

## Comparison between non-parametric indexes in the selection of biofortified curly lettuce

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### Abstract

Selection indexes can be applied to simultaneous analysis of traits, increasing the efficiency of breeding in choosing the genotypes that meet the needs of both the market and the consumer. While it is a technic applied to several cultures, there are few studies about it in lettuce lines. For this reason, the aim of this study was to compare assess the efficiency of different selection indexes for selecting biofortified leaf lettuce lines with good agronomic traits. The experiment was carried out at the Estação Experimental de Hortaliças of Federal University of Uberlândia (UFU), campus Monte Carmelo, in 2018. The experiment was designed in full randomized blocks of 3 repetitions with 25 leaf lettuce genotypes (22 lines from cultivars Pira 72 versus Uberlândia 10000 and 3 commercial cultivars – cv. Grand Rapids, UFU-Biofort and Uberlândia 10000). The lines are part of the UFU biofortified lettuce breeding program and were assessed for the total green mass (g), the stem diameter (cm), commercial leaves count, plant diameter (cm), foliar temperature (°C), SPAD index, anthocyanin content (mg 100g<sup>-1</sup> sample), and bolting (days after sowing). To estimate selection gains, 10 genotypes were selected using both direct and indirect selection, as well as the traditional index, the sum of ranks index, Willians base index, and the multiplicative index. Mulamba and Mock sum of ranks index and Subandi multiplicative index provided the biggest total gains for the traits assessed in biofortified leaf lettuce. Both indexes were similar in selecting genotypes.

**Keywords:** biofortified foods, *Lactuca sativa* L., selection gains, selection indexes

### Introduction

The lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable in Brazil and worldwide. *Per capita* consumption of lettuce salad is lower only than that of potatoes (Brzezinski et al., 2017; Kim et al., 2018). Leaf lettuce (*Lactuca sativa*, var. *crispa*) is economically more important when compared to the iceberg, butterhead, and romaine ones (Sala & Costa, 2012; Kim et al., 2018).

Lettuce is a rich source of nutrients such as carotenoids, ascorbic acid, and phenolic compounds (López et al., 2014). Carotenoids help to prevent several diseases related to the oxidative stress. In addition, they are vitamin A precursors (Rocha & Reed, 2014; Cassetari et al., 2015). The changing in population eating habits, and the search for a healthful diet and a better quality of life directly affect the market demand for a better nutrition quality lettuce.

Few researches have been conducted into

the biofortified lettuce (Cassetari et al., 2015; Jacinto et al., 2019; Silveira et al., 2019; Sousa et al., 2019). One of the main obstacles is to feasibly get an individual with both good agronomic traits and high level of carotenoids (Maciel et al., 2019a). Overall, the selection of lettuce lines has been done by univariate analysis (Son & Oh, 2013; Resende et al., 2017; Tezza & Minuzzi, 2019), and the product was superior in only one or few traits. With such limitation, it is necessary to appraise possible improving methodologies capable increase the efficiency in genetic selection. Alternatively, one possibility to increase the success chance in breeding programs is to make simultaneous selection of some traits with economic relevance, applying selection indexes (Vasconcelos et al., 2010; Terres et al., 2015; Bizari et al., 2017; Tassone et al., 2019). These indexes can enable the optimal linear combination within a set of information from the experimental unit, and, therefore, the efficient

simultaneous selection of traits (Cruz et al., 2014).

Nonparametric indexes may help to select lettuce lines that combine good agronomic traits with a better nutrition quality. Many pieces of research confirm the efficiency of applying such indexes to the selection of superior individuals from breeding programs (Terres et al., 2015; Bizari et al., 2017; Vieira et al., 2017), except for lettuce. For this reason, the aim of this study was to compare assess the efficiency of different selection indexes for selecting biofortified leaf lettuce lines with good agronomic traits.

### Material and Methods

The experiment was conducted at the Vegetable Experimental Station at the Federal University of Uberlândia-UFU, Monte Carmelo campus (18°42'43.19"S; 47°29'55.8"W; 873 meters above sea level), in 2018. The temperature during the experiment ranged from 16.23 °C minimum and 27.49 °C maximum, with an average relative humidity of 75.47% and average precipitation of 1.27 mm.

The study covered 22 lettuce lines, obtained by pedigree method. These lines were obtained through hybridization performed between cultivars Pira 72 and Uberlândia 10000 (rich in carotenoids) (Souza et al., 2008), after six successive self-pollination procedures, from 2013 to 2017. The lines are part of the UFU biofortified lettuce breeding program, and all genealogy was stored in the software "BG a BIOFORT" INPI patent number BR512019002403-6 (Maciel et al., 2019b). Three following cultivars were used as control: cv. Grand Rapids, UFU-Biofort and Uberlândia 10000 (Souza et al., 2008), totaling 25 treatments.

Sowing was carried out on March 28th, 2018. The seedlings were grown in 200-cell EPS trays with commercial coconut fiber substrate. After sowing, the trays were placed in a 5 m x 6 m, arch-type greenhouse, built with 3.5 m ceiling, and covered with UV-resistant, 150-micron transparent polyethylene film and side curtains of white, anti-aphid screen. Lettuce seedlings were transplanted to definitive field seedbeds 29 days after sowing. Seedbeds were 1.30 m long and were tilled using a rototiller.

The experiment soil showed the following features: pH (H<sub>2</sub>O) = 5.9; available P = 30.1 mg dm<sup>-3</sup>; K = 0.22 cmolc dm<sup>-3</sup>; Ca = 2.8 cmolc dm<sup>-3</sup>; Mg = 1.0 cmolc dm<sup>-3</sup>; H<sup>+</sup> exchangeable Al = 3.40 cmolc dm<sup>-3</sup>; organic matter = 4.2 dag Kg<sup>-1</sup>; SMP index = 3.40; aluminum = 0.0 cmolc dm<sup>-3</sup>; CEC pH 7.0 = 7.42 cmolc dm<sup>-3</sup>; CEC pH 7.0 base saturation = 54%; effective CEC aluminum saturation = 0%; copper = 2.3 mg dm<sup>-3</sup>; zinc = 6.6 mg dm<sup>-3</sup>; and manganese = 6.6 mg dm<sup>-3</sup>. The handling followed the procedures for lettuce

culture (Filgueira, 2013).

The plots of the experiment comprised 16 plants, which were distributed in four rows per seedbed distant 0.25 m x 0.25 m from each other. The eight middle plants of each plot were assessed. The plants were harvested 35 days after transplanting, and were assessed for total green mass (g), stem diameter (cm), leaves count, plant diameter (cm), foliar temperature (°C), soil plant analysis development (SPAD) index, anthocyanin content (mg 100 g<sup>-1</sup> sample), and bolting (days after sowing).

### Traits Assessed

The total green mass (GM) was assessed by weighing all the outer leaves. Stem diameter (SD) was calculated with calipers. Commercial leaves count (LC) was assessed by counting the number of leaves longer than 5 cm. Plant diameter (PD) was calculated in centimeters, with the aid of a graduated ruler. The measurement was performed from one edge of the plant to the other. Foliar temperature (FT) was measured with an infrared thermometer (model 4000.4GL, Everest Interscience, Tucson, AZ, USA) by pointing the device sensor to the central part of foliar surface of the sampled outer leaves.

The total of anthocyanin content (ANT) was calculated using Francis (1982) method. The concentration of anthocyanin pigments was determined by absorbance, according to the equation:

$$At = \frac{(Abs * f)}{\epsilon}$$

where: *At* = anthocyanins (mg of anthocyanin 100 g<sup>-1</sup> of fresh mass); *Abs* = absorbance; *f* = dilution factor;  $\epsilon$  = molar attenuation coefficient of cyanidin (98.2).

The SPAD index (Cassetari et al., 2015) was calculated by the mean value of the data from the four central plants of each plot. The hand-held chlorophyll meter model Minolta SPAD-502 CFL1030 was used. The days until the flower stalk appeared were also assessed (plant bolting was counted in days from sowing - EB).

### Statistical analysis

The experiment was designed in full randomized blocks of 3 repetitions. The analysis of variance (p-value ≤ 0.05) was performed to obtain the trait mean squares (TMS) and the coefficient of variation (CV). The coefficient of genetic variation (CVg), the ratio between the coefficient of genetic variation (CVg) and the coefficient of environmental variation (CVe) – CVg/CVe, and the coefficient of genotypic determination (H<sup>2</sup>) for each variable were also assessed. The H<sup>2</sup> was calculated using the following estimator

$$H^2 = \frac{\hat{\sigma}_g}{TMS/r} \quad \hat{\sigma}_g = \left( \frac{TMS - RMS}{r} \right),$$

where:  $H^2$  is the coefficient of genotypic determination;  $\hat{\sigma}_g$  is the genetic quadratic component; TMS is the genotype mean square; RMS is the residual mean square; and  $r$  is the number of repetitions.

Both the phenotypic ( $r_p$ ) and genotypic ( $r_g$ ) correlations, as well as the selection indexes were determined for the traits which showed significant result in analysis of variance. The phenotypic and genotypic correlations estimators were

$$r_p = \frac{TMP_{xy}}{\sqrt{TMS_x \cdot TMS_y}} \text{ and } r_g = \frac{\hat{\sigma}_{gxy}}{\sqrt{\hat{\sigma}_{g_x}^2 \cdot \hat{\sigma}_{g_y}^2}}, \text{ respectively,}$$

where: TMP is the treatment mean product; TMS is the treatment mean square; X and Y are the traits analyzed;  $\hat{\sigma}_{gxy}$  is the genotypic covariance estimator between X and Y; and  $\hat{\sigma}_{g_x}^2$  and  $\hat{\sigma}_{g_y}^2$  are the genotypic variances estimators of X and Y, respectively (Cruz et al., 2012). The significance of the phenotypic correlation was estimated by the t test ( $p \leq 0.05$ ). For genotypic correlation, the bootstrap method was applied ( $p \leq 0.05$ ).

To estimate the selection gains, 10 genotypes (40% of genotypes studied) were selected using both the direct and indirect methodologies (Cruz et al., 2012); Smith (1936) and Hazel (1943) traditional selection index (SH); Mulamba & Mock (1978) sum of ranks index (MM); Willians (1962) base index (W); and the multiplicative index of Subandi et al. (1973). The selection criteria for all selection indexes were to increase GM, SD, LC, SPAD and EB and to decrease PD. The economic weight comprises the coefficient of genetic variation of each variable, according to Cruz et al. (2012). The software Genes v. 2015.5.0 (Cruz, 2013) was used in the statistical analysis.

The estimate gain via direct selection (Cruz et al., 2012) at the  $i$ -th trait ( $GS_i$ ) was denoted by

$$GS_i = (X_{si} - X_{oi})h_i^2 = DS_i h_i^2,$$

where:  $X_{si}$  is the mean of lines selected for  $i$  trait;  $X_{oi}$  is the original population mean;  $DS_i$  is the population selection differential; and  $h_i^2$  is the trait  $i$  genotypic determination coefficient.

The indirect gain (Cruz et al., 2012) for  $j$  trait, by selecting trait  $i$ , was given by

$$GS_{j(i)} = DS_{j(i)} h_i^2,$$

where:  $DS_{j(i)}$  is the indirect selection differential from the mean of the individuals' traits whose superiority had been highlighted by another trait to which the direct selection was applied.

The traditional selection index held by Smith (1936) and Hazel (1943) was determined by the linear combination of the traits assessed. It was estimated by the selection index ( $I$ ) and the aggregate genotype ( $H$ ) as follows:

$$I = b_1 y_1 + b_2 y_2 + \dots + b_n y_n = \sum_{i=1}^n b_i y_i = y' b$$

$$H = a_1 g_1 + a_2 g_2 + \dots + a_n g_n = \sum_{i=1}^n a_i g_i = g' a$$

where:  $n$  is the number of traits analyzed;  $b$  is the  $1 \times n$  vector for the weighting factors of the estimated selection index;  $y'$  is the  $n \times p$  matrix for the traits phenotypic values;  $a$  is the  $1 \times n$  vector for economic weights previously established;  $g$  is the  $n \times p$  matrix for unknown genetic values of the  $n$  traits in question;  $p$  is the number of families or progenies analyzed.

The  $n \times n$  matrix for phenotypic variances and covariances is represented by  $P$ . The  $n \times n$  matrix for traits genetic covariances is given by  $G$ . The vector  $b$  is estimated by  $b = P^{-1} G a$ , and  $P^{-1}$  is the traits phenotypic variances and covariances inverse matrix. The expected gain for trait  $j$  was calculated by

$$\Delta g_{j(i)} = DS_{j(i)} h_j^2,$$

where:  $\Delta g_{j(i)}$  is the trait  $j$  expected gain, and selection based on index  $I$ ;  $DS_{j(i)}$  is the trait  $j$  selection differential, and selection based on index  $I$ ; and  $h_j^2$  is the trait  $j$  heritability.

The sum of ranks index (Mulamba & Mock, 1978) dispenses with both the genotypic and phenotypic variance and covariance estimates and the economic weights to the traits studied (Cruz et al., 2014). It sorts the genotypes hierarchically by assigning higher absolute values to those with better performance. The process is done for each trait studied. After that, the sort number of each genotype related to each trait studied is added up, giving the selection index as follows:

$$I = r_1 + r_2 + \dots + r_n,$$

where:  $I$  is the index value for a given individual or family;  $r_j$  is the rank of an individual related to the  $j$ -th variable; and  $n$  is the number of variables the index comprises.

According to the breeder's specifications, the method can assign different weights to sort the variables rank. Thus, the economic weights were given by:

$$I = p_1 r_1 + p_2 r_2 + \dots + p_j r_n$$

where:  $p_j$  is the economic weight assigned to the  $j$ -th trait.

The Willians (1962) base index comprises the linear combination of the traits mean phenotypic values, which are weighted by their respective economic weights. The index is given by:

$$I = a_1 y_1 + a_2 y_2 + \dots + a_n y_n = \sum_{i=1}^n a_i y_i = y' a,$$

where:  $y$  is the mean; and  $a$  is the economic weight for the traits analyzed.

In the multiplicative index (Subandi et al.,

1973), the index is assumed to be directly or inversely proportional to the variable analyzed, according to the breeder's criteria. The index is given by:

$$I_{Ei} = \log \prod_{j=1}^m (X_{ij} - K_j) = \log[(X_{i1} - K_1)(X_{i2} - K_2) \dots (X_{im} - K_m)],$$

where:  $I_{Ei}$  is the multiplicative index;  $X_{ij}$  is the mean of the trait  $j$  measured in the genotype  $i$ ;  $K_j$  is the lowest possible value:  $[K_j = \frac{n(\min.X_{ij}) - \max.X_{ij}}{n-1}]$ ;  $n$  is the number of genotypes;  $\min.X_{ij}$  and  $\max.X_{ij}$  are the trait  $j$  lowest and highest means, respectively.

Mean, maximum and minimum values of each variable were estimated for the genotype-ideotype distance index (Cruz, 2006).  $X_{ij}$  was considered the mean phenotypic value of the  $i$ -th genotype related to the  $j$ -th trait.  $Y_{ij}$  is the transformed mean phenotypic value, and  $C_j$  is a constant related to the depreciation of the genotype mean. Thus,  $L_{ij}$  is the genotype lower limit related to the trait  $j$ ,  $LS_j$  is the genotype higher limit, and  $VO_j$  is the genotype optimal value.

If  $L_{ij} < X_{ij} < LS_j$ , so  $Y_{ij} = X_{ij}$ ;

If  $X_{ij} < L_{ij}$ ,  $Y_{ij} = X_{ij} + VO_j - L_{ij} - C_j$ ;

If  $X_{ij} > LS_j$ ,  $Y_{ij} = X_{ij} + VO_j - LS_j + C_j$ .

The methodology considers  $C_j = LS_j - L_{ij}$ . The value  $C_j$  proved that any  $X_{ij}$  within the optimal interval of variation would result in a  $Y_{ij}$  with magnitude near the optimal value ( $VO_j$ ). The  $X_{ij}$  transformation aimed at reducing the phenotypic values outside the interval. The transformed values of  $Y_{ij}$  were standardized and weighted by the weight assigned to each trait, as follows:

$$y_{ij} = \sqrt{a_j} \frac{y_{ij}}{s(y_j)}$$

where:  $s(y_j)$  is the standard deviation of the transformed mean phenotypic values; and  $a_j$  is the trait economic weight or value.  $VO_j$  was standardized and weighted as well, as follows:

$$VO_{ij} = \sqrt{a_j} \frac{vo_j}{s(y_j)}$$

The values of the genotype-ideotype distance (GID) index were calculated as follows:

$$I_{DGI} = \sqrt{\frac{1}{n} \sum_{j=1}^n (y_{ij} - vo_j)^2}$$

## Results and Discussion

The genotypes of biofortified leaf lettuce showed genetic variability for the traits GM, SD, LC, PD, SPAD and EB at the 1% and 5% levels by F-test (Table 1), demonstrating the possibility of selecting superior lines. The traits FT and ANT did not show significant differences, because the genotypes were under the same environmental condition. Steidle Neto et al. (2017) found different ANT content in lettuce genotypes grown in different environments.

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

Characters <sup>1</sup>	TMS <sup>2</sup>	H <sup>2</sup> (%)	CVg (%)	CVg/CVe	CV (%)
GM	7759.92**	82.76	23.86	1.27	18.86
SD	0.15*	51.00	7.06	0.59	11.99
LC	50.82**	91.42	18.42	1.88	9.77
PD	14.52**	72.77	7.54	0.94	7.99
FT	2.30 <sup>ns</sup>	-	-	-	8.40
SPAD	48.26**	95.96	17.57	2.81	6.24
ANT	26.55 <sup>ns</sup>	-	-	-	28.84
EB	410.23**	92.60	13.81	2.04	6.76

<sup>1</sup>GM= green mass (g); SD= stem diameter (cm); LC = commercial leaves count; PD = plant diameter (cm); FT = foliar temperature (°C); SPAD= SPAD index; ANT= total of anthocyanin content (mg 100g<sup>-1</sup> sample); EB = early bolting (days after sowing). <sup>2</sup>TMS: trait mean squares; H<sup>2</sup>: coefficient of genotypic determination; CVg: coefficient of genetic variation; CVe: coefficient of environmental variation; CV: general coefficient of variation; \*\*: significant at the level of 1% and 5% probability, respectively, by the F test; ns: not significant; -: non-significant variables in the analysis of variance.

The coefficients of variation (CV) fluctuated between 6.24% (SPAD) and 28.84% (ANT). The distribution of CV makes it possible to define ranges of values that guide the researcher regarding the credibility and validity of the experiments (Storck et al., 2011). In field experiments, CV value below 10% is considered low; medium, if between 10% and 20%; high, if between 20% and 30%; and very high, if above 30% (Pimentel-Gomes, 2009). In this study, 62.5% of the variables had low CV, demonstrating higher experimental precision and lower residual error value in the analysis of variance. Pinto et al. (2017) analyzing the development and production of curly lettuce found CV values close to those observed in this work for PD SD, LC and GM.

The coefficient of genetic variation (CVg) values ranged between 7.06% (SD) and 23.86% (GM) (Table 1). The CVg is an important parameter, making it possible to know the extent of genetic variability in the population for all the studied traits (Leite et al., 2016). Assessing heat resistant lettuce progenies, Souza et al. (2008) reported the CVg values: PD 6.39%; LC 6.97%; leaf fresh weight 13.45%; and EB 25.94%. In this study, CVg values were PD = 7.54%; EB = 13.81% and LC = 18.42% (Table 1). The difference in CVg values of both studies may be due to the genotypes and the conditions under which the experiment was conducted.

The CVg does not allow a complete estimate of the heritable variations and, therefore, it is necessary to estimate the coefficient of genotypic determination (H<sup>2</sup>) or the trait heritability (Thakur et al., 2016). The H<sup>2</sup> makes it possible to estimate the genetic gain to be achieved and to establish the best strategy of the breeding program (Baldissera et al., 2014). The values of H<sup>2</sup> vary according to the variables analyzed, and the results over 0.70 are considered high (Ramalho et al., 2012).

The highest values of H<sup>2</sup> were those of SPAD, EB, LC, and GM, which were 95.96%, 92.60%, 91.42% and

82.76%, respectively, all considered high. Queiroz et al. (2014) and Silva et al. (2019) also found high heritability values (95.65% and 96%, respectively) for leaf count. The results suggested a successful perspective in phenotypic selection, confirmed by the CVg/CVe ratio for the same variables, whose values were over one (Table 1). When the CVg/CVe ratio is greater than or equal to one, the genetic variation available is the main responsible for the

estimate variation of the experimental data (Leite et al., 2016).

The traits correlations contribute to the comprehension of the gene action. The results of this study showed that the genotypic correlation is greater than the phenotypic one. The traits that showed positive genotypic correlation were GM and PD (0.80), LC and EB (0.48), and SPAD and EB (0.44) (Table 2).

**Table 2.** Genotypic (G) and phenotypic (F) correlations in six characters evaluated in lettuce genotypes.

Characters <sup>1</sup>		GM	SD	LC	PD	SPAD	EB
GM	G	1.00	0.48	-0.12	0.80**	-0.29	-0.50*
	F	1.00	0.38	-0.03	0.77**	-0.26	-0.47*
SD	G		1.00	0.15	0.50	-0.16	-0.30
	F		1.00	0.10	0.38	-0.15	-0.18
LC	G			1.00	-0.02	0.30	0.48*
	F			1.00	0.05	0.27	0.43*
PD	G				1.00	-0.47*	-0.57**
	F				1.00	-0.40*	-0.49*
SPAD	G					1.00	0.44*
	F					1.00	0.39*
EB	G						1.00
	F						1.00

<sup>1</sup>GM= green mass (g); SD= stem diameter (cm); LC = commercial leaves count; PD = plant diameter (cm); SPAD= SPAD index; EB = early bolting (days after sowing). \*\*Significant phenotypic correlation at 1 and 5% probability, respectively, by the t-test. \*\*\*Significant genotypic correlation at 1% and 5% probability, respectively, by the bootstrap method with 5000 iterations.

The positive genotypic correlation between LC and EB and between SPAD and EB (Table 2) may help the selection process, because the selection of plants with higher LC and SPAD index allows to get plants more resistant to early bolting. The slight difference between the estimates of genotypic and phenotypic correlation provides the knowledge of how environmental factors impact the traits analyzed (Thakur et al., 2016). The results obtained for both correlations were close. Thus, the small difference between the values of genotypic and phenotypic correlations displayed by some of the variables analyzed in this study indicates that

environmental factors have minor impact on such traits. The selection on the basis of those traits will certainly improve lettuce's desirable agronomic characteristics. Comparable results were reported by Souza et al. (2008) and Thakur et al. (2016), who reported higher values of genotypic correlation of traits when compared to those of phenotypic correlation.

The direct selection provides maximum gains in a single trait that the breeder selects (Cruz, 2013; Cruz et al., 2014). In this study, the direct selection provided individual gains bigger than those of the indirect selection (Table 3).

**Table 3.** Selection gain estimates (SG%) obtained for six evaluated characters, by direct selection (values in bold) and indirect in lettuce genotypes.

Characters <sup>1</sup>	SG (%)					
	GM	SD	LC	PD	SPAD	EB
GM	<b>21.84</b>	10.6	0.03	-12.36	-1.42	-12.15
SD	1.34	<b>4.90</b>	-0.21	-0.96	0.64	-1.39
LC	1.45	1.59	<b>18.17</b>	-2.30	8.40	9.25
PD	4.25	1.68	-0.06	<b>-5.27</b>	-1.21	-2.42
SPAD	-3.17	-1.69	7.64	6.04	<b>16.15</b>	5.46
EB	-4.39	-2.04	4.48	3.38	5.88	<b>12.09</b>
Total	21.32	15.04	30.05	-11.47	28.44	10.84

<sup>1</sup>GM= green mass (g); SD= stem diameter (cm); LC = commercial leaves count; PD = plant diameter (cm); SPAD = SPAD index; EB = early bolting (days after sowing). Favorable selection criteria: increase for GM, SD, LC, SPAD and EB and reduction for PD.

The biggest gains by means of direct selection were those of the variables GM (21.84%), LC (18.17%), SPAD (16.15%) and EB (12.09%). The trait SD showed the smallest individual gain (4.90%) by direct selection (Table 3), which matched with its low CVg (7.06%) (Table 1). The SD relates to the increase of plant lodging resistance

(Chen et al., 2014). The SPAD index is directly associated to the chlorophyll content in the plants leaves, which is positively related to plant photosynthetic capacity (Klooster et al., 2012; Son & Oh, 2013; Cassetari et al., 2015), increasing productivity.

The LC direct selection provided indirect gains for

GM (1.45%), SD (1.59%), SPAD (8.40%), increased EB time (9.25%) and reduced PD (-2.30%) (Table 3). This was the best strategy of indirect selection, because LC reduced PD without negatively affecting the rest of the traits. In Brazil, as well as in the USA and Europe, people tend to consume packaged, processed lettuce leaves (Sala & Costa, 2012). Thus, plants with higher commercial LC are preferable to meet such demand (Suinaga et al., 2013).

The early EB, induced by high temperatures of Brazilian summer, results in lettuce losses, causing raise to the prices and reduction to the supply. Lettuce is usually sold in plastic or wooden box, almost without any cooling method to maintain the produce quality (Sala & Costa, 2012). Thus, plants with lower PD is preferable aiming at preventing quality damage e losses during storage

process.

Different from direct selection, the selection indexes promote simultaneous selection by combining several traits, increasing the chances of success in breeding program (Cruz et al., 2014). Mulamba and Mock index and Subandi multiplicative index showed comparable results, as well as the biggest gains for LC (13.68%), SPAD index (12.04%), EB (6.11%) and reduction for PD (-1.44%). Both indexes also resulted in the highest total gain value (30.42%) (Table 4), besides selecting the same genotypes. The LC direct selection was similar to 70% of the genotypes (UFU-206#3#1#1; UFU-189#3#1#1; UFU-197#1#1; UFU-7#1#2#1; UFU-189#3#2#1; Uberlândia 10000, and UFU-189#3#4#1) provided by the previous indexes (Table 5).

**Table 4.** Selection gain estimates (SG%) obtained by the classic index proposed by Smith (1936) and Hazel (1943) (SH), Mulamba & Mock (1978) (MM) ranks sum index, Willians base index (1962) (W), Subandi's multiplicative index (1973) (M) and index based on genotype-ideotype distance (Cruz, 2006) (GI) in 25 lettuce genotypes.

Index	SG (%) <sup>1</sup>						
	GM	SD	LC	PD	SPAD	EB	Total
SH	21.84	1.34	1.45	4.25	-3.17	-4.39	21.32
MM	-1.07	1.10	13.68	-1.44	12.04	6.11	30.42
W	21.39	0.94	2.73	4.54	-0.15	-3.48	25.97
M	-1.07	1.10	13.68	-1.44	12.04	6.11	30.42
GI	-7.0	-1.74	-4.40	-1.61	-2.99	0.92	-16.82

<sup>1</sup>GM= green mass (g); SD= stem diameter (cm); LC = commercial leaves count; PD = plant diameter (cm); SPAD = SPAD index; EB = early bolting (days after sowing).

Maciel et al. (2020) analyzed through univariate analysis the same germplasm bank featured in the present study. These authors observed the genotypes selected by the Mulamba & Mock and Subandi indexes are among those that showed the best agronomic and nutritional characteristics.

Results from Terres et al. (2015), showed comparable genetic gains by means of selection indexes in potato hybrid populations. They reported that the total gain estimates were superior with Mulamba and Mock index and Subandi multiplicative index. Those indexes struck a balance in the distribution of selection gains for most of the lettuce genotypes assessed in this study.

SH index showed the biggest GM gain (21.84%) (Table 4), result comparable to that of direct selection (Table 3). In second place, the W index caused GM to gain 21.39%. Both indexes showed the second and third highest total gain: 21.32% and 25.97%, respectively (Table 4). By contrast, both indexes showed negative gain values for SPAD traits (SH -3.17% and W -0.15%) and EB traits (SH -4.39% and W -3.48%) (Table 4).

SH index selected 90% of the genotypes pointed by W index (Table 5). The difference was that SH index selected the genotype UFU-125#1#1#1, whereas W index selected UFU-189#3#2#1.

**Table 5.** Indication of the top ten lettuce genotypes by the classic index proposed by Smith (1936) and Hazel (1943), Mulamba & Mock's (1978) sum of ranks index, Willians base index (1962), multiplicative index of Subandi et al. (1973) (M), and index based on genotype-ideotype (GI) distance.

Selection indexes	Selected lettuce genotypes
Direct selection for number of commercial leaves	UFU-206#3#1#1; UFU-189#3#1#1; UFU-197#1#1; UFU-7#1#2#1; UFU-189#3#2#1; Uberlândia 10000; UFU-189#3#4#1; UFU-189#1#2#1; UFU-155#1#1#1; UFU-125#2#2#1
Smith (1936) and Hazel (1943)	Grand Rapids; Uberlândia 10000; UFU Biofort; UFU-155#1#1#1; UFU-155#1#2#1; UFU-206#1#5#1; UFU-206#3#1#1; UFU-206#1#6#1; UFU-7#1#2#1 e UFU-125#1#1#1
Willians (1962)	Grand Rapids; Uberlândia 10000; UFU Biofort; UFU-155#1#1#1; UFU-155#1#2#1; UFU-206#1#5#1; UFU-206#3#1#1; UFU-206#1#6#1; UFU-7#1#2#1 e UFU-189#3#2#1
Mulamba & Mock (1978)	UFU-206#3#1#1; UFU-189#3#1#1; UFU-197#1#1; UFU-7#1#2#1; UFU-189#3#2#1; Uberlândia 10000; UFU-189#3#4#1; UFU-197#2#2#1; UFU-189#1#2#1 e UFU-206#1#5#1
Subandi et al. (1973)	UFU-206#3#1#1; UFU-189#3#1#1; UFU-197#1#1; UFU-7#1#2#1; UFU-189#3#2#1; Uberlândia 10000; UFU-189#3#4#1; UFU-197#2#2#1; UFU-189#1#2#1 e UFU-206#1#5#1
Genótipo-ideótipo	UFU-7#1#2#1; UFU-125#1#1#1; UFU-189#3#2#1; UFU-206#1#6#1; UFU-189#3#1#1; UFU-199#3#1#1; UFU-206#1#4#1; UFU-120#1#1#1; UFU-206#1#1#1 e UFU-197#3#1#1

Willians (1962) base index corresponds to that of Smith & Hazel (1943), when phenotypic variances and covariances are determined mainly by genetic factors (Cruz et al., 2014). Analyzing Mulamba and Mock, Elston and Schwarzbach indexes applied to lettuce, Cândido et al. (2017) reported positive gains for the traits assessed by the first index. The authors attributed the positive result with Mulamba and Mock index to its strong correlation to the rest of the indexes and to the fact it can be easily measured. Such index has been suggested by other authors (Vasconcelos et al., 2010; Terres et al., 2015; Bizari et al., 2017).

GI index showed positive selection gains only for EB (0.92%) (Table 4). Fifty percent of the 10 selected genotypes were not found in the selection of the other indexes in this study: UFU-199#3#1#1; UFU-206#1#4#1; UFU-120#1#1#1; UFU-206#1#1#1 and UFU-197#3#1#1. The lettuce line common to all selection indexes was UFU-7#1#2#1 (Table 5). It shows that the same line was selected, regardless of the selection index. Dealing with strawberry hybrids, Vieira et al. (2017) found similarities in the genotypes selected by Mulamba & Mock (1978) sum of ranks index, the genotype-ideotype distance-based index (Cruz, 2006), and Smith (1936) and Hazel (1943) traditional selection index.

## Conclusions

Mulamba and Mock sum of ranks index and Subandi multiplicative index promoted the biggest total gains for the traits assessed. These indexes enabled positive gains for DC, NF, SPAD and EB, reducing the DP.

Both indexes were mutually consistent regarding the selected genotypes.

LC direct selection promoted the biggest SPAD and bolting indirect gains, reducing PD.

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