promoted indirect expected gains for the chlorophyll index, plant diameter, and stem diameter (Table 3). This was the best indirect selection strategy, as it enabled positive gains for all characters evaluated, in addition to promoting the greatest total selection gain. Peixoto et al. (2020) also concluded that direct selection of the number of leaves was the best indirect selection strategy for biofortified lettuce germplasm.

Diamante et al. (2013) reported the importance of the characteristic number of leaves in growing lettuce as being relevant for the producer, both for indicating adaptation of the genetic material to the environment, and for the commercialization of lettuce. Higher concentrations of chlorophyll enhance photosynthetic activity and may lead to agronomic trait increments (Silva et al., 2014).

Selection indices, unlike direct selection, make it possible to carry out simultaneous selection of several characteristics of economic importance, increasing the chance of success in genetic breeding (Tassone et al., 2019).

The selection indices of Smith-Hazel, Williams, and Mulamba & Mock enabled good direct gains for all evaluated characters, showing comparable results and promoting balanced gain distribution (Table 4).

Candido et al. (2017), when evaluating strains of loose-leaf lettuce in different locations, found that the Mulamba & Mock index enabled better direct gains for the traits evaluated, with total gains ranging from 24.56–46.85%. On the other hand, in

Table 3. Selection gain estimates (20% of the selection intensity) obtained by direct selection and indirect selection for four evaluated variables in 91 lettuce genotypes

Variables	Selection gain (%)			
	Chlorophyll	Plant diameter	Stem diameter	Number of leaves
Chlorophyll	32.17*	-1.65	2.58	4.92
Plant diameter	-1.20	22.89*	5.60	6.43
Stem diameter	2.04	6.10	23.73*	15.27
Number of leaves	4.33	7.80	17.01	27.20*
Total	37.34	35.14	48.92	53.82

* - Direct selection

Table 4. Selection gain estimates (%) (20% of the selection intensity) obtained by the classical index proposed by Smith-Hazel (SH), Mulamba & Mock (MM) ranks sum index, and Williams base index (WI) for four variables evaluated in 91 lettuce genotypes

Index	Selection gain (%)				
	Chlorophyll	Plant diameter	Stem diameter	Number of leaves	Total
SH	17.98	13.96	11.76	19.98	63.68
MM	15.40	12.31	17.31	18.30	63.32
WI	17.98	13.96	11.76	19.98	63.68

SH - Smith (1936) and Hazel (1943); MM - Sum of ranks—Mulamba & Mock (1978); WI - Base-index—Williams (1962)

this study the indices struck a balance in the distribution of the selection gains for most of the lettuce genotypes assessed.

Peixoto et al. (2020) compared nonparametric indices in the selection of biofortified curly lettuce, and the Mulamba & Mock index also resulted in the highest total gain value (30.42%). In contrast to this study, we found superior selection gains for all indices of all the analyzed variables, probably due to the greater number of genotypes evaluated and higher values of heritability, since it is directly related to the genetic gain and greater genetic variability of the germplasm.

For the estimation of the selection gains, 17 individuals were selected using the Smith-Hazel, Mulamba & Mock, and Williams index (Table 5).

The same 17 genotypes were selected using the Smith (1936) and Hazel (1943) and Williams indices, and 13 genotypes (76.47%) also stood out for Mulamba & Mock index (Table 5). The Williams index is similar to the index proposed by Smith-Hazel, when the phenotypic variances and covariance are defined predominantly by genetic factors (Cruz et al., 2014). Supporting this fact, Cruz et al. (2012) highlighted that estimates of gains by the same indices do not always show similar results because of the genotype-environment interaction and the accuracy of the matrices of variances and covariances.

Dealing with lettuce lines, Peixoto et al. (2020) found similarities in the genotypes selected by the Mulamba & Mock index and the Smith-Hazel index. The same fact was observed by Vieira et al. (2017) working with strawberry hybrids.

Table 5. Indication of the top 17 lettuce genotypes, selected by the classic indexes proposed by Smith (1936) and Hazel (1943) (SH), Mulamba & Mock (1978) sum of ranks index (MM), and Williams base index (1962) (WI)

Selection indexes	Selected lettuce genotypes
Classic index (SH)	UFU 66#10; UFU 215#12; UFU 104#3; UFU 184#2; UFU 184#1; UFU 215#4; UFU 215#2; UFU 215#1; UFU 217#5; UFU 217#4; UFU 217#1; UFU 189#1; UFU 199#6; UFU 199#5; UFU 199#3; UFU 106#1; UFU 210#1
Sum of ranks (MM)	UFU 66#10; UFU 215#12; UFU 104#4; UFU 104#2; UFU 184#2; UFU 184#1; UFU 215#4; UFU 215#2; UFU 215#1; UFU 217#5; UFU 217#4; UFU 217#1; UFU 189#2; UFU 189#1; UFU 199#6; UFU 106#1; UFU 210#1
Base index (WI)	UFU 66#10; UFU 215#12; UFU 104#3; UFU 184#2; UFU 184#1; UFU 215#4; UFU 215#2; UFU 215#1; UFU 217#5; UFU 217#4; UFU 217#1; UFU 189#1; UFU 199#6; UFU 199#5; UFU 199#3; UFU 106#1; UFU 210#1

Sousa et al. (2020) evaluated the genetic dissimilarity of the same germplasm bank of the biofortified lettuce samples examined in the present study, and showed that seven genotypes stood out, revealing excellent agronomic traits and high levels of carotenoids. Five of these were also among those selected in the present study, confirming the potential of the biofortified lettuce genotypes in this breeding program.

CONCLUSIONS

1. There is a considerable genetic component in the phenotypic expression of all traits studied, with a high probability of genetic gains in additional selection cycles based on the phenotype in the breeding program.

2. The selection indices of Smith-Hazel, Williams, and Mulamba & Mock are efficient in showing good direct gains for the evaluated traits in biofortified lettuce.

3. Thirteen genotypes were selected by all indices, thus presenting suitable agronomic traits that are promising for advancing generations within the breeding program aimed at obtaining biofortified lettuce strains.

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