

## Indirect selection for content of carotenoid in pumpkin (*Cucurbita moschata* Duch) accessions

### Selección indirecta del contenido de carotenoides en accesiones de calabaza (*Cucurbita moschata* Duch)

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#### ABSTRACT

Carotenoid quantification in food can be performed by various techniques, such as spectrophotometry, mass spectrometry, high performance liquid chromatography, gas chromatography, and colorimetry. The objective of this study was to verify the feasibility of indirect selection for assessment of total carotenoids in pumpkin accessions. The experimental design comprised of complete randomized blocks with two replications and three plants per plot. The treatments consisted of 51 *Cucurbita moschata* Duch accessions from the DCAF/UFERSA Cucurbitaceae germplasm collection and a commercial *C. maxima* Duch cultivar. After maturation, the fruits were harvested, and colorimetric and total carotenoid contents were evaluated. Considering the effect of accessions as random, variance components were estimated, and the genotypic values were predicted by maximum restricted likelihood (REML) or best linear unbiased prediction (BLUP). Both direct and indirect genetic gains were estimated from a selection intensity of 50%. Pulp color intensity parameters and  $h^\circ$  angle indicated the possibility of indirect selection of accessions with higher total carotenoid contents. The ABO22 accession presented higher total carotenoid content under the evaluation conditions of the experiment.

#### Keywords

*Cucurbita moschata* Duch • colorimetry • correlation • variability

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## RESUMEN

La cuantificación de los carotenoides en los alimentos se puede realizar mediante diversas técnicas, como la espectrofotometría, la espectrometría de masas, la cromatografía líquida de alto rendimiento, la cromatografía de gases y la colorimetría. El objetivo de este estudio fue verificar la viabilidad de la selección indirecta para evaluar los carotenoides totales en las accesiones de calabaza. El diseño experimental fue bloques completos al azar, con dos repeticiones y tres plantas por parcela. Los tratamientos consistieron en 51 accesiones de *Cucurbita moschata* Duch de la colección de germoplasma DCAF/UFERSA Cucurbitaceae y un cultivar comercial de *C. maxima* Duch. Después de la maduración, se cosecharon los frutos y se evaluó el contenido colorimétrico y total de carotenoides. Considerando el efecto de las accesiones como aleatorio, se estimaron los componentes de la varianza y se predijeron los valores genotípicos mediante la máxima verosimilitud restringida (REML) o una mejor predicción lineal e imparcial (BLUP). Las ganancias genéticas directas e indirectas se estimaron con base en una intensidad de selección del 50%. Los parámetros de intensidad de color de la pulpa y el ángulo  $h^\circ$  indicaron la posibilidad de selección indirecta de accesiones con un contenido de carotenoides total más alto. La adhesión a ABO22 mostró un mayor contenido de carotenoides totales en las condiciones de evaluación del experimento.

### Palabras clave

*Cucurbita moschata* Duch • colorimetría • correlación • variabilidad

## INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch) is considered to be an important source of carotenoids, which are the  $\beta$ -carotene precursors of vitamin A the most abundant with antioxidant activity (38). Carotenoids belong to the most commonly used group of natural dyes with colors ranging from yellow to red which are used in the food, pharmaceutical, and cosmetic industries (29, 30). The use of natural dyes has increased because they add nutritional value to food and are healthy when compared with artificial dyes (25, 43).

Concern about the growing increase in malnutrition worldwide has led to the development of studies on food biofortification with the aim of improving the quality of agricultural products through conventional plant breeding. Food biofortification programs intend to reduce malnutrition and ensure greater food security through increased iron, zinc, and vitamin A levels in the diets of the poor (9, 33). Pumpkin, considered a traditional vegetable in the diet of the Brazilian population, especially in the Northeast, constitutes an important focus of study in the field of food biofortification (32, 42).

Pumpkin has a wide genetic variability across the regions of Brazil. It is cultivated mainly by family producers who select seeds over the year, which allows the identification of parents with important breeding characteristics. Thus, evaluation of the largest number of accessions could lead to the identification of superior genotypes in terms of nutritional characteristics, as well as other components that add value to the product (21).

The most commonly used methods for carotenoid quantification are the extraction, characterization, and identification of carotenoid pigments by spectrophotometry, mass spectrometry, high performance liquid chromatography (HPLC), and the extremely accurate gas chromatography. However, these methods are expensive and time-consuming due to their use of many reagents. Therefore, alternatives for indirect quantification of carotenoids have been sought; in this context, colorimetry has been used because it is an instrumental method capable of measuring color characteristics in objects, in addition to being faster and less expensive when compared with other methods (6, 7).

When one considers that in the initial phase of breeding programs the evaluation of a large number of samples is common, the use of less expensive methodologies could result in greater germplasm coverage. Some studies involving pumpkin, tomato, and carrot have observed a high correlation between colorimetric methods and the content of carotenoids, demonstrating their great potential to replace spectrophotometric and chromatographic methods (4, 12, 23, 45).

On the other hand, the scientific literature dealing with the genus *Cucurbita* have reported variability of morphological and quality characteristics (5, 8, 34). Thus, the efficiency of the method must be proven in the germplasm that is intended to be used as the basis for a breeding program, because if there is no correlation between colorimetric values and carotenoid content, selection may be compromised. Therefore, using the methodology of mixed linear models, the objective of the present work was to verify the viability of indirect selection for assessment of the total content of carotenoids in pumpkin accessions.

## MATERIALS AND METHODS

The experiment was conducted at the experimental vegetable garden of the Departamento de Ciências Agronômicas e Florestais (DCAF), Universidade Federal Rural do Semi-Árido, Mossoró-RN (latitude 5° 11' S, 37° 20' long WGr and altitude 18 m) from September 2017 to February 2018. The soil of the experimental area is classified as Eutrophic Red Yellow Ultisol (14).

The experimental design comprised of complete randomized blocks with two replications and three plants per plot. The treatments consisted of 51 *C. moschata* Duch accessions from the DCAF/CCA/UFERSA Cucurbitaceae germplasm collection and a commercial *C. maxima* Duch cultivar obtained from a local wholesaler. The species *C. maxima* Duch was used as a new object in the study; it is commonly used in diversity studies with nearby species (15, 31). The spacing used was 4.00 x 3.00 m between rows and between plants, respectively.

The seeds were sown in 128-cell polystyrene trays with a commercial substrate in order to obtain the seedlings; transplanting took place when the seedlings had two to three definite leaves.

Tillage consisted of plowing and harrowing. The irrigation system used was a drip; an adjustable drip was placed in each plant with a flow rate of 18 liters/hour, providing the slide according to the phenological stage of the crop (3). Branch combing and weed control were performed manually.

After maturation, the fruits were harvested and sent to the postharvest laboratory located at the DCAF Centro de Pesquisas Vegetais do Semiárido (CPVSA). Then, pulp color and total carotenoid content were evaluated using one fruit per plant.

Pulp staining was determined at four equidistant points (fruit region around to the sun, fruit region exposed to the soil, fruit region exposed to the stalk, and fruit region exposed to inflorescence), followed by the arithmetic mean of staining. A Color Reader CR-400 Konica Minolta tri-stimulant manual colorimeter was used, with the following parameters: L\*, luminosity; a\*, contribution of red; and b\*, the contribution of yellow. Chromaticity or saturation (C\*) and hue (h°) were calculated based on the equations described by Itle and Kabelka (23).

The pulp color intensity was obtained from the visual analysis of the pulp of the fruits using grade descriptors, where fruits with light-colored pulp received grade 3, medium colored pulp, grade 5, and dark-colored pulp, grade 7. The total carotenoid content was measured using a UV Vis spectrophotometer FEMTO model 800 XI, according to the analytical methodology of separation and extraction of compounds with organic solvents of Rodriguez-Amaya and Kimura (2004) in duplicate.

Statistical analysis was performed according to statistical model 21 of the Selegen-REML/BLUP software (36). The correlation between the variables was determined by Pearson's correlation coefficient (16).

The selection gain for the variables was estimated using the formula  $GS = h^2 mg \times Ds$ , where  $h^2 mg$  is the average heritability of the genotype and  $Ds$  is the selection differential,  $Ds = Ms - Mo$ , where  $Ms$  is the selected average and  $Mo$  the original average. The percentage gain with selection (GS%) was obtained through  $GS (\%) = \frac{GS}{Mo} \times 100$  (44). Selection was performed considering 50% of accessions, because it is an initial selection.

**RESULTS AND DISCUSSION**

The effect of accessions was significant at the 5% probability level by the Chi-square test for chromaticity (C\*) and total carotenoid (TC) characteristics. For the other characteristics, no significance was verified (table 1).

**Table 1.** Deviance analysis and estimates of variance components for pulp color characteristics: luminosity (L\*), saturation (C\*), hue (h°), pulp color intensity (PCI), and total carotenoids (TC) in accessions of *Cucurbita* sp1.

**Tabla 1.** Análisis de desviación y estimaciones de los componentes de varianza para las características del color de la pulpa: luminosidad (L\*), saturación (C\*), tonalidad (h°), intensidad del color de la pulpa (ICP) y carotenoides totales (CT) en las accesiones de *Cucurbita* sp1.

Model	Deviance				
	L*	C*	h°	PCI	TC
Completo	189.92	243.88	356.75	110.28	788.04
Accessions	190.54	248.07	358.80	111.36	792.76
LRT	0.62 <sup>ns</sup>	4.19*	2.05 <sup>ns</sup>	1.08 <sup>ns</sup>	4.72*
$\hat{\sigma}_g^2$	0.33	1.73	3.89	0.18	950.91
$\hat{\sigma}_e^2$	2.47	3.60	14.27	0.98	1805.73
IPV	2.80	5.33	18.16	1.16	2756.64
$\hat{h}_g^2$	11.0	32.0	21.0	15.38	34.0
$\hat{h}_{mg}^2$	21.0	49.0	35.0	26.66	51.0
Acgen	0.46	0.70	0.59	0.52	0.72
CVgi	0.74	4.50	3.52	8.50	33.22
CVe	2.01	6.50	6.74	19.93	45.78
CVr	0.37	0.69	0.52	0.43	0.73
Average	78.03	29.21	56.02	4.98	92.82

<sup>1</sup> Accessions of *C. moschata* Duch from the DCAF/UFERSA cucurbitaceae germplasm collection and *C. maxima* Duch cultivar obtained from the local Mossoró market - RN. RLT: Likelihood ratio test.

$\hat{\sigma}_g^2$ : genotypic variance.  $\hat{\sigma}_e^2$ : residual variance.  $\hat{\sigma}_p^2$ : individual phenotypic variance.  $\hat{h}_g^2$  (%): heritability of individual plots in the broad sense, i.e., total genotypic effects.  $\hat{h}_{mg}^2$  (%): heritability of the genotype mean, assuming complete survival. Acgen: accuracy of genotype selection, assuming complete survival. CVg%: coefficient of genotypic variation. CVe%: residual coefficient of variation. CVr: relative coefficient of variation. Average: overall experiment average.

<sup>1</sup> Acciones de *C. moschata* Duch de la colección de germoplasma DCAF/UFERSA cucurbitaceae y del cultivar *C. maxima* Duch obtenido en el mercado local de Mossoró - RN. RLT: Prueba de razón de verosimilitud.  $\hat{\sigma}_g^2$ : varianza genotípica.  $\hat{\sigma}_e^2$ : varianza residual.  $\hat{\sigma}_p^2$ : varianza fenotípica individual.  $\hat{h}_g^2$  (%): heredabilidad de parcelas individuales en un sentido amplio. es decir, efectos genotípicos totales.  $\hat{h}_{mg}^2$  (%): heredabilidad del promedio del genotipo. suponiendo una supervivencia completa. Acgen: precisión de la selección del genotipo. suponiendo una supervivencia completa. % CVg: coeficiente de variación genotípica. CVe%: coeficiente de variación residual. CVr: coeficiente de variación relativo. Promedio: promedio general de experiencia.

The average heritability of accessions obtained average magnitude values for the variables C\* (49%) and TC (51%) (table 1). Heritability measures the level of correspondence between the phenotype value and the genetic value when heritability is high. Selection in the early generations of self-fertilization is effective; however, if its value is low, selection should be practiced only in the most advanced generations to determine the procedures and strategies to be adopted in the development stages of the cultivar (17, 39). For accuracy, high magnitude values were observed for C\* (0.70) and TC (0.72) (table 2, page 16).

For the relative coefficient of variation, the variables presented values lower than one unit (table 1, page 15). Thus, the values found demonstrate that there was greater environmental influence for the variables, in accordance with Vencovsky (1987). Estimates of genetic parameters are important indicators for breeding programs, because they predict genetic values and maximize selection, where the significance of accession effects indicates the existence of genetic variability (11, 13, 28). According to the results of the parameters, it is evident that the adoption of breeding methods capable of returning considerable gains is necessary (1).

Recurrent selection is a methodology that allows for gains in traits with quantitative inheritance, resulting in a new population higher than the original, and providing better performance in selected individuals (19). Recurrent intrapopulation selection may be an appropriate methodology to obtain significant and consistent gains across generations. The procedure leads to improvements in genetic parameters, especially heritability, because it continuously and progressively improves population performance by increasing favorable allele frequencies of the characters under selection through the repeated selection and recombination cycles (10, 22).

The L\* variable presented negative correlations of medium magnitude for the INTP (-0.54) and TC (-0.47) variables, where an increase in one variable meant a decrease in another (table 2). Thus, pumpkin fruits with lighter pulp (yellow) have higher L\* values and lower PCI and TC values, and darker pulps (intense orange) have lower L\* values and higher PCI and TC values.

**Table 2.** Genetic correlation coefficient for pulp color characteristics: luminosity (L\*), chromaticity (C\*), hue (h°), pulp color intensity (PCI), and total carotenoids (TC) in accessions of *Cucurbita* sp.

**Tabla 2.** Coeficiente de correlación genética para las características del color de la pulpa: luminosidad (L\*), cromaticidad (C\*), tono (h°), intensidad del color de la pulpa (ICP) y carotenoides totales (CT) en las accesiones de *Cucurbita* sp.

	L*	C*	h°	PCI	TC
L*	-	0.05 <sup>ns</sup>	0.63*	-0.54*	-0.47*
C*		-	-0.62*	0.53*	0.58*
h°			-	-0.85**	-0.82**
PCI				-	0.71**
TC					-

\*\* , \* , and <sup>ns</sup>: significant at 1%, 5% probability, and non-significant by t-test, respectively.

\*\* , \* y <sup>ns</sup>: significativo al 1%, 5% de probabilidad y no significativo por la prueba t, respectivamente.

C\*, which is directly linked to the element concentration and represents a quantitative attribute for intensity (20), presented a positive mean correlation for PCI (0.53) and TC (0.58), and a high magnitude negative correlation to h° in the same variables (-0.85 and -0.82 respectively). Here, the increase in pulp saturation, C\*, causes an increase for PCI and TC variables, and a reduction in h° angle, resulting in fruits with orange and very bright pulp which are more attractive to the consumer market (table 2). Aquino *et al.* (2016) studied ripe banana pulp and found that the h° index was negatively correlated with C\* (-0.71), which is similar to the results of the current study.

The PCI variable showed a high positive correlation with TC of 0.71 (table 2), demonstrating that total carotenoid content is directly related to pulp color. The darker pulp indicates a higher content of carotenoid. Although visual assessment shows a higher variation in grades due to the subjectivity of the evaluator, it can be used with favorable results at the beginning of selection in breeding programs, where there are a large number of accessions to be evaluated. However, as selection advances, this method will have low efficiency.

The orange coloration of the pulp is a desirable component of quality in pumpkin fruits, and the angle h° is the variable that best defines this characteristic, where values above 30° and below 75° represent the orange color; the lower the values in this range, the more intense the pulp coloring (18, 27, 40). In the present study, evaluation of the angle h° was not verified by the chi-square test between the accessions, but showed high correlation with PCI (-0.85) and TC (-0.82), demonstrating a difference in the content of carotenoids between accessions.

The results found by Itle and Kabelka (2009) show a strong correlation between colorimetric values and carotenoid content, indicating that indirect selection for the high carotenoid index in pumpkin can be successful, easy to perform, and low cost.

The total carotenoid content in the different accessions ranged from 52.65  $\mu\text{g g}^{-1}$  for ABO40 accession and 155.78  $\mu\text{g g}^{-1}$  for ABO22 (table 3, page 17-18). Amariz *et al.* (2009) studied *C. moschata* accessions belonging to the "Embrapa Semi-Árido" Cucurbitaceae Germplasm Active Bank and found variation from 21.3 to 78.5  $\mu\text{g g}^{-1}$  in the total carotenoid content. When studying other accessions of "Embrapa Semi-Árido" Cucurbitaceae Germplasm Active Bank grown in Petrolina-PE (42), the same authors found total carotenoid contents ranging from 14.93 to 290.62  $\mu\text{g g}^{-1}$ . Variation in the results of different authors may occur due to the processes of preparation of raw materials, the different cultivars, the places of cultivation, and the ripening states of the fruit (26, 41, 45).

**Table 3.** Genotypic means of pumpkin accessions.  
**Tabla 3.** Media genotípica de accesiones de calabaza.

Accessions	Genotypic averages				
	L*	C*	h°	PCI	TC ( $\mu\text{g g}^{-1}$ )
ABO22	77.59	29.80	53.70	5.43	155.78
ABO55	77.85	29.88	54.64	5.29	138.23
ABO38	77.99	30.53	54.77	5.12	130.95
ABO05	78.04	30.31	54.94	5.16	129.29
ABO03	77.66	29.49	54.34	5.25	128.10
ABO28	77.68	29.35	54.49	5.34	125.54
ABO36	77.77	28.46	54.42	5.25	121.72
ABO34	78.29	30.11	56.02	4.72	119.87
ABO11	77.96	29.41	55.64	4.85	117.77
ABO43	78.06	30.08	55.33	4.99	111.48
ABO18	77.58	29.35	53.97	5.38	107.25
ABO37	78.04	29.67	55.36	4.99	105.52
ABO09	77.52	30.65	54.37	5.38	103.82
ABO39	78.13	30.28	55.62	4.99	101.44
ABO50	78.23	30.50	55.67	5.12	100.95
ABO13	77.95	28.87	56.00	4.98	99.13
ABO30	77.85	29.60	55.28	4.98	95.71
ABO35	78.50	30.63	56.04	4.98	94.20
ABO21	77.71	28.05	56.25	4.98	94.17
ABO42	78.26	30.41	55.76	4.99	94.13
ABO19	78.12	29.52	55.68	4.98	91.88
ABO33	77.99	29.23	55.97	4.98	91.03
ABO47	78.01	29.28	55.89	5.12	90.46
ABO24	78.31	30.15	55.97	5.07	89.66
ABO51	77.96	28.92	56.62	4.85	88.86
ABO10	77.34	28.15	55.06	4.98	87.86
ABO04	78.30	29.80	56.35	4.90	87.47
ABO12	77.97	28.78	56.22	5.03	86.68
ABO25	78.05	29.20	56.47	4.94	86.55
ABO31	78.10	29.11	56.14	5.07	86.33

**Table 3 (cont.).** Genotypic means of pumpkin accessions.**Tabla 3 (cont.).** Media genotípica de accesiones de calabaza.

Accessions	Genotypic averages				
	L*	C*	h°	PCI	TC ( $\mu\text{g g}^{-1}$ )
ABO27	78.12	29.17	56.46	4.99	84.43
ABO23	78.00	28.75	56.66	4.88	83.11
ABO29	78.02	28.47	56.59	4.85	83.09
AB044	78.06	29.88	55.54	4.81	83.08
ABO56	78.43	29.98	56.62	5.12	82.17
ABO08	77.92	28.80	56.01	4.98	81.81
ABO14	78.07	28.95	55.96	4.98	81.08
ABO52	78.32	29.66	56.37	4.72	80.95
ABO26	78.08	28.45	57.46	5.12	80.22
ABO06	78.10	29.59	56.47	4.85	79.03
ABO16	78.32	29.07	56.72	4.72	78.17
ABO17	77.95	29.48	56.65	4.89	77.40
ABO49	78.13	29.31	56.77	4.78	77.36
ABO07	78.26	28.32	56.51	4.72	77.04
ABO41	78.13	28.44	56.83	4.88	76.10
ABO48	77.92	28.60	56.67	4.72	75.10
ABO15	77.99	27.76	57.23	4.88	72.71
ABO01	78.10	27.69	56.74	4.72	69.57
ABO02	78.27	28.89	55.84	4.98	69.34
ABO20	77.82	27.24	57.42	4.76	67.59
CC	78.66	26.77	60.48	4.45	52.99
ABO40	78.30	27.96	58.13	4.58	52.65
Avarage	78.03	29.21	56.02	4.98	92.82

Taking into account the results obtained for the estimation of genetic parameters in the evaluated accessions and using a selection intensity of 50% because it was an initial selection, a selection of 26 accessions was performed with a prediction of genetic gains for the characters L\*, C\*, h°, PCI, and total carotenoid content (table 4, page 19).

The L\* variable presented the smallest gain with indirect selection at 4.76%. The h° variable presented the largest indirect selection gain of 6.76%, with a selected average of 105.12  $\mu\text{g g}^{-1}$ ; this variable obtained the average closest to direct selection of total carotenoids from evaluation using a spectrophotometer (108.26  $\mu\text{g g}^{-1}$ ) (table 4, page 19).

The selection gain estimate obtained a low percentage, which can be explained by the heritability with a maximum estimate of 51% (TC). Heritability represents the reliability with which the phenotypic value represents the genotypic value; therefore, characteristics with low heritability reflect a greater influence of the environment, reducing their discriminatory power (24, 35).

Direct selection of total carotenoids obtained the highest estimate for selection gain (8.48%) compared with other variables (table 4, page 19). From the selection obtained, it can be observed that the ten accessions with the highest total carotenoid contents may be the most promising for improvement, with averages above 110  $\mu\text{g g}^{-1}$ , whereas the ABO22 accession obtained the highest average of 155.78  $\mu\text{g g}^{-1}$  (table 3, page 17-18). The accessions were collected in the cities of Rio do Fogo, Touros, Ipanguaçu, Assú, and some unknown sites. No relation was found between the accession collection site and the total carotenoid content because of the variability of the accessions collected.

**Table 4.** Estimation of indirect selection in pumpkin accessions for total carotenoids with a selection level of 50%.**Tabla 4.** Estimación de la selección indirecta en accesiones de calabaza para carotenoides totales con un nivel de selección del 50%.

	Indirect Selection <sup>1</sup>				Direct selection <sup>2</sup>
	L*	C*	h°	PCI	TC
<b>MS(μg g<sup>-1</sup>)</b>	101.49	104.19	105.12	103.44	108.26
<b>MO (μg g<sup>-1</sup>)</b>	92.82	92.82	92.82	92.82	92.82
$\hat{h}_{mg}^2$	0.51	0.51	0.51	0.51	0.51
<b>GS</b>	4.42	5.80	6.27	5.42	7.87
<b>GS(%)</b>	4.76	6.25	6.76	5.84	8.48

<sup>1</sup> Indirect selection: selection made on the basis of color, lightness (L\*), chromaticity (C\*), hue (h°), and pulp color intensity (PCI). <sup>2</sup> Direct selections: selection of total carotenoids (TC) from spectrophotometer analysis. MS: genotypic mean total carotenoids from accessions selected indirectly by the characters L\*, C\*, h°, and PCI. Selection level: 51%. MO: original mean total carotenoids.  $\hat{h}_{mg}^2$ : average heritability of total access carotenoids. GS: selection gain.

<sup>1</sup> Selección indirecta: selección realizada en los caracteres de color: luminosidad (L\*), cromaticidad (C\*), tonalidad (h°) e intensidad de color de la pulpa (ICP). <sup>2</sup> Selección directa: selección de carotenoides totales (CT) a partir del análisis espectrofotométrico. EM: carotenoides totales medios genotípicos de accesiones seleccionadas indirectamente por los caracteres L\*, C\*, h° e ICP. Nivel de selección: 51%. MO: carotenoides totales promedio originales.  $\hat{h}_{mg}^2$ : heredabilidad promedio de los carotenoides de acceso completo. GS: ganancia de selección.

## CONCLUSIONS

The results associated with the parameters for the chromaticity and content of carotenoids present heritability and accuracy varying from medium to high magnitude, respectively, and obtained better results by using the recurrent selection method. Pulp color intensity parameters and h° angle indicate the possibility of indirect selection of accessions with a higher total carotenoid content. The ABO22 accession presented higher total carotenoid content under the evaluation conditions of the experiment.

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