



Acta Scientiarum. Agronomy
ISSN: 1807-8621
Editora da Universidade Estadual de Maringá -
EDUEM

Genetic diversity among Brazilian okra landraces detected by morphoagronomic and molecular descriptors

Massucato, Luana Rainieri; Nakamura, Karina Kazue; Ruas, Paulo Mauricio; Zeffa, Douglas Mariani; Silva, Derly José Henrique da; Gonçalves, Leandro Simões Azeredo

Genetic diversity among Brazilian okra landraces detected by morphoagronomic and molecular descriptors

Acta Scientiarum. Agronomy, vol. 42, 2020

Editora da Universidade Estadual de Maringá - EDUEM

Available in: <http://www.redalyc.org/articulo.oa?id=303062597009>

DOI: 10.4025/actasciagron.v42i1.43426

Genetic diversity among Brazilian okra landraces detected by morphoagronomic and molecular descriptors

Luana Rainieri Massucato¹

Universidade Estadual de Londrina, Brazil

ORCID: <http://orcid.org/0000-0003-2057-0943>

Karina Kazue Nakamura¹

Universidade Estadual de Londrina, Brazil

Paulo Mauricio Ruas²

Universidade Estadual de Londrina, Brazil

Douglas Mariani Zeffa³

Universidade Estadual de Maringá, Brazil

Derly José Henrique da Silva⁴

Universidade Federal de Viçosa, Brazil

Leandro Simões Azeredo Gonçalves^{1*}

Universidade Estadual de Londrina, Brazil

Acta Scientiarum. Agronomy, vol. 42,
2020

Editora da Universidade Estadual de
Maringá - EDUEM

Received: 22 June 2018
Accepted: 28 September 2018

DOI: 10.4025/actasciagron.v42i1.43426

CC BY

ABSTRACT. : The conservation of okra landraces [*Abelmoschus esculentus* (L.) Moench] in gene banks is essential for the success of their use in breeding programmes. This study evaluated the genetic diversity among okra landraces in Brazil based on morphoagronomic descriptors and AFLP markers. We studied 30 accessions of the vegetable gene bank of the *Universidade Federal de Viçosa*. To this end, 17 morphoagronomic descriptors and five combinations of AFLP primers were used. Genetic parameters were estimated for the quantitative traits and the accessions were grouped by Ward's method, using the Gower's and Jaccard's distance measures, respectively, for the morphoagronomic and molecular data. Polymorphisms were observed for all qualitative traits, while the quantitative traits were significant by deviance analysis. The genetic parameters confirmed the existence of variability among accessions, and high accuracy and heritability indices were found for the traits related to fruit and plant height. Ward's grouping showed no relationship between the clusters formed with the morphoagronomic and molecular data and the geographical origin of the accessions. No association between morphoagronomic descriptors and AFLP markers was observed. The lack of correlation suggests that both approaches of characterization are important to understand and differentiate the okra accessions.

Keywords: genetic variability, molecular markers, phenotype, bayesian analysis.

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is a popular vegetable in tropical and subtropical regions (Patil et al., 2015). In several countries in the world, it is considered a staple food for the population, with high socioeconomic and nutritional relevance (Hughes, 2009; Gemede, Ratta, Haki, Woldegiorgis, & Beyene, 2015; Kumar et al., 2017a). Okra fruits

and seeds are edible, and immature fruits are a rich source of dietary fibre, carbohydrates, vitamins, minerals, and antioxidant substances (Kamalesh, Subrata, Asraf, & Pranabesh, 2016; Petropoulos, Fernandes, Barros, & Ferreira, 2018). The seeds are rich in high-quality edible oil (13-22%) and proteins (20-24%), and the latter contain balanced amounts of two important amino acids, lysine and tryptophan (Hughes, 2009).

In Brazil, okra production represents a highly dynamic activity, mostly carried out in small agricultural areas by family farmers. This crop species is usually grown from seeds acquired from relatives or neighbours and conserved in the region over several generations. However, environmental changes (e.g., frequent drought, low soil fertility and occurrence of pests and diseases), consumer preference, urbanization, environmental exodus and abandonment of rural properties have resulted in a loss of genetic diversity of landraces in Brazil over the years (Moulin et al., 2012; Santos, Rodrigues, Gonçalves, Sudré, & Pereira, 2012). In this sense, the conservation of biodiversity in gene banks becomes highly relevant for the conservation of this variability.

In the late 1960s, the *Universidade Federal de Viçosa* (UFV) established a vegetable gene bank (BGH-UFV) that preserves collections from cities or regions of long-standing colonization and places where varieties are said to have been developed by farmers (Silva, Moura, & Casali, 2001). Moreover, BGH-UFV also exchanged germplasm with more than 100 countries (<http://www.bgh.ufv.br>). In this way, the characterization and evaluation of these accessions is essential for their conservation and successful use in breeding programmes.

Phenotypic descriptors, based on morphological and agricultural traits, were widely used in the characterization of okra gene banks (Reddy, Haribabu, Ganesh, & Chandrasekhar, 2012; Yonas, Garedew, & Debela, 2014; Asare, Asare-Bediako, Agyarko, Taah, & Osei, 2016; Ramgiry & Singh, 2017). However, although the genetic basis of okra is considered broad (Saifullah, Rabbani, & Garvey, 2010), the use of only phenotypic descriptors in studies of genetic diversity is restrictive, since phenotypes may be influenced by environmental factors or stages of plant development (Akash, Shiyab, & Saleh, 2013; Kumar et al., 2017b).

Molecular markers are seen as an important additional tool in germplasm characterization studies, since they are minimally influenced by environmental conditions or plant development factors (Schafleitner, Kumar, Lin, Hegde, & Ebert, 2013). In the case of okra, several molecular markers have been used for germplasm characterization, e.g., random amplified polymorphic DNA (RAPD; Martinello, Leal, Amaral Júnior, Pereira, & Daher, 2003), inter-simple sequence repeat (ISSR; Yuan et al., 2014), amplified fragment length polymorphism (AFLP; Kyriakopoulou et al., 2014), and simple sequence repeats (SSR; Kumar et al., 2017a). The AFLP markers, in spite of being dominant, have several advantages, such as broad genome coverage, cost-effectiveness, reproducibility, and independence of sequence information (Zhang, van Parijs, & Xiao, 2014).

The aim of this study was to evaluate the genetic diversity among okra landraces in Brazil by means of morphoagronomic descriptors and AFLP markers and to identify possible relationships between groups of accessions and their respective origins.

Material and methods

Plant material

A total of 30 okra accessions from the vegetable gene bank of the *Universidade Federal de Viçosa* (BGH-UFV) (Figure 1) were evaluated. Twenty-seven accessions were landraces from four regions of Brazil (North, Northeast, Southeast and Midwest) and nine Brazilian states (Amazonas, Bahia, Distrito Federal, Espírito Santo, Goiás, Minas Gerais, Pernambuco, Rio de Janeiro, and Sergipe), two accessions were from Turkey, and one was a commercial cultivar (cv. Santa Cruz 47).

Morphoagronomic characterization

The okra accessions were evaluated in an experimental area of the *Universidade Estadual de Londrina* (UEL) in Londrina, Paraná, Brazil (23° 19' 42" S, 51° 12' 11" W), from September to December 2016. The design consisted of randomized blocks with three replications. The plots consisted of two 4.0 m rows, spaced with 1 m between rows and 0.4 m between plants. Weeds were controlled by weeding, irrigation and spraying with insecticides and fungicides to control and prevent pests and diseases.

The morphoagronomic traits of the plants were characterized based on 17 descriptors established by the International Plant Genetic Resources Institute (IPGRI, 1991), currently named Biodiversity International, with 10 qualitative and seven quantitative descriptors. The following qualitative descriptors were evaluated: colour of the leaf internerve spaces (CLI), lobule incision (LI), leaf serration (LS), fruit colour (FC), fruit surface (FS), fruit apex shape (FAS), constriction of the basal part of the fruit (CBF), number of locules (NL), stem colour (SC), and branching degree (BD). The quantitative descriptors consisted of number of seeds per fruit (SN), fruit length (FL, in cm), fruit diameter (FD, cm), leaf length (LL, cm), leaf width (LW, cm), plant height (PH, in m), and mean weight of five fruits (FW, in g).

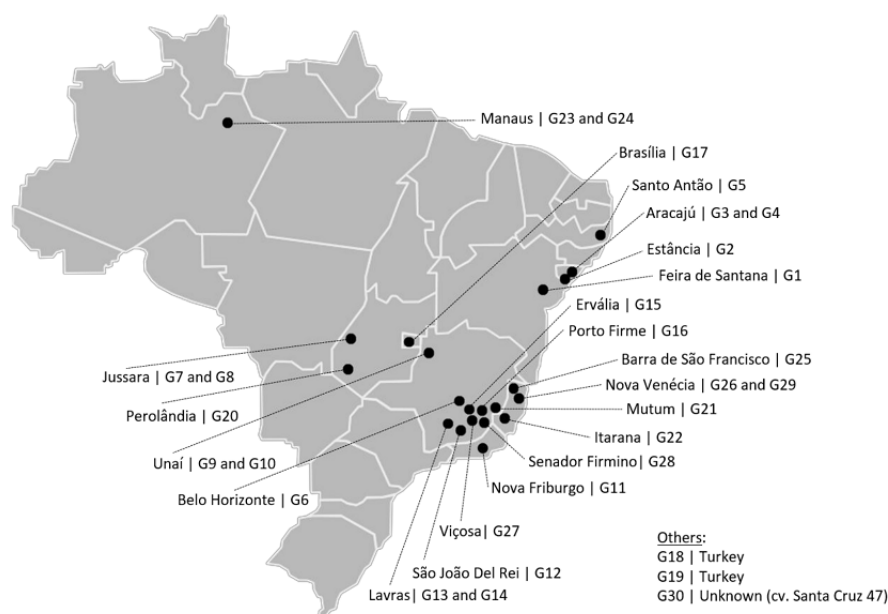


Figure 1
 Identification and origin of 30 okra accessions (*Abelmoschus esculentus* L.) from the vegetable gene bank of the Universidade Federal de Viçosa (BGH-UFV).

Molecular characterization

For the molecular analysis, the DNA was extracted individually from young leaves of five plants per accession. The samples were ground in crucibles with liquid nitrogen, followed by extraction according to the 2% CTAB method (cetyltrimethylammonium bromide, Sigma-Aldrich, Missouri-USA). The quality and integrity of the DNA was assessed by 1% agarose gel electrophoresis. The DNA concentration was estimated with a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, California-USA).

The AFLP technique was conducted according to the protocol proposed by Vos et al. (1995) with modifications. The DNA extracted from each of the five plants per accession was mixed proportionally. Approximately 700 ng of this DNA was double-digested with 1 U *Mse*I and 5 U *Eco*RI (Thermo Scientific, California-USA) and ligated to *Eco*RI (0.5 μ M) and *Mse*I (5 μ M) linkers in a reaction containing: T4 DNA ligase (2 U); 1X T4 DNA ligase buffer; NaCl (0.05 M); BSA (50 μ g μ L⁻¹); and completed with DTT (0.25 mM) to a final volume of 10 μ L. The programme established for the digestion-binding step consisted of: 37°C for 4 hour, 22°C for 1 hour and 70°C for 10 min. The digestion/binding pattern was visualized on a 1% agarose gel. Once digestion was confirmed, the amplified product was diluted 1:4 with ultrapure water.

Pre-selective amplification was performed using 3.5 μ L of the GoTaq[®] Green Master Mix (Promega, Winchester-USA), 0.58 μ L of the pre-selective primers *Eco*RI + A and *Mse*I + C (4.75 μ M), 3.0 μ L of the dilution of the restriction enzyme and ultra-pure water to bring the

volume to 10 μ L. Pre-selective amplification consisted of a programme of 1 cycle at 72°C for 2 min., 20 cycles at 94°C for 1 s, 56°C for 30 s and 72°C for 2 min., and one final cycle of 60°C for 30 min. Pre-selective PCR amplification was confirmed on a 2% agarose gel, and the amplified product was diluted 1:8 in ultrapure water. For selective amplification, an initial screening was performed with 24 combinations of selective *EcoRI*/*MseI* primers. The five most polymorphic combinations (*EcoRI*(FAM)/-ACC/*MseI*-CTAG, *EcoRI*(NED)-ACC/*MseI*-CAG, *EcoRI*(VIC)-ACC/*MseI*-CAC, *EcoRI*(PET)-ACT/*MseI*-CAAG, and *EcoRI*(VIC)-AGC/*MseI*-CTTC) were chosen for selective amplification.

The selective reactions were carried out in a volume of 10 μ L containing 3.5 μ L of master mix for PCR (GoTaq[®] Green Master Mix, Promega, Winchester, USA), 0.54 μ L of each primer for *MseI* (5 μ M) and *EcoRI* (1 μ M), 2.5 μ L of the diluted pre-amplification reaction and 2.92 μ L of ultrapure water. The amplification programme consisted of 1 cycle at 94°C for 2 min., 65°C for 30 s and 72°C for 2 min.; 8 cycles at 94°C for 1 s, 64°C for 30 s and 72°C for 2 min.; 23 cycles at 94°C for 1 s, 56°C for 30 s and 72°C for 2 min.; and 1 final cycle at 60°C for 30 min. The DNA fragments were resolved by capillary electrophoresis using the automated DNA sequencer model 3500xL (Applied Biosystems, California-USA). The results of the electrophoresed fragments were combined in a binary matrix by the software GeneMapper[®] v.4.1 (Applied Biosystems).

Data analysis

The quantitative traits were analysed by the restricted maximum likelihood (REML) and best linear unbiased predictor (BLUP) methods with the Selegen-REML/BLUP software (Resende, 2016). The predicted genotypic values of the quantitative traits were used for Pearson's correlation analyses and to determine the relative importance of the traits by the Singh's method (1981). The genetic distance matrix of the morphoagronomic data between the accessions was estimated by combined analysis of qualitative and quantitative traits obtained with Gower's distance (1971). Subsequently, Ward's cluster analysis was performed. The relative importance of the traits was calculated by the software Genes (Cruz, 2016), while the other analyses were performed with the R software (<http://www.r-project.org>) using the packages agricolae, corrgram and Nbclust.

For the molecular data, Jaccard's distance matrix was calculated and then Ward's clustering was used. The correlation between molecular and morphological distances was established using the Mantel test with 1000 permutations. These analyses were performed with the R software and the agricolae, ade4 and Nbclust packages. Based on the molecular data, Bayesian clustering was also performed using Structure V 2.3.4 software (Pritchard, Stephens, & Donnelly, 2000) based on the methods described by Evanno, Regnaut, and Goudet (2005) with 200,000 iterations (Monte Carlo Markov Chain) with a burn-in of 100,000 iterations assuming a mixed cluster or admixture model and correlated allele frequencies.

Values of k from 1 to 30 were tested, with 10 independent interactions for each k value. The k -number was determined using the Structure Harvester v0.6.92 program (Earl & von Holdt, 2012), and the bar plots were drawn by the Structure 2 program (Ramasamy, Ramasamy, Bindroo, & Naik, 2014).

Results and discussion

The morphoagronomic characterization of the okra accessions detected polymorphism for all evaluated traits (Table 1). In terms of fruit traits, 36.7% of the accessions had green fruits, 33.3% had a light green colour and 30% dark green. With regard to the fruit surface, 33.3% of the accessions had a flat surface, 50% were convex and 27.3% were concave. Most of the accessions had a very pointed fruit apex (56.7%), and only the accessions G19, G24, and G26 had a less pointed fruit apex. The presence or absence of basal constriction in the fruits was observed in 56.7 and 43.3% of the accessions, respectively. Fruits with five lobes were reported in 66.7% of the accessions, while the other fruits had more than five lobes.

For the leaf traits, 93.3% of the accessions showed light green internerval leaf colour and only the G28 and G25 had green and dark green colour, respectively. Regarding the leaf edge, the leaves of 20% of the accessions had weakly serrated leaf edges, 66.6% were moderately serrated, and only accessions G21, G26 and G29 had strongly serrated leaf edges. For the trait lobe incision, the incision of the accessions was grouped into medium, shallow and deep incisions (43.3, 10 and 46.7%, respectively). The accessions showed a weak, medium or strong degree of branching of the plants (26.7, 46.6 and 26.7%, respectively). A light green stem colour was predominant among the accessions (46.7%), followed by green and red (33.3 and 10.0%, respectively) stem colours.

Table 1

Morphoagronomic characterization based on 10 qualitative descriptors of 30 okra (*Abelmoschus esculentus* L.) accessions from the vegetable gene bank of the Universidade Federal de Viçosa (BGH-UFV).

Accessions	Morphoagronomic traits ^{1/}									
	CLI	LI	LS	FC	FS	FAS	CBF	NL	SC	BD
G1	Light green	Medium	Medium	Dark green	Flat	Pointed	Presence	> 5	Green	Medium
G2	Light green	Medium	Medium	Light green	Convex	Very pointed	Presence	5	Red	Medium
G3	Light green	Medium	Medium	Dark green	Flat	Very pointed	Presence	5	Green	Strong
G4	Light green	Shallow	Weak	Dark green	Concave	Pointed	Absence	5	Light red	Medium
G5	Light green	Medium	Weak	Dark green	Convex	Very pointed	Presence	5	Light green	Medium
G6	Light green	Medium	Medium	Dark green	Convex	Very pointed	Presence	5	Green	Strong
G7	Light green	Medium	Medium	Green	Convex	Very pointed	Presence	5	Light green	Medium
G8	Light green	Medium	Medium	Dark green	Concave	Very pointed	Absence	> 5	Green	Weak
G9	Light green	Deep	Medium	Light green	Concave	Very pointed	Absence	5	Red	Medium
G10	Light green	Deep	Medium	Dark green	Flat	Very pointed	Presence	5	Light green	Medium
G11	Light green	Medium	Medium	Green	Convex	Very pointed	Absence	5	Green	Medium
G12	Light green	Deep	Medium	Light green	Convex	Very pointed	Presence	5	Light red	Medium
G13	Light green	Medium	Weak	Dark green	Concave	Pointed	Absence	5	Green	Medium
G14	Light green	Medium	Weak	Light green	Convex	Pointed	Presence	5	Light green	Strong
G15	Light green	Medium	Medium	Light green	Convex	Very pointed	Absence	> 5	Light green	Medium
G16	Light green	Deep	Medium	Green	Convex	Very pointed	Presence	5	Light green	Weak
G17	Light green	Deep	Weak	Green	Convex	Very pointed	Presence	5	Light green	Strong
G18	Light green	Medium	Medium	Green	Flat	Very pointed	Absence	5	Light green	Medium
G19	Light green	Shallow	Medium	Green	Concave	Very pointed	Absence	> 5	Green	Strong
G20	Light green	Deep	Medium	Light green	Convex	Very pointed	Presence	> 5	Light green	Weak
G21	Light green	Deep	Strong	Light green	Convex	Pointed	Absence	> 5	Light green	Medium
G22	Light green	Deep	Weak	Dark green	Flat	Pointed	Presence	5	Light red	Weak
G23	Light green	Deep	Medium	Light green	Flat	Very pointed	Absence	5	Light green	Weak
G24	Light green	Medium	Weak	Light green	Convex	Less pointed	Absence	> 5	Light green	Weak
G25	Dark green	Shallow	Medium	Light green	Flat	Pointed	Presence	> 5	Red	Weak
G26	Light green	Deep	Strong	Green	Flat	Less pointed	Absence	5	Green	Strong
G27	Light green	Deep	Medium	Green	Flat	Pointed	Absence	5	Light green	Medium
G28	Green	Deep	Medium	Green	Flat	Pointed	Presence	> 5	Light red	Weak
G29	Light green	Deep	Strong	Green	Convex	Pointed	Presence	> 5	Green	Strong
G30	Light green	Deep	Medium	Green	Convex	Very pointed	Presence	5	Green	Strong

1/CLI: leaf color internerve spaces; LI: lobule incision; LS: leaf serration; FC: fruit color; FS: fruit surface; FAS: fruit apex shape; CBF: constriction of the basal fruit part; NL: number of locules; SC: stem color; and BD: branching degree.

This variability observed among the accessions may be related to the wide geographical distribution, since most of these accessions are from nine Brazilian states and two are from Turkey. In Brazil, okra is a widely cultivated and consumed vegetable that was introduced by slaves. This crop is grown by small farmers who usually store some of the seeds to sow the next crop, generation after generation, while the rest is sold and/or exchanged with other farmers in the region, indicating the likely influence of farmers on the dispersal of genetic variability.

For the quantitative morphoagronomic descriptors, significance ($p < 0.05$) was detected by the deviance analysis for all traits evaluated (Table 2). The SN ranged from 64.22 to 73.07 seeds, while FL and FD ranged from 13.94 to 17.12 cm and 2.36 to 2.94 cm, respectively. Regarding the FW, values ranging from 23.49 to 30.51 g were observed. The accessions G10, G12 and G16 had the heaviest fruits, and G9, G28, and G6 the lightest. The LL and LW ranged from 21.92 to 22.25 cm and 28.74 to 29.90 cm, respectively, while PH ranged from 1.85 to 2.8 m.

For most traits, broad-sense heritability (h^2_g) and accuracy of genotype selection (AS_g) were high, except for LL and LW with low h^2_g (0.17 and 0.28, respectively) and moderate AS_g (0.41 and 0.53, respectively).

AS_g is a highly relevant parameter in the evaluation of experimental quality, since not only the number of replications and the environmental variation are considered but also the relationship between the genetic and residual variations; it is therefore considered the most important parameter with regard to selective evaluation (Henderson, 1984). In this context, fruit-related traits (FL, FD and FW) and PH, with high values of accuracy, also obtained high h^2_g values (Table 2). The parameter h^2_g is essential in breeding programmes for measuring the phenotypic variation caused by genetic factors, i.e., it reflects the proportion of the inherited phenotypic variance (Falconer & Mackay, 1996). Studying genetic parameters in okra, Das, Chattopadhyay, Chattopadhyay, Dutta, and Hazra (2012) reported high h^2_g values for all studied traits, varying between 0.80 and 0.98, in agreement with the results obtained in this study for the SN, PH, FL, FD, and FW traits.

The correlation analysis did not detect linear correlations between most of the evaluated traits (Figure 2). Positive correlations were only observed between the LL and LW traits ($r = 0.73$), while negative correlations were found for FL \times LL ($r = -0.48$). This lack of correlation between most of the evaluated traits disagrees with the findings reported in the literature, mainly for the fruit-related traits. In a study on genetic (r_g) and phenotypic correlations (r_p) between the main agricultural traits of okra, Rashwan (2011) reported significant and positive correlations for FW \times FL (r_g and $r_p = 0.54$) and negative correlations for FW \times FD ($r_g = -0.91$ and $r_p = -0.90$) and FL \times FD ($r_g = -0.82$ and $r_p = -0.81$). On the other hand, Ibrahim, Abed, and Moghazy (2013) reported significant and positive correlations for FL \times FD ($r_g = 0.61$ and $r_p = 0.30$).

Table 2

Estimation of the variance components (REML) and genetic parameters of seven quantitative descriptors of 30 okra accessions (*Abelmoschus esculentus* L.) from the vegetable gene bank of the *Universidade Federal de Viçosa* (BGH-UFV).

Parameters	Morphoagronomic traits ^{1/}						
	SN	FL	FD	LL	LW	PH	FW
Deviance	453.76*	218.70*	238.25*	274.52*	320.87*	241.44*	420.14*
σ^2_g	38.49	5.27	0.12	0.50	1.53	0.07	21.64
σ^2_e	37.77	1.93	0.02	7.20	11.65	0.04	8.75
σ^2_p	76.26	7.20	0.14	7.70	13.18	0.11	30.39
h^2	0.75	0.89	0.93	0.17	0.28	0.82	0.88
AS_g	0.87	0.94	0.96	0.41	0.53	0.90	0.93
CV _g (%)	9.58	16.29	16.50	3.24	4.26	15.39	19.83
CV _e (%)	9.49	9.86	7.63	12.29	11.75	12.61	12.60
Mean	64.72	14.09	2.13	21.83	29.04	1.66	23.45

1/SN: seed number, FL: fruit length, FD: fruit diameter, LL: leaf length, LW: leaf width, PH: plant height, and FW: fruit weight. 2/ σ^2_g : genotypic variance; σ^2_e : residual variance; σ^2_p : individual phenotypic variance; h^2_g : broad-sense heritability; AS_g : accuracy of selection of genotypes; CV_g: coefficient of genotype variation; and CV_e: coefficient of residual variation. *significant by the Chi-square test with 1 degree of freedom, at 5% probability.

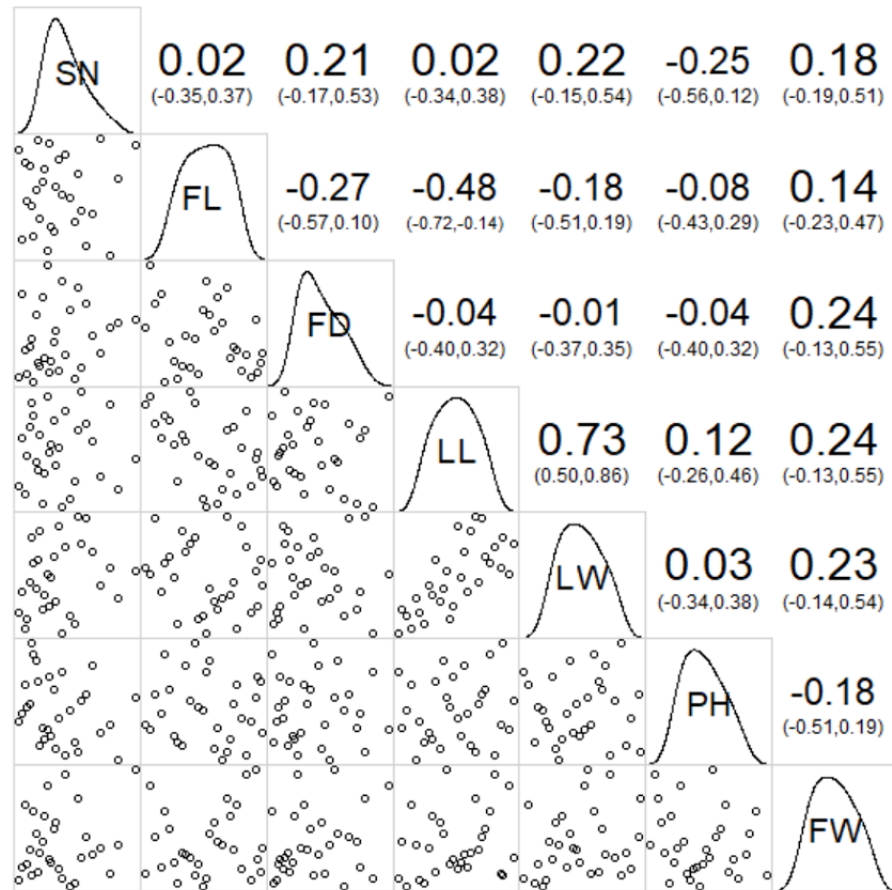


Figure 2

Estimation of genotypic correlation coefficients with their respective confidence intervals (95%) among seven quantitative morphoagronomic descriptors in 30 okra accessions (*Abelmoschus esculentus* L.) from the vegetable gene bank of the *Universidade Federal de Viçosa* (BGH-UFV). SN: seed number per fruit; FL: fruit length; FD: fruit diameter; LL: leaf length; LW: leaf width; PH: plant height; and FW: fruit weight.

The analysis of Singh (1981) indicated that all traits contributed moderately to the discrimination of accessions since the values of relative importance ranged from 11.84 (SN) to 16.72% (FL) (Figure 3). By Ward's method, three distinct groups were formed which could not be related to the geographical origin of the accessions (Figure 4a). The disagreement of groupings based on morphoagronomic descriptors and the geographical origin of the accessions was also reported by Gulsen, Karagul, and Abak (2007) and Kyriakopoulou et al. (2014). On the other hand, in an evaluation of the phenotypic diversity of 11 Turkish okra landraces based on 21 morphoagronomic traits, Düzyaman (2005) observed the formation of four distinct groups related to the geographical distribution of the accessions.

Group I consisted of nine accessions (G4, G8, G9, G10, G12, G16, G22, G25, and G28) characterized by heavier fruits with a higher seed number. For the qualitative traits, this group had predominantly dark green fruits with a concave surface and presence of constriction of the basal part of the fruit. Eleven accessions (G1, G2, G3, G5, G7, G7, G14,

G15, G17, G20, and G30) constituted group II which had common traits of medium LI and convex FS. The quantitative traits indicated longer fruits with smaller diameters and shorter PH. Group III comprised 10 accessions (G11, G13, G18, G19, G21, G23, G24, G26, G27, and G29) with low FL, high LW and LL, and no CBF.

Amplified fragment length polymorphism markers proved efficient in detecting genetic variability among okra accessions. The five EcoRI/MseI primer combinations used (E-ACC/M-CTAG, E-ACC/M-CAG, E-ACC/M-CAC, E-ACT/M-CAAG, and E-AGC/M-CTTC) generated a total of 688 markers, of which 534 (77.61%) were polymorphic. These results were superior to those obtained by Kyriakopoulou et al. (2014) who investigated the genetic diversity of 50 okra landraces in Greece based on 33 AFLP primer combinations and found a total of 1,438 amplified bands, of which only 175 were polymorphic (12.17%). Similarly, Akash et al. (2013) evaluated the genetic divergence among 22 okra accessions with AFLP markers and amplified 722 bands, of which 227 were polymorphic (31.6%). Among the primers used, the E-ACC/M-CAG combination identified the highest number of polymorphisms (164 bands), while E-ACC/M-CAC detected the fewest polymorphisms (49 bands). The combinations of E-ACC/M-CTAG, E-ACT/M-CAAG and E-AGC/M-CTTC generated 58, 117 and 146 polymorphic markers, respectively.

The genetic dissimilarity among okra accessions ranged from 0.20 to 0.51 with a mean distance of 0.32 (± 0.09). The shortest distance was observed between accessions G28 and G29 derived from the municipalities of Senador Firmino and Nova Venécia (states Minas Gerais and Espírito Santo, respectively). The greatest distance was observed between G14 (Lavras, state Minas Gerais) and G25 (Barra de São Francisco, state Espírito Santo).

By Ward's method and based on the Jaccard index, two groups were formed which could not be related to their accessions' geographical origins (Figure 4b). Group I was constituted of 19 accessions from the states of Minas Gerais, Espírito Santo, Goiás, Sergipe, Amazonas, Bahia, and Rio de Janeiro, and one accession from Turkey and cv. Santa Cruz (Figure 1). Group II also contained accessions from different Brazilian states (states Minas Gerais, Sergipe, Pernambuco, Distrito Federal, and Amazonas) and one accession from Turkey. The absence of an association between the geographical origin and the molecular markers can be attributed to the practice of seed exchange among farmers and non-restricted fruit transport between the different regions of Brazil. Evaluating the genetic diversity among *Capsicum chinense* accessions from different regions of Brazil, Baba et al. (2016) also observed a lack of relation among the groups formed and the geographical origin of the accessions, indicating a wide seed dispersion.

The two groups formed by the molecular data identified wide variability in the morphoagronomic traits and no correlations between the distance matrices of the morphoagronomic and molecular data. These results indicate that both approaches of characterization are

important for a clearer differentiation among okra accessions. Several studies of characterization of *A. esculentus* gene banks have indicated the importance of morphoagronomic and molecular characterization for a better understanding of the variability (Gulsen et al., 2007; Kyriakopoulou et al., 2014; Yıldız, Ekbiç, Düzyaman, Serçe, & Abak, 2016).

The simulations performed by the ΔK value method (Evanno et al., 2005) also clustered into two groups (Figure 5). Group I (red) consisted of 19 accessions and group II (green) of 10 accessions. The landrace G24 was classified as an admixture since the coefficient of adhesion was lower than 0.6, allowing the inclusion of common loci from both groups. The groups formed by Bayesian analysis were consistent with the groups formed by Ward's clustering, with the exception of accessions G4 and G19 which were allocated in different groups by the two methodologies.

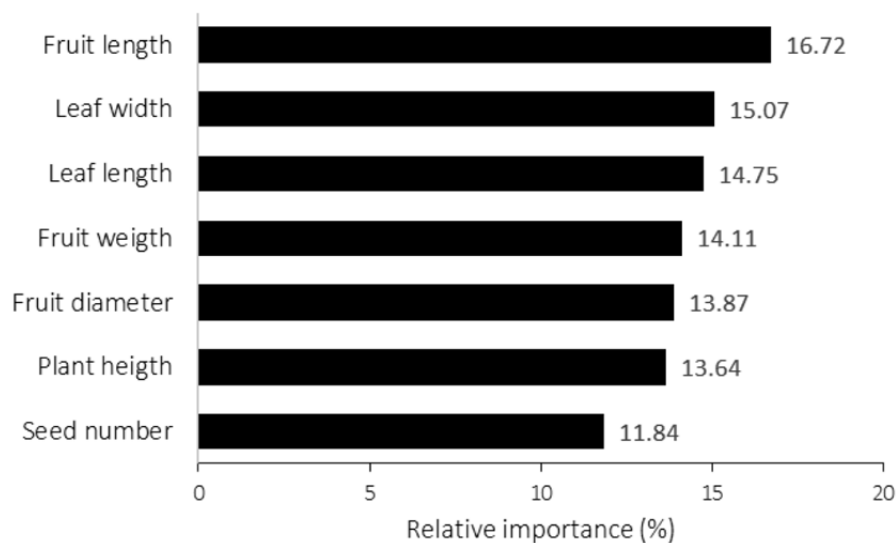


Figure 3

Relative importance of seven quantitative morphoagronomic descriptors by the Singh's method (1981) in 30 okra (*Abelmoschus esculentus* L.) accessions from the vegetable genebank of the *Universidade Federal de Viçosa* (BGH-UFV).

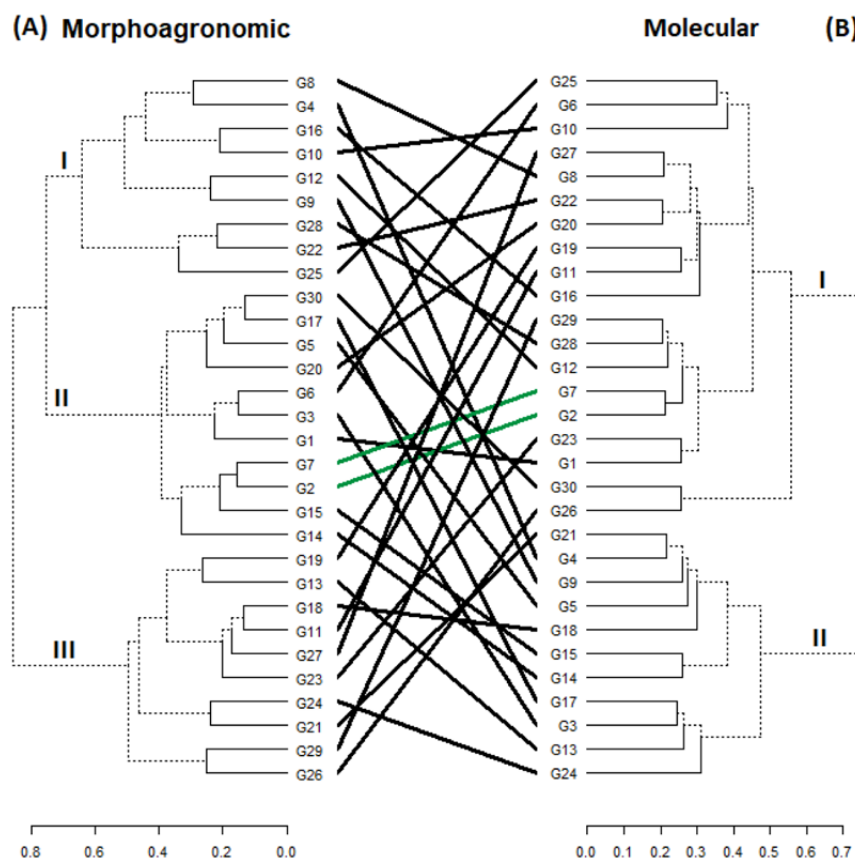


Figure 4
Dissimilarity dendrogram of 30 okra (*Abelmoschus esculentus* L.) accessions established by the Ward's method, based on the distances of Gower (morphoagronomic) (A) and Jaccard (molecular) (B).

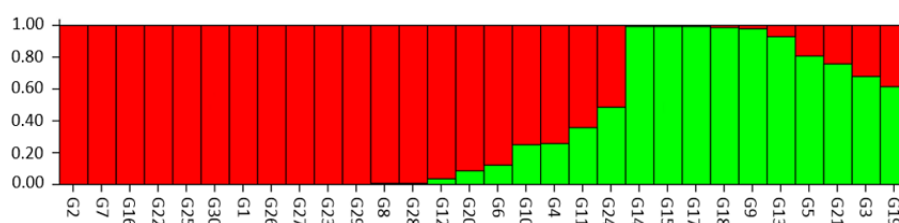


Figure 5
Assignment of 30 okra accessions by bar plots, based on five AFLP primer combinations (E-ACC/M-CTAG, E-ACC/M-CAG, E-ACC/M-CAC, E-ACT/M-CAAG, and E-AGC/M-CTTC). The two colors represent different clusters. The y-axis indicates the estimated membership percentage of each accession in a determined cluster.

Conclusion

The morphoagronomic traits and AFLP markers identified wide genetic variability among the 30 okra accessions, indicating a successful exploitation in breeding programmes. Cluster analysis was ineffective at detecting relationships between the groups formed with the morphoagronomic and molecular data and the geographical origin of

the accessions. No association was detected between morphoagronomic descriptors and AFLP markers.

References

- Akash, M. W., Shiyab, S. M., & Saleh, M. I. (2013). Yield and AFLP analyses of inter-landrace variability in okra (*Abelmoschus esculentus* L.). *Life Science Journal*, 10(2), 2771-2779.
- Asare, A. T., Asare-Bediako, E., Agyarko, F., Taah, K., & Osei, E. O. (2016). Phenotypic traits detect genetic variability in Okra (*Abelmoschus esculentus* L. Moench). *African Journal of Agricultural Research*, 11(33), 3169-3177. DOI: 10.5897/AJAR2016.11160
- Baba, V. Y., Rocha, K. R., Gomes, G. P., Ruas, C. F., Ruas, P. M., Rodrigues, R., & Gonçalves, L. S. A. (2016). Genetic diversity of *Capsicum chinense* accessions based on fruit morphological characterization and AFLP markers. *Genetic Resources and Crop Evolution*, 63(8), 1371-1381. DOI: 10.1007/s10722-015-0325-4
- Cruz, C. D. (2016). Genes Software-extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum. Agronomy*, 38(4), 547-552. DOI: 10.4025/actasciagron.v38i4.32629
- Das, S., Chattopadhyay, A., Chattopadhyay, S. B., Dutta, S., & Hazra, P. (2012). Genetic parameters and path analysis of yield and its components in okra at different sowing dates in the Gangetic plains of eastern India. *African Journal of Biotechnology*, 11(95), 16132-16141. DOI: 10.5897/AJB12.545
- Düzyaman, E. (2005). Phenotypic diversity within a collection of distinct okra (*Abelmoschus esculentus*) cultivars derived from Turkish land races. *Genetic Resources and Crop Evolution*, 52(8), 1019-1030. DOI: 10.1007/s10722-004-6118-9
- Earl, D. A., & von Holdt, B. M. (2012). Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359-361. DOI: 10.1007/s12686-011-9548-7
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14(8), 2611-2620. DOI: 10.1111/j.1365-294X.2005.02553.x
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*. Edinburgh, GB: Addison Wesley Longman.
- Gemedie, H. F., Ratta, N., Haki, G. D., Woldegiorgis, A. Z., & Beyene, F. (2015). Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): a review. *Journal Food Process Technology*, 6(458), 1-6. DOI: 10.4172/2157-7110.1000458
- Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 27(4), 857-871. DOI: 10.2307/2528823
- Gulsen, O., Karagul, S., & Abak, K. (2007). Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biologia*, 62(1), 41-45. DOI: 10.2478/s11756-007-0010-y

- Henderson, C. R. (1984). *Applications of linear models in animal breeding*. Guelph, CA: University of Guelph Google Scholar.
- Hughes, J. (2009). Just famine foods? What contributions can underutilized plants make to food security? *Acta Horticulturae*, 806(1), 39-48. DOI: 10.17660/ActaHortic.2009.806.2
- Ibrahim, E. A. A., Abed, M. Y., & Moghazy, A. M. (2013). Genetic behavior of families selected from some local okra (*Abelmoschus esculentus* L. Moench) populations in Egypt. *Plant Breeding and Biotechnology*, 1(4), 396-405. DOI: 10.9787/PBB.2013.1.4.396
- International Plant Genetic Resources Institute [IPGRI]. (1991). *Okra descriptor, diversity for development*. Rome, IT: International Plant Genetic Resource Institute.
- Kamalesh, P., Subrata, D., Asraf, A. K., & Pranabesh, C. (2016). Phytochemical investigation and hypoglycaemic effect of *Abelmoschus esculentus*. *Research Journal of Pharmacy and Technology*, 9(2), 162-164. DOI: 10.5958/0974-360X.2016.00028.7
- Kumar, M., Sharma, V. R., Kumar, N., Sirohi, U., Naresh, R. K., & Chaudhary, V. (2017b). Screening of microsatellite markers for genetic diversity assessment and conservation of germplasm in okra (*Abelmoschus esculentus* L. Moench). *International Journal of Current Microbiology and Applied Sciences*, 6(6), 509-520. DOI: 10.20546/ijcmas.2017.606.060
- Kumar, S., Parekh, M. J., Fougat, R. S., Patel, S. K., Patel, C. B., Kumar, M., & Patel, B. R. (2017a). Assessment of genetic diversity among okra genotypes using SSR markers. *Journal of Plant Biochemistry and Biotechnology*, 26(2), 172-178. DOI: 10.1007/s13562-016-0378-2
- Kyriakopoulou, O. G., Arens, P., Pelgrom, K. T., Karapanos, I., Bebeli, P., & Passam, H. C. (2014). Genetic and morphological diversity of okra (*Abelmoschus esculentus* [L.] Moench.) genotypes and their possible relationships, with particular reference to Greek landraces. *Scientia Horticulturae*, 171(1), 58-70. DOI: 10.1016/j.scienta.2014.03.029
- Martinello, G. E., Leal, N. R., Amaral Júnior, A. T. D., Pereira, M. G., & Daher, R. F. (2003). Genetic diversity in okra using RAPD markers. *Horticultura Brasileira*, 21(1), 20-25. DOI: 10.1590/S0102-05362003000100004
- Moulin, M. M., Rodrigues, R., Gonçalves, L. S., Sudré, C. P., Santos, M. H., & Silva, J. R. P. (2012). Collection and morphological characterization of sweet potato landraces in north of Rio de Janeiro state. *Horticultura Brasileira*, 30(2), 286-292. DOI: 10.1590/S0102-05362012000200017
- Patil, P., Sutar, S., Joseph, J. K., Malik, S., Rao, S., Yadav, S., & Bhat, K. V. (2015). A systematic review of the genus *Abelmoschus* (Malvaceae). *Rheedea*, 25(1), 14-30.
- Petropoulos, S., Fernandes, Â., Barros, L., & Ferreira, I. C. (2018). Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage. *Food Chemistry*, 242, 466-474. DOI: 10.1016/j.foodchem.2017.09.082
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959. DOI: 10.1111/j.1471-8286.2007.01758.x
- Ramasamy, R. K., Ramasamy, S., Bindroo, B. B., & Naik, V. G. (2014). Structure plot: a program for drawing elegant structure bar plots in user friendly interface. *Springer Plus*, 3(1), 431. DOI: 10.1186/2193-1801-3-431

- Ramgiry, M., & Singh, S. (2017). Genetic divergence analysis in okra (*Abelmoschus esculentus* (L.) Moench). *International Journal of Pure & Applied Bioscience*, 5(2), 981-986. DOI: 10.18782/2320-7051.2753
- Rashwan, A. M. A. (2011). Study of genotypic and phenotypic correlation for some agro-economic traits in okra (*Abelmoschus esculentus* (L.) Moench). *Asian Journal of Crop Science*, 3(2), 85-91. DOI: 10.3923/ajcs.2011.85.91
- Reddy, M. T., Haribabu, K., Ganesh, M., & Chandrasekhar, K. (2012). Genetic divergence analysis of indigenous and exotic collections of okra (*Abelmoschus esculentus* (L.) Moench). *Journal of Agricultural Technology*, 8(2), 611-623.
- Resende, M. D. V. D. (2016). Software Selegen-REML/BLUP: a useful tool for plant breeding. *Crop Breeding and Applied Biotechnology*, 16(4), 330-339. DOI: 10.1590/1984-70332016v16n4a49
- Saifullah, M., Rabbani, M. G., & Garvey, E. J. (2010). Estimation of genetic diversity of okra (*Abelmoschus esculentus* L. Moench) using RAPD markers. *SAARC Journal of Agriculture*, 8(2), 19-28. DOI: 10.5829/idosi.ajaes.2014.14.02.12289
- Santos, M. H. D., Rodrigues, R., Gonçalves, L. S. A., Sudré, C. P., & Pereira, M. G. (2012). Agrobiodiversity in Cucurbita spp. landraces collected in Rio de Janeiro assessed by molecular markers. *Crop Breeding and Applied Biotechnology*, 12(2), 96-103. DOI: 10.1590/S1984-70332012000200001
- Schafleitner, R., Kumar, S., Lin, C. Y., Hegde, S. G., & Ebert, A. (2013). The okra (*Abelmoschus esculentus*) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene*, 517(1), 27-36. DOI: 10.1016/j.gene.2012.12.098
- 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. *Horticultura Brasileira*, 19(2), 108-114. DOI: 10.1590/S0102-05362001000200002
- Singh, D. (1981). The relative importance of characters affecting genetic divergence. *Indian Journal of Genetics and Plant Breeding*, 41(2), 237-245.
- Vos, P., Hogers, R., Bleeker, M., Reijmans, M., Lee, T. V. D., Hornes, M., ... Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407-4414. DOI: 10.1093/nar/23.21.4407
- Yıldız, M., Ekbiç, E., Düzyaman, E., Serçe, S., & Abak, K. (2016). Genetic and phenotypic variation of Turkish Okra (*Abelmoschus esculentus*). *Journal of Plant Biochemistry and Biotechnology*, 25(3), 234-244. DOI: 10.1007/s13562-015-0330-x
- Yonas, M., Garedew, W., & Debela, A. (2014). Multivariate analysis among okra (*Abelmoschus esculentus* (L.) Moench) collection in South Western Ethiopia. *Journal of Plant Sciences*, 9(2), 43-50. DOI: 10.3923/jps.2014.43.50
- Yuan, C. Y., Zhang, C., Wang, P., Hu, S., Chang, H. P., Xiao, W. J., ... Guo, X. H. (2014). Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers. *Genetics and Molecular Research*, 13(2), 3165-3175. DOI: 10.4238/2014.April.25.1
- Zhang, Z., van Parijs, F. R., & Xiao, B. (2014). The status of AFLP in the genomics era and a pipeline for converting AFLPs into single-

locus markers. *Molecular Breeding*, 34(3), 1245-1260. DOI: 10.1007/s11032-014-0113-4

Author notes

*

Author for correspondence. E-mail: leandrosag@uel.br