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GENETIC AND MORPHOLOGICAL DESCRIPTORS TO ACCESS BRAZILIAN OKRA GENOTYPES DIVERSITY[1]

FÁBIO JANONI CARVALHO[2]*, THIAGO FELLIPE NUNES DE MENDONÇA[3], ANA CAROLINA SILVA SIQUIEROLI[4], GABRIEL MASCARENHAS MACIEL[3], ANDRESSA ALVES CLEMENTE[3]

ABSTRACT - Information of the variation for important morphological and physiological traits of okra is still limited. Molecular analysis is an important additional tool in germplasm characterization studies. The study aimed to evaluate the performance of the growth and yield of 20 pre-commercial okra accessions to identify molecular markers' association with morphological traits. Nineteen morphological traits were measured with five qualitative and 14 quantitative descriptors. For analysis of genetic patterns Random Amplified Polymorphic DNA (RAPD) markers were used with nine primers and 24 usable bands. The genetic dissimilarity was evaluated based in morphological and genetic matrices. Also, graphical representation of genetic distances was obtained by UPGMA and Tocher's optimization method. The morphological characterization of the accessions detected polymorphism for all evaluated traits. RAPD markers were efficient in detecting genetic variability among okra accessions. For the primers used in the experiment, only OPE10 did not amplify the DNA strand. The other eight primers produced a total of 35 bands, in which 25 were polymorphic and ten were monomorphic. The morphological traits and molecular markers identified wide genetic variability among the 20 okra accessions, indicating successful crosses in breeding programs and isolating some interesting materials. Morphological and molecular cluster analyses were complementary and helped in the genotype selection. Molecular analysis indicated some divergent accessions that were not found in morphological analysis, which could highlight some materials that have a desirable trait, that is difficult and highly costly to access in field experiments.

Keywords: *Abelmoschus esculentus* L. Genetic variability. Molecular markers. Phenotype.

DESCRITORES GENÉTICOS E MORFOLÓGICOS PARA AVALIAR A DIVERSIDADE GENÉTICA DE ACESSOS DE QUIABO BRASILEIRO

RESUMO – Informações da variação de importantes características morfológicas e fisiológicas do quiabo são limitadas. A análise molecular é uma ferramenta adicional importante nos estudos de caracterização de germoplasma. O estudo teve como objetivo avaliar o desempenho do crescimento e da produção de 20 acessos pré-comerciais de quiabo para identificar a associação de marcadores moleculares com características morfológicas. Dezenove caracteres morfológicos foram medidos com cinco descritores qualitativos e 14 quantitativos. Para a análise dos padrões genéticos, foram utilizados marcadores RAPD com nove primers e 24 bandas utilizáveis. A dissimilaridade genética foi avaliada com base em matrizes morfológicas e genéticas. Além disso, a representação gráfica das distâncias genéticas foi realizada pelo método UPGMA e otimização de Tocher. A caracterização morfológica dos acessos detectou polimorfismo para todas as características avaliadas. Os marcadores RAPD foram eficientes na detecção da variabilidade genética entre os acessos de quiabo. Para os primers usados no experimento, apenas OPE10 não amplificou a fita de DNA. Os outros oito primers produziram um total de 35 bandas, sendo 25 polimórficas e dez monomórficas. Os caracteres morfológicos e marcadores moleculares identificaram ampla variabilidade genética entre os 20 acessos de quiabo, indicando a possibilidade de cruzamentos bem-sucedidos em programas de melhoramento e o isolando genótipos interessantes. As análises morfológica e molecular foram complementares e auxiliaram na seleção dos genótipos. A análise molecular indicou alguns acessos divergentes que não foram encontrados na análise morfológica, o que poderia destacar alguns materiais que apresentam uma característica desejável, sendo interessantes para detectar características de difícil mensuração em campo.

Palavras-chave: *Abelmoschus esculentus* L. Variabilidade Genética. Marcadores Moleculares. Fenótipo.

*Corresponding author
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[2]General Coordination of Research and Innovation, Instituto Federal de Educação Ciência e Tecnologia do Triângulo Mineiro, Uberaba, MG, Brazil; fabiojanoni@iftm.edu.br – ORCID: 0000-0002-0327-1821.
[3]Institute of Agricultural Sciences, Universidade Federal de Uberlândia, Monte Carmelo, MG, Brazil; thiagofellipe@live.com – ORCID: 0000-0003-0773-750X, gabrielmaciel@ufu.br – ORCID: 0000-0002-3004-9134, andressalves50@gmail.com – ORCID: 0000-0002-0927-1435.
[4]Institute of Biotechnology, Universidade Federal de Uberlândia, Monte Carmelo, MG, Brazil; carol@ufu.br – ORCID: 0000-0003-4713-1262.

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench.) is one of the most consumed vegetables in tropical and subtropical regions (PATIL et al., 2015). Its fruits and seeds are edible, and the fruits are a rich source of dietary fiber, carbohydrates and vitamins (PETROPOULOS et al., 2018). In Brazil, there is no reliable records on okra production area and yield, but its cultivation is a highly dynamic activity, mostly carried out in small agricultural areas by family farmers (MASSUCATO et al., 2020).

Several factors have resulted in a loss of genetic diversity of landraces in Brazil over the years, like environmental changes, consumer preference, urbanization, environmental exodus and abandonment of rural properties (MOULIN et al., 2012). Because of that, the conservation of agrobiodiversity with gene banks becomes highly important and a lot of okra breeding programs started in Brazil in the last years. However, information on the level of variation for important morphological and physiological traits of okra is still limited. Knowledge on genetic diversity plays an important role in breeding programs to improve agronomic traits and resistance to environmental stresses (ABD EL-FATTAH; HARIDY; ABBAS, 2020). Some cultivars have been released recently, including hybrids, but one open-pollinated cultivar ('Santa Cruz 47') has been dominating the market over 45 years (SILVA et al., 2021).

Phenotypic descriptors, based on morphological and agricultural traits, were widely used in the characterization of okra gene banks (YONAS; GAREDEW; DEBELA, 2014; ASGHAR, 2016; MACIEL et al., 2018). These traits are not adequate for the development of gene pools since they are influenced by environmental conditions and stage of plant development or detect only finite variation (TERZOPOULOS; BEBELI, 2008).

Molecular analysis is an important additional tool in germplasm characterization studies because it is minimally influenced by environmental conditions or plant development factors (SCHAFLEITNER et al., 2013). Molecular markers are powerful tools to assess the genetic diversity among different genotypes (CONG-YING et al., 2015). Many types of DNA marker systems were used to evaluate the genetic variation and relationships among okra genotypes, identifying unique genotypes and markers that are associated with or linked to important morphological traits (ABD EL-FATTAH; HARIDY; ABBAS, 2020). Those molecular techniques present a fast methodology to access the genetic variability in plant breeding programs.

Some DNA-based techniques have already been used to detect okra diversity: inter-simple sequence repeat (CONG-YING et al., 2014; EL-SHERBENY et al., 2018), random amplified polymorphic DNA (PATEL; JAPDA; DHRUVE, 2018), amplified fragment length polymorphism (KYRIAKOPOULOU et al., 2014), and simple sequence repeats (OUEDRAOGO et al., 2018; RAVISHANKAR et al., 2018). These techniques were used for genome coverage with cost-effectiveness, reproducibility, and independence of sequence information.

There are few reports on okra germplasm diversity in Brazil (MATTEDI et al., 2015; MACIEL et al., 2017, 2018; SILVA et al., 2021). Embrapa Hortaliças has one of the biggest okra germplasm banks in Brazil, which may hold important and strategic genetic diversity, but has not been characterized yet (SILVA et al., 2021). Characterization of plant material enables the exploitation of genetic resources in breeding programs (ZANKLAN et al., 2018). The present study aimed to evaluate the performance of the growth and yield of 20 okra accessions in Brazil to identify molecular markers' association with morphological traits.

MATERIAL AND METHODS

Twenty pre-commercial okra lineages from the Okra Germplasm Bank of Universidade Federal de Uberlândia (UFU) were evaluated. The experiment was conducted at the Horticulture Experimental Station, Monte Carmelo, Brazil (18º42'43.19" S, 47º29'55.8" W, 873m altitude). Sowing was performed on March 7th, 2016. The germplasm bank consisted of 20 homozygous accessions obtained after hybridization of 'Veloce' x 'Santa Cruz 47' cultivars followed by five successive self-pollinations. The plants were cultivated in 10-liter pots with coconut fiber substrate. A nutrient solution was used for the plants development. The electrical conductivity (μS cm^{-1}), pH and temperature of the nutrient solution (°C) were monitored using a TEC-RL0C portable conductivity meter. The average electrical conductivity and pH along the experiment were 1.48 μS cm^{-1} and 6.32. After transplanting, between the first and second week, commercial macronutrients (NPK) were used in the proportion of 1.0:1.2:1.0. After the third week, fertigation protocol was changed to the NPK ratio of 1.0:0.7:2.0. The fertigation was carried out with SPAGHETTI LDPE microtube drippers.

Four replications of each accession were planted in a randomized complete block design (RCBD), with five plants per replication disposed sequentially and spaced by 0.4 x 1.0 m. The morphological traits began to be evaluated at flowering stage. Nineteen morphological traits were evaluated (Table 1) established by the International Plant Genetic Resources Institute (IPGRI, 1991), with five qualitative and 14 quantitative descriptors. Three harvests were made on June 3, June 9 and June 28 of 2016.

Table 1. Morphological traits used to analyze genetic diversity in twenty pre-commercial okra accessions.

Trait	Type	Description*
Fruit Color (FC)	Qualitative	Classified as light green / green / dark green
Texture of rind (TR)		Classified as very folded / folded / smooth
Fruit pilosity (FP)		Classified as high / low / no
Plant color (PC)		Classified as light / intermediary / dark
Leaf limb shape (LLS)		Classified as rounded / slightly rounded / peaked /very peaked
Fruit length (FL) Fruit width (FW)	Quantitative	Measured in centimeters
Chlorophyll A (ChlA) Chlorophyll B (ChlB) Total chlorophyll (TChl)		Measured at the first flowering stage with the portable meter CLOROFILOG CFL1030 (FALKER®)
Glandular trichomes (GT)		Trichomes per cm² counted with stereoscopic loupe in the fruit middle third
Number of fruits in first harvest (NF1)		Number of fruits per plant in the first harvest
Fruit weight in first harvest (FW1)		Fruit weight per plant in the first harvest
Number of fruits in second harvest (NF2)		Number of fruits per plant in the second harvest
Fruit weight in second harvest (FW2)		Fruit weight per plant in the second harvest
Number of fruits in third harvest (NF3)		Number of fruits per plant in the third harvest
Fruit weight in third harvest (FW3)		Fruit weight per plant in the third harvest
Total number of fruits (TNF)		Sum of NF1, NF2 and NF3
Total fruit weight (TFW)		Sum of FW1, FW2 and FW3

*Description established by the International Plant Genetic Resources Institute (IPGRI, 1991).

For the molecular data, young leaves from the top of the plant were collected in bulk, selecting five leaves from five plants, totaling 100 leaves per accession. The leaves were wrapped in aluminum foil and dipped in liquid N_2 to prevent DNA degradation. DNA extraction was performed using the CTAB method. Due to the precipitate being too large and viscous, an additional wash with 1M NaCl was performed. The solution was incubated at 65 ºC for 5 minutes and then at 4 ºC for 30 minutes. The samples were centrifuged for 10 minutes at 11,750 x g and the supernatant was collected. After that, the DNA was sedimented by adding two thirds of the volume with isopropanol (-20 ºC) and performing two subsequent washes with 70% ethanol. The extracted DNA was resuspended in 150 µL TE (1M TrisHCl, 500 mM EDTA pH 8.0) with RNAse (10 µg / mL) and incubated at 37 ºC, for 30 minutes to two hours (FERREIRA; GRATTAPAGLIA, 1998). The amount of DNA was determined by reading a spectrophotometer at 260 nm.

The Random Amplified Polymorphic DNA (RAPD) reactions were performed according to a protocol of Williams et al. (1990), in a volume of 25 µL containing 2.4 Mmol/L of $MgCl_2$ 100 µM of dATP, dCTP, dGTP and dTTP; 0.3 µM of primer; 20 ng of genomic DNA; 1 unit of Taq DNA polymerase and 1X PCR buffer. For the primers' selection, a large number of polymorphic bands and interspecific polymorphism were adopted as selection criteria. Thus, nine primers were selected (Operon Technologies series - Alameda, CA, USA) with the following sequence (5'-3'): OPA15 (TTCCGAACCC); OPB08 (GGACCCTTAC); OPB20 (TGGACCGGTG); OPC08 (GTCCACACGG); OPC11 (AAAGCTGCGG); OPD03 (TGAGCGGACA); OPD05 (GTCGCCGTCA); OPE10 (CCTCTAGACC); OPF19 (CACCAGGTGA).

The PCR reactions were carried out in a thermocycler under the following conditions: 95 ºC for 1 minute, followed by 45 cycles (1 minute at 94 ºC, 1 minute at 36 ºC and 2 minutes at 72 ºC), and a final step of 7 minutes at 72 ºC for extension, using the fastest temperature transition mode (1 ºC/sec). The amplified fragments were separated on agarose gel (1.5%) in TBE 1X buffer (1M of Tris base, 500mM of Boric Acid and EDTA), stained with fluorescent dyes. To estimate the size of the fragments, a 100 base pair DNA Ladder marker was used. The gels were submitted to ultraviolet light in a photo-documenter for analysis. With the PCR results, a binary matrix was created, where the number one corresponded to the presence of the band and the zeros to the absence, for each accession. A total of 38 bands were produced, but only 24 bands showed differences among the materials.

Genetic dissimilarity was evaluated based on two matrices: Morphological (Method 1 with 19 traits) and Genetic (Method 2 with 24 traits). The matrices based on multicategorical variables were estimated by Gower's algorithm, because of the efficiency of the technique to analyze quantitative and qualitative data simultaneously, or only qualitative data (MOURA et al., 2010). The dissimilarity between genotypes was expressed as:

$$S_{ijk} = \frac{\sum_{k=1}^{p} W_{ijk}.S_{ijk}}{\sum_{k=1}^{p} W_{ijk}},$$

where: k is the number of variables (k = 1, 2, …, p); i and j are two individuals representing the accessions; W_{ijk} is the weight given to ijk comparison (one for valid comparisons and zero for invalid comparisons); S_{ijk} is the variable contribution k in the similarity between i and j individuals.

Graphical representation of genetic distances was obtained by Unweighted Pair-Group Method using an Arithmetic Mean (UPGMA). Tocher's optimization method was also established to group the accessions. Cophenetic correlation coefficient (CCC) was performed for UPGMA and Tocher's methods to identify the clustering quality from both. UPGMA clusters were compared using a cutoff of 32.5%, established where an abrupt change was visualized in the branches present in the dendrogram (CRUZ; REGAZZI; CARNEIRO, 2012).

Mean Decrease Impurity Importance (MDI) was measured to evaluate the importance of variables (LOUPPE et al., 2013) and Pearson's correlation was measured for the quantitative morphological traits with a *t*-test to validate the correlations. Data were analyzed with R software version 3.5.0, using the packages *clusters* to estimate dissimilarity matrix, *randomForest* to measure MDI (LIAW; WIENER, 2002); *stats* for UPGMA, its CCC and Pearson's Chi-Square; *biotools* for Tocher and its CCC (SILVA; DIAS, 2013).

RESULTS AND DISCUSSION

Morphological characterization of the accessions detected polymorphism for all evaluated traits (Tables 2 and 3). When analyzing the fruit, 75% of the lineages showed a green color and 25% a light green color. The color results contrasted with those of the accessions studied by Massucato et al. (2020), where 36.7% had green fruits, 33.3% had fruits with a light green color and 30% dark green. For the texture of rind, 75% had folded rind, 20% very folded, and only one accession had a smooth texture (UFU-10). For pilosity, 70% of the accessions had low and 30% had high pilosity (Table 2). An intermediary plant color was predominant among the accessions (60%), followed by light color (25%). UFU-03, UFU-09 and UFU-17 showed dark plant color. The results were diverse from those found in another study, where 46.7% of the accessions were light and 33.3 intermediary (MASSUCATO et al., 2020). For the leaves, the most common shape was A (45%) and B (45%). UFU-07 had the C shape and UFU-10 had the D shape. The parent 'Santa Cruz 47' has fruits with high pilosity, whereas 'Veloce' has few aculeus. Six accessions preserved the high amount of aculeus, which is not a desirable trait for okra harvest.

Table 2. Morphological characterization based on five qualitative descriptors of 20 okra (*Abelmoschus esculentus* L.) accessions.

Accession	FC	TR	FP	PC	LLS
UFU-01	Green	Folded	Very	Intermediary	A
UFU-02	Green	Folded	Very	Intermediary	A
UFU-03	Light green	Folded	Very	Dark	B
UFU-04	Light green	Folded	Very	Intermediary	A
UFU-05	Green	Folded	Few	Intermediary	A
UFU-06	Green	Very Folded	Few	Light	A
UFU-07	Green	Folded	Few	Intermediary	C
UFU-08	Light green	Very Folded	Few	Light	A
UFU-09	Green	Very Folded	Very	Dark	A
UFU-10	Light green	Smooth	Few	Light	D
UFU-11	Green	Folded	Few	Light	A
UFU-12	Green	Very Folded	Few	Intermediary	B
UFU-13	Green	Folded	Very	Intermediary	B
UFU-14	Green	Folded	Few	Intermediary	B
UFU-15	Light green	Folded	Few	Light	B
UFU-16	Green	Folded	Few	Intermediary	B
UFU-17	Green	Folded	Few	Dark	B
UFU-18	Green	Folded	Few	Intermediary	B
UFU-19	Green	Folded	Few	Intermediary	A
UFU-20	Green	Folded	Few	Intermediary	B

FC: Fruit Color; TR: Texture of rind; FP: Fruit pilosity; PC: Plant color; LLS: Leaf limb shape.

For the quantitative morphoagronomic traits (Table 3), there was significant difference (*p-value*<0.05) between the lineages for all variables evaluated. Fruit length ranged from 9.5 (UFU-15) to 15.3 (UFU-09) cm, and fruit width from to 15.18 (UFU-07) to 20.73 (UFU-05) cm. The number of glandular trichomes on the fruit varied from 1.0 (UFU-15) to 32.5 (UFU-09) per cm². The content of Chlorophyll A ranged between 12.14 (G15) and 34.86 (UFU-20), and for Chlorophyll B between 3.40 (UFU-15) and 15.14 (UFU-8). The total number of fruits varied from eight (UFU-15) to 70 (UFU-07) and the total weight from 0.322 g (UFU-10) to 1.843 g (UFU-07). UFU 07 had the highest number of fruits and fruit weight in the first harvest (25; 0.712 g) followed by UFU-16 (21; 0.656 g), indicating the earliness of both, another desirable trait of 'Veloce' hybrid.

Table 3. Morphological characterization of 20 okra (*Abelmoschus esculentus* L.) accessions based on 14 quantitative descriptors.

Accession	FL	FW	ChlA	ChlB	TChl	GT	NF1	FW1	NF2	FW2	NF3	FW3	TNF	TFW
UFU-01	13.0	17.52	31.92	9.78	41.70	25.0	13	0.336	9	0.149	23	0.415	45	0.900
UFU-02	13.7	18.94	33.42	11.58	45.00	22.8	14	0.298	3	0.039	22	0.409	39	0.746
UFU-03	14.5	17.28	30.94	11.22	42.16	28.6	4	0.106	3	0.045	15	0.440	22	0.591
UFU-04	14.3	17.41	31.94	12.00	43.94	19.8	4	0.106	5	0.068	20	0.474	29	0.648
UFU-05	15.1	20.73	31.48	9.56	41.04	17.4	3	0.092	3	0.041	30	0.687	36	0.820
UFU-06	14.8	19.11	32.82	11.42	44.24	20.3	16	0.42	6	0.096	27	0.528	49	1.044
UFU-07	10.6	15.18	34.12	13.16	47.28	18.2	25	0.712	9	0.155	36	0.976	70	1.843
UFU-08	14.0	18.79	34.70	15.14	49.84	19.8	13	0.332	8	0.124	28	0.671	49	1.127
UFU-09	15.3	20.52	33.66	12.20	45.86	32.5	7	0.282	2	0.038	18	0.658	27	0.978
UFU-10	11.0	15.35	12.42	3.48	15.90	19.0	20	0.132	0	0.000	1	0.190	21	0.322
UFU-11	14.0	16.48	25.84	8.54	34.38	16.3	2	0.054	0	0.000	10	0.284	12	0.338
UFU-12	11.8	18.63	34.58	13.66	48.24	14.5	14	0.344	4	0.062	21	0.447	39	0.853
UFU-13	11.8	16.57	30.86	11.40	42.26	20.8	17	0.476	5	0.081	27	0.583	32	1.140
UFU-14	9.8	17.26	37.74	14.50	52.24	26.8	19	0.504	6	0.071	24	0.506	49	1.081
UFU-15	9.5	15.47	12.14	3.40	15.54	1.0	4	0.07	2	0.024	2	0.510	8	0.604
UFU-16	14.0	19.38	30.36	10.04	40.40	23.0	21	0.656	2	0.044	17	0.437	40	1.137
UFU-17	14.2	18.56	32.14	10.98	43.12	18.3	8	0.206	3	0.043	14	0.345	25	0.594
UFU-18	10.2	19.92	33.38	12.94	46.32	12.0	12	0.308	5	0.064	9	0.225	26	0.597
UFU-19	13.0	16.96	32.76	11.70	44.46	16.0	3	0.074	2	0.032	18	0.362	23	0.468
UFU-20	13.8	18.02	34.86	14.26	49.12	16.5	16	0.346	6	0.077	13	0.249	35	0.672

FL: Fruit length (cm); FW: Fruit width (cm); ChlA: Chlorophyll A; ChlB: Chlorophyll B; TChl: Total chlorophyll; GT: Glandular trichomes (GT/cm²); NF1: Number of fruits in first harvest; FW1: Fruit weight in first harvest (kg); NF2: Number of fruits in second harvest; FW2: Fruit weight in second harvest (kg); NF3: Number of fruits in third harvest; FW3: Fruit weight in third harvest (kg); TNF: Total number of fruits; TFW: Total fruit weight (kg).

The correlation analysis detected linear correlation between some quantitative traits. Total number of fruits and total fruit weight showed significant positive correlation with ten other variables, which could be discarded in further essays (Table 4). Chlorophyll A and B were also highly correlated with total chlorophyll, revealing the necessity to evaluate only one type of chlorophyll. Additionally, it was noted that the number of fruits had a positive correlation with fruit weight in each harvest (0.82 for the first, 0.87 for the second and 0.75 for the third). Number of fruits was also strongly correlated with fruit weight in each harvest, suggesting that only fruit weight could have been used to detect the divergence. Ahiakpa et al. (2013), studying genetic diversity of 30 okra accessions found strong correlation for other morphological traits, like maximum number of internode and stem diameter at base (0.93), and stem diameter at base and maximum number of internode (0.93).

When the accessions were clustered by morphoagronomic traits, five groups were formed by UPGMA's method (Figure 1a) and seven groups by Tocher's method (Table 5). Tocher's optimization isolated the accessions UFU-03, UFU-07, UFU-08, UFU-09 and UFU-010. UPGMA's method grouped accession UFU-06 with UFU-08 and created an isolated cluster for UFU-07 and UFU-10. Using morphological traits, the lineages UFU-03, UFU-07,

UFU-08, UFU-09 and UFU-010 were the most divergent accessions and should be selected in the breeding process to explore heterosis. UFU-07 has desirable traits like earliness and high fruit pilosity in contrast to the other genotypes that were not early.

The other accessions were clustered together (a total of 65% for Tocher and 80% for UGMA of the lineages). UPGMA dendrogram had expressed cophenetic correlation coefficient of 79.36% and Tocher's of 85.00%. Maciel et al. (2018) also obtained a CCC of 79.00% for UPGMA dendrogram studying genetic diversity in 47 okra hybrids using morphological traits. When compared with the molecular traits (Figure 1b and Table 5), the accessions UFU-01, UFU-05 and UFU-06 were isolated. This could indicate that the morphological traits used to detect genetic diversity were not sufficient to separate those accessions that could have some desirable traits like disease resistance and rusticity. However, UFU -07 was also isolated from the other accessions, indicating that some of the outstanding traits of this accession were detected with the primers.

Table 4. Estimation of genotypic correlation coefficients among fourteen quantitative morphoagronomic descriptors in 20 okra (*Abelmoschus esculentus* L.) accessions.

FL	-0.23	0.02	0.08	0.04	-0.02	0.17	0.34	0.25	0.25	0.07	0.11	0.12	0.29
	FW	0.52*	0.41	0.49*	0.30	-0.15	0.05	0.00	-0.18	0.23	-0.01	0.11	0.04
		chlA	0.95***	0.99***	0.49*	0.14	0.41	0.55*	0.45*	0.70**	0.25	0.60**	0.48*
			chlB	0.98***	0.27	0.21	0.42	0.60**	0.56*	0.62**	0.24	0.59**	0.49*
				TChl	0.46*	0.16	0.42*	0.57**	0.49*	0.68***	0.25	0.60**	0.49*
					GT	0.17	0.27*	0.13	-0.02	0.38	0.18	0.33	0.27
						NF1	0.82***	0.44	0.56*	0.29	0.14	0.70***	0.62*
							FW1	0.49*	0.55*	0.52*	0.4	0.74***	0.85***
								NF2	0.87***	0.66**	0.43	0.79***	0.68***
									FW2	0.56*	0.38	0.73***	0.66***
										NF3	0.75***	0.83***	0.78***
											FW3	0.57**	0.78***
												TNF	0.85***
													TFW

FL: Fruit length; FW: Fruit width; ChlA: Chlorophyll A; ChlB: Chlorophyll B; TChl: Total chlorophyll; GT: Glandular trichomes; NF1: Number of fruits in first harvest; FW1: Fruit weight in first harvest; NF2: Number of fruits in second harvest; FW2: Fruit weight in second harvest; NF3: Number of fruits in third harvest; FW3: Fruit weight in third harvest; TNF: Total number of fruits; TFW: Total fruit weight. The values were estimated by Pearson's correlation. *Significant at 5% probability; **Significant at 1% probability; ***Significant at 0.01% probability.

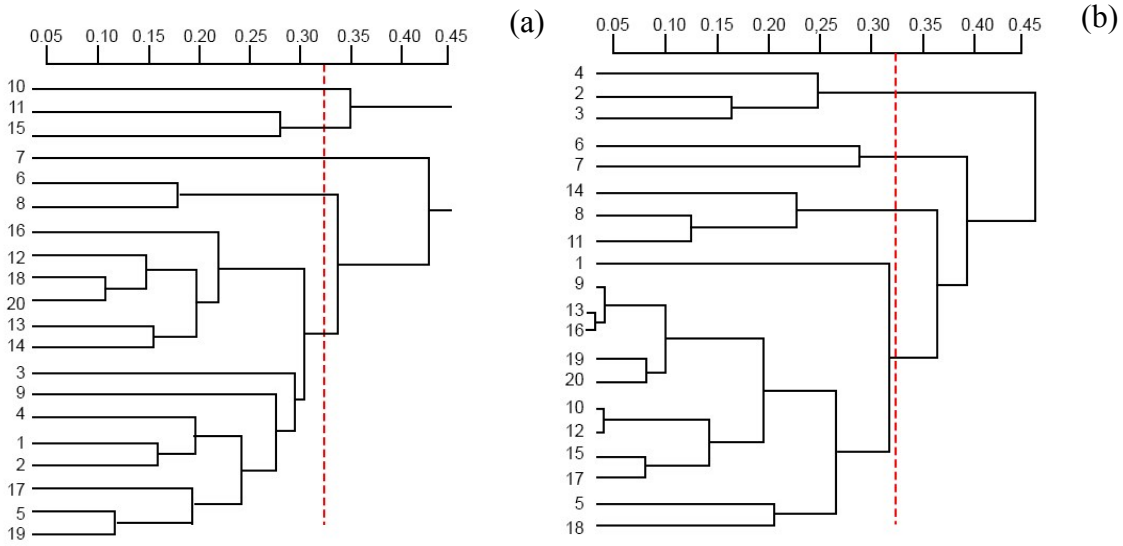

Figure 1. Dendrogram of the genetic divergence among 20 okra lineages achieved by the UPGMA method. Method 1 – Morphological traits (a); Method 2 – Molecular traits (b).

Table 5. Clustering of 20 okra accessions by the Tocher's optimization procedure obtained from different traits.

	Morphological traits	Molecular traits
Cluster 1	UFU-01 UFU-02 UFU-04 UFU-05 UFU-06 UFU-12 UFU-13 UFU-14 UFU-16 UFU-17 UFU-18 UFU-19 UFU-20	UFU-09 UFU-10 UFU-12 UFU-13 UFU-15 UFU-16 UFU-17 UFU-18 UFU-19 UFU-20
Cluster 2	UFU-03	UFU-02 UFU-03 UFU-04
Cluster 3	UFU-11 UFU-15	UFU-08 UFU-11 UFU-14
Cluster 4	UFU-07	UFU-07
Cluster 5	UFU-10	UFU-01
Cluster 6	UFU-08	UFU-05
Cluster 7	UFU-09	UFU-06

Random Amplified Polymorphic DNA markers were efficient in detecting genetic variability among okra accessions (Figure 2). For the primers used in the experiment, only OPE10 did not amplify the DNA strand. The other eight primers produced a total of 35 bands, in which 25 were polymorphic (71.43%) and ten were monomorphic (28.57%). The primers OPD19 and OPB08 had the highest polymorphism value (100%). Summarizing, each primer produced 4.37 bands, being 1.25 monomorphic and 3.13 polymorphic. Thus, it could be observed that there was a higher number of lineages with the *loci* tending to heterozygosis or dominant homozygosis.

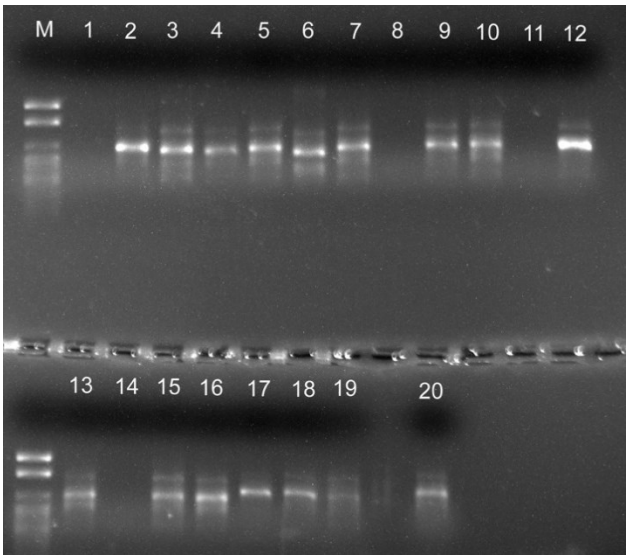

Figure 2. Amplification products generated by OPA15 primer 100 bp DNA Ladder; (UFU-01 to UFU-20).

The results obtained were similar to those found by Massucato et al. (2020) with 77.6% of polymorphism with five EcoRI/MseI primer combinations in 30 okra accessions, and by Kumar et al. (2017) with 60.66% with 30 SSR primers in 96 okra accessions. However, some results also revealed a small percentage of polymorphism, as for Kyriakopoulou et al. (2014) with 12.17% of polymorphism in 50 okra landraces in Greece based on 33 AFLP primer combinations, and Akash, Shiyab and Saleh (2013) with 31.6% of polymorphism among 22 okra accessions with AFLP markers.

Twenty-five bands were able to be used in the multivariate analysis, but one band was eliminated because it produced the same results of another. The UPGMA dendrogram of the molecular traits indicated the cluster of four groups (Figure 1). However, UFU-02, UFU-03 and UFU-04 formed a new cluster and were removed from the bigger cluster. Accession UFU-08 was grouped with UFU-11 and UFU-14. Tocher's optimization method also formed seven clusters compared to the first method. The smaller clusters had a similarity to the

UPGMA dendrogram, with the exception that UFU-06 and UFU-07 formed isolated clusters and UFU-01 and UFU-05 were also isolated. Seven accessions (UFU-12, UFU-13, UFU-16, UFU-17, UFU-18, UFU-19 and UFU-20) were included in the bigger cluster for both methods, indicating a high similarity between them. UPGMA dendrogram had expressed cophenetic correlation coefficient of 87.29% and Tocher's of 87.38%.

Our results were aligned with what was found by those researchers, showing that molecular analysis is efficient to determinate genetic divergence in okra populations. Ikran-ul-Haq and Azmat (2013) studied genetic diversity in 39 accessions in Pakistan using 20 primers, and these authors' results were also efficient to cluster the analyzed accessions. Kumar et al. (2017) found that SSR markers helped to reveal hidden variability in okra and its wild relatives. In another study with six okra varieties in Egypt, Abd El-Fattah, Haridy and Abbas (2020) used different marker systems: agro-morphological, morpho-physiological and molecular parameters (ISSR, SRAP and SSR) to generate pre-breeding data that were helpful for choosing the appropriate parents.

It was noticed that both approaches of characterization are important for a clearer differentiation among okra accessions and should be used in congruence to study the genetic diversity. Morphological characterization could be more efficient to detect more divergences from the germplasm bank if more plant traits were collected. Several studies of okra gene banks have indicated the importance of morphologic and molecular characterization to better understand the variability (GULSEN; KARAGUL; ABAK, 2007; KYRIAKOPOULOU et al., 2014; YILDIZ et al., 2016; MASSUCATO et al., 2020).

Mean Decrease Impurity (MDI) exhibits desirable properties for assessing the relevance of a variable: it is equal to zero only if the variable is irrelevant and it depends only on the relevant variables (LOUPPE et al., 2013). The amplitude of MDI was not high, showing that all the traits contributed somehow to the dissimilarity of the bank. For the morphological descriptors, the traits with less impact on MDI, and respectively with less impact to detect genetic divergence among the accessions, were FC, TR, FP, PC and LLS. On the other hand, FW, GT, ChlA and ChlB had a great contribution to distinguish the accessions, showing higher values for MDI (Figure 3).

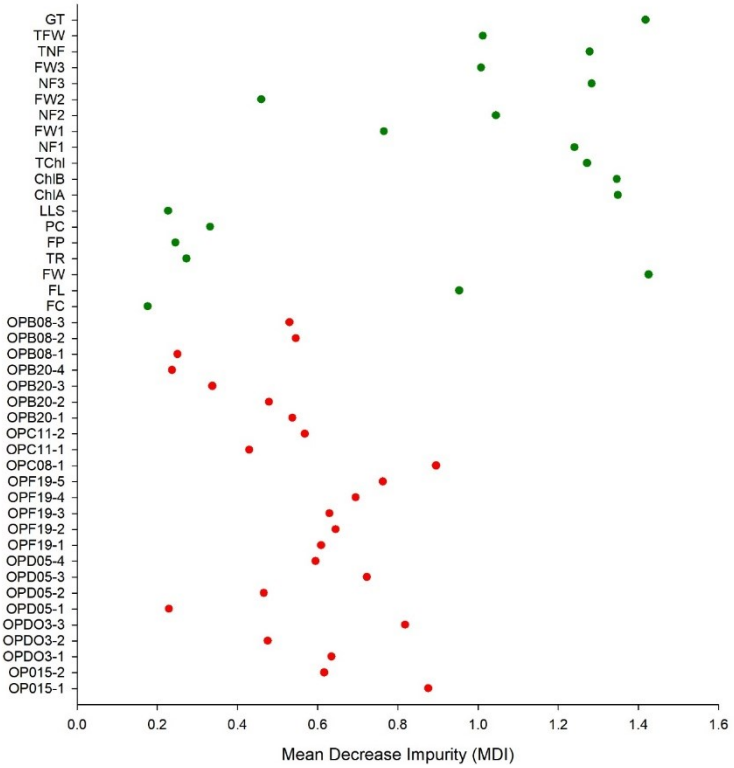

Figure 3. Mean Decrease Impurity of 19 morphological descriptors (green dots) and 24 molecular descriptors (red dots) among 20 okra accessions. FC: Fruit Color; TR: Texture of rind; FP: Fruit pilosity; PC: Plant color; LLS: Leaf limb shape; FL: Fruit length; FW: Fruit width; ChlA: Chlorophyll A; ChlB: Chlorophyll B; TChl: Total chlorophyll; GT: Glandular trichomes; NF1: Number of fruits in first harvest; FW1: Fruit weight in first harvest; NF2: Number of fruits in second harvest; FW2: Fruit weight in second harvest; NF3: Number of fruits in third harvest; FW3: Fruit weight in third harvest; TNF: Total number of fruits; TFW: Total fruit weight.

For quantitative morphoagronomic traits, Massucato et al. (2020) found that all traits contributed moderately to the discrimination of okra accessions using Singh's criterion. In contrast, Maciel et al. (2018) found that the trait that contributed the most to genetic divergence was productivity (74.08%), followed by the number of fruits (21.71%) and earliness index (3.42%). For the molecular analysis, the dissimilarity matrix is composed of the presence or absence of the band, which may have contributed to the low variance in the Mean Decrease Impurity among the descriptors.

CONCLUSIONS

The quantitative and qualitative morphological traits and molecular markers identified wide genetic variability among the 20 Brazilian okra accessions, indicating successful crosses in breeding programs, and also isolated some materials which could indicate an interesting genetic variability to explore. UFU-07 appears to be an excellent genotype to be used in breeding programs, with low fruit pilosity and earliness. Molecular analysis also highlighted UFU-07 as a contrasting accession to others (UFU-01, UFU-05 and UFU-06). These other materials could have been highlighted because of another desirable trait (disease resistance, for example) that is difficult and highly costly to access in field experiments.

For the morphological traits, glandular trichomes and fruit width contributed the most to the genetic variability of the accessions. Morphological and molecular cluster analysis were complementary and helped in the genotype selection and should be use together to ensure the best selection. This will be helpful for breeders to plan breeding program, to understand species relationships and to establish core collections of *Abelmoschus* species.

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