Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Identification of high seed oil yield and high oleic acid content in Brazilian germplasm of winter squash (*Cucurbita moschata* D.)

Ronaldo Silva Gomes^{a,*}, Ronaldo Machado Júnior^a, Cleverson Freitas de Almeida^a, Rebeca Lourenço de Oliveira^a, Rafael Ravaneli Chagas^a, Ednângelo Duarte Pereira^a, Fabio Teixeira Delazari^b, Derly José Henriques da Silva^a

^a Agronomy Department, Federal University of Viçosa-UFV, PH Rolfs Avenue, Zip Code 36570-000, Viçosa, MG, Brazil ^b National Service of Rural Learning- SENAR, Marcino dos Santos Street, number 401, Zip Code 79042-140, Campo Grande, MS, Brazil

ARTICLE INFO

Article history: Received 27 June 2021 Revised 18 November 2021 Accepted 28 November 2021 Available online 3 December 2021

Keywords: Bioactive compound Genetic correlation Clustering *Cucurbita moschata* Genetic parameter Seed oil



Cucurbita moschata D. seed oil contains approximately 75% unsaturated fatty acids, with high levels of monounsaturated fatty acids and antioxidant compounds such as vitamin E and carotenoids, constituting a promising food in nutritional terms. In addition, the Brazilian germplasm of C. moschata exhibits remarkable variability, representing an important source for the genetic breeding of this vegetable and other cucurbits. The present study evaluated the productivity and profile of the seed oil of 91C. moschata accessions from different regions of Brazil maintained in the Vegetable Germplasm Bank of the Federal University of Vicosa (BGH-UFV). A field experiment was conducted between January and July 2016. The accessions showed high genetic variability in terms of characteristics related to seed oil productivity (SOP), such as the weight of seeds per fruit and productivity of seeds, providing predicted selection gains of 29.39 g and 0.26 t ha⁻¹, respectively. Based on the phenotypic and genotypic correlations, a greater SOP can be achieved while maintaining a high oleic acid concentration and low linoleic acid concentration, providing oil of better nutritional and chemical quality. In the variability analysis, the accessions were clustered into five groups, which had different averages for SOP and fatty acid concentration of seed oil, an approach that will guide the use of appropriate germplasm in programs aimed at genetic breeding for SOP and seed oil profile. Per se analysis identified BGH-4610, BGH-5485A, BGH-6590, BGH-5556A, BGH-5472A, and BGH-5544A as the most promising accessions in terms of SOP, with an average $(\mu + g)$ of approximately 0.20 t ha⁻¹. The most promising accessions for a higher oleic acid concentration of seed oil were BGH-5456A, BGH-3333A, BGH-5361A, BGH-5472A, BGH-5544A, BGH-5453A, and BGH-1749, with an average (μ + g) of approximately 30%, almost all of which were also the most promising in terms of a lower linoleic acid concentration of the seed oil, with an average (μ + g) of approximately 45%. Part of the C. moschata accessions evaluated in the present study can serve as a promising resource in genetic breeding programs for SOP and fatty acid profile, aiming at the production of oil with better nutritional and physicochemical quality.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Abbreviations: NFP, number of fruits per plant; WSF, weight of seeds per fruit, PS, productivity of seeds; SOC, total seed oil content; SOP, seed oil productivity; UFV, Federal University of Viçosa; BLUPs, best unbiased linear predictions; BLUEs, best unbiased linear estimates; PUFAs, polyunsaturated fatty acids; RML, restricted maximum likelihood; MUFA, monounsaturated fatty acids.

* Corresponding author at: PH Rolfs Avenue, Genetic Resources Management Laboratory, Zip Code 36570-900, Viçosa, MG, Brazil.

E-mail address: ronaldo.s.gomes@ufv.br (R. Silva Gomes).

Peer review under responsibility of King Saud University.

FLSEVIER

Production and hosting by Elsevier

Winter squash (*Cucurbita moschata* D.) is one of the cucurbit vegetables of great socioeconomic importance owing to the high nutritional value of its fruits and seeds. Cultivated mainly for fruit production, *C. moschata* has been strategically used in biofortification programs for vitamin A because of its high content of carotenoids, such as β - and α -carotene—the major precursors of vitamin A (Carvalho et al., 2012; Saltzman et al., 2013). In addition, winter squash fruits are an excellent source of minerals such as K, Ca, P, Mg, and Cu (Nagar et al., 2018; Priori et al., 2017). Associated to this, the cultivation area and worldwide production of *C. moschata*, which is estimated considering data from other cucurbits such as *C*.

https://doi.org/10.1016/j.sjbs.2021.11.064

1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.







This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

pepo and C. maxima, was nearly 2 million hectares and 27.6 million tons, respectively, in 2018 (Food and Agriculture Organization-FAO, 2020), highlighting the socioeconomic importance of C. moschata.

Furthermore, C. moschata seed oil can serve as an excellent product because of its nutritional and physicochemical properties, which are associated with the high seed production potential of this vegetable. Lipids account for up to 49% of C. moschata seed components (Jarret et al., 2013; Patel, 2013), and studies on the germplasm of this cucurbit have already identified accessions that can produce up to 0.58 t \cdot ha⁻¹ of seeds (Gomes et al., 2020). C. moschata seed oil contains approximately 75% unsaturated fatty acids, with a high content of monounsaturated fatty acids (MUFAs), such as oleic acid (Jarret et al., 2013; Sobreira, 2013; Veronezi and lorge, 2015). Thus, it is an excellent substitute for vegetable lipid sources that contain high levels of saturated fatty acids, which are harmful to human health. Associated with this, some studies have reported that C. moschata seeds and seed oil contain high levels of antioxidant compounds, such as vitamin E and carotenoids, which are beneficial to human health (Veronezi and Jorge, 2012; Dash and Ghosh, 2017) and also protect the seed oil from oxidative processes that may lead to rancidity. In this regard, C. moschata seed oil may serve as a health food in the cultivation regions of this vegetable, particularly in less developed regions, and in the family farming context (Gomes et al., 2020).

Studies on the Brazilian germplasm of C. moschata have emphasized the evaluation of agromorphological characteristics and reported remarkable variability in these traits (De Lima et al., 2016; Ferreira et al., 2016; Oliveira et al., 2016; Gomes et al., 2020). As an allogamous species, variability in the C. moschata germplasm is associated with the occurrence of natural hybridization across different populations. Already present in the diet of native Latin American people (Dillehay et al., 2007; Piperno and Stothert, 2003) and with a widespread cultivation on the American continent, the variability of C. moschata may also be related to anthropogenic actions, such as the frequent exchange of seeds among family farmers (Gomes et al., 2020). Additionally, the variability of the Brazilian germplasm of C. moschata reflects its adaptation to a broad ecological range, constituting different edaphoclimatic conditions; thus, these accessions represent an important source for the genetic breeding of this vegetable and other cucurbits.

The Vegetable Germplasm Bank of the Federal University of Viçosa (BGH-UFV) maintains approximately 350 accessions of C. moschata (Silva et al., 2001). The C. moschata collection maintained in the BGH-UFV constitutes a substantial sample of the Brazilian germplasm, which is one of the largest collections of this species in the country (Fonseca et al., 2015). A preliminary assessment of the seed oil fatty acid profile of a small part of the C. moschata germplasm maintained in the BGH-UFV revealed high variability among 54 accessions in terms of its oleic acid content (Sobreira, 2013). In this study, BGH-7765, with an oil oleic acid content of 28.39%, was identified as a promising accession for use as a parent germplasm in breeding programs aimed at improving C. moschata seed oil. With the present study, the agromorphological characterization of the collection of C. moschata maintained in the BGH-UFV is approaching its conclusion, comprising a large part of this collection (Moura, 2003; Lima Neto, 2013). The present study aimed to identify promising accessions in terms of the seed oil productivity and fatty acid profile, meeting the demand from breeding programs for germplasm with these characteristics. This study will support the establishment of a core collection of *C. moschata* from BHG-UFV, which is a fundamental approach for optimizing the use and conservation of this germplasm.

To this end, the objectives of the present study were (a) to evaluate the seed oil productivity (SOP) and oil fatty acid profiles of 91*C. moschata* accessions from different regions of Brazil maintained in the BGH-UFV, (b) to analyze the correlations between these characteristics, and (c) to examine the variability of this germplasm to identify accessions with a high SOP and high oleic fatty acid content but a low linoleic acid content in seed oil.

2. Material and methods

2.1. Germplasm origin

The present study evaluated 91*C. moschata* accessions maintained in BGH-UFV. These accessions, mostly landraces, have been collected by the BGH-UFV over a period of more than five decades (Silva et al., 2001) from different geographical regions of Brazil (Fig. 1). These genotypes have been maintained from controlled crosses involving chain-crossing between plants of the same accession. These crossings are carried out between a set of approximately 20 plants in order to obtain an adequate effective population size (Ne) for this species (Vencovsky and Crossa, 2003). The accessions were evaluated with four genotypes used as controls, the cultivars "Jacarezinho" and "Maranhão" and the hybrids Jabras and Tetsukabuto, which are widely cultivated and commercialized in Brazil.

2.2. Location and conduct of the experiment

A field experiment was conducted between January and July 2016 at Horta Velha, an experimental unit of the Department of Agronomy of UFV (20°4524″S, 42°5045″W; altitude, 648.74 m).

The genotype seedlings were cultivated in expanded polystyrene trays with 72 cells containing a commercial substrate (Topstrato[®]). Subsequently, the seedlings were transplanted in the experimental area following the augmented block design proposed by Federer (1956), with five replicates for each control. The 91 accessions were replicated once and were randomly assigned to all blocks in the experiment, according to the methodology described by Federer (1956). The plants were distributed with 3×3 m spacing between plants and rows, resulting in a stand of 1,111 plants ha⁻¹. Each experimental plot contained five plants, and all evaluations of fruits, seeds, and seed oil were performed on three fruits each from three central plants in a plot.

2.3. Assessment of seed oil productivity

The genotypes were evaluated for the number of fruits per plant (NFP), total weight of seeds per fruit (WSF), productivity of seeds (PS), and total seed oil concentration (SOC). The PS, SOC, and SOP estimates were obtained using the following equations (Eqs. (1), (2), and (3), respectively):

$$PS = NFP \times WSF \times 1,111^* \tag{1}$$

$$SOC = \frac{\text{Weight of oil extracted from the seed sample(mg)}}{\text{Total weight of the seed sample(mg)}} \times 100$$
(2)

$$SOP = \frac{PS \times SOC}{100}$$
(3)

where PS is the productivity of seeds (t ha⁻¹), NFP is the number of fruits per plant, WSF is the weight of seeds per fruit (g), *1111 is the number of plants per hectare, SOC is the oil concentration expressed as the percentage of the seed weight on a dry basis (%), and SOP is the seed oil productivity (t ha⁻¹). The variables SOC and SOP addressed in the present study are common to the work of Gomes et al., (2020), which, in turn, aimed at evaluating



Fig. 1. Brazilian map displaying the regions of origin of the Cucurbita moschata accessions tested in the present study.

the agromorphological characteristics of the *C. moschata* germplasm from the BGH-UFV, namely aspects such as cycle, fruit production, and nutritional aspects of the fruit pulp, with a main objective of analyzing the genetic variability of this germplasm.

Initially, the seeds were dried in a forced air circulation oven for 72 h at 30 °C. Next, 20 g of seeds from each genotype were ground in a Willey knife mill with a 1 mm sieve. SOC was determined in an extractor (ANKOM XT15) following extraction with petroleum ether using a standard AOAC method (Thiex et al., 2003). Before loading in the extractor, the ground seed samples were dried in an unventilated oven at 105 °C for 2 h. Then, approximately 2 g of each sample was transferred to filter envelopes (XT4; ANKOM technology), which were sealed and placed in the extractor. Oil was extracted from the samples with ether circulation for 30 min at 90 °C, and the percentage of oil was calculated as the difference between the sample weight before and after extraction. SOC was expressed in grams per 100 g of seeds on a dry basis.

2.4. Analysis of the fatty acid profile of seed oil

The seed oil extracted by cold pressing was subjected to gas chromatography. Oil was extracted from approximately 50 g of seeds using a hydraulic press aid, according to the methodology described by Gomes et al. (2020).

The composition of the methyl esters of oil fatty acids was determined according to the methodology described by Bubeck et al. (1989), with some modifications in terms of seed oil extraction and gas chromatography. Unlike the protocol by Bubeck et al. (1989), the oil samples in the present study were obtained from the mechanical pressing of seeds, according to Gomes et al. (2020). After obtaining the oil samples, the process of transesterification of the oil to methyl esters was carried out similar to that described by Bubeck et al. (1989). Gas chromatography was performed using a Shimadzu GC-17A gas chromatograph equipped with an automatic insertion platform, a flame ionization detector, and a Carbowax capillary column (30 m \times 0.25 nm). Chromatography was performed at an injector temperature of 230 °C and a

detector temperature of 250 °C. The column operation was programmed to start at 200 °C, with an increase of 3 °C·min⁻¹ until a final temperature of 225 °C was reached. Nitrogen was used as the carrier gas at a flow rate of 1.3 L·min⁻¹. The concentration of each methyl ester of fatty acids was expressed as a percentage of the relative peak area.

2.5. Obtaining of best unbiased linear predictions (BLUPs), best unbiased linear estimates (BLUEs), variance components, and geneticstatistical parameters

Data were analyzed using a mixed model based on the predictions of the restricted maximum likelihood (RML) and BLUPs. The "Ime4" package in R version 3.6.1 was used (Bates et al., 2015). The variance components were obtained based on RML, and the genotypic values were obtained based on BLUPs and BLUE using the following model:

$$\mathbf{y} = Wb + Xa + Zt + e$$

where y represents the vector comprising the phenotypic values of variables, *b* represents the vector comprising the effect of blocks (random effect), *a* represents the vector comprising the effect of accessions (random effect), *t* represents the vector comprising the control effect (fixed effect), and *e* represents the error vector. W, X, and Z represent the incidence matrices of parameters *b*, *a*, and *t*, respectively, with the data vector y. All statistical analyses were performed based on the genotypic values.

The genetic parameters were obtained based on the following estimators:

$$h^2 = 1 - (Pev/2\sigma_g^2)$$

where h^2 represents the broad-sense heritability,

Pev represents the prediction of error variance (Cullis et al., 2006),

and
$$\sigma_{g}^{2}$$
 represents the genotypic variance.

$$\Lambda = \sqrt{1 - (Pe \nu / \sigma_g^2)}$$

 $DS = h^{2*}$

where *DS* represents the selection differential estimated from an average of 15% of the most promising accessions for each characteristic.

$$CV_{g} \% = (\sigma^{2}_{g}/\mu) \times 100$$

$$CV_p \% = (\sigma_p^2/\mu) \times 100$$

 $\text{CV}r \% = (\sigma_{r}^{2}/\mu) \times 100.$

where CV_g %, CV_p %, and CVr % represent the coefficient of genotypic, phenotypic, and residual variation, respectively. The parameters σ_g^2 , σ_p^2 , and σ_r^2 represent the phenotypic, genotypic, and residual variances, respectively. The parameter μ represent the phenotypic average.

2.6. Analysis of correlations between characteristics

The correlations between characteristics were analyzed based on the following model:

$$rg = Co v(x,y) / \sqrt{\sigma_g^2(x)\sigma_2^2(y)}$$

where *rg* represent the correlation. *Cov* (*x*, *y*) represents the genetic covariance between two variables, X and Y, and $\sigma_g^2(x)$ and e $\sigma_g^2(y)$ represent the genetic variances corresponding to the variables X and Y, respectively.

The significance of correlations was analyzed in GENES (Cruz, 2013) using the Mantel test (Z statistic) at 1% and 5% probability.

2.7. Analysis of the germplasm variability

The matrix of distances between the genotypes was obtained based on the genotypic values of characteristics related to the SOP and seed oil profile. The distance between the genotypes was estimated as the average Euclidean distance with data standardization. Based on this, variability was assessed using Tocher's clustering in GENES (Cruz, 2013).

Principal component analysis was used to identify the contribution of the characteristics to genotype variability using GENES (Cruz, 2013).

2.8. Identification of promising accessions

Promising accession clusters were identified, and a *per se* analysis of the most promising accessions for each characteristic was performed. *Per se* analysis was performed based on the ranking of the respective genotypic values and the environmental interaction-free genotypic value (μ + g), considering 15% of the most promising accessions.

3. Results

3.1. Variance components and genetic-statistical parameters of characteristics related to SOP and fatty acid profiles

MSF was associated with SOP, with the highest genotypic variance (Table 1). Regarding the oil fatty acid profile, oleic acid concentration exhibited the highest genotypic variance. Linoleic acid and polyunsaturated fatty acid (PUFA) concentrations exhibited genotypic variances of 10.66% and 10.65%, respectively. All these variances were significant (p < 0.01), as shown in Table 1.

Most of the tested characteristics showed very high heritability (>0.70) (Table 1), according to the classification of Resende (1995).

The heritability estimates for PS, SOP, oleic acid, and linoleic acid concentrations were 0.705, 0.667, 0.857, and 0.729, respectively.

The predicted selection gains for PS and SOP were 0.266 and 0.10 t ha⁻¹, respectively (Table 1). Among the characteristics related to the oil fatty acid profile, oleic acid concentration achieved the greatest selection gain (6.99%), followed by the linoleic acid concentration (-5.12%) and PUFA concentration (-5.11%), which also achieved considerable selection gains (Table 1). The phenotypic amplitude was up to 0.90 t ha⁻¹ (phenotypic mean, 0.26 t ha⁻¹) for PS, and up to 0.36 t ha⁻¹ (phenotypic mean, 0.050 t ha⁻¹) for SOP (Table 1). The oleic acid and linoleic acid concentration ranged from 16.01 to 40.18% (phenotypic mean, 24.55%) and from 36.58 to 58.33% (phenotypic mean, 50.66%), respectively.

3.2. Correlations of characteristics related to SOP and fatty acid profiles

For convenience, NFP, MSF, and PS are assumed to be directly related to the SOP. The phenotypic correlations of these first variables with SOP ranged from 0.51 (SOP × MSF) to 0.99 (SOP × PS), with all correlations being significant (p < 0.01). The phenotypic correlations between SOP × PS (0.99) and SOP × NFP (0.75) were classified as very strong and strong, respectively, according to Shimakura and Ribeiro Júnior (2012). The genotypic correlations between characteristics directly related to SOP were similar to the phenotypic correlations in terms of direction and significance, although their magnitudes were slightly smaller (Fig. 2).

SOP showed the strongest positive correlation with oleic acid concentration (0.23) and oleic acid:linoleic ratio (0.22) and strongest negative correlations with linoleic (-0.21) and PUFA (-0.21) concentrations. SOP showed weak correlations with fatty acids, according to Shimakura and Ribeiro Júnior (2012), ranging from 0.20 to 0.39. Most of the genotypic correlations between SOP and oil fatty acids were similar to the phenotypic correlations in terms of direction and significance, although their magnitudes were smaller (Fig. 2).

Among fatty acids, linoleic and PUFA concentrations showed the strongest positive correlation (1; p < 0.01) (Fig. 2). The correlation between the oleic concentration and oleic acid:linoleic ratio (0.98; p < 0.01) was classified as very strong (Fig. 2), according to Shimakura and Ribeiro Júnior (2012). Oleic acid:linoleic ratio × linoleic acid concentration and oleic acid:linoleic ratio × PUFA acid concentration showed very strong negative correlations (both -0.96; p < 0.01). Similarly, oleic acid concentration × linoleic acid concentration showed a very strong negative correlation (-0.95; p < 0.01). Most of the genotypic correlations between fatty acids were similar to the phenotypic correlations in terms of direction, significance, and magnitude (Fig. 2).

3.3. Clustering and variability of accessions based on characteristics related to SOP and fatty acid profiles

In the clustering analysis, the tested accessions and controls formed five groups. Group 1 included 83 accessions (91.20%) and the controls Jabras, Jacarezinho, Maranhão, and Tetsukabuto. Group 2 comprised eight accessions (8.79%), corresponding to the second largest group. Group 3 comprised two accessions, and the remaining groups (4 and 5) comprised a single accession (Table 2).

Group 5 presented the highest average $(\mu + g)$ value for SOP, estimated at 0.277 t ha⁻¹. Group 2 also presented a high average $(\mu + g)$ value for SOP, estimated at 0.19 t ha⁻¹ (Tables 2 and 3). Regarding the fatty acid concentration of seed oil, group 3 presented the highest average $(\mu + g)$ value for oleic acid concentration (34.18%), followed by group 2 (30.62%). Group 3 had the lowest average $(\mu + g)$ value for linoleic acid concentration (43.16%) (Tables 2 and 3).

Table 1

|--|

Fruit trait												
Trait	$\sigma^{2}{}_{p}$	σ^{2}_{g}	$\sigma^2{}_b$	σ^2_r	Α	h ²	SG	Phenotypic range	μ	CVg%	CV _p %	CV _r %
NFP	8.683	2.876 ns	0.585	5.222	0.600	0.361	1.961	1–15	4.783	35.456	61.607	47.776
Seed traits Traits WSF PS SOC SOP	σ_{p}^{2} 491.465 0.042 11.840 0.007	σ^2_{g} 427.158 ^{**} 0.025 ^{ns} 1.988 ^{ns} 0.004 ^{ns}	σ_b^2 16.394 0.006 0.000 0.001	σ ² _r 47.913 0.010 9.851 0.002	A 0.943 0.839 0.425 0.816	h ² 0.891 0.705 0.181 0.667	SG 29.399 0.266 0.797 0.102	Range 4.4–119.3 0.01–0.9 25.57–48.89 0.003–0.36	μ 51.929 0.269 18.516 0.050	CV _g % 39.800 58.778 7.614 126.49	CV _p % 42.691 76.185 18.583 167.332	CV _r % 13.329 37.174 16.950 89.442
Traits of seed o	oil profile											
Traits Palmitic Stearic Oleic Linoleic Linolenic Oleic/linoleic Saturated PUFA	σ_p^2 1.064 1.159 24.863 17.914 0.001 0.023 2.074 17.906	σ ² g 0.000 ns 17.182** 10.664** 0.001 ns 0.016 ns 0.016 ns 10.653**	σ_b^2 0.077 0.066 5.268 3.629 0.000 0.004 0.151 3.617	σ^2_r 0.987 1.093 2.412 3.620 0.000 0.001 1.923 3.634	A 0.000 0.925 0.853 0.992 0.937 0.000 0.853	h ² 0.000 0.857 0.729 0.986 0.879 0.000 0.728	SG 0.000 0.000 6.999 -5.121 -0.013 0.232 0.000 -5.116	Phenotypic range 12.04–18.14 6.31–12.3 16.01–40.18 36.58–58.33 0.01–0.33 0.27–1.09 21.03–28.14 36.60–58.34	$\mu \\ 15.16 \\ 9.60 \\ 24.55 \\ 50.66 \\ 0.18 \\ 0.049 \\ 24.76 \\ 50.68 \\$	$CV_g\%$ 0.000 16.884 6.446 17.568 258.14 0.000 6.440	CV _p % 6.804 11.214 20.310 8.354 17.568 309.505 5.816 8.349	CV,% 6.553 10.890 6.326 3.755 0.000 0.000 5.600 3.761

Number of fruits per plant (NFP), weight of seeds per fruit (WSF), productivity of seeds (PS), seed oil concentration (SOC), seed oil productivity (SOP), and polyunsaturated fatty acid (PUFA) concentration. Components of phenotypic (σ^2_p), genotypic (σ^2_g), and residual (σ^2_r) variances, as well as the variance associated with the block effect (σ^2_b). Accuracy (*A*), broad-sense heritability (h^2), selection gain (*SG*), phenotypic range, phenotypic average (μ), coefficient of genotypic (CV_g %), phenotypic (CV_p %), and residual (CV_r %) variation. Not significant (ns); **p < 0.05, likelihood-ratio test.



Fig. 2. Phenotypic (A) and genotypic (B) correlations of characteristics related to seed oil productivity and fatty acids. Number of fruits per plant (NFP), weight of seeds per fruit (WSF), productivity of seeds (PS), seed oil concentration (SOC), seed oil productivity (SOP), oleic acid (OA) concentration, linoleic acid (LA) concentration, linolenic acid (LNA) concentration, OA:LA ratio, and polyunsaturated fatty acid (PUFA) concentration.

Seed oil productivity (SOP), and polyunsaturated fatty acid (PUFA) concentration. The genotypic averages followed by the same letters in the column do not differ from one another based on Tocher's method of average grouping.

3.4. Principal components analysis

The first three principal components (PCs) explained 82.13% of the total variation among accessions in terms of the characteristics related to SOP and the fatty acid profiles. PC1 accounted for 47.16% of the total variation. The oleic acid:linoleic acid ratio and oleic acid concentration were the characteristics with the highest positive loading, while linoleic acid and PUFA concentrations had the highest negative loading on PC1 (Fig. 3).

PC2 explained 24.14% of the total variation, and PS, SOP, and WSF were the characteristics with the highest positive loading on this PC. PC3 explained 10.83% of the total variation, and SOC and MSF were the characteristics with the highest loading on this PC.

Furthermore, PCA revealed the relationships between the characteristics. Along PC1, SOP was strongly and positively correlated with PS and NFP, and along the same PC, there was a strong positive correlation between oleic acid concentration and oleic acid:linoleic acid ratio and a strong negative correlation between linoleic acid concentration and PUFA concentration (Fig. 3).

3.5. Identification of promising accessions in terms of SOP and fatty acid profiles

Among the tested accessions, the $(\mu + g)$ estimate for SOP ranged from 0.14 to 0.27 t ha⁻¹, which was much higher than the general average for accessions (0.05 t ha⁻¹). Notably, the accessions BGH-4610A, BGH-5485A, BGH-6590, BGH-5556A, BGH-5472A, and BGH-5544A were the most promising in terms of SOP, with values close to 0.20 t ha⁻¹ (Table 4).

Among the fatty acids of seed oil, the oleic acid concentration showed the highest (μ + g) amplitude (17.71 to 37.20%) (Fig. 4). Associated with this, the (u + g) estimates for oleic acid concentra-

Table 2

Tocher's clustering of accessions based on the genotypic values of characteristics related to seed oil productivity and oil fatty acid profiles.

Clusters	Accessions
Clusters 1	Accessions BGH-5224 BCH-5240 BGH-5560A BGH-5301 BGH-6099 BGH-315 BGH-5603 BGH-1004 BGH-5499A BGH-5548A BGH-95 BGH- 5554A BGH-5593 BGH-5638 BGH-5494A BGH-5530A BGH-5551 BGH-5597 BGH-5606A BGH-5522 BGH-900 BGH-6115 BGH- 4598A BGH-5559A BGH-4590A BGH-5247A BGH-6096 BGH- 5616A BGH-5553 BGH-5493A BGH-7668 BGH-6096 BGH- 5616A BGH-5553 BGH-5493A BGH-7668 BGH-6594 BGH-5659A BGH-4607A BGH-1461A BGH-6594 BGH-6794 BGH-5559A Maranhão BGH-6749 BGH-4516 BGH-5497 BGH-1927 BGH-4681A GBH-5694 BGH-117 BGH-6587A BGH-6117A BGH-6595 BGH-291 BGH-5648 BGH-5455A BGH-6116 BGH-5528 BGH-5639 BGH- 5248 BGH-4287A BGH-4454A BCH-5624A BGH-6593 BGH-5541
	Tetsukabuto BGH-5442 BGH-5538 BGH-5591A BGH-5051 BGH- 4459A Jabras BGH-1992 BGH-305A BGH-5630A BGH-7219A BGH- 5598A BGH-4453 BGH-5466 Jacarezinho BGH-4281 BGH-5473A BGH-1961 BGH-5649A BGH-5653 BGH-6155 BGH-5440A
2	BGH-5472A BGH-5544A BGH-5556A BGH-5361A BGH-3333A BGH-5453A BGH-6590 BGH-5485A
3	BGH-1749 BGH-5456A
4	BGH-1945A
5	BGH-4610

tion among the selected accessions ranged from 27.28 to 37.20% (Table 4). The accessions BGH-5456A, BGH-3333A, BGH-5361A, BGH-5472A, BGH-5544A, BGH-1749, BGH-5653, and BGH-5453A were the most promising in terms of oleic acid concentration, with values close to 30.00%.

Among the selected accessions, the (μ + g) estimate for linoleic concentration ranged from 41.09 to 48.77%. The accessions BGH-5456A, BGH-3333A, BGH-5361A, BGH-1749, BGH- 5544A, and BGH-5556A had the lowest linoleic acid concentration estimates with values close to 40.00% (Table 4).

The (μ + g) estimates for the oleic acid:linoleic acid ratio ranged from 1.00 to 0.57 among the selected accessions, and the accessions BGH-5456A, BGH-5361A, BGH-3333A, BGH-1749, BGH-5472A, and BGH-5544A had the lowest estimates, with values close to 0.75. The (μ + g) estimates for PUFA concentration ranged from 41.12 to 48.78%, and the accessions with the lowest estimates were BGH-5456A, BGH-3333A, BGH-5361A, BGH-1749, and BGH-5544A, with values close to 40% (Table 4).

4. Discussion

4.1. Variance components and genetic-statistical parameters of characteristics related to SOP and fatty acid profiles

The greatest genotypic variances for MSF and PS, which were associated with their high heritability (0.89% and 0.70%, respectively), confirmed the marked genetic variability in these two characteristics in the germplasm, particularly for MSF (Table 1). From these results, the predicted selection gains were significant up to 29.39 g for MSF and 0.26 t ha⁻¹ for PS (Table 1). Consistent with our results, remarkable variability in characteristics related to seed

Table 3

Grouping of genotypic averages of the accession groups based on Tocher's method of average grouping.

Clusters	SOP	Oleic	Linoleic	PUFA
1	0.087b	22.996b	51.850b	51.864b
2	0.198a	30.626a	46.482a	46.506a
3	0.041b	34.184a	43.168a	43.186a
4	0.046b	22.012b	51.873b	52.121b
5	0.277a	22.759b	51.820b	51.835b

production, such as MSF and PS, in the *C. moschata* germplasm has been reported previously (Lima Neto, 2013; Darrudi et al., 2018; Oliveira et al., 2021).

Regarding the fatty acid profile of seed oil, the concentration of oleic acid had the highest genotypic variance and a very high heritability, demonstrating the high genetic variability and feasibility of identifying *C. moschata* accessions that can produce oil with higher oleic acid concentrations. As a result, the predicted selection gain for oleic acid was 6.99%, which corresponded to the greatest selection gain among the components of seed oil (Table 1). Furthermore, the linoleic acid and PUFA concentrations exhibited high genetic variability, also demonstrating the feasibility of identifying *C. moschata* accessions that can produce oil with a lower linoleic acid concentration, providing predicted selection gains of -5.12% for this first fatty acid (Table 1).

The results for the fatty acid profiles obtained in the present study are consistent with previously reported profiles (Sobreira, 2013; Jarret et al., 2013). For instance, Jarret et al. (2013) evaluated the fatty acid profile of 38 seed samples corresponding to C. moschata accessions in the Plant Germplasm Collection of the United States Department of Agriculture, Griffin, and reported that oleic acid presented the greatest amplitude (10 to 53.80%), followed by linoleic acid (24.70 to 61.70%). Previous studies also corroborate high linoleic and oleic acid concentrations, low palmitic acid concentrations, and trace stearic and linolenic acid concentrations in C. moschata seed oil (Applequist et al., 2006; Kim et al., 2012; Veronezi and Jorge, 2015). The fatty acid analysis in the present study did not detect other fatty acids besides linoleic, oleic, palmitic, stearic, and linolenic acids. To the best of our knowledge, however, no previous study on C. moschata has analyzed the genetic parameters associated with the components of the fatty acid profile of seed oil, such as genetic variance, heritability, and selection gain.

Studies on other oilseeds, such as soybean, rapeseed, and sunflower have often reported variations in the fatty acid profiles of germplasm samples and attributed such variations to genetic factors (Hemingway, et al., 2015; Yol et al., 2017). Given the quantitative nature of the fatty acid profile, it is reasonable to assume that this characteristic is also influenced by environmental factors, and some studies have shown the strong influence of environmental factors, such as temperature, on the fatty acid profiles of various oilseeds (Werteker et al., 2010). Corroborating the findings of Gomes et al. (2020), most of the accessions evaluated in the present study were acquired from family farmers who do not typically select accessions for the characteristics of seed or seed oil, which may have contributed to the maintenance of high variability in these traits.

4.2. Correlations of characteristics related to SOP and fatty acid profiles

The phenotypic correlations of NFP, MSF, and PS with SOP ranged from moderate to very strong (p < 0.001), corroborating the notion that these characteristics are directly related to SOP (Fig. 2). The genotypic correlations of the first three variables with SOP were very similar to the phenotypic correlations, indicating that the relationship of the variables NFP, MSF, and PS with SOP may be attributed to genetic factors (Fig. 2). These trends were repeated for correlations of other variables, which tended to express genotypic correlations similar to the phenotypic.

In the present study, we expected the variables NFP, MSF, and PS to be strongly correlated with SOP, consistent with previous reports of strong correlations between seed yield and oil productivity (Assefa et al., 2018). Thus, in addition to PS and MSF, NFP appears to be a determinant of greater SOP in *C. moschata*. These correlations of SOP with its directly associated characteristics can be strategic to guide indirect selection aimed at increasing *C*.



Fig. 3. Dispersion of characteristics related to seed oil productivity and fatty acid profile in relation to the first three principal components (PCs 1, 2, and 3). Number of fruits per plant (NFP), weight of seeds per fruit (WSF), productivity of seeds (PS), seed oil concentration (SOC), seed oil productivity (SOP), and polyunsaturated fatty acid (PUFA).

Table 4			
Estimates of genotypic values (g) and	environmental interaction-free genotypic valu	les $(\mu + g)$ for seed oil product	tivity and fatty acid profiles.

Accessions	SOP		Accessions	Oleic		Accessions	Linoleic	
	g	μ + g		g	μ + g		g	μ + g
BGH-4610A	0.227	0.277	BGH-5456A	12.651	37.201	BGH-5456A	-9.563	41.097
BGH-5485A	0.203	0.253	BGH-3333A	9.458	34.008	BGH-3333A	-7.354	43.306
BGH-6590	0.176	0.226	BGH-5361A	8.987	33.537	BGH-5361A	-6.129	44.531
BGH-5556A	0.174	0.224	BGH-5472A	7.965	32.515	BGH-1749	-5.421	45.239
BGH-5472A	0.166	0.216	BGH-5544A	6.913	31.463	BGH-5544A	-4.821	45.839
BGH-5544A	0.152	0.202	BGH-1749	6.617	31.167	BGH-5556A	-4.663	45.997
BGH-5440A	0.133	0.183	BGH-5653	5.904	30.454	BGH-5453A	-4.262	46.398
BGH-5630A	0.126	0.176	BGH-5453A	5.702	30.252	BGH-5472A	-4.021	46.639
BGH-4281	0.126	0.176	BGH-6155	5.128	29.678	BGH-6155	-3.976	46.684
BGH-5473A	0.126	0.176	BGH-5466	5.042	29.592	BGH-5653	-3.408	47.252
BGH-5361A	0.123	0.173	BGH-5556A	4.515	29.065	BGH-5648	-2.367	48.293
BGH-5453A	0.103	0.153	BGH-6590	3.384	27.934	BGH-5466	-2.315	48.345
BGH-4287A	0.096	0.146	BGH-5639	2.752	27.302	BGH-5639	-1.938	48.722
BGH-5598A	0.093	0.143	BGH-5648	2.733	27.283	BGH-5624A	-1.889	48.771
AO		0.050	OA		24.55	OA		50.66
AS		0.195	SA		30.818	SA		46.222
Accessions	Linolenic		Accessions	Oleic/Linoleic		Accessions	PUFAs	
	g	μ + g		g	μ + g		g	μ + g
BGH-5455A	-0.180	0.000	BGH-5456A	0.959	1.008	BGH-5456A	-9.555	41.125
BGH-4598A	-0.175	0.005	BGH-5361A	0.789	0.838	BGH-3333A	-7.348	43.332
BGH-1749	-0.175	0.005	BGH-3333A	0.768	0.817	BGH-5361A	-6.131	44.549
BGH-5301	-0.174	0.006	BGH-1749	0.707	0.756	BGH-1749	-5.433	45.247
BGH-1927	-0.173	0.007	BGH-5472A	0.684	0.733	BGH-5544A	-4.821	45.859
BGH-5240	-0.173	0.007	BGH-5544A	0.676	0.725	BGH-5556A	-4.661	46.019
BGH-4287A	-0.172	0.008	BGH-5453A	0.636	0.685	BGH-5453A	-4.261	46.419
BGH-5630A	-0.171	0.009	BGH-5653	0.612	0.661	BGH-5472A	-3.989	46.691
BGH-5051	-0.171	0.009	BGH-6155	0.597	0.646	BGH-6155	-3.974	46.706
BGH-5248	-0.170	0.010	BGH-5556A	0.595	0.644	BGH-5653	-3.407	47.273
BGH-5591A	-0.170	0.010	BGH-5466	0.584	0.633	BGH-5648	-2.368	48.312
BGH-4454A	-0.169	0.011	BGH-6590	0.527	0.576	BGH-5466	-2.311	48.369
BGH-5224	-0.169	0.011	BGH-5648	0.527	0.576	BGH-5639	-1.940	48.740
BGH-5485A	-0.169	0.011	BGH-5639	0.521	0.570	BGH-5624A	-1.895	48.785
AO		0.180	OA		0.049	OA		50.68

Seed oil productivity (SOP), polyunsaturated fatty acids (PUFAs), original average (OA), and average of selected genotypes (AS).

moschata oil productivity. Thus, the selection of genotypes with higher PS and MSF may be a promising alternative to obtain greater SOP in *C. moschata*.

The positive correlations of SOP with oleic acid concentration and oleic acid:linoleic acid ratio observed in the present study suggest the feasibility of obtaining greater oil productivity while maintaining a desirable oil profile, with a high oleic acid concentration and oleic acid:linoleic acid ratio (Fig. 2). Additionally, the negative correlations of SOP with linoleic acid and PUFA concentrations indicate the feasibility of obtaining a greater SOP



Fig. 4. Box plots showing the variability in the oleic and linoleic fatty acid concentration in the seed oil of the tested accessions.

while maintaining lower PUFAs, such as linoleic acid. To the best of our knowledge, no study on *C. moschata* has addressed the relationship between SOP and components of the seed oil fatty acid profile. Meanwhile, studies on other oilseeds, such as peanut, have reported weak phenotypic correlations between the SOP and oleic acid concentration (-0.034) (Yol et al., 2017), suggesting a small change in the fatty acid content with an increase in oil productivity in this crop. In contrast to the present study, Yol et al. (2017) reported a weak but positive correlation between SOP and oleic acid content.

Regarding the correlations among the components of the oil fatty acid profile, the strong, negative, but significant correlations of oleic acid concentration with linoleic acid and PUFAs concentration indicated an antagonistic relationship between these components of *C. moschata* seed oil (Fig. 2). The phenotypic correlations among these characteristics may be attributed to environmental and genetic factors. For instance, environmental factors such as temperature may strongly influence the correlations among fatty acids, such as oleic and linoleic acids (Abdelghany et al., 2020). In line with this, studies on other oilseeds, such as soybean, have shown that the oleic acid content of oil tends to decrease with increasing temperature, contrary to that observed for linoleic acid content (Bachlava et al., 2008), corroborating the negative correlations between these two fatty acids observed in the present study.

The metabolic pathways of fatty acids may be the key genetic factors responsible for the correlations among the components of the oil fatty acid profile. In this context, desaturases in the plastids and endoplasmic reticulum play a central role in fatty acid synthesis and catalyze their conversion to MUFAs or PUFAs (Long et al., 2018). Among these enzymes, delta-12 fatty acid desaturase 2 (Δ 12-FAD2) converts oleic acid precursors into linoleic acid precursors (Ohlrogge and Browse, 1995), and according to Dehghan and Yarizade (2014), the *FAD2* gene family is rather ubiquitous and diverse in plants. Thus, the strong negative correlation between oleic and linoleic acid concentrations observed in the present study may also be related to the action of FAD2 during the biosynthesis of these two fatty acids.

The analysis of correlations among characteristics is an important approach in plant breeding, which must contemplate several variables simultaneously (Dias et al., 2017), proving very useful when determining selection strategies. As shown in the present study, the plant germplasms maintained in banks commonly constitute a representative sample of the species gene pool, thus providing comprehensive information on the relationships among germplasm characteristics, which makes the correlation analysis very useful during the initial evaluation of plant germplasms conserved in banks. To the best of our knowledge, this study is the first to provide information on the relationships between components of the fatty acid profile of *C. moschata* seeds, and it will be essential to guide selection strategies aimed at improving seed oil quality in *C. moschata*. Thus, the information on correlations among the components of seed oil observed in the present study may be particularly meaningful in breeding programs aimed at improving the fatty acid profile of *C. moschata* seed oil, considering the feasibility of increasing oleic acid concentration while decreasing PUFA concentration, given the strong negative correlation between these two components.

4.3. Clustering and variability of accessions based on characteristics related to SOP and fatty acid profiles

The clustering of accessions confirmed the variability in characteristics related to SOP and fatty acid profiles among the studied accessions (Table 2). This clustering is consistent with the high estimates of genotypic variance and heritability for the evaluated characteristics related to SOP, including MSF and PS, as well as those related to the fatty acid profile of seed oil, including oleic and linoleic acid concentrations (Table 1). The variability observed among the accessions tested in the present study also corroborates previous reports emphasizing remarkable variability in both agromorphological and molecular characteristics in the *C. moschata* germplasm (Ferriol et al., 2004; Barboza et al., 2012; Ferreira et al., 2016).

The clustering of accessions in the present study did not reflect a greater similarity between accessions from the same state or geographic region, consistent with the reports of agromorphological characteristics of the C. moschata germplasm from different geographic regions (Moura, 2003; Gomes et al., 2020). Unlike the present study, previous studies involving the analysis of fatty acid profile of the seeds of other oilseed crops, such as soybean, have reported that germplasms from regions at higher latitudes tended to express a higher palmitic, stearic, and oleic acid content than germplasms from lower latitudes (Wu et al., 2017; Abdelghany et al., 2020), suggesting a greater similarity between the germplasms from the same region. In this line, Bachlava et al. (2008) observed that the oleic acid content of soybean tended to increase from lower to higher latitudes, indicating an increase in the content of this fatty acid with a decrease in temperature, contrary to the trends for linoleic and linolenic acid. Similarly, Song et al. (2016) observed that the oleic acid content of soybean seeds was negatively correlated with the duration of sunlight incidence. Thus, the greater similarity between the soybean germplasms from the same region may reflect regional ecogeographic characteristics, which is different from the results of the present study.

Already present in the diet of native peoples (Piperno and Stothert, 2003; Dillehay et al., 2007), *C. moschata* is widely cultivated in Latin America, which is an important center of diversity for this vegetable. In addition, previous studies have highlighted the variability in the Brazilian germplasm of *C. moschata* (De Lima et al., 2016; Ferreira et al., 2016; Gomes et al., 2020), possibly as a result of the adaptation of this germplasm to a wide ecological range, consisting of diverse edaphoclimatic conditions. Additionally, the intrinsic characteristics of *C. moschata*, such as the occurrence of natural hybridization across populations, associated with the processes of selection and seed exchange practiced by populations involved in its cultivation, also contribute to the variability in the Brazilian germplasm of this vegetable (Gomes et al., 2020).

The similarity between accessions of different geographic regions observed in the present study suggests the adaptability of the germplasm, indicating the feasibility of its cultivation under edaphoclimatic conditions different from those in its regions of origin. Compared with other crops, such as soybean (Abdelghany et al., 2020), the possible adaptability of *C. moschata* germplasm observed in the present study represents an opportunity to meet the diverse demands of various cultivation regions and systems of this vegetable, specifically in Brazil, which is characterized by its continental dimension.

Additionally, crossbreeding between divergent genotypes has been conveniently explored in *C. moschata*, aiming at the exploitation of hybrid vigor for aspects related to growth habit, fruit, seed production, and fruit chemical-nutritional aspects (El-Tahawey et al., 2015; Kumar et al., 2018). Based on genotypic data and environmental interaction-free genotypic values, the results of the variability analysis will be particularly useful in assisting the crossing of promising genotypes aimed at the exploration of hybrid vigor for characteristics related to SOP and fatty acid profiles.

4.4. Principal components analysis

In consonance with their respective variance components and genetic parameters (Table 1), the oleic acid, linoleic acid, and PUFA concentrations made the greatest contributions to the discrimination of the genotypes in PCA, confirming the high variability of these characteristics (Fig. 3). The results of PCA regarding the variability of accessions were consistent with the results of the clustering analysis (Table 2), also corroborating the strong positive correlations between PS and SOP, as well as the positive correlation between the SOP and oleic acid concentration (Fig. 2).

4.5. Identification of promising accessions in terms of SOP and fatty acid profiles

Based on their highest genotypic averages for SOP, groups 5 and 2 were identified as the most promising for this characteristic (Table 3). Consistent with this result, *per se* analysis identified accession BGH-4610 from group 5 as the most promising in terms of SOP, with a (μ + g) estimate of 0.27 t ha⁻¹ (Table 4). In addition, accessions BGH-5485A, BGH-6590, BGH-5556A, BGH-5472A, and BGH-5544A from group 2 were also identified as promising in terms of the SOP *per se* analysis, with the (μ + g) estimate of ~ 0. 20 t ha⁻¹ (Table 4).

Groups 3 and 2 were identified as the most promising in terms of SOP with higher oleic acid concentrations (Table 4). Associated with this, the accession BGH-5456A from group 3 was identified as the most promising in terms of high oleic acid concentration in the *per se* analysis, with a (μ + g) estimate of 37.20% (Table 4). The accessions BGH-3333A, BGH-5361A, BGH-5472A, BGH-5544A, and BGH-5453A from group 2 and the accession BGH-1749 from group 3 were also identified as promising in terms of high oleic acid concentration, with a (μ + g) estimate of ~ 30.00%

(Table 4). The identification of promising groups and the identification of accessions with a high oleic acid concentration in the seed oil is associated with the high amplitude of $(\mu + g)$ estimates for this characteristic (Fig. 4).

Groups 3 and 2 also presented the lowest linoleic acid and PUFAs average concentrations, confirming that they are the most promising in terms of SOP with lower a PUFA (Table 4). Consistent with this result, the accessions BGH-5456A, BGH-3333A, BGH-5361A, BGH-5544A, BGH-5556A, and BGH-1749 were identified as promising in terms of low linoleic acid concentration, with an $(\mu + g)$ estimate of ~ 45% (Table 4). In *per se* analysis, the accessions identified as the most promising in terms of low linoleic acid were also the most promising in terms of low PUFA concentration, demonstrating the predominance of linoleic acid among PUFAs.

Recently, the development of cultivars with an oil profile that is more suited to human nutrition and health has been emphasized in the genetic breeding of oilseed crops. This is in agreement with a series of studies demonstrating the association between the consumption of lipid sources predominantly comprising saturated fatty acids and the high risk of cardiometabolic pathologies, particularly cardiovascular diseases and type II diabetes mellitus (Harris et al., 2009; Keys et al., 2017; Wu et al., 2019). This has encouraged the replacement of saturated lipids in human food with unsaturated fatty acids, with a particular focus on vegetable oils, the main source of unsaturated fatty acids in the human diet.

Associated with high levels of unsaturated fatty acids, vegetable oils should ideally have a high stability against environmental stressors, such as humidity, light, heat, and oxygen. These oils must also be resistant to oxidative actions, which are related to the production of secondary components responsible for triggering allergic responses and cardiovascular diseases, such as atherosclerosis (Yanishlieva and Marinova, 2001; Garbin et al., 2013), as well as being responsible for a deterioration in the sensory quality (Choe and Min, 2006) and a reduction in the shelf life of oils (Xie et al., 2019). Therefore, breeding programs for oilseed crops such as soybean, rapeseed, and corn have emphasized the development of cultivars that produce more stable oils, prioritizing the increase in the content of oleic acid, a MUFA, which has a greater oxidative stability (Burton et al., 2006; Bachlava et al., 2008; Wang et al., 2009; Long et al., 2018). Some studies have confirmed the higher oxidative stability of oleic acid (C18: 1 (Δ^9)) compared to PUFAs such as linoleic acid [C18: 2 ($\Delta^{9, 12}$)] and linolenic acid [C18: 3 ($\Delta^{9, 12}$, ¹⁵), indicating that oleic acid is 10 times more stable than linoleic acid and 20 times more stable than linolenic acid (Liu and White, 1992). Thus, genetic breeding to improve the fatty acid profile of C. moschata seed oil should simultaneously target an increase in oleic acid content and decrease in polyunsaturated fatty acids, particularly the linoleic acid content, aiming to increase the nutritional and physicochemical quality of oil.

The identification of *C. moschata* accessions with high oleic acid concentrations in seed oil indicates the feasibility of identifying accessions that produce seed oil with a higher nutritional quality and stability. Notably, *C. moschata* seed oil contains high levels of bioactive components, such as vitamin E and carotenoids (Veronezi and Jorge, 2012), which are important antioxidants in the human diet and protect the oil against oxidative processes. Despite their high nutritional value, a large portion of seeds produced during *C. moschata* cultivation are still discarded (Li, 2019), particularly in Brazil. As highlighted by Gomes et al. (2020), the use of *C. moschata* seeds for oil production represents an alternative strategy to complement the diet, in addition to increasing the income of farmers involved in the production of this vegetable.

To the best of our knowledge, the present study is the first to analyze the SOP and fatty acid profile of *C. moschata* based on a relatively large number of accessions representative of different geographic regions of Brazil. Our results demonstrate the marked potential of some *C. moschata* accessions for oil production, as confirmed by the *per se* identification of accessions with a high SOP and high oleic acid concentration in the oil. Our results corroborate the productive potential as well as the nutritional and physicochemical quality of seed oil from the Brazilian germplasm of *C. moschata*.

5. Conclusions

The tested accessions expressed high genetic variability in terms of WSF and PS, providing predicted selection gains of 29.39 g and 0.26 ha^{-1} , respectively.

Phenotypic and genotypic correlations indicated that a greater *C. moschata* SOP could be achieved by selecting for higher PS and WSF. Correlations also indicated that a greater SOP can be obtained while maintaining a high oleic fatty acid concentration and low linoleic acid concentration, providing oil with a better nutritional and chemical quality.

In the analysis of variability, the 91 accessions tested in this study were clustered into five groups, allowing the identification of the most promising groups in terms of a greater SOP and higher oleic acid concentration in the oil, an approach that will guide the use of this germplasm in breeding programs aimed at improving the SOP and fatty acid profile.

Per se analysis identified the accessions BGH-4610, BGH-5485A, BGH-6590, BGH-5556A, BGH-5472A, and BGH-5544A as the most promising in terms of SOP, with a (μ + g) estimate of ~ 0.20 t ha ⁻¹. Accessions BGH-5456A, BGH-3333A, BGH-5361A, BGH-5472A, BGH-5544A, BGH-5453A, and BGH-1749 were identified as the most promising in terms of higher oleic concentration in oil, with a (μ + g) estimate of ~ 30%, most of which were also the most promising in terms of a lower linoleic acid concentration in the oil, with a (μ + g) estimate of ~ 40%. Therefore, part of the *C. moschata* germplasm evaluated in the present study is a promising source for the genetic improvement of SOP and the fatty acid profile, aiming at the production of oil with better nutritional and physicochemical quality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). This work was supported by the Coordenação de Aperfeiçoamento de Pessoal e Nível Superior (CAPES) (grant number 001). This work was supported by the National Council of Technological and Scientific Development (CNPq), which corresponded to a doctorate scholarship (doctorate-GD grant), granted to the first author, Ronaldo Silva Gomes. No additional external funding was received for this study.

References

- Abdelghany, A.M., Zhang, S., Azam, M., Shaibu, A.S., Feng, Y., Li, Y., Tian, Y.u., Hong, H., Li, B., Sun, J., 2020. Profiling of seed fatty acid composition in 1025 Chinese soybean accessions from diverse ecoregions. The Crop J. 8 (4), 635–644. https:// doi.org/10.1016/j.cj.2019.11.002.
- Applequist, W.L., Avula, B., Schaneberg, B.T., Wang, Y.H., Khan, I.A., 2006. Comparative fatty acid content of four *Cucurbita* species grown in a common (shared) garden. J. Food Compos. Anal. 19, 606–611. https://doi.org/10.1016/j. jfca.2006.01.001.

- Assefa, Y., Bajjalieh, N., Archontoulis, S., Casteel, S., Davidson, D., Kovács, P., Naeve, P., Ciampitti, I.A., 2018. Spatial characterization of soybean yield and quality (amino acids, oil, and protein) for United States. Sci Rep-UK 8, 1–11 https://doi: 10.1038/s41598-018-32895-0.
- Bachlava, E., Burton, J.W., Brownie, C., Wang, S., Auclair, J., Cardinal, A.J., 2008. Heritability of oleic acid content in soybean seed oil and its genetic correlation with fatty acid and agronomic traits. Crop Sci. 48 (5), 1764–1772. https://doi. org/10.2135/cropsci2008.01.0049.
- Barboza, N., Albertazzi, F.J., Sibaja-Cordero, J.A., Mora-Umana, F., Astorga, C., Ramírez, P., 2012. Analysis of genetic diversity of *Cucurbita moschata* (D.) germplasm accessions from Mesoamerica revealed by PCR SSCP and chloroplast sequence data. Sci. Hortic-Amsterdam. 134, 60–71. https://doi.org/10.1016/ i.scienta.2011.10.028.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48 https://doi.org/10.18637/jss.v067. i01.
- Bubeck, D.M., Fehr, W.R., Hammond, E.G., 1989. Inheritance of palmitic and stearic acid mutants of soybeans. Crop Sci. 29 (3), 652–656.
- Burton, J.W., Wilson, R.F., Rebetzke, G.J., Pantalone, V.R., 2006. Registration of N98– 4445A mid-oleic soybean germplasm line. Crop Sci. 46 (2), 1010–1012. https:// doi.org/10.2135/cropsci2004-0769.
- Carvalho, L.M.J.De., Gomes, P.B., Godoy, R.Ld.O., Pacheco, S., do Monte, P.H.F., de Carvalho, J.L.V., Nutti, M.R., Neves, A.C.L., Vieira, A.C.R.A., Ramos, S.R.R., 2012. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (*Cucurbita moschata* Duch): A preliminary study. Food Res. Int. 47 (2), 337–340. https://doi.org/10.1016/j.foodres.2011.07.040.
- Choe, E., Min, D.B., 2006. Mechanisms and factors for edible oil oxidation. Compr. Rev. Food Sci. F. 5 (4), 169–186. https://doi.org/10.1111/j.1541-4337.2006.00009.x.
- Cruz, C.D., 2013. GENES—a software package for analysis in experimental statistics and quantitative genetics. Acta Sci-Agron. 35, 271–276. https://doi.org/10.4025/ actasciagron.v35i3.21251.
- Cullis, B.R., Smith, A.B., Coombes, N.E., 2006. On the design of early generation variety trials with correlated data. J Agric Biol. Envir. S. 11 (4), 381–393. https:// doi.org/10.1198/108571106X154443.
- Dash, P., Ghosh, G., 2017. Proteolytic and antioxidant activity of protein fractions of seeds of *Cucurbita moschata*. Food Biosc. 18, 1–8. https://doi.org/10.1016/j. fbio.2016.12.004.
- Darrudi, R., Nazeri, V., Soltani, F., Shokrpour, M., Ercolano, M.R., 2018. Genetic diversity of *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne accessions using fruit and seed quantitative traits. J. Appl. Res. Med. Arom. Plant. 8, 60–66. https://doi.org/10.1016/j.jarmap.2017.11.003.
- De Lima, G.K.L., De Queiróz, M.A., Silveira, L.M.da., 2016. Rescue of Cucurbita spp. germplasm in Rio Grande do Norte. Rev. Caatinga. 29 (1), 257–262. https://doi. org/10.1590/1983-21252016v29n130rc.
- Dehghan, N.F., Yarizade, K., 2014. Bioinformatics study of delta-12 fatty acid desaturase 2 (FAD2) gene in oilseeds. Mol. Biol. Rep. 41 (8), 5077–5087. https:// doi.org/10.1007/s11033-014-3373-5.
- Dias, F. dos S., dos Santos Silva, F.C., Gomes, R.S., da Silva, D.C., de Asevedo Soares, D., Rosa, D.P., da Silva, A.F., 2017. Path analysis in fruits of the buriti (*Mauritia flexuosa* L. f) in populations from the Brazilian Middle North Region. Tree Genet Genomes. 13 (6). https://doi.org/10.1007/s11295-017-1205-1.
- Dillehay, T., Rossen, J., Andres, T.C., Williams, D.E., 2007. Preceramic Adoption of peanut, squash, and cotton in Northern Peru. Science. 316, 1890–1893 https:// www.science.org/doi/pdf/10.1126/science.1141395.
- El-Tahawey, M.A.F.A., Kandeel, A.M., Youssef, S.M.S., Abd El-Salam, M.M.M., 2015. Heterosis, potence ratio, combining ability and correlation of some economic traits in diallel crosses of pumpkins. Egypt J. Plant Breed. 19 (2), 419–439.
- Federer, W.T., 1956. Augmented or (Hoonuiaku) designs. Hawaiian Planter's Record. 55, 191–208.
- Ferreira, M.G., Salvador, F.V., Lima, M.NR., Azevedo, A.M., Lima Neto, I.S., Sobreira, F. M., Silva, D.JH., 2016. Parâmetros genéticos, dissimilaridade e desempenho per se em acessos de abóbora. Hortic. Bras. 34 (4), 537–546. https://doi.org/ 10.1590/s0102-053620160413.
- Ferriol, M., Picó, B., Córdova, P.F., Nuez, F., 2004. Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. Crop Sci. 44 (2), 653–664.
- Food and Agriculture Organization of the United Nations- FAO STATE, 2020. Accessed 15 February 2021. https://www.fao.org/faostat/en/#data/QCL.
- Fonseca, M. A., Queiroz, M. A. de., Lopes, J. F., Assis, J. G. de A., Romão, R. L., Ramos, S. R.R., Do Carmo, C. A. S., Moura, M. C. L., Dias, R. C. S., Silveira, L. M. Da., Barbieri, R. L., Da Silva, D. J. H., Noronha, S., 2015. Geographical distribution and conservation of *Cucurbita* in Brazil. Magistra. 27, 432–442.
- Garbin, U., Baggio, E., Stranieri, C., Pasini, A., Manfro, S., Mozzini, C., Vallerio, P., Lipari, G., Merigo, F., Guidi, G., Cominacini, L., Pasini, A.F., 2013. Expansion of necrotic core and shedding of Mertk receptor in human carotid plaques: a role for oxidized polyunsaturated fatty acids? Cardiovasc. Res. 97, 125–133. https:// doi.org/10.1093/cvr/cvs301.
- Gomes, R.S., Machado Júnior, R., de Almeida, C.F., Chagas, R.R., de Oliveira, R.L., Delazari, F.T., da Silva, D.J.H., Niedz, R.P., 2020. Brazilian germplasm of winter squash (*Cucurbita moschata* D.) displays vast genetic variability, allowing identification of promising genotypes for agro-morphological traits. Plos One. 15 (6), e0230546. https://doi.org/10.1371/journal.pone.0230546.
- Harris, W.S., Mozaffarian, D., Rimm, E., Kris-Etherton, P., Rudel, L.L., Appel, L.J., Engler, M.M., Engler, M.B., Sacks, F., 2009. Omega-6 fatty acids and risk for cardiovascular disease. A science advisory from the American Heart Association

R. Silva Gomes, R. Machado Júnior, C. Freitas de Almeida et al.

Nutrition Subcommittee of The Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation. 119 (6), 902–907. https://doi.org/10.1161/CIRCULATIONAHA.108.191627.

- Hemingway, J., Eskandari, M., Rajcan, I., 2015. Genetic and environmental effects on fatty acid composition in soybeans with potential use in the automotive industry. Crop Sci. 55 (2), 658–668. https://doi.org/10.2135/ cropsci2014.06.0425.
- Jarret, R.L., Levy, I.J., Potter, T.L., Cermak, S.C., Merrick, L.C., 2013. Seed oil content and fatty acid composition in a genebank collection of *Cucurbita moschata* Duchesne and *C. argyrosperma* C. Huber. Plant Genet. Resour-C. 11 (2), 149–157. https://doi.org/10.1017/S1479262112000512.
- Keys, A., Menotti, A., Karvonen, M.J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B.S., Dontas, A.S., Fidaza, F., Key, M.H., Kromhout, D., Nedeljkovic, S., Punsar, S., Seccareccia, F., Toshima, H., 2017. The diet and 15-year death rate in the seven countries study. Am. J. Epidemiol. 185, 1130–1142. https://doi.org/ 10.1093/aje/kwx101.
- Kim, M.Y., Kim, E.J., Kim, Y.-N., Choi, C., Lee, B.-H., 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (*Cucurbitaceae*) species and parts. Nutr. Res. Pract. 6, 21–27. https://doi.org/10.4162/nrp.2012.6.1.21.
- Kumar, V., Mishra, D.P., Yadav, G.C., Yadav, S., 2018. Exploitation of heterobeltiosis and economic heterosis for horticultural yield, and its attributes and biochemical traits in pumpkin (*Cucurbita moschata* Duch. ex. Poir) under salt affected soil. Curr. Sci. India. 115, 1550–2156 https://www.currentscience.ac.in/ Volumes/115/08/1550.pdf.
- Li, H., 2019. Evaluation of bioactivity of butternut squash (*Cucurbita moschata* D.) seeds and skin. Food Sci. Nutr. 8 (7), 3252-3261. https://doi.org/10.1002/ fsn3.1602.
- Lima Neto, I. S., 2013. Pré- melhoramento de abobra (*Cucurbita moschata*) visando a biofortificacao em carotenoides. D. Sc. Thesis, Universidade Federal de Viçosa. Available from: <u>https://www.locus.ufv.br/handle/123456789/1367</u>.
- Liu, H.-R., White, P.J., 1992. Oxidative stability of soybean oils with altered fatty acid. J. Am. Oil Chem. Soc. 69, 528–532.
- Long, W., Hu, M., Gao, J., Chen, S., Zhang, J., Cheng, L., Pu, H., 2018. Identification and functional analysis of two new mutant BnFAD2 alleles that confer elevated oleic acid content in rapeseed. Front. Genet. 9, 1–12. https://doi.org/10.3389/ fgene.2018.00399.
- Moura, M.C.C.L., 2003. Identificação de fontes de resistência ao potyvirus ZYMV e diversidade genética e ecogeográfica em acessos de abóbora. D. Sc. Thesis. Universidade Federal de Viçosa. Available from https://www.locus.ufv.br/ handle/123456789/10265.
- Nagar, A., Sureja, A.K., Kar, A., Bhardwaj, R., Krishnan, S.G., Munshi, A.D., 2018. Profiling of mineral nutrients and variability study in pumpkin (*Cucurbita* moschata) genotypes. Agr. Res. 7 (2), 225–231. https://doi.org/10.1007/s40003-018-0329-3.
- Ohlrogge, J., Browse, J., 1995. Lipid biosynthesis. Plant Cell. 7, 957–970. https://doi. org/10.2307/3870050.
- Oliveira, R.L., Gomes, R.S., Almeida, C.F., Machado Júnior, R., Rocha, J.R.A.S.d.C., Silva, D.J.H., Carneiro, P.C.S., 2021. Multitrait selection of pumpkin genotypes aimed at reducing the growth habit and improving seed production. Crop Sci. 61 (3), 1620–1629. https://doi.org/10.1002/csc2.20386.
- Oliveira, R.L.O., Gonçalves, L.S.A., Rodrigues, R., Baba, V.Y., Sudré, C.P., Santos, M.H., Aranha, F.M., 2016. Genetic divergence among pumpkin landraces. Semin-Cienc Agrar. 37, 1–10. https://doi.org/10.5433/1679-0359.2016v37n2p547.
- Patel, S., 2013. Pumpkin (*Cucurbita* sp.) seeds as nutraceutic: A review on status quo and scopes. Mediterr. J. Nutr. Metab. 6 (3), 183–189. https://doi.org/10.1007/ s12349-013-0131-5.
- Piperno, D.R., Stothert, K.E., 2003. Phytolith evidence for early Holocene Cucurbita domestication in Southwest Ecuador. Science. 299, 1054–1057 https:// www.science.org/doi/10.1126/science.1080365.

- Priori, D., Valduga, E., Villela, J.C.B., Mistura, C.C., Vizzotto, M., Valgas, R., Barbieri, R. L., 2017. Characterization of bioactive compounds, antioxidant activity and minerals in landraces of pumpkin (*Cucurbita moschata*) cultivated in Southern Brazil. Food Sci. Tech-Brazil. 37, 33–40. https://doi.org/10.1590/1678-457X.05016.
- Resende, M.D.V., 1995. Delineamento de experimentos de seleção para maximização da acurácia seletiva e do progresso genético. Rev. Arvore. 19, 479–500.
- Saltzman, A., Birol, E., Bouis, H.E., Boy, E., De Moura, F.F., Islam, Y., Pfeiffer, W.H., 2013. Biofortification: progress toward a more nourishing future. Glob. Food Secur. 2 (1), 9–17. https://doi.org/10.1016/j.gfs.2012.12.003.
- Shimakura, S.E., Ribeiro Júnior, P.J., 2012. Estatística descritiva: interpretação do coeficiente de correlação. Universidade Federal do Paraná, Curitiba.
- Silva, D.J.H., Moura, M.C.C.L., Casali, V.W.D., 2001. Recursos genéticos do banco de germoplasma de hortaliças da UFV: histórico e expedições de coleta. Hortic. Bras. 19 (2), 108–114. https://doi.org/10.1590/S0102-05362001000200002.
- Sobreira, F. M., 2013. Divergência genética entre acessos de abóbora para estabelecimento de coleção nuclear e pré-melhoramento para óleo funcional. D. Sc. Thesis, Universidade Federal de Viçosa. Available from: https://www. locus.ufv.br/handle/123456789/1367.
- Song, W., Yang, R., Wu, T., Wu, C., Sun, S., Zhang, S., Jiang, B., Tian, S., Liu, X., Han, T., 2016. Analyzing the effects of climate factors on soybean protein, oil contents, and composition by extensive and high-density sampling in China. J. Agric. Food Chem. 64 (20), 4121–4130. https://doi.org/10.1021/acs.jafc.6b00008.
- Thiex, N. J., Anderson, S., Gildemeister, B., 2003. Crude fat, hexanes extraction, in feed, cereal grain, and forage (Randall/Soxtec/Submersion Method): Collaborative study. Journal of AOAC International, 86, 899-908.
- Vencovsky, R., Crossa, J., 2003. Measurements of representativeness used in genetic resources conservation and plant breeding. Crop Sci. 43 (6), 1912–1921.
- Veronezi, C.M., Jorge, N., 2015. Chemical characterization of the lipid fractions of pumpkin seeds. Nutr. Food Sci. 45, 164–173. https://doi.org/10.1108/NFS-01-2014-0003.
- Veronezi, C., Jorge, N., 2012. Bioactive compounds in lipid fractions of pumpkin (*Cucurbita* sp) seeds for use in food. J. Food Sci. 77, 53–57. https://doi.org/ 10.1111/j.1750-3841.2012.02736.x.
- Wang, H., Wu, B., Song, T., Chen, S., 2009. Effects of long-term selection for kernel oil concentration in KYHO, a high-oil maize population. Crop Sci. 49, 459–466. https://doi.org/10.2135/cropsci2008.06.0336.
- Werteker, M., Lorenz, A., Johannes, H., Berghofer, E., Findlay, C.S., 2010. Environmental and varietal influences on the fatty acid composition of rapeseed, soybeans and sunflowers. J. Agron. Crop Sci. 196, 20–27. https://doi. org/10.1111/j.1439-037X.2009.00393.x.
- Wu, J. H. Y., Micha, R., Mozaffarian, D., 2019. Dietary fats and cardiometabolic disease: mechanisms and effects on risk factors and outcomes. 16, 1-21. <u>https://doi.org/10.1038/s41569-019-0206-1</u>.
- Wu, T., Yang, X., Sun, S., Wang, C., Wang, Y., Jia, H., Man, W., Fu, L., Song, W., Wu, C., Yan, H., Jiang, B., Hou, W., Ren, G., Han, T., 2017. Temporal-Spatial Characterization of Seed Proteins and Oil in Widely Grown Soybean Cultivars across a Century of Breeding in China. Crop Science 57 (2), 748–759.
- Xie, Y., Jiang, S., Li, M., Guo, Y., Cheng, Y., Qian, H., Yao, W., 2019. Evaluation on the formation of lipid free radicals in the oxidation process of peanut oil. LWT-Food Sci. Technol. 104, 24–29. https://doi.org/10.1016/j.lwt.2019.01.016.
- Yanishlieva, N.V., Marinova, E.M., 2001. Stabilisation of edible oils with natural antioxidants. Eur. J. Lipid Sci. Tech. 103, 752–767. https://doi.org/10.1002/ 1438-9312(200111)103:11<752::AID-E/LT752>3.0.CO;2-0.
- Yol, E., Ustun, R., Golukcu, M., Uzun, B., 2017. Oil content, oil yield and fatty acid profile of groundnut germplasm in Mediterranean climates. J. Am. Oil Chem. Soc. 94 (6), 787–804. https://doi.org/10.1007/s11746-017-2981-3.