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Prediction of genetic and selection parameters in pumpkin (*Cucurbita moschata* Duch.) progenies for morphoagronomic characteristics and pulp quality

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Abstract

The estimation of genetic parameters allows for the selection of superior individuals for important traits in plant breeding. The objectives of the present study were to estimate and predict the gains in genetic parameters, as well as to select superior individuals for morphoagronomic and pulp quality characteristics in *C. moschata*. Ten progenies derived from accessions collected in the northeast of Brazil were evaluated for 17 morphoagronomic characteristics and chemical quality of the pulp, including total carotenoids and β-carotene. The low values of additive genetic variance denote the need for additional selection cycles for the evaluated characteristics. Heritability greater than 30% was observed for 13 of the 17 variables analysed, indicating success in selection. Likewise, accuracy values between 74 and 93% were obtained for 15 of the evaluated variables, demonstrating the existence of high genetic variance. Individual ranking was conducted for the variables fruit weight (FRW), soluble solid content (SSC), titratable acidity (TA), total carotenoid content (CBT) and β-carotene (β-Car), identifying five individuals of progeny 10 that were promising for advancement in selection for SSC, CBT and β-car. In ranking the simultaneous evaluations for all the characteristics and taking into account the formats piriform and 'moranga', two individuals of progeny 10, which coincided with the top ranking for SSC, CBT and β-car, were identified. This identification of more than one promising individual enhances the potential of the progenies evaluated for the development of commercial and productive lines in semi-arid conditions.

Keywords: *Cucurbita moschata*, heritability, selection index.
Abbreviations: Sibling plants (SIB); Genetic Bank (GB); Genebank of Cucurbits (GBC); characteristics of fruits: fruit weight (FRW); length (LEN); larger diameter (LD); smaller diameter (SD); longitudinal internal cavity diameter (LICD); median internal cavity diameter (MICD); apical thickness of the skin (APS); equatorial thickness of the skin (EQS); apical thickness of the pulp (APP); equatorial thickness of the pulp (EQP); luminosity (L); chroma (C); hue angle (H); soluble solid content (SSC); titratable acidity (TA); total carotenoid content (TCC); β-carotene content (β-car); high-performance liquid chromatography (HPLC).

Introduction

Pumpkin (*Cucurbita moschata*) is one of the most consumed species in the Cucurbits family (Talukdar and Hossain, 2014). Phylogenetic and evolutionary studies using molecular tools show that it originated from Mexico and countries of Central and South America (Piperno and Pearsall, 1998; Smith, 2006; Kistler et al., 2015).

For centuries, popular medicine has used pumpkin for the prevention of various diseases, such as diabetes (Wang et al., 2017). However, its main activity is associated with its antioxidant function, making it a functional food due to its high content of β-carotene (Saini et al., 2015; Mezzomo and Ferreira, 2016). This pigment presents antioxidant activity and can be converted to vitamin A, which is essential for the maintenance of ocular retinal epithelial cells (Bai et al., 2011) and acts in the prevention of chronic diseases such as diabetes (Dhillon et al., 2015). Therefore, pumpkin can help combat against vitamin A deficiency, which affects the normal functioning of the visual system and is the main cause of blindness in children (Unscn, 2010) in low-income populations.

Cyril et al. (2014) reported that the success of a breeding programme depends on the available genetic variability, genetic advancements and indirect effects on yield and its attributes. Moreover, in a breeding programme, the main objective is to determine the additive genetic variance that is sufficient to indicate that selection, either from a single plant or between progenies, is effective for the parameters of interest (Hallauer et al., 2010).

The use of heritability estimates and heritability variances in plant breeding has been recommended to predict the genetic variation of a population and to identify superior parental species to promote the continuity of the cycle and the efficiency of the method (Dudley and Moll, 1969). Therefore, the prediction of these genetic parameters

provides information about the degree to which a characteristic can be transmitted in successive generations of the improved species (Bello et al., 2012). The prediction allows researchers to evaluate and to identify the responses of the genotypes to the measures associated with the genetic variance (Rutkoski, et al., 2015), guiding a breeding programme more effectively.

In Brazil, pumpkin is considered a naturalized species that is perfectly adapted to the semiarid region, where it has developed great variability, as evidenced by the wide variation in characteristics such as the colouring of the bark and pulp and the size and shape of the fruit (Ramos et al., 2000). This variability has been conserved in the Genetic Bank (GB) of Embrapa Semiárido, in Petrolina, Pernambuco state, Brazil, and has allowed for several research projects, including the characterization of accessions of the GB (Borges et al., 2011) for agronomical traits of commercial fruit quality, as well as total carotenoid content, in particular, β-carotene.

The objectives of the present study were to estimate and predict gains in genetic parameters, as well as to select superior individuals for morphoagronomic and pulp quality characteristics, in *C. moschata*.

Results

Analysis of variance indicated significant differences between the progenies evaluated ($P < 0.01$), except for the variables longitudinal internal cavity diameter (LICD) and chroma (C). The minimum and maximum values of coefficients of variation ranged from 2.03 for luminosity (L) to 52.39 for smaller diameter (SD), respectively (Table 2S).

For the additive genetic variance, all estimates were positive and different from zero (Table 1). The values ranged from 0.0040 for TA to 34.00 for APP, with the lowest values observed for FRW, apical thickness of the skin (APS), C, TA, TCC and β-car (Table 1).

Individual heritability values, in the restricted sense, adjusted for plot effect for the variables analysed, ranged from 0.0048 in C to 0.7379 in SS. Heritability values greater than 30% were obtained for characteristics FRW, length (LEN), larger diameter (LD), SD, median internal cavity diameter (MICD), APS, equatorial thickness of the skin (EQS), apical thickness of the pulp (APP), equatorial thickness of the pulp (EQP), SSC, TA, TCC and β-car (Table 1).

The best linear unbiased prediction (BLUP) analysis in the present study maximized the accuracy, reflecting the correlation between the true genotypic value and those estimated values (Resende and Duarte, 2007). The values of accuracy for almost all characteristics were very high or high, except APB, which had the intermediate r value of 68%, and EQP, which had the low r value of 17%, according to Resende and Duarte (2007) (Table 1).

Regarding the coefficients of variation, except for LICD, C and hue angle (H), the additive genetic variation coefficient (CV_{gi}) was higher than the environmental variation coefficient (CV_e), with values ranging from 106.63 to 0.3316% (Table 1). The residual variation coefficient (CV_r), which means the ratio between the additive genetic variation coefficient and environmental variation, was thus also lower for the characteristics with lower CV_{gi} (Table 1).

Considering the results obtained for the estimation of genetic parameters in the evaluated progenies and using a selection intensity of 10%, the best 17 individuals were selected to predict their genetic gains for the next breeding cycles based on characteristics associated with the commercial quality of fruit (FRW, SSC and TA) and those associated with pumpkin's status as a functional food (TCC and β-car). Table 2 refers to the prediction of the genetic gains for these variables from the average of the selected plants, indicating the progeny number (Table 1) and plant number (Table 2). The individuals 10/1, 10/3, 10/5, 10/12, and 10/13 coincided in the rankings of variables SSC, TCC and β-car, although they had different positions in each ranking. The predicted additive genetic gain in percentage was 35.58, 60.38, 56.25, 52.61 and 61.83 for the FRW, SSC, TA, TCC and β-car, respectively.

The seventeen best individuals for all characteristics were also determined, differentiating the ranking according to the interest in obtaining lines with fruits of piriform and 'moranga' types, through the index of selection of Mulamba and Mock (1978) (Tables 3 and 4). The results of differential and gain selection were represented in both rankings for piriform and 'moranga' types. Variables whose estimates had negative values were predicted to decrease with the selection programme used. The individuals 10/1 and 10/12, with a piriform fruit shape, had high genetic values for more than one commercial variable (Table 2) and presented high performance when considering all variables analysed (Table 3). However, no individual with this same performance was observed for the 'moranga' type (Table 4).

Discussion

The existence of variability between the progenies evaluated was confirmed by ANOVA as well as by the estimates of the variances for the evaluated characteristics (Tables 1S and 1). These results show that existence of heterogeneity between families indicates good prospects for obtaining genetic gains by selection (Ndukauba, et al., 2015; Neves et al., 2011).

Low values for additive genetic variance were expected because the genetic constitution of the evaluated progenies resulted of a crossing between sibling plants from inbred lines. The lack of negative values and values different from zero denotes the possibility of success in the selection of superior individuals (Hallauer et al., 2010) for the characteristics studied, but it also indicates the need for additional cycles of selection in the pumpkin breeding programme to obtain uniform lines for the characteristics in question.

Heritability reflects the proportion of phenotypic variation inherited (Falconer and Mackay, 1996). Heritability is essential to delineate the genetic population of interest in a breeding programme (Dudley and Moll, 1969) and indicates the efficacy of the selection process. The heritability values found in the present study, over 30% for 13 analysed variables, indicate a strong possibility of success in a programme that aims to select among and within progenies of pumpkin. The characteristics LICD, L, C and H had heritabilities below 30%, indicating a higher environmental influence (Table 1).

Regarding L, C and H, recent studies have made substantial progress to characterize the mechanisms that interfere in the synthesis of waxes present on the surface of skin in vegetables. The responses in wax production are influenced by abiotic factors, and thus, there is a strong relationship between cuticle permeability and water absorption, as the variations in the waxy surface can promote changes in

luminosity and pulp colour (Fernández et al., 2016; Vargas et al., 2008). For the variable H, the environmental influence is due to the relationship of the yellow colour with β-carotene biosynthesis, and this pigment is directly involved in the capture of the light energy during photosynthesis. In a study about the effect of plant-environment interactions on the synthesis of secondary metabolites and on β-carotene synthesis, Santos (2015) reported that *Gracilariopsis tenuifrons* exposed to high luminosity showed a reduction in pigment levels, which decreased energy absorption. According to the same author, this is a compensation mechanism that does not affect either the photosynthetic apparatus or the biomass synthesis of the plant and could be a protective strategy.

Accuracy refers to the systematic errors of experimental data, and measuring accuracy allows for the identification of such errors in experimental analysis (Shaikh and Karim, 2015). The high accuracy values obtained also allowed us to confirm the existence of genetic variance in the progenies studied for the characteristics in question, as well as the reliability in the obtained results.

Most variables had a higher coefficient of genetic variation than coefficient of environmental variation, except for LICD, C and H (Table 1), indicating that the observed variations are influenced by genetic factors, a favourable situation for selection (Georgieva et al., 2016).

Most variables had a coefficient of residual variation (CVr) above 1, except for LICD, C and H (Table 1), indicating a favourable situation for selection within the progenies evaluated, as defined by Vencovsky (1987).

Considering information regarding selection of the best 17 individuals (selection intensity of 10%), it was observed that in regard to FRW, there was a predominance of individuals of progeny 5. The general mean obtained in the present study was 2.67 kg (Table 2), whereas in the individuals selected within progeny 5, the prediction of genetic gain values ranged from 3.37 to 4.08 kg (Table 2). In studies related to the fruit mass of pumpkin, the values are quite variable: Han et al. (2015), for example, assessing characteristics in 41 varieties of *C. moschata* in China, observed 20 varieties with fruit mass varying from 0 to 4 kg. Values varying from 0.33 to 6.84 kg were estimated by Tamil Selvi et al. (2012) when assessing performance *per se* in 15 other genotypes.

For SSC, the value obtained for the general mean in the evaluated progenies was 11.5 °Brix, and the prediction of genetic gain values was 18.4 °Brix (Table 2), much higher than the values and variations found for pumpkin in previous studies (Gajewski et al. 2008; Zinash et al., 2013; Zhao et al., 2015). Loy (2004) defines the acceptability of fresh pumpkin fruits as one of the main factors in the equilibrium of the quantity of sugars, emphasizing that pumpkin fruits have SSC contents between 11 and 13 °Brix, a range that contained the general mean of the progenies studied here and was surpassed by the genetic value (Table 2). TA presented a general mean of 0.16% citric acid, while the predicted additive genetic value was 0.25% for citric acid. For this characteristic, in the ranking carried out, there was the predominance of individuals of progeny 7 (Table 2). These values are lower than those found by Zinash et al. (2013).

The mean total carotenoid and β-carotene contents of 261.69 μg g^{-1} and 215.10 μg g^{-1}, respectively (Table 2), are higher than those reported by Azevedo-Meleiro and Rodriguez-Amaya (2007) for the commercial varieties of pumpkin 'Menina Brasileira' and 'Goianinha', whose total carotenoid contents (the sum of β-carotene, α-carotene, lutein, violaxanthin and neoxanthin) were 118.7 μg g^{-1} and 105.1 μg g^{-1}, respectively, and whose β-carotene contents were 66.7 μg g^{-1} and 56.7 μg g^{-1}. Nakkanong et al. (2012), comparing the levels of carotenoids between fruits of *C. moschata*, *C. maxima* and the interspecific hybrid *C. moschata* x *C. maxima*, observed 110.20 μg g^{-1} of total carotenoids in fresh and mature fruits in the first species. This value represented the sum of the levels of β-carotene, α-carotene, lutein, violaxanthin and neoxanthin. They measured 10.52 μg g^{-1} of β-carotene in this species, almost twenty times less than the mean value obtained in the present study.

For total carotenoid content, individuals of progenies 10 and 1 presented similar amounts, with predicted values ranging from 427.1 to 370.3 μg g^{-1} of pulp (Table 2). For β-carotene content, in addition to the similarity of the distribution of individuals of progenies 10 and 1, a progeny individual 9 was also observed, with values ranging from 377.6 to 313.8 μg g^{-1} of pulp (Table 2). These results are particularly interesting because they indicate the superiority of the progenies in β-carotene content, which can easily satisfy the nutritional requirement of this carotenoid for humans.

We also observed the coincidence in the ranking of individuals for SSC, TCC and β-car (Table 2). Gajewski et al. (2008), studying quality characteristics in different species of pumpkins, reported the existence of a strong direct correlation between sweet taste intensity (high SSC value) and high total carotenoids. These same authors reported that high soluble solid content corresponded to a high sugar content and was an important quality factor in pumpkin, as fruits with higher soluble solid content had higher β-carotene and total carotenoid contents.

On the other hand, aggregation of multiple pieces of information in the experimental unit, as well as the use of selection indexes based on a set of variables, is a strategy used in plant breeding to minimize negative correlations among characteristics (Cruz and Regazzi, 2002). The ranking for multiple variables allowed for the use of 34 promising progenies to improve the breeding programme, taking into account the piriform and ‘moranga’ types, which were predominant in the studied individuals (Tables 3 and 4).

The ranking of individuals, both by variables of greater commercial relevance and by the selection index for all characteristics assessed, indicated the individuals 10/1 and 10/12 will be potentially interesting to the next selection cycle in pumpkin of the piriform format. For the ‘moranga’ format, the individual 6/3 had a good W value as well as a good joint assessment of variables (Table 4).

The results indicate the superiority of the progenies in the total carotenoid and β-carotene variables. In addition, the selection considering all the variables shows the possibility of selection for different formats: piriform and 'moranga'. Finally, the use of selected progenies through genetic parameters creates good prospects for the development of lines with superior nutritional and productive characteristics.

Material and Methods

Experimental site

The experiment was carried out from June to October 2013 in the experimental field of Embrapa Semiárido, in Petrolina, Pernambuco state, Brazil, (09° 09' S, 40° 22' W, 365.5 m asl).

Table 1. Genetic parameters estimated for characteristics evaluated in pumpkin progenies (*Cucurbita moschata*).

Characteristics	Genetic parameters									
	$\hat{\sigma}_a^2$	$\hat{\sigma}_{fi}^2$	$\hat{\sigma}_c^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$	$r_{\hat{a}a}$ (%)	CV_{gi}(%)	CV_e(%)	CV_r	Mean ($\hat{u}$)
FRW (kg)	0.3439	1.0500	0.0823	0.5487	0.3265 ± 0.1262	87.0	21.95	12.33	1.78	2.67
LEN (cm)	10.5900	22.5600	1.3300	9.3200	0.4693 ± 0.1513	90.0	16.08	6.57	2.45	20.24
LD (cm)	4.6900	10.3800	0.5623	4.4900	0.4520 ± 0.1485	90.0	12.02	4.88	2.46	18.03
SD (cm)	7.0600	13.1200	0.4473	4.9100	0.5377 ± 0.1939	92.0	106.63	33.13	3.20	2.49
LICD (cm)	1.2900	10.5100	1.0500	7.1500	0.1231 ± 0.0775	74.0	8.89	9.21	0.97	12.80
MICD (cm)	1.4200	4.0400	0.1091	2.1900	0.3517 ± 0.1310	89.0	9.83	3.81	2.58	12.12
APB (mm)	0.5993	1.3500	0.0064	0.6552	0.4424 ± 0.1469	68.0	21.65	5.42	3.99	3.57
EQB (mm)	1.0700	1.7800	0.0660	0.5666	0.5997 ± 0.1710	78.0	25.07	7.39	3.39	4.12
APP (mm)	34.0000	74.0000	0.1815	34.8100	0.4581 ± 0.1495	85.0	24.00	5.60	4.28	24.31
EQP (mm)	1.2500	1.9400	0.0429	0.5656	0.6451 ± 0.1774	17.0	36.39	8.59	4.24	30.76
L	2.2900	9.4600	0.6773	5.6800	0.2423 ± 0.1087	77.0	2.25	1.45	1.55	67.19
C	0.0542	11.3100	2.9000	7.3200	0.0048 ± 0.0153	93.0	0.33	2.57	0.13	70.20
H	1.7400	9.7000	1.7300	5.4500	0.1795 ± 0.0936	87.0	2.07	2.21	0.94	63.79
SSC (°Brix)	6.1200	8.3000	0.4956	1.4700	0.7379 ± 0.1897	88.0	21.59	6.56	3.29	11.46
TA (% of citric acid)	0.0040	0.0010	0.0013	0.0041	0.3800 ± 0.1362	88.0	38.50	24.60	1.56	0.16
TCC (µg g^{-1} of pulp)	0.0057	0.0161	0.0011	0.0081	0.3542 ± 0.1314	93.0	28.83	14.69	1.96	261.80
β-car (µg g^{-1} of pulp)	0.0052	0.0140	0.0010	0.0068	0.3708 ± 0.1345	92.0	33.45	16.96	1.97	215.10

Fruit weight (FRW), expressed in kg; length (LEN), larger diameter (LD), smaller diameter (SD), longitudinal internal cavity diameter (LICD), and median internal cavity diameter (MICD), all expressed in cm; apical thickness of the skin (APS), equatorial thickness of the skin (EQS), apical thickness of the pulp (APP), equatorial thickness of the pulp (EQP), all expressed in mm; luminosity (L); chroma (C); hue angle (H); soluble solid content (SSC), expressed in °Brix; titratable acidity (TA), expressed in g of citric acid·100 mL^{-1}; total carotenoid content (TCC) and β-carotene content (β-car), expressed in µg g^{-1} and determined by high-performance liquid chromatography (HPLC); ($\hat{\sigma}_{\hat{a}}^2$) = additive genetic variance; ($\hat{\sigma}_{fi}^2$) = individual phenotypic variance; ($\hat{\sigma}_c^2$) = environmental variance among plots; ($\hat{\sigma}_{\hat{e}}^2$) = Residual variance (environmental + non-additive); ($\hat{h}^2$) = Individual heritability in the narrow sense, adjusted for plot effect; (r) = accuracy (%); (CV_{gi}) = individual additive genetic variation coefficient (%); (CV_{ge}) = environmental variation coefficient (%); (CV_r) = residual variation coefficient; ($\hat{u}$) = general mean for each variable measured

Table 2. Phenotypic value (*f*), additive effect ($\hat{a}$) and additive genetic value ($\hat{u} + \hat{a}$) predicted for individuals selected for FRW, SSC, TA, TCC and β-carotene content.

FRW (kg)				SSC (ºBrix)				TA (% of citric acid)			
Progeny/Plant	f	$\hat{a}$	$\hat{u} + \hat{a}$	Progeny/Plant	f	$\hat{a}$	$\hat{u} + \hat{a}$	Progeny/Plant	f	$\hat{a}$	$\hat{u} + \hat{a}$
5/14	5.52	1.41	4.08	**10/3**	**19.10**	**9.67**	**21.13**	9/6	0.82	0.29	0.45
6/15	5.87	1.34	4.01	**10/1**	**18.50**	**8.42**	**19.88**	7/18	0.38	0.12	0.28
3/18	5.68	1.23	3.90	9/18	16.57	8.28	19.74	7/12	0.36	0.11	0.27
5/18	4.67	1.17	3.84	9/7	16.30	7.84	19.30	7/17	0.32	0.10	0.26
5/21	4.35	1.07	3.74	10/16	17.45	7.79	19.25	7/11	0.32	0.09	0.25
3/1	4.95	1.04	3.71	**10/12**	**18.25**	**7.75**	**19.21**	9/15	0.24	0.09	0.25
5/4	4.21	0.98	3.65	**10/5**	**18.00**	**7.38**	**18.84**	7/14	0.30	0.08	0.24
6/3	5.03	0.91	3.58	9/9	15.70	6.83	18.29	9/16	0.22	0.08	0.24
5/6	3.95	0.90	3.57	10/8	17.80	6.81	18.27	7/16	0.28	0.08	0.24
3/12	4.01	0.90	3.57	7/12	16.40	6.74	18.20	8/18	0.28	0.08	0.24
6/2	4.76	0.82	3.49	7/15	16.20	6.58	18.04	8/12	0.30	0.07	0.23
5/17	3.46	0.80	3.47	10/17	16.80	6.44	17.90	7/1	0.20	0.07	0.23
4/20	4.80	0.80	3.47	9/14	15.40	6.21	17.67	7/15	0.26	0.07	0.23
6/11	3.75	0.73	3.40	8/15	15.95	6.10	17.56	9/9	0.26	0.07	0.23
5/9	3.31	0.72	3.39	**10/13**	**16.95**	**5.04**	**16.50**	7/6	0.19	0.06	0.22
5/13	3.24	0.70	3.37	8/11	14.83	4.91	16.37	5/13	0.28	0.06	0.22
3/16	3.86	0.70	3.37	10/14	16.83	4.79	16.25	5/15	0.25	0.06	0.22
Mean of selected plants	4.44	0.95	3.62	Mean of selected plants	16.88	6.92	18.38	Mean of selected plants	0.31	0.09	0.25
Predicted additive genetic gain		0.95		Predicted additive genetic gain		6.92		Predicted additive genetic gain		0.09	
Predicted additive genetic gain (%)		35.58		Predicted additive genetic gain (%)		60.38		Predicted additive genetic gain (%)		56.25	
Predicted improved mean			3.62	Predicted improved mean			18.38	Predicted improved mean			0.25
General mean ($\hat{u}$)	2.67			General mean ($\hat{u}$)	11.46			General mean ($\hat{u}$)	0.16		

Individuals marked in bold are those coincident for the characteristics SSC, TCC and β-car.

Table 2. Phenotypic value (*f*), additive effect (*â*) and additive genetic value (*û + â*) predicted for individuals selected for fruit weight, soluble solid content, total acidity, total carotenoid content and β-carotene content.

TCC				β-car			
Progeny/Plant	f	â	û + â	Progeny/Plant	f	â	û + â
10/3	570.0	165.4	427.1	10/1	0.50	162.5	377.6
1/5	560.0	165.2	426.9	1/5	0.50	155.7	370.8
10/1	550.0	158.4	420.1	10/3	0.48	154.9	370.0
10/12	560.0	155.6	417.3	10/12	0.50	154.3	369.4
1/17	490.0	153.0	414.7	10/9	0.48	146.7	361.8
10/9	530.0	145.0	406.7	10/16	0.38	145.8	360.9
10/16	430.0	143.7	405.4	1/17	0.40	135.7	350.8
1/2	480.0	137.0	398.7	10/15	0.35	134.4	349.5
1/11	460.0	136.8	398.5	1/11	0.41	131.5	346.6
1/12	460.0	136.8	398.5	1/12	0.40	127.7	342.8
10/15	400.0	133.1	394.8	1/2	0.42	125.3	340.4
1/6	460.0	129.9	391.6	10/5	0.40	124.4	339.5
10/13	470.0	123.9	385.5	10/13	0.42	123.9	339.0
10/5	440.0	119.6	381.3	1/6	0.41	121.5	336.6
10/7	430.0	116.1	377.7	10/7	0.38	116.8	331.9
1/14	390.0	112.1	373.8	1/14	0.33	101.1	316.2
1/8	380.0	108.6	370.3	9/18	0.42	98.7	313.8
Mean of selected plants	474.12	137.67	399.35	Mean of selected plants	422.35	132.99	348.09
Predicted additive genetic gain		137.67		Predicted additive genetic gain		132.99	
Predicted additive genetic gain (%)		52.61		Predicted additive genetic gain (%)		61.83	
Predicted improved mean			399.35	Predicted improved mean			348.09
General mean (û)	261.692			General mean (û)	215.1		

Individuals marked in bold are those coincident for the characteristics SSC, TCC and β-car.

Table 3. Ranking of individuals in terms of fruit with piriform format in the pumpkin breeding programme of Embrapa Semiárido for characteristics evaluated in pumpkin progenies (*Cucurbita moschata*).

	Means																	
	FRW	LEN	LD	SD	LICD	MICD	APB	EQB	APP	EQP	L	C	H	SSC	TA	TCC	β-car	Sum of ranks
Progeny/Plant																		
10/1	3.44	30.2	16.3	9.7	15.0	10.8	4.34	4.29	23.90	68.2	64.88	70.91	60.2	18.5	0.12	550,00	500,00	817
2/7	3.37	23.7	19.5	8.5	17.2	12.8	1.54	2.45	26.81	35.9	64.70	68.29	62.7	12.3	0.16	440.15	386.9	851
2/8	2.21	22.25	16.9	7.4	12.9	11.35	2.56	3.35	24.15	39.4	63.19	73.7	60.7	11.65	0.14	314.08	246.47	870
5/18	4.67	14.2	23.8	0	8.00	13.2	3.63	2.88	47.97	24.45	64.55	71.28	62.1	12.5	0.24	307.18	193.84	883
2/17	3.23	17.3	20.7	0	10.5	13.6	2.95	4.36	31.33	28.0	63.16	72.87	60.9	12	0.15	388.5	338.98	914
10/12	3.43	29.25	17.2	9.5	16.9	11.5	5.44	5.84	22.78	53.6	63.15	71.11	59.05	18.25	0.12	555.24	503.71	939
2/5	2.77	21.73	17.5	3.27	13.53	10.63	2.71	2.74	33.16	34.1	64.8	70.58	61.73	10.73	0.15	261.65	224.62	953
2/19	4.46	17.6	23.33	0	10.77	15.53	3.32	3.60	35.63	29.7	62.79	70.31	61.13	9.63	0.16	349.88	308.30	960
9/18	2.26	23.7	14.53	3.37	15.33	9.6	4.62	5.04	19.92	35.1	64.61	72.5	60.27	16.57	0.18	468.14	417.93	962
2/18	2.79	22.2	18.5	8.8	13.00	12.6	3.67	3.96	26.88	40.0	61.95	70.63	59.8	11.2	0.18	219.47	187.13	965
6/21	3.6	14.87	24.23	0	8.23	13.83	2.88	3.44	47.53	24.8	64.44	71.63	61.9	10.23	0.21	244.36	183.01	986
10/4	3.74	32.3	18.80	9.7	16.00	13.2	4.6	6.65	23.40	74.1	64.93	74.93	61.3	16.3	0.12	329.48	273.09	996
10/3	2.23	28.1	14.30	8.00	12.7	9.7	3.59	5.81	17.62	70.0	66.10	70.96	60.6	19.1	0.12	574.27	484.52	1007
2/10	2.37	17.1	18.20	3.75	9.9	12.1	2.27	3.04	25.38	23.8	64.89	69.66	59.95	11.4	0.15	352.16	304.12	1013
2/11	2.34	15.1	18.70	0	11.3	12.5	2.42	2.22	25.25	35.1	63.09	69.95	61.9	11.2	0.14	469.10	404.19	1026
10/14	2.63	31.13	14.38	8.58	15.05	9.95	3.96	5.51	16.25	73.3	65.42	72.71	61.58	16.83	0.12	403.85	354.88	1042
6/20	1.88	19.70	24.80	0	12.00	15.00	3.38	3.38	41.10	29.2	61.8	70.71	60.8	10.9	0.20	247.22	218.91	1046
Mean selected ($\hat{u}$)	3.02	22.37	18.92	4.73	12.84	12.22	3.40	4.03	28.76	42.27	64.02	71.33	60.97	13.48	0.15	380.85	325.12	
General mean ($\hat{u}_s$)	2.67	20.24	18.03	2.49	12.8	12.12	3.57	4.12	24.31	30.76	67.19	70.2	63.79	11.46	0.16	261.80	215.1	
Selection differential (SD)	0.3547	2.13	0.8917	2.24	0.0417	0.1088	-0.1673	-0.0891	4.457	11.51	-3.16	1.137	-2.81	2.02	-0.003	119.85	110.12	
Heritability ($\hat{h}^2$)	0.3265	0.4693	0.452	0.5377	0.1231	0.3517	0.4424	0.5997	0.4581	0.6451	0.2423	0.0048	0.1795	0.7379	0.3800	0.3542	0.3708	
Selection Gain (SG)	0.1158	1.003	0.4030	1.2095	0.0051	0.0382	-0.0740	-0.0534	2.04	7.42	-0.7665	0.0054	-0.5049	1.49	-0.0013	0.0424	0.0408	
(SG) %	4.33	4.95	2.23	48.57	0.0401	0.3157	-2.07	-1.29	8.39	24.14	-1.14	0.0077	-0.7915	13.05	-0.8382	16.26	18.99	

Table 4. Ranking of individuals in terms of fruit with 'moranga' format in the pumpkin breeding programme of Embrapa Semiárido for characteristics evaluated in pumpkin progenies (*Cucurbita moschata*).

	Means																	Sum of ranks
Progeny/Plant	FRW	LEN	LD	SD	LICD	MICD	APB	EQB	APP	EQP	L	C	H	SSC	TA	TCC	β-car	
5/18	4.67	14.20	23.80	0	8.00	13.20	3.63	2.88	47.98	24.50	64.55	71.28	62.10	12.50	0.24	307.18	193.84	673
2/17	3.23	17.30	20.70	0	10.50	13.60	2.95	4.36	31.33	28.00	63.16	72.87	60.90	12.00	0.15	388.50	338.98	778
6/21	3.60	14.87	24.23	0	8.23	13.83	2.88	3.44	47.53	24.80	64.44	71.63	61.90	10.23	0.21	244.36	183.01	783
2/11	2.34	15.10	18.70	0	11.30	12.50	2.42	2.22	25.25	35.10	63.09	69.95	61.90	11.20	0.14	469.10	404.19	827
6/3	5.03	13.90	24.30	0	7.50	13.00	4.04	2.28	49.86	30.30	65.06	72.93	64.00	9.90	0.14	240.84	213.72	834
2/19	4.46	17.60	23.33	0	10.77	15.53	3.32	3.60	35.63	29.70	62.79	70.31	61.13	9.63	0.16	349.88	308.30	839
9/9	1.75	16.80	15.00	0	11.00	8.70	3.26	4.96	28.57	21.30	64.55	70.55	59.30	15.70	0.26	488.50	443.67	904
5/16	2.29	12.65	18.90	0	7.60	12.15	2.88	2.97	30.14	20.60	64.51	73.13	62.35	7.85	0.19	278.57	234.22	939
2/14	3.8	18.20	22.75	0	10.90	15.60	3.44	3.00	30.16	29.90	66.21	73.63	62.95	11.20	0.14	268.89	236.98	952
6/1	3.74	15.40	21.90	0	10.40	15.60	2.59	3.67	26.31	22.80	65.50	72.38	63.30	11.30	0.16	294.07	239.76	953
6/5	3.76	14.9	21.15	0	10.35	13.35	3.03	2.71	36.20	24.90	65.42	73.74	63.60	10.50	0.17	191.09	124.70	957
6/4	2.92	15.65	19.25	0	11.10	12.55	3.83	3.03	30.22	24.40	61.56	68.51	60.30	11.40	0.24	208.12	179.13	969
6/20	1.88	19.70	24.80	0	12.00	15.00	3.38	3.38	41.10	29.20	61.80	70.71	60.80	10.90	0.20	247.22	218.91	973
2/13	2.55	18.20	17.90	0	8.80	12.20	3.17	3.58	35.85	25.60	65.06	68.86	60.40	9.30	0.26	299.30	267.86	976
3/19	2.79	15.70	19.50	0	9.00	11.60	3.86	3.98	31.67	28.00	64.79	73.36	63.70	10.90	0.12	250.56	208.54	977
6/13	2.73	13.73	20.37	0	8.60	13.93	2.66	2.89	30.69	23.20	65.16	73.27	63.57	10.50	0.14	227.35	185.56	983
5/15	3.13	16.2	20.30	0	8.50	13.80	4.89	4.18	28.35	30.20	65.81	76.38	62.30	8.70	0.25	279.36	224.10	998
Mean selected ($\hat{u}$)	3.21	15.88	20.99	0	9.67	13.30	3.30	3.35	34.51	26.61	64.32	71.97	62.02	10.80	0.18	296.05	247.38	
General mean ($\hat{u}_s$)	2.67	20.24	18.03	2.49	12.80	12.12	3.57	4.12	24.31	30.76	67.19	70.20	63.79	11.46	0.16	261.00	215.10	
Selection differential (SD)	0.5458	-4.35	2.962	-2.49	-3.12	1.18	-0.2638	-0.7608	10.20	-4.14	-2.86	1.77	-1.76	-0.6535	0.0264	35.05	32.28	
Heritability ($\hat{h}^2$)	0.3265	0.4693	0.4520	0.5377	0.1231	0.3517	0.4424	0.5997	0.4581	0.6451	0.2423	0.0048	0.1795	0.7379	0.3800	0.3542	0.3708	
Selection Gain (SG)	0.1782	-2.04	1.33	-1.33	-0.3841	0.4158	-0.1167	-0.4563	4.67	-2.67	-0.6951	0.0084	-0.3160	-0.4822	0.0100	0.0124	0.0119	
(SG) %	6.67	-10.09	7.42	-53.77	-3.00	3.43	-3.26	-11.07	19.23	-8.70	-1.03	0.0121	-0.4954	-4.20	6.28	4.75	5.56	

The climatic variables during the experiment were characterized by an average temperature of 25.6 °C, with a minimum of 19.6 °C and a maximum of 34.5 °C, average relative humidity of 55% and cumulative rainfall of 13.3 mm (Embrapa Semiárido, 2013).

Plant material and field procedures

Ten progenies resulting from crossing between sibling plants (SIB) of three pumpkin accessions belonged to the Genebank of Cucurbits (GBC) for northeast Brazil at Embrapa Semiárido (Petrolina – PE - Brazil). Of these 10, two progenies from the accession GBC569, four progenies from the accession GBC567 and four progenies from the accession GBC545 were previously selected for commercial characteristics and high levels of total carotenoids (Table 1S). In the presentation of the results in Tables 2, 3 and 4, information on individuals agreed with the identification of the progeny to which they belonged, as well as the plant whose fruit was evaluated.

Sowing was carried out in polystyrene trays with commercial substrate for vegetables, based on vermiculite and vegetable ashes, on June 21, 2013. Transplanting was carried out in line 13 days after the emergence of the seedlings, maintaining a plant hole at a spacing of 4.0 m x 2.5 m. The soil was prepared by harrowing and ploughing. Fertilization followed previous recommendations after soil analysis (Cavalcanti, 2008). The control of invasive plants was carried out by hand-weeding. Drip irrigation was applied three times a week at levels of approximately 10 mm, defined based on the Class A pan evaporation. Preventive and curative phytosanitary control of the whitefly (*Bemisia argentifolii*) and powdery mildew (*Podosphaera xantii*) were applied, common to this culture in the region.

Controlled pollination (self-fertilization) was carried out between the first male and female flowers in the morning period. Flowers were tied up with wool threads and insulated with paper bags to prevent the pollen detaching from the male flower; in the female flower, there was no contamination by pollinating insects, favouring controlled self-fertilization.

At the end of flowering, the percentage of plants that produced self-fertilized fruits was quantified. Controlled pollination resulted in ~78.09% success: from 210 pollinated plants (21 plants/progeny), 164 had at least one self-fertilized fruit with the possibility of selection (Tables 1S). The harvest was carried out 115 days after planting, using as indicator of maturity the reduction of brightness, increase in hardness of the skin and drying of the peduncle. Fruits obtained through self-fertilization were evaluated in each plant of the plot.

Fruit variables

Harvested fruits were analysed in the Laboratory of Post-harvest Physiology from Embrapa Semiárido.

The evaluated characteristics were FRW, in kg, weighing each fruit individually on a semi-analytical balance (model PBK989-AB30) with a capacity of 30 kg; b) LEN, LD, SD, LICD and MICD of fruits, determined by digital callipers (LeroyMerlin), with values expressed in cm; c) APS, EQS, APP and EQP, also measured by digital callipers, with values expressed in mm; d) pulp colour, determined from the L, C and H values, which were measured using a digital colourimeter (CR400 Konica Minolta); e) SSC, expressed as °Brix and measured in the Atago - PAL - 1 digital refractometer; f) TA, in g of citric acid·100 mL^{-1}, measured with a digital burette (Jencons-Digitrate Pro; 50-mL capacity) by titration with 0.1 N NaOH solution and with phenolphthalein as the indicator; g) TCC, in µg g^{-1}, for the extraction, followed by the method recommended by Rodriguez-Amaya and Kimura (2004), from 5 g of pulp of each fruit; readings were carried out in a visible ultraviolet spectrophotometer (Cary 50 Bio) at 850 nm; and h) β-car, in µg g^{-1}, from the extract used for the determination of total carotenoids (Rodriguez-Amaya and Kimura, 2004). Quantification was performed by high-performance liquid chromatography (HPLC) using a Waters Alliance e2695 coupled to a diode array detector (DAD) 2998 at 450 nm. The extracts were processed in a column YMC Carotenoid-C 30 (4.6 × 150 mm, 3 µm) using a gradient method: 0 min, 80% methanol + 20% tert-butyl methyl ether; 0.5 min, 75% methanol + 25% tert-butyl methyl ether; 15 min, 15% methanol + 85% tert-butyl methyl ether; 15.05 min, 10% methanol + 90% tert-butyl methyl ether; 16.5 min, 10% methanol + 90% tert-butyl methyl ether; 16.55 min, 80% methanol + 20% tert-butyl methyl ether; and 22 min, 80% methanol + 20% tert-butyl methyl ether, with flow of 0.8 mL min^{-1} and oven temperature of 33 °C.

Experimental design and statistical analysis

The experimental design was arranged in a randomized block design with three replications and seven plants per plot, with 210 plants. The normality test of Lilliefors (Ghasemi and Zahediasl, 2012) was used, followed by analysis of variance (ANOVA).

For estimation and prediction of genetic parameters, the progenies were considered as S_1 due to the genetic makeup of plants resulting from crosses between sib, parent, and non-homozygous plants, with a similar behaviour to that of progenies from selfing of allogamous plants. Data were analysed using mixed REML/BLUP models, in which REML (restricted maximum likelihood) and BLUP (best linear unbiased prediction) were used to estimate the genetic parameters and predict the additive and genotypic values, respectively, ordering both families as individuals in terms of the evaluated variables.

The general formula of the mixed model followed the format: $y = \mathrm{X_b} + \mathrm{Z_a} + \mathrm{W_c} + \mathrm{e}$, where *y* is the data vector, *b* is the vector of repetition effects (assumed as fixed) added to the general mean, *a* is the vector of individual additive genetic effects (random), *c* is the effect vector of plots (random), and *e* means the vector errors or residues (random). X, Z and W represent the incidence matrix for effects *b*, *a* and *c*, respectively (Resende, 2000). The mean distributions and structures were:

$y|\mathrm{b}, \mathrm{V} \sim \mathrm{N}\ (\mathrm{X_b}, \mathrm{V})$; $a|\mathrm{A}, \sigma_a^2 \sim N\ (0, A\sigma_a^2)$; $c|\sigma_\mathrm{c}^2 \sim \mathrm{N}\ (0, I\sigma_\mathrm{c}^2)$; $e|\sigma_\mathrm{e}^2 \sim N\ (0, I\sigma_\mathrm{e}^2)$

Cov (a, c') = 0; Cov (a, e') = 0; Cov (c, e') = 0, showing that:

$$E\begin{bmatrix} y \\ a \\ c \\ e \end{bmatrix} = \begin{bmatrix} X_b \\ 0 \\ 0 \\ 0 \end{bmatrix} \text{ e Var} \begin{bmatrix} y \\ a \\ c \\ e \end{bmatrix} = \begin{bmatrix} V & ZG & WC & R \\ GZ' & G & 0 & 0 \\ CW' & 0 & C & 0 \\ R & 0 & 0 & R \end{bmatrix}$$

where:

$G = A\hat{\sigma}_a^2;\ R = I\hat{\sigma}_c^2;\ C = I\hat{\sigma}_e^2;\ V = ZA\hat{\sigma}_a^2 Z' + WI\hat{\sigma}_c^2 W' + I\hat{\sigma}_e^2 = ZGZ' + WCW' + R$

The mixed-model equation was:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z' + A^{-1}\lambda_1 & Z'W \\ W'X & W'Z & W'W + 1\lambda_2 \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y_1 \\ W'y \end{bmatrix}$$

where:

$\lambda_1 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_a^2} = \frac{1-\hat{h}^2-\hat{c}^2}{\hat{h}^2}$; $\lambda_2 = \frac{\hat{\sigma}_e^2}{\hat{\sigma}_c^2} = \frac{1-\hat{h}^2-\hat{c}^2}{\hat{c}^2}$

$\hat{h}^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2+\hat{\sigma}_c^2+\hat{\sigma}_e^2}$ meaning narrow-sense heritability of the block;

$\hat{c}^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^{2+}+\hat{\sigma}_c^2+\hat{\sigma}_e^2}$ correlation due to the common environment of the plot, where:

$\hat{\sigma}_a^2$ = additive genetic variance; $\hat{\sigma}_c^2$ = variance among plots; $\hat{\sigma}_e^2$ = residual variance (environment within plots + non-additive); and A is the additive genetic correlation matrix between the assessed plants.

According to Resende (2000), the multi-effect index is equivalent to:

$$I = b_1(Y_{ijk} - \bar{Y}_{ij}) + b_2(\bar{Y}_{i...} - \bar{Y}_{...}) + b_3(\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{...})$$

where:

$b_1 = \frac{(1{,}5-\rho_a)\hat{\sigma}_a^2}{\hat{\sigma}_{dp}^2}$; $b_2 = \frac{\frac{[1{,}5+(nb-1)\rho_a]}{n}\hat{\sigma}_a^2}{\hat{\sigma}_{fa}^2+\hat{\sigma}_c^2 \Big/ b+\hat{\sigma}_{dp}^2 \Big/ nb}$;

and ρ_a = additive genetic interclass correlation; $\hat{\sigma}_{fa}^2$ = variance among families; $\hat{\sigma}_c^2$ = variance among plots; $\hat{\sigma}_{dp}^2$ = variance within plots; Y_{ijk}= individual phenotypic value; $\bar{Y}_{ij.}$ = plot mean and $\bar{Y}_{i..}$ = progeny mean; $\bar{Y}_{.j.}$ = block mean; and $\bar{Y}_{...}$= general mean.

The interactive estimators of variance components by the REML and algorithm EM are:

$$\hat{\sigma}_e^2 = \left[y'y - \hat{b}'X' - \hat{a}'Z'y - \hat{c}'W'y\right] \Big/ [N - r(x)];$$

$$\hat{\sigma}_a^2 = \left[\hat{a}'A^{-1}\hat{a} + \hat{\sigma}_e^2 tr(A^{-1}C^{22})\right] \Big/ q$$

$$\hat{a}_c^2 = \hat{c}'c + \hat{\sigma}_e^2 trC^{33} \Big/ s$$

where C^{22} and C^{33} come from:

$$C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix} = \begin{bmatrix} C^{11} & C^{12} & C^{13} \\ C^{21} & C^{22} & C^{23} \\ C^{31} & C^{32} & C^{33} \end{bmatrix}$$

and C = coefficient matrix of the mixed model equations; tr = matrix trace operator; $r(x)$ = rank of matrix x; and $N, q; s$ = total number of data, of plants and of plots, respectively.

The accuracy of genetic gain prediction was obtained by $r\,(\%) = \sqrt{h^2}$

A 10% selection rate was applied for the ranking of the best individuals with prediction of the genetic gains in the next cycles of lineage selection for the characteristics associated with the commercial quality of the fruit (W, SSC and TA) and for those associated with the status of the pumpkin as a functional food (TCC and β-car).

The ranking was also carried out to select the best individuals in the joint evaluation of all the variables for progress in the cycles, aiming to obtain lines with pyriform shape (presence of regions with different diameters: SD and LD) or the 'moranga' shape (where regions with different diameters did not differentiate). Therefore, we used the index of the sum of ranks or sum of posts proposed by Mulamba and Mock (1978), in which individuals are classified in relation to each one of the characteristics, according to the interests of the programme of the improved species. This method does not require estimations of variances, phenotypic covariance or genotypic covariance, nor the establishment of economic weights. We have

$DS = \hat{u}_s - \hat{u}$

where $\hat{u}_s$ = average of selected plants and $\hat{u}$ = general average. The selection gain was obtained through the product of the heritability value and the value of the selection differential, and the results are presented as percentage values. The data were analysed by the SELEGEN, GENES (Cruz, 2006) and Excel software programs.

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