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Genetic dissimilarity between lettuce genotypes with different levels of carotenoids biofortification

Aline José da SILVEIRA[1], Rafael Resende FINZI[1*], Luciano Dias CABRAL NETO[1], Gabriel Mascarenhas MACIEL[1], Igor Forigo BELOTI[1], Ana Carolina Pires JACINTO[1]

[1]Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Monte Carmelo, MG, Brasil.
*E-mail: rafaelfinzi@hotmail.com

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ABSTRACT: Vegetables with high carotenoids content can help to prevent many diseases. Lettuce is one of the most consumed vegetables all over the world that present potential for biofortification investment. However, there are few genotypes of lettuce biofortificated on the market and for direct use in plant breeding. Evaluate new lettuce genotypes that are rich in carotenoids are essential in order to know the commercial potential and their usefulness as possible parents in breeding programs. Therefore, the experiment aimed to evaluate the agronomic potential and genetic dissimilarity between lettuce genotypes with different levels of carotenoids. It was conducted in a randomized block design, with 83 treatments and two replications. During the course of the trial, were evaluated quantitative characters: leaf chlorophyll content (a, b and total), canopy diameter and number of leaves; and qualitative characters: size, color and shape of the leaves. The genetic diversity was obtained by multivariate analyzes using the sum of quantitative and qualitative data and, after that, using different grouping methods (UPGMA and Tocher). The genotypes showed genetic dissimilarity and agronomic potential for different lettuce segments and high carotenoids content. Among the evaluated characters, total chlorophyll content represented the main contribution for genetic variability. There was no coherence between the hierarchical method of UPGMA and the optimization method of Tocher when used for determinate the genetic diversity between lettuce genotypes with different levels of carotenoids.
Keywords: biofortification; genetic variability; *Lactuca sativa*; vitamin A.

Diversidade genética entre genótipos de alface com diferentes níveis de biofortificação por carotenóides

RESUMO: Alimentos ricos em carotenóides podem auxiliar na prevenção de diversas doenças. A alface é uma das hortaliças mais consumidas no mundo apresentando potencial para investimento em biofortificação. No entanto, existem poucos genótipos de alface biofortificado disponíveis no mercado, inclusive, para uso direto no melhoramento genético. Avaliar novos genótipos de alface ricos em carotenóides é essencial, a fim de se conhecer o potencial comercial e sua utilidade como potenciais genitores em programas de melhoramento. Portanto, o objetivo do trabalho foi avaliar o potencial agronômico e diversidade genética em genótipos de alface com diferentes níveis de carotenóides. O experimento foi conduzido em campo no delineamento de blocos casualizados, com 83 tratamentos e duas repetições. Foram avaliados dados quantitativos: teor de clorofila foliar a, b e total; diâmetro de copa e número de folhas; e dados qualitativos: tamanho, cor e formato de folha da alface. A divergência genética foi obtida por meio de análises multivariadas utilizando-se a soma de matrizes de dados qualitativos e quantitativos, e posteriormente empregando-se diferentes métodos de agrupamento (UPGMA e Tocher). Os genótipos apresentaram diversidade genética e potencial agronômico para diferentes segmentos de alfaces, aliado ao alto teor de carotenóides. Entre as variáveis respostas avaliadas, clorofila total representou maior contribuição para a variabilidade genética. Não houve coerência entre o método hierárquico UPGMA e de otimização Tocher para caracterização da divergência genética em genótipos de alfaces com diferentes níveis de carotenoides.
Palavras-chave: biofortificação; *Lactuca sativa*; variabilidade genética; vitamina A.

1. INTRODUCTION

The lettuce (*Lactuca sativa* L.) is the most cultivated leaf vegetable in Brazil (SALA; COSTA, 2012). Generally cultivated by family farmers the crop highlighted for presenting a high socioeconomic importance in the South American country and for its benefits to human health. Allied to the fact that consumers are increasingly demanding healthier foods associated with the amounts of bioactive compounds (CRUZ et al., 2012), it is known that the vegetables consume, such as lettuce, helps to prevent many diseases due to the presence of antioxidant compounds (MAIANI et al., 2009; PIENIZ et al., 2009). Beyond that, the leaf vegetable can be an important source of vitamin A.

Vitamin A is found directly in animal foods, while in vegetables such as lettuce, the vitamin is obtained from carotenoids that are precursors of it. The deficiency of vitamin

A is a serious problem all over the world, which can increase the risk of mortality, morbidity and blindness (MILAGRES et al., 2007; RAMALHO et al., 2008).

The practice of increasing the concentration of minerals and vitamins, in edible plants, is known as biofortification and it can be used as an alternative to reduce the risk of deficiency (BOUIS et al., 2011). There are reports of a lettuce genotype (Uberlândia 10000), of smooth type that was biofortified, being rich in carotenoids (SOUZA et al., 2007) and, because of that, it could be used in order to get Vitamin A.

In this context, it is known that the development of promising genotypes depends on the genetic variability available in germplasm banks (LEBEDA et al., 2014). From the cross-breeding between divergent parents it is possible to select superior plants in segregante populations, and with that, develop new cultivars (DAMERUM et al., 2015). The variability between genitors might be estimated using measures of genetic dissimilarity (AZEVEDO et al., 2013; SILVA et al., 2010; TREUREN; HINTUN, 2009).

The possibility of indirect selection, for β-carotene, through chlorophyll evaluation, has also been recommended, making easier to select superior genotypes (CASSETARI et al., 2015; MOU, 2005).

Therefore, the objective of this work was to evaluate the agronomic potential and genetic diversity in lettuce genotypes with different levels of carotenoids.

2. MATERIAL AND METHODS

The experiment was conducted from 2013 to 2016, at the Experimental Horticulture Station of the Federal University of Uberlândia (UFU) (18º42'43,19"S e 47º29'55,8", 873 m above sea level), in Monte Carmelo city. The region presents humid temperate climate, hot summer and dry winter. During the experiment, it was found that the maximum, average and minimum temperature, were, respectively, 38, 22 and 18 ºC.

Eighty two genotypes, obtained after hybridization between cultivars Pira 72 versus Uberlândia 10000, rich in carotenoid (SOUZA et al., 2007) followed by two successives self-fertilizations from the genealogic method, were evaluated. All the germplasm used is part of the biofortificate lettuce breeding program of the UFU. In addition, the cultivar Uberlândia 10000 was also evaluated. This cultivar has smooth green leaves and high vitamin A content (SOUZA et al., 2007).

The genotypes were sowed on February 10, 2016. The seedlings were produced in polystyrene trays, with 200 cells, filled with commercial substrate of coconut fiber. They were grown on a greenhouse, measuring 5 x 6 m and ceiling 3.5 m, covered with transparent polyethylene film of 150 micron, additivated against ultraviolet rays and curtain side of white and anti-aphid scream.

Twenty eight days after sowing, the seedlings were transplanted to flowerbeds, measuring 1.3 m, in the field.

The soil presented the following characteristics: pH (H_2O) = 5.9; P disponible = 30.1 mg.dm^{-3}; K = 0.22; Ca^{+2} = 2.8 cmolc.dm^{-3}; Mg = 1.0 cmolc.dm^{-3}; H^+ Al exchangeable = 3.40 cmolc.dm^{-3}; organic matter = 4.2 dag Kg^{-1}; SMP index = 3.4; Al = 0.0 cmolc.dm^{-3}; CTC pH 7.0 = 7.42 cmolc.dm^{-3}; Copper; 2.3 mg.dm^{-3}; Zinc = 6.6 mg.dm^{-3} and Manganese = 6.6 mg.dm^{-3}. The cultural traits were done based on the recommendation for lettuce cropping (FILGUEIRA, 2008).

The experiment was set up in a randomized complete block design, with two replications, using the following statistical model: $Y_{ij} = \mu + b_j + t_i + e_{ij}$. Each plot was consisted of twenty plants, where only the six central plants were evaluated.

After forty days of the transplanting, the following evaluations were done: Canopy diameter, that was done measuring six plants in each plot and, after that, it was obtained an average; Number of leaves, obtained in six plants per plot and an average was done; Leaf chlorophyll a and b (IFC – Index of Falker Chlorophyll), when the data were measured utilizing a portable digital chlorophyllometer (Clorofilog – CFL 1030 Falker), on the distance of 0.02 m from the edge and 0.05 m from the central nervure, in six leaves of each plot; and Total chlorophyll, which was consisted of the sum of chlorophyll a and b, in six leaves of each plot. The values of total chlorophyll were used, as indirect form, in order to estimate the carotenoids content. It was done because they have a high correlation in the lettuce crop, suggesting an efficient use of the chlorophyll content aiming to estimate the carotenoids (CASSETARI et al., 2015; MOU, 2005). The qualitative data evaluated were: size (1-normal, 2-mini-vegetable), leaf format (1-smooth, 2-curly) and color (1-green, 2-purple, 3-variegated).

The genetic dissimilarity between the access was made utilizing multivariate analyzes. The qualitative data were grouped by multicasts variables and, the dissimilarity matrix is calculated using the Cole-Rodgers distance and the matrix for the quantitative data was obtained by Mahalanobis distance. Both matrix (qualitative and quantitative) were sum and the genetic difference was represented by a dendrogram, which was done using the hierarchical method Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) and the Tocher method. Grouping validation by UPGMA method was obtained by the coefenetic coefficient of correlation (CCC), calculated by the Mantel test (1967). The relative contribution of the quantitative characters was calculated according to Singh criterion (1981). All the data were analyzed on the statistical software GENES (CRUZ, 2013).

3. RESULTS

The lettuce genotypes presented genetic dissimilarity and formed different groups, according to the UPGMA and Tocher grouping methods (Figure 1 and Table 1, respectively).

The Characteristics of the 83 evaluated lettuce genotypes, considering the grouping formed by UPGMA method and the Relative contribution of the quantitative characteristics in the genetic diversity are present in Table 2 and Table 3, respectively.

4. DISCUSSION

Using the hierarchical grouping method UPGMA (CCC = 0.80, $p<0.01$), it was found 10 different groups. The group I was consisted of 12 genotypes and the commercial cultivar Uberlândia 10000, being all materials of normal size, with smooth and green leaves. Uberlândia 10000 stands out for present high carotenoids content, which able us to infer that all the genotypes that stay in the same group of this commercial cultivar possibly have a high carotenoids content.

The average of the most divergent groups, for chlorophyll total, presented a variation of 6.7, which shows a high discrepancy among the genotypes of group X and VIII (Table 2). Nowadays, there is no lettuce germplasm showing high genetic variability for chlorophyll content. Besides that, it is known a correlation of 80%, in lettuce crops, between

chlorophyll content and carotenoids (CASSETARI et al., 2015).

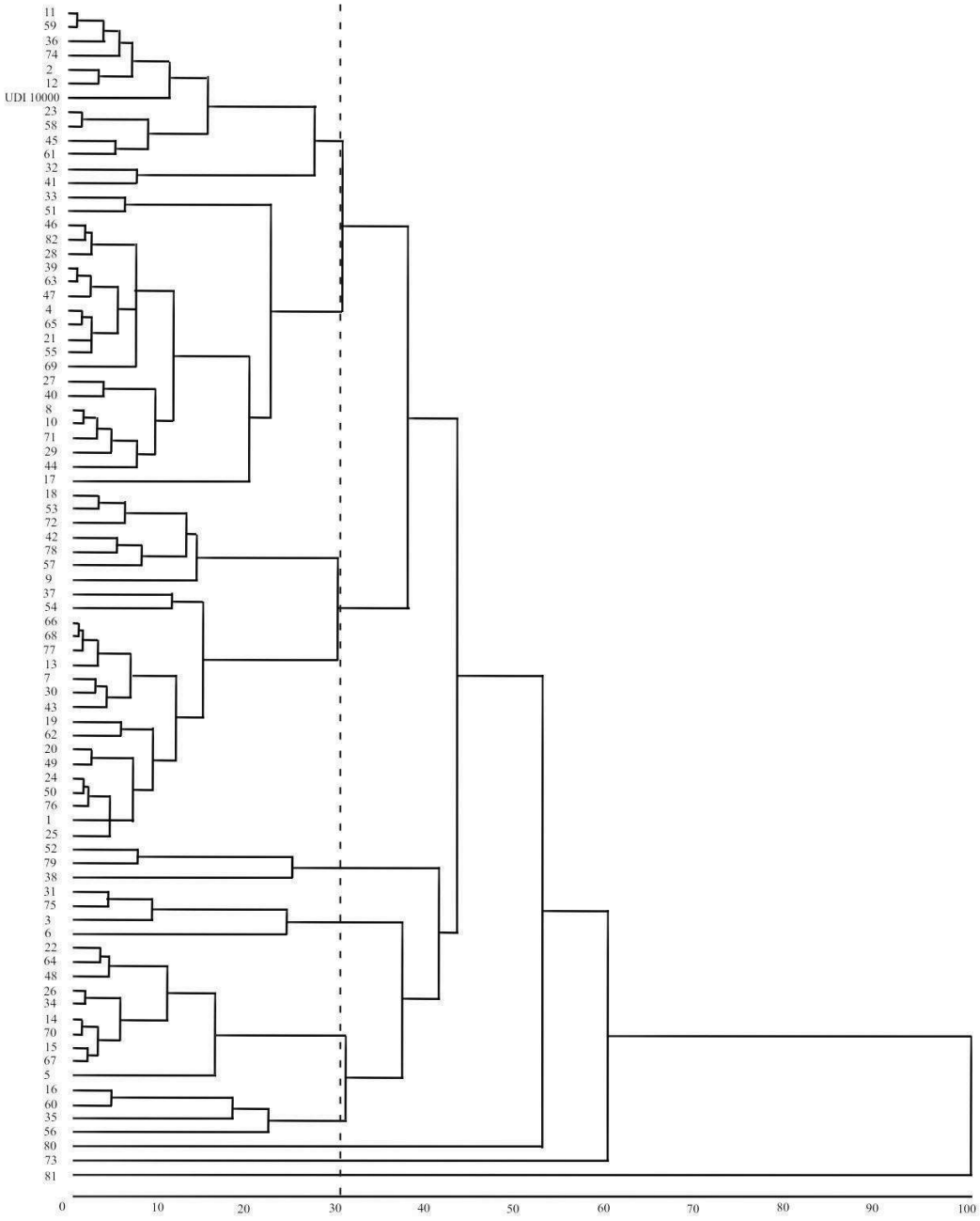

Figure 1.Dendrogram of the genetic diversity between the 83 lettuce genotypes, obtained by the hierarchic method of UPGMA as dissimilarity measurement. The numerals indicate the genotypes UFU-A.
Figura 1. Dendograma da diversidade genética entre os 83 genótipos de alface, obtidos pelo método hierarquizado de UPGMA como medida de dissimilaridade. Os numerais indicam os genótipos UFU-A.

Table 1. Grouping formed by 83 lettuce genotypes, obtained by the method of Tocher.
Tabela 1. Agrupamento formado por 83 genótipos de alface, obtidos pelo método de Tocher.

Group	Access
I	UFU-A1, UFU-A2, UFU-A3, UFU-A4, UFU-A5, UFU-A6, UFU-A7, UFU-A8, UFU-A9, UFU-A10, UFU-A11, UFU-A12, UFU-A13, UFU-A14, UFU-A15, UFU-A16, UFU-A17, UFU-A18, UFU-A19, UFU-A20, UFU-A21, UFU-A22, UFU-A23, UFU-A24, UFU-A25, UFU-A26, UFU-A27, UFU-A28, UFU-A29, UFU-A30, UFU-A31, UFU-A32, UFU-A33, UFU-A34, UFU-A35, UFU-A36, UFU-A37, UFU-A38, UFU-A39, UFU-A40, UFU-A41, UFU-A42, UFU-A43, UFU-A44, UFU-A45, UFU-A46, UFU-A47, UFU-A48, UFU-A49, UFU-A50, UFU-A51, UFU-A52, UFU-A53, UFU-A54, UFU-A55, UFU-A56, UFU-A57, UFU-A58, UFU-A59, UFU-A60, UFU-A61, UFU-A62, UFU-A63, UFU-A64, UFU-A65, UFU-A66, UFU-A67, UFU-A68, UFU-A69, UFU-A70, UFU-A71, UFU-A72, UFU-A73, UFU-A74, UFU-A75, UFU-A76, UFU-A77, UFU-A78, UFU-A79, UFU-A80, UDI 10000
II	UFU-A81

Table 2. Characteristics of the 83 evaluated lettuce genotypes, considering the grouping formed by UPGMA method, based on the Mahalanobis distance.
Tabela 2. Características dos 83 genótipos de alface avaliados, considerando o agrupamento formado pelo método UPGMA, baseado na distância de Mahalanobis.

Groups	Nº	C.a[1]	C.b[1]	T.C[1]	N.L[1]	D[1]	Type[2]	Color[2]
I	13	28.8±4.1	5.9±1.5	34.6±5.6	33.8±9.4	33.4±4.4	smooth	green
II	21	28.5±3.8	5.9±1.6	34.5±5.3	37.7±8.0	33.1±4.0	curly	green
III	25	29.3±4.0	6.5±1.9	35.9±5.7	33.6±6.1	32.3±3.7	curly	variegate
IV	3	30.1±3.2	6.4±1.3	36.5±4.4	43.6±11.1	29.7±3.5	mini/smooth	variegate
V	4	31.2±3.3	7.3±1.3	38.6±4.6	49.8±7.0	32.0±3.3	smooth	green
VI	10	28.7±4.0	6.0±1.4	34.6±5.2	38.4±7.6	34.6±2.6	smooth	variegate
VII	4	28.0±4.8	6.0±1.8	33.9±6.6	37.2±9.4	35.8±5.1	smooth	purple
VIII	1	27.1	5.3	32.4	36.5	42.8	smooth	variegate
IX	1	31.7	7.4	39.0	54.1	43.0	curly	green
X	1	31.2	7.4	39.1	33.5	30.7	mini/smooth	purple

[1]For the groups composed by more than one genotype, the standard deviation were measured and appears after the averages. Nº: number of genotypes in each group; C.A: chlorophyll a (IFC); C.B: chlorophyll b (IFC); C.T: total chlorophyll (IFC); N.L: number of leaves (leaves plant^{-1}); D: canopy diameter (cm). [2]Values obtained from the mode of each group's genotypes.

Table 3. Relative contribution of the quantitative characteristics in the genetic diversity of the 83 evaluated lettuce genotypes, according to Singh (1981).
Tabela 3. Contribuição relativa das características quantitativas na diversidade genética dos 83 genótipos de alface avaliados, segundo Singh (1981).

Characteristics	S.j	S.j (%)
Chlorophyll A content	376.68	0.77
Chlorophyll B content	6485.02	13.28
Leaf chlorophyll total	20688.52	42.37
Canopy diameter	8908.39	18.24
Number of leaves	12367.43	25.33

During the experiment, groups II and III presented curly leaves of normal size, differing from each other by coloring. It was also observed, that the group II presented green lettuces and group III variegate leaves. This two groups, along with the I, corresponded to 72% of evaluated lettuces. In lettuce breeding programs, for almost all tropical countries, materials with curly leaves are less important, even though, these crops are more consummated in Brazil (SALA; COSTA, 2012). However, nowadays there are no lettuce genotypes of curly type, showing high carotenoids content, in Brazil. It is important to emphasize that due to a higher Brazilian consumption, the groups II and III must be prioritized.

The groups IV and X highlighted for having mini-lettuces of smooth leaves, with purple and variegate color, respectively, and the lowest canopy diameter. Even though, mini-lettuces of group IV presented the highest average number for number of leaves per plant. The biofortificate crops development is recent in Brazil and all over the world, showing an increasing during the years, but it is still less explored (SALA; COSTA, 2012). Nowadays, there are no biofortificate mini-lettuce genotypes, with high carotenoids content, for direct use in breeding programs.

The groups V, VI, VII and VIII, presented smooth leaves with different colors, green, variegate, purple and variegate, respectively (Table 2). However, the genotypes of group V highlighted, showing the highest average number of leaves per plant (49.8) and, the genotypes of group VIII (UFU-A80) also highlighted, showing one of the highest average for canopy diameter (42.8 cm). Following the same reasoning, the lineage UFU-A73 (group IX) also stood out for canopy diameter (43.0 cm). During the experiment it was possible to verify that these genotypes combine agronomic potential and high carotenoids level in the leaves.

During the experiment, many groups were formed by UPGMA method; on the other hand, using the method of Tocher, only two groups were formed. Group I was composed by most all genotypes and, group II was composed by only the genotype UFU-A81. One of the factors that can interfere on lettuce genotypes grouping is the amount of descriptors used (KŘÍSTKOVÁ et al., 2008). For quantitative and qualitative descriptors used in this study, the UPGMA method was more efficient, because it shows a higher diversity in the formed groups. It is noteworthy that the leaf chlorophyll content and, consequently, the carotenoids content, was the descriptor that most contributed with genetic variability (42.7%) of the evaluated genotypes (Table 3).

Consumers are becoming increasingly interested in innovative products, in color, flavor or size, as well as consume foods functional properties - antioxidant activity (MATTOS et al., 2009). Following this thought, the lettuce genotypes of groups IX and X, by UPGMA method, highlights as potential commercial materials. Besides that, they can be used as genitors in lettuce breeding programs.

5. CONCLUSIONS

The genotypes showed genetic dissimilarity and agronomic potential for different lettuce segments and high carotenoids content. Among the evaluated characters, total chlorophyll content represented the main contribution for genetic variability. There was no coherence between the hierarchical method of UPGMA and the optimization method of Tocher when used for determinate the genetic diversity between lettuce genotypes with different levels of carotenoids.

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