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Scientific Article

Variability in germplasm of sponge gourd by morphological and molecular descriptors

Variabilidade em germoplasma de bucha vegetal por descritores morfológicos e molecular

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ABSTRACT - *Luffa cylindrica*, known as sponge gourd, stands out for its potential for use in several cosmetic, domestic and aircraft upholstery segments stands out. However, each segment requires specificity, so that genetic variability of the species can become a limiting factor in its exploitation. Thus, this study aimed to morphologically characterize 13 accessions and molecularly characterize 24 accessions of sponge gourd belonging to the Cucurbit Germplasm Collection of the Center for Agricultural Sciences of the Federal Rural University of the Semi-Arid Region. A field experiment was installed.. At ripening, were evaluated for 15 descriptors. For molecular characterization, ISSR primers were used. The data obtained with the morphological and molecular characterization separated the accessions into different cluster configurations, denoting wide genetic variability. Considering the morphological descriptors, the medial circumference of the fruit (26.7%) had the most significant contribution to the divergence found. Accessions A12, A19, A23 and A24 are indicated for the dermo-cosmetic sector, while accessions A19 and A23 are the most indicated for the vegetable sponge market since light fibers with soft texture are indicated for the cosmetic and cleaning sector. For the molecular data, 103 bands were amplified, 80% polymorphic, and the polymorphic information content showed an average of 0.28, classifying them as moderately informative. The accessions were considered distinct, which indicates that the cucurbit germplasm collection contains loofah genotypes with significant genetic diversity.

Keywords: *Luffa cylindrica* (L.) Roem. Genetics. Breeding. Potentialities.

RESUMO - *Luffa cylindrica*, conhecida como bucha vegetal, destaca por seu potencial de utilização em segmentos como: uso cosmético, doméstico e estofados de aeronaves entre outros. Entretanto, cada segmento exige uma especificidade, de forma que a variabilidade genética da espécie pode se tornar fator limitante a sua exploração. Assim, o objetivo do presente trabalho foi caracterizar morfologicamente 13 acessos e molecularmente 24 acessos de bucha vegetal pertencentes a Coleção de Germoplasma de Cucurbitáceas do Centro de Ciências Agrárias da Universidade Federal Rural do Semi-Árido. Para caracterização morfológica foi instalado um experimento em campo Quando da maturação, os frutos foram avaliados para 15 descritores. Para caracterização molecular, foram utilizados *primers* ISSR. Os dados obtidos com a caracterização morfológica e molecular conseguiram separar os acessos em diferentes configurações de agrupamento, denotando ampla variabilidade genética. Considerando os descritores morfológicos, a circunferência medial do fruto (26,7%) apresentou maior contribuição na divergência encontrada. Os acessos A12, A19 A23 e A24 são indicados para o setor dermocosmético, já os acessos A19 e A23 são os mais indicados para o mercado de esponja vegetal, visto que fibras claras com textura macia são indicadas ao setor cosmético e de limpeza. Para os dados moleculares, foram amplificadas 103 bandas, 80% polimórficas, e o conteúdo de informação polimórfica apresentou uma média de 0,28, classificando-os como medianamente informativos. Os acessos foram considerados distintos, o que indica que a coleção de germoplasma de cucurbitácea detém genótipos de bucha vegetal com expressiva diversidade genética.

Palavras-chave: *Luffa cylindrica* (L.) Roem. Genética. Melhoramento. Potencialidades.

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INTRODUCTION

Luffa cylindrica (L.) M.Roem., known as sponge gourd, belongs to the cucurbit family. Its origin is pointed out as the tropical regions of Asia and it has dispersed in several territories. It is an allogamous, domesticated species, widely distributed in the tropics and subtropics as a cultivated and naturalized plant (AL-SNAFI, 2019).

Sponge gourd fruits, of the berry type, when dried, due to their fibrous composition, are widely used in the industry as a cleaning sponge, whether for body or domestic use (BLIND, 2016; BLIND et al., 2018). Considered natural, biodegradable, and non-toxic when treated, it requires low production costs compared to other materials (SIQUEIRA; BRAS; DUFRESNE, 2010; SILVA; RANIL; FONSEKA, 2012; AZEEZ; BELLO; ADEDEJI, 2013).

Although the greatest visibility for the species is given about the various uses for its fruit (PAULA et al. 2024; COSTA, 2018; BLIND, 2016; BLIND et al., 2018), the species has also been reported as a source of resistance to various plant pathogens and can be used in various direct works for the development of pure lines resistant to pathogens that may affect the species or indirect works, such as the development of rootstocks for species of economic importance that belong to the same family as sponge gourd (GALATTI et al., 2013; ITO et al., 2014).

Despite its significant commercial importance, one of the limitations of exploring new uses and potentialities of the loofah, whether from the natural fiber of the fruit or the plant, is the variability shown by the species. Although there are reports in the scientific literature and popular culture about its use, variability is observed even in commercial crops for the most diverse characteristics of interest (FERREIRA et al., 2010; MEDEIROS et al., 2015).

Thus, it is necessary to know and measure this variability so crop improvement programs can direct the selections to meet the various market niches the crop can occupy. On the other hand, it is worth noting that characterization works can also identify uniform genotypes for traits of interest, enabling their immediate use and adding value to the crop due to the uniformity of the final product. In this case, in addition to the economic increase to the production chain, it assumes social importance since family farmers are the ones who predominantly cultivate loofah (EIRA AGUIAR et al., 2014).

Given the above, it is important to highlight that different methodologies can measure a species' genetic variability, and the researcher is responsible for determining the one that best meets its needs. Morphological characterization, although exposing the evaluated characteristics to the environment, allows the visualization of the expression of the phenotype of the individuals in this environment. The environment does not influence the molecular characterization; however, depending on the marker used, it does not allow us to know the characteristics responsible for the observed variability.

By associating morphological characterization with molecular characterization, it is possible to obtain knowledge about the genetic variability of a species to support the choice of individuals or populations with desirable characteristics for a given purpose (GHALI et al., 2011). Thus, the objective of the present work was to perform morphological and molecular characterization and to analyze the genetic variability of sponge gourd accessions through morphological and molecular descriptors.

MATERIAL AND METHODS

A total of 25 accessions from the cucurbit germplasm collection of the Department of Agronomic and Forestry Sciences, Center for Agrarian Sciences, Federal Rural University of the Semi-Arid Region, Mossoró, Brazil, were characterized. Morphological characterization was carried out in 13 accessions from 06/21 to 11/16/2023. Molecular characterization was performed from 01/18/2023 to 12/19/2023 in 24 accessions, as shown in Table 1. Only A24 was not molecularly characterized among the morphologically characterized accessions.

For the morphological characterization, 13 accessions were used (A1, A2, A12, A18, A19, A20, A21, A22, A23, A24, A25, A31 and A32). Twenty seeds from each accession were sown in polyethylene trays filled with substrate to produce vegetable seedlings. After seedling emergence, when they had two expanded leaves, they were transplanted to the field at a spacing of 2.00 m between rows and 4.20 m between plants. The cultivation was conducted in a trellis system until the fruits matured. Drip irrigation was performed once a day. The cultural practices carried out were weeding and directing of branches. No fertilization was performed.

During the fruiting period, the fruits were protected with polyethylene nets until harvest. Protecting the fruits is necessary to prevent the loss of seeds since some fruits tend to release the skin at the end of the flower scar. Harvest was carried out when the fruits were dry and with brittle skin; these fruits were sent to the Plant Genetic Resources Laboratory of the Center for Plant Research of the Semi-Arid Region, where the descriptors listed below were measured: fruit type (FT), fruit peel (FP), apical fruit shape (AFS), basal fruit shape (BFS), fruit shape (FS), natural fiber color (NFC), fiber softness (FS), fruit fiber dry mass (FM), medial diameter of the fruit (MDF), basal circumference (BC), medial circumference (MC), apical circumference (AP), minimum fiber thickness (MFT), maximum fiber thickness (MXFT), fruit length (FL) (Table 2) (JOSHI et al. 2004; FERREIRA et al. 2010).

The qualitative descriptors were classified through visual analysis (BLIND, 2016; JOSHI et al., 2004). For the quantitative descriptors, a caliper and a tape measure were used to measure the circumference, diameter, thickness and length, expressed in centimeters (cm). In contrast, a digital scale was used to determine fruit fiber mass, expressed in grams (g). These were measured after removing seeds and husks.

Molecular characterization was performed in 24 accessions. The accessions were sown in trays as described for the accessions used for morphological characterization. Considering the impossibility of extracting the DNA from all plants simultaneously, when the seedlings had two expanded leaves, they were transplanted to polyethylene pots with a capacity of 1.0 liter, filled with a substrate for producing vegetable seedlings. After transplanting, one plant per pot was cultivated. These pots were kept in a greenhouse until DNA extraction was carried out according to the methodology described by Doyle and Doyle (1990) with modifications based on Faleiro et al. (2002). DNA was extracted from 10 plants from each accession, and the extraction was carried out individually, always using the youngest leaves. DNA was quantified and revealed by electrophoresis in 1% agarose gel stained with ethidium bromide, visualized in a photodocumenter (Proteinsimple, Alphaimager HP) in the presence of UV light (DOYLE; DOYLE, 1990).

Table 1. Accessions of sponge gourd (*Luffa cylindrica*) preserved in cold chamber and characterized morphologically and molecularly.

Accessions[1]	Collection date	Collection location
A1	-[2]	Mossoró/RN
A2	Dec/2015	Mossoró/RN
A5	Nov/2012	Governador Dix-Sept Rosado/RN
A6	-[2]	Mossoró/RN
A7	Feb/2012	Petrolina/PE
A8	Sep/2012	Mossoró/RN
A9	Feb/2012	Santa Maria da Boa Vista/PE
A10	Oct/2012	Apodi/RN
A11	Oct/2013	Mon Senhor Tabosa/CE
A12	Oct/2012	Apodi/RN
A13	Nov/2012	São José do Belmonte/PE
A14	Sep/2013	Oroco/PE
A16	Jan/2010	Apodi/RN
A18	Oct/2006	Mossoró/RN
A19	-[2]	Apodi/RN
A20	Oct/2008	Mossoró/RN
A21	Out/2006	Mossoró/RN
A22	2010	Apodi/RN
A23	2010	Apodi/RN
A24	-[2]	Passagem/RN
A25	Oct/2008	Mossoró/RN
A26	Oct/2008	Mossoró/RN
A27	-[2]	Serra Talhada/PE
A31	-[2]	Apodi/RN
A32	Dec/2014	Serra Talhada/PE

[1] Number of accessions refers to the original identification in the Germplasm Collection.
[2] Date of collection unknown.

Table 2. Qualitative and quantitative descriptors used for morphological characterization of sponge gourd (Luffa cylindrica).

Qualitative descriptors	Quantitative descriptors
Fruit type	Fruit medial diameter (cm)
Fruit skin	Fruit basal circumference (cm)
Fruit apical shape	Fruit medial circumference (cm)
Fruit basal shape	Fruit apical circumference (cm)
Fruit shape	Minimum fiber thickness (cm)
Natural fiber color	Maximum fiber thickness (cm)
Fiber softness	Fruit length (cm)
-	Fruit medial diameter (cm)

After DNA quantification, a composite sample (Bulk) with the DNA of the 10 plants was prepared for each accession. To this end, an aliquot of 10 µL of DNA from each plant was collected from the accession to compose the Bulk.

Polymerase chain reaction (PCR) was used to amplify the DNA of the accessions, using 28 Inter Simple Sequence Repeat (ISSR) Primers (Table 3). The reactions occurred in a thermal cycler (Amplitherm), with the following steps: initial denaturation of the double strands, temperature of 94 ºC for four minutes, followed by 35 cycles, contemplating the stages of denaturation (94 ºC for 30 seconds), annealing (46 to 50 ºC for 45 seconds, varying with the primer) and extension (72 ºC for 10 minutes).

Table 3. Base sequences of ISSR primers used for DNA amplification of sponge gourd (*Luffa cylindrica*) accessions.

Order number	Primer name	Primer sequence (5’....3’)
1	UBC-808	AGAGAGAGAGAGAGAGC
2	UBC-810	GAGAGAGAGAGAGAGAT
3	UBC-811	GAGAGAGAGAGAGAGAC
4	UBC-12	GAGAGAGAGAGAGAGAA
5	UBC-23	TCTCTCTCTCTCTCTCC
6	UBC-825	ACACACACACACACACT
7	UBC-826	ACACACACACACACACC
8	UBC-828	TGTGTGTGTGTGTGTGA
9	UBC-834	AGAGAGAGAGAGAGAGYT
10	UBC-835	AGAGAGAGAGAGAGAGYC
11	UBC-836	AGAGAGAGAGAGAGAGYA
12	UBC-840	GAGAGAGAGAGAGAGAYT
13	UBC-841	GAGAGAGAGAGAGAGAYC
14	UBC-842	GAGAGAGAGAGAGAGAYG
15	UBC-846	GAGAGAGAGAGAGAGAA
16	UBC-847	CACACACACACACACARC
17	UBC-848	CACACACACACACACARG
18	UBC-850	GTGTGTGTGTGTGTGTYC
19	UBC-852	TCTCTCTCTCTCTCTCCRA
20	UBC-855	ACACACACACACACACYT
21	UBC-856	ACACACACACACACACYA
22	UBC-857	ACACACACACACACACYG
23	UBC-858	TGTGTGTGTGTGTGTGRT
24	UBC-861	ACCACCACCACCACCACC
25	UBC-866	CTCCTCCTCCTCCTCCT
26	UBC-868	GAAGAAGAAGAAGAAGAA
27	UBC-888	DBBCACCACACACACA
28	UBC-889	DBDACACACACACACAC

To estimate the dissimilarity matrices, the complement of the coincidence index was used for the qualitative variables and the standardized Euclidean distance for the quantitative traits. For the molecular data, the complement of the Jaccard index was used. All analyses were performed using the "MultivariateAnalysis" Package (AZEVEDO, 2021 of the R software version 4.3.1 (R DEVELOPMENT CORE TEAM, 2023).

From the dissimilarity matrices, hierarchical grouping was performed using the UPGMA (Unweighted Pair Group Method using Arithmetic Average). The "pheatmap" package (KOLDE, 2019) of the R software version 4.3.1 (R DEVELOPMENT CORE TEAM, 2023) was used.

The cut-off point was defined based on Mojena’s (1977) criterion. The degree of fit between the dendrogram elaborated and the original dissimilarity matrix, i.e., the dissimilarity matrix and the matrix originated by the UPGMA technique, was measured using the cophenetic correlation coefficient (SOKAL; ROHLF, 1962).

For the molecular characterization, a binary matrix was constructed according to the absence (0) and presence (1) of the specific DNA fragments that were amplified, and variance analysis was performed by means of AMOVA.

RESULTS AND DISCUSSION

Morphological characterization

As can be seen in Table 4, variability was observed for all descriptors evaluated. Considering the descriptor fruit type, the cylindrical type predominated among the accessions, with variability being observed only for the A25 accession, with cylindrical and triangular fruits. For fruit peel, variation was observed between and within the accessions, with accessions A1 and A23 showing only rough skin, A2, A12, A18, A19, 21 and A31 smooth type, A20, A 24 and 32, depressed type. A22 had smooth or depressed bark, and A25 had depressed or rough bark. The apical shape of the fruit varied between depressed (A1), oval (A2 and A25), and pointy (A12, A18, A19, A21, A22, A23, A24, and A32). A20 showed fruits in both classes, pointy and oval. Variation was also observed for the descriptor basal shape of the fruit, where the classes oval (A2, A18, A20 and A32), pointy (A12, A19, A21, A22, A23, A24, and A31), depressed (A25), as well as variation within the accession, with the depressed and oval (A1) shapes, were observed. For fruit shape, the round (A1 and A2), ellipsoid (A12, A23, A24, A31 and A32) and elongated (A18) classes were observed. Variation was also observed within the accessions for this feature, such as ellipsoid or elongated (A19 and A22), ellipsoid or round (A20 and A25) and ellipsoid or pyriform (A21). The natural color of the fiber varied between cream and yellow. Accessions A1, A2, A18, A20, A23, A31 and A32 were uniform, showing only the cream color. Likewise, accessions A12 and A24 were uniform for yellow. Accessions A19, A2, A22 and A25 were cream or yellow. For the fiber softness descriptor, only accessions A31 and A32 were uniform, showing only the hard class. The other accessions evaluated showed variability within the accessions, showing the soft or hard classes (A1, A2, A20, A21, A31).

Table 4. Characterization of sponge gourd (*Luffa cylindrica*) fruits through quantitative descriptors.

Accessions	FT	FP	AFS	BFS	FS	NFC	FS
A1	Cylindrical	Rough	Depressed	Depressed or Oval	Round	Cream	Soft or Hard
A2	Cylindrical	Smooth	Oval	Oval	Round	Cream	Soft or Hard
A12	Cylindrical	Smooth	Pointy	Pointy	Ellipsoid	Yellow	Very Soft
A18	Cylindrical	Smooth	Pointy	Oval	Elongated	Cream	Hard
A19	Cylindrical	Depressed	Pointy	Pointy	Ellipsoid or Elongated	Cream or Yellow	Soft or Very Soft
A20	Cylindrical	Smooth	Pointy or Oval	Oval	Ellipsoid or Round	Cream	Soft or Hard
A21	Cylindrical	Smooth or Depressed	Pointy	Pointy	Ellipsoid or Piriformis	Cream or Yellow	Soft or Hard
A22	Cylindrical	Rough	Pointy	Oval	Ellipsoid or Elongated	Cream or Yellow	Soft, Very Soft or Hard
A23	Cylindrical	Depressed	Pointy	Pointy	Ellipsoid	Cream	Very Soft
A24	Cylindrical	Depressed or Rough	Pointy	Pointy	Ellipsoid	Yellow	Very Soft
A25	Cylindrical or Triangular	Smooth	Oval	Pointy	Ellipsoid or Round	Cream or Yellow	Soft, Very Soft or Hard
A31	Cylindrical	Depressed	Pointy	Depressed	Ellipsoid	Cream	Hard
A32	Cylindrical	Rough	Pointy	Pointy	Ellipsoid	Cream	Hard

FT: fruit type; FP: fruit peel; AFS: apical fruit shape; BFS: basal fruit shape; FS: fruit shape; NFC: natural fiber color; FS: fiber softness.

Although a scale to measure the ease of peeling the fruits was not established, it was observed that fruits with smooth peel were more accessible. Similarly, when the fiber was classified as soft or very soft, its fibrils were relatively thin, while when hard, they were thicker.

Harder fibers are commonly used in handicraft manufacturing because they are firmer and more durable. They are also suggestively indicated for use in industrial composites such as insoles and oil filters. Soft fibers are used for dermo-cosmetic purposes (BLIND, 2016; BLIND et al., 2018).

Fibers with a soft texture, light color, and cylindrical shape are commonly used to produce bath sponges. Hard, light-colored, and cylindrical fibers are used to produce sponges for domestic cleaning (BLIND, 2016; BLIND et al., 2018).

Some characteristics of sponge gourd plants are of interest in any production sector, such as higher production of fruits and fibers; however, the definition of the ideal trait to be shown by the fruit will depend on the sector to which it will be destined (GHALI et al., 2011).

In a study with *Luffa cylindrica* to identify thermal comfort for seats and backrests (COSTA, 2018), it was found that softer fibers are preferred to provide greater comfort since, according to Medeiros et al. (2015), excess hardness increases pressure, compressing blood vessels and making circulation difficult. Seats made with vegetable fiber caused greater discomfort, which is associated with the inhomogeneity of the blanket made with fiber. Therefore, the study of genetic improvement is necessary to promote the homogeneity of the fruits.

Establishing groups based on a simple visual inspection through phenotypic and biometric combinations can expand genetic diversity and hereditary possibilities among the accessions studied (BLIND, 2016).

Also, considering the qualitative characteristics, it was possible to separate the accessions into two groups (Figure 1). The first group formed by accessions A1, A2, A18, A20, A25 and A32, and the second group formed by accessions A12, A19, A21, A22, A23, A24 and A31. The high estimate of cophenetic correlation (0.71, $p<0.05$) indicates that the original and final distance matrices are similar.

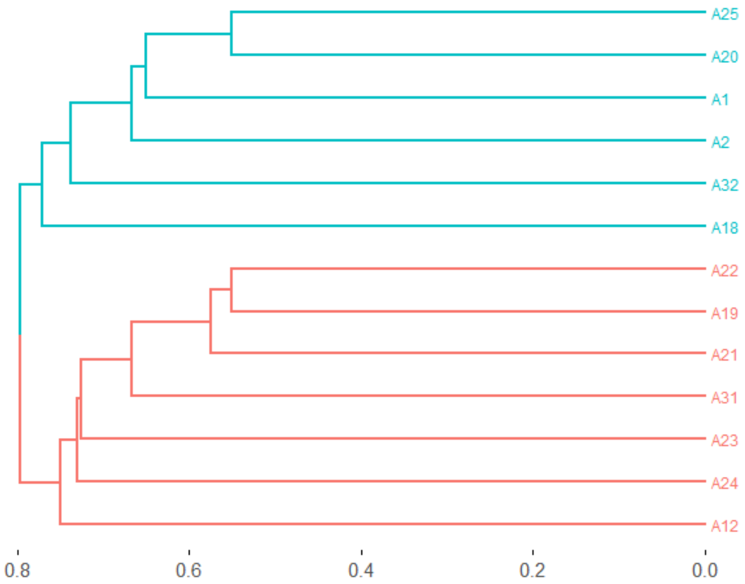

Figure 1. UPGMA dendrogram based on the matrix of Euclidean distances obtained from qualitative morphological variables evaluated in accessions of sponge gourd (*Luffa cylindrica*). (Cophenetic correlation = 0.71, $p<0.05$).

In group 1, five accessions (A1, A2, A18, A20 and A25) come from Mossoró/RN, and one (A32) from Serra Talhada/PE. In group 2, five accessions (A12, A19, A22, A23, and A31) were collected in Apodi/RN, with seven accessions coming from the state of RN (Figure 1). This can result from specific management practices, natural selection, interaction between environmental factors and the influence of geographical distance.

It can be seen that group 1 was formed by accessions with apical and basal fruit shapes and various classifications, while group 2 grouped accessions with basal and apical fruit shapes classified as pointy. Accession A18 stands out as the most different from the others (Figure 1), and it was the only one that had elongated FS (Table 4). Both groups contained subgroups in their formation, indicating that despite the variation in traits, there is genetic similarity between the grouped accessions.

The fruits and fibers evaluated showed a significant variability in quantitative characteristics (Table 5). Accessions A2 and A32 had the highest values of fiber mass (FM), medial diameter of the fruit (MDF), basal (BC), medial (MC) and apical (AC) circumference of the fruit, and minimum thickness (MFT) and maximum thickness (MXFT) of the fiber. For fruit length (FL), accession A32 had the highest value, followed by accessions A19 and A22, with the same average length (Table 5).

Using the quantitative traits, it was possible to form two groups of accessions: group 1 with accessions A1 and A2, and group 2 formed by the other 11 accessions characterized morphologically (A12, A18, A19, A20, A21, A22, A23, A24, A25, A31, A32). The high estimate of cophenetic correlation (0.92, $p<0.05$) indicates that the matrices of original and final distances are similar (Figure 2).

Group 1 was composed of the accessions (A1 and A2) that had the fruits with the largest circumferences, diameters, and thicknesses (Table 5), and they were the only ones with a round shape (Table 4).

Two well-defined subgroups formed group 2. The accessions with the lowest means for FL and FM formed its first subgroup (A12, A21, A23, A24 and A31), while the second subgroup (A18, A19, A20, A22, A25 and A32) had higher values for these variables.

The most similar accessions are A21 and A31 (Figure 2), and Table 5 shows that they had similar means for all the variables studied.

Table 5. Means of quantitative descriptors measured in fruits of sponge gourd (*Luffa cylindrica*) accessions.

Accessions	FM	MDF	BC	MC	AC	MFT	MXFT	FL
A1	15.11	10.25	29.25	32.50	26.95	0.60	0.95	18.50
A2	18.45	11.20	27.25	32.50	27.25	0.90	1.10	24.50
A12	2.73	3.90	10.50	11.50	7.50	0.10	0.20	14.00
A18	11.31	5.70	16.00	17.50	9.50	0.30	0.40	26.60
A19	11.17	6.55	16.00	18.25	12.25	0.20	0.50	27.00
A20	15.68	7.73	21.33	23.17	16.00	0.43	0.67	26.17
A21	7.74	5.33	13.83	15.83	10.67	0.30	0.40	19.83
A22	9.17	5.18	13.25	16.38	9.75	0.13	0.23	27.00
A23	5.98	5.80	15.00	17.00	17.00	0.10	0.10	20.50
A24	7.01	4.30	11.50	12.00	8.50	0.10	0.30	18.00
A25	11.02	7.27	18.17	21.17	18.00	0.33	0.47	21.00
A31	7.74	5.60	13.50	16.00	9.00	0.20	0.30	20.50
A32	16.42	7.20	18.00	22.50	13.00	0.50	0.60	27.50

FM: fiber mass (g); MDF: medial diameter of the fruit (cm); BC: basal circumference (cm); MC: medial circumference (cm); AC: apical circumference (cm); MFT: minimum fiber thickness (cm); MXFT: maximum fiber thickness (cm); FL: fruit length.

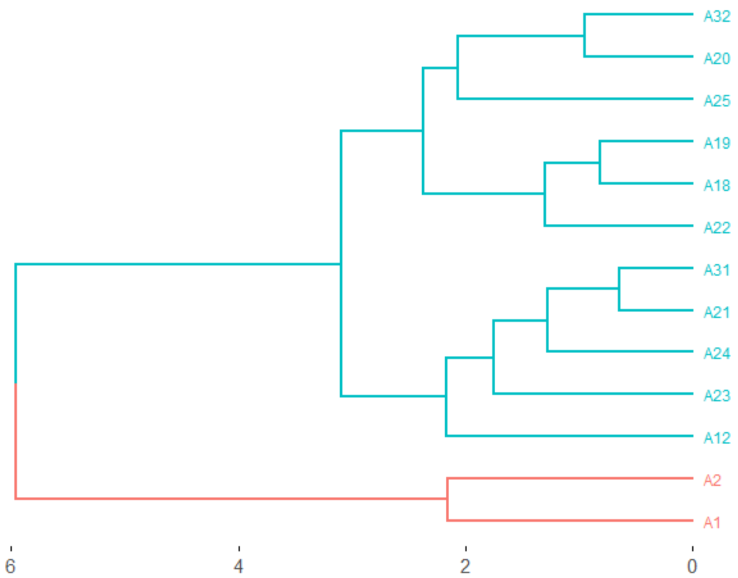

Figure 2. UPGMA dendrogram from the matrix of Euclidean distances obtained from quantitative morphological variables evaluated in accessions of sponge gourd (*Luffa cylindrica*). (Cophenetic correlation = 0.92, $p<0.05$).

Pearson's correlation estimates were positive, with values ranging from 0.219 (FL x BC) to 0.991 (MC x MDF) among the variables studied. The FL variable was the only one that did not show significance among the evaluated variables: MDF, MC, AC, BC, MFT, and MXFT. Blind et al. (2018) also did not find significance between FL, MFT, and number of fruits.

There was a difference between the variables fruit length and fiber mass, with a value of 0.622. This correlation is common in agronomic species since the larger the fruit, the greater the mass. In addition, it is an important characteristic in different agroindustry sectors, which favors their use in indirect selection (BLIND, 2016; BLIND et al., 2018).

In the principal component analysis, the first two eigenvalues explained 77.90% and 15.10% of the variation, totaling 93%, fitting a two-dimensional representation. Cruz, Ferreira and Pessoni (2011) stated that a reliable discrimination of accessions is possible when the first two canonical variables explain at least 80% of the total variation.

Blind (2016) found a lower variation result. In his studies of characterization of the genetic diversity of sponge gourd, he found a total variation of 81.12% using two principal components.

The variables apical, medial and basal circumference of the fruit, medial diameter of the fruit, and maximum and minimum thickness of the fiber were the ones that contributed the most to the first principal component. In contrast, fruit length and fiber mass were the ones that contributed the most to the second principal component.

Based on the scores of the first two principal components, the accessions were divided into two groups. The first group consisted of accessions A1 and A2, and the second group consisted of the other accessions, confirming the result obtained by the UPGMA group.

The contribution of the studied traits was evaluated. It was observed that the variable that most contributed to the genetic divergence was the medial circumference of the fruit (26.6%), followed by apical circumference (26.3%) and basal circumference (19.7%), fiber mass (13%), fruit length (11.4%), medial diameter of the fruit (2.8%), maximum thickness of the fruit (0.1%) and finally, minimum thickness of the fruit (0%).

Molecular characterization

As observed in Table 6, of the 28 primers used, 23 showed a good amplification pattern and generated a total of 103 fragments (Table 6), with approximately 77% of these composed of polymorphic bands. The number of total fragments (Table 6) ranged from one (UBC-12; UBC-840; UBC-841; UBC-846) to nine (UBC-888). And the number of polymorphic fragments (Table 6) ranged from one (UBC-12; UBC-25; UBC-840; UBC-841; UBC-846; UBC868) to seven (UBC-825; UBC-889), with a mean percentage of polymorphism (P%) of 80% (Table 6), thus confirming that the ISSR markers have the ability to detect polymorphism and genetic diversity among sponge gourd accessions.

This result is higher than that found by Tyagi et al. (2016), who studied 45 accessions of *L. cylindrica* present in different geographic regions of India from SRAP markers and obtained a polymorphism of 72.8%. Likewise, Blind (2016) studied the diversity of sponge gourd accessions from seven states of Brazil from AFLP markers and obtained 66.8% of total polymorphism.

The polymorphic information content (PIC), which indicates the degree of polymorphism of a genetic marker in a population, i.e., the existence of variability, ranged from 0.15 (UBC-826; UBC-846; UBC-855; UBC-857) to 0.41 (UBC-834) (Table 6).

Table 6. ISSR primers used for characterization of loofah (*Luffa cylindrica*), total number of amplified fragments (NAF), number of polymorphic fragments (NPF), percentage of polymorphism (%P), polymorphic content index (PCI), effective multiple ratio (EMR) and marker index (MI).

Primer	NAF	NPF	%P	PCI	EMR	MI
UBC-808	4	3	75	0.35	2.25	0.79
UBC-810	5	3	60	0.38	1.80	0.68
UBC-811	4	3	75	0.33	2.25	0.74
UBC-12	1	1	100	0.22	1.00	0.22
UBC-23	2	1	50	0.28	0.50	0.14
UBC-825	7	7	100	0.28	7.00	1.94
UBC-826	7	4	57	0.15	2.29	0.35
UBC-834	5	5	100	0.41	5.00	2.07
UBC-835	5	4	80	0.33	3.20	1.06
UBC-836	3	2	67	0.25	1.33	0.33
UBC-840	1	1	100	0.28	1.00	0.28
UBC-841	1	1	100	0.22	1.00	0.22
UBC-842	3	2	67	0.33	1.33	0.44
UBC-846	1	1	100	0.15	1.00	0.15
UBC-847	6	5	83	0.37	4.17	1.55
UBC-848	7	5	71	0.33	3.57	1.18
UBC-855	7	6	86	0.15	5.14	0.79
UBC-856	5	4	80	0.22	3.20	0.70
UBC-857	7	6	86	0.15	5.14	0.79
UBC-866	3	2	67	0.33	1.33	0.44
UBC-868	2	1	50	0.27	0.50	0.14
UBC-888	9	5	56	0.22	2.78	0.62
UBC-889	8	7	88	0.32	6.13	1.95
Total	103	79				
Mean			80	0.28	2.25	0.68

According to the classification of Botstein et al. (1980), markers with PCI values higher than 0.5 are considered very informative, those with values between 0.25 and 0.5 are considered moderately informative, while those with values below 0.25 are considered uninformative. Therefore, in the present study, eight primers showed PCI values lower than 0.25 (Table 6) and were considered uninformative. In comparison, the other fifteen primers showed PCI between 0.25 and 0.5 (Table 6), hence being considered moderately informative, thus more suitable for variability studies (RIBEIRO, 2011). The genetic similarity values, estimated from the Jaccard coefficient, ranged from 0.408 to 0.914.

The UPGMA cluster (Figure 3) had a Cophenetic Correlation Coefficient (CCC) of 0.774. This CCC allows for estimating the fit between the dissimilarity matrix and the clustering. This result is lower than those reported by Blind et al. (2020), who obtained a cophenetic correlation of 0.9606 in a study of genetic diversity with *Luffa cylindrica*. As the cophenetic correlation coefficient increases, there is a decrease in the distortion induced when grouping individuals (CRUZ; FERREIRA; PESSONI, 2011). According to Patto et al. (2004), CCC values higher than 0.56 represent a good alignment between the dissimilarity and clustering matrices, while Sokal and Rohlfe (1995) comment that the ideal fit between the matrix and the cluster is evaluated by CCC values greater than 0.80.

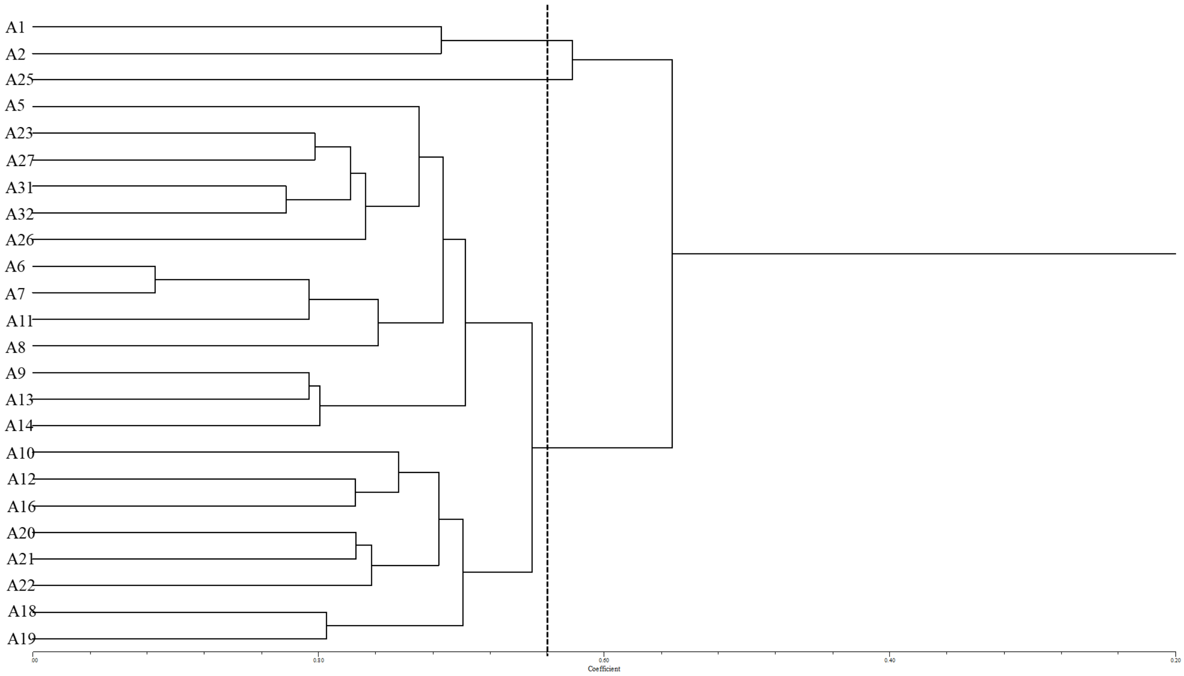

Figure 3. Dendrogram obtained by the UPGMA method and arithmetic complement of the Jaccard index as the mean of similarity, in 24 accessions of sponge gourd (Luffa cylindrica) based on 21 ISSR primers. Cut-off point = 0.66%.

The dendrogram (Figure 3) represents the genetic relationship between the accessions of sponge gourd. Three different groups were identified, with a mean distance of 0.66 between the groups.

The first group was formed by two accessions (A1 and A2), while the second group was formed by only one accession (A25). The third group contains the other 21 accessions studied, which represents approximately 83.33% of the accessions, namely: A5; A6; A7; A8; A9; A10; A11; A12; A13; A14; A16; A18; A19; A20; A21; A22; A23; A26; A27; A31; A32 (Figure 3).

The first two groups come from the city of Mossoró-RN. The third group was the only one that had subgroups within its group (Figure 3). The presence of subgroups may indicate genetic heterogeneity within specific regions or groups. This can result from complex interactions between environmental factors, gene flow, natural selection, and specific management practices.

The first subgroup of group 3 can be divided into two groups, one containing accessions A9, A13 and A14, all from Pernambuco, and another containing 11 more accessions (Figure 3). However, it is still possible to observe the formation of two more groups decreasing the cut-off point: the groups of accessions A6, A7, A11 and A8, which contain accessions from various origins grouped (PE; CE; RN), and the group containing accessions A5, A23, A27, A31, A32 and A26, which has accessions from two states, 33.3% from Pernambuco and 66.6% from Rio Grande do Norte (Figure 3).

The second subgroup of group 3 had all accessions from the same state (Rio Grande do Norte), with five of the eight accessions (62.5%) from the city of Apodi and the other three (37.5%) from the city of Mossoró (Figure 3).

Blind (2016) obtained a similar result, verifying a positive result in which the dispersion and grouping of the accessions of sponge gourd corresponded to their origin. The trend of this grouping suggests that the genetic structure of these accessions may have been influenced by geographic distance and the limitation of gene flow between different areas. This may have occurred due to the sharing of genetic materials due to social interactions and common agricultural practices.

However, considering the subgroup containing different states (Figure 3), this may have occurred due to the dispersion and flow of cultivation since the loofah is a species with a mode of reproduction by allogamy, cultivated in an incipient way, without previous screening for the use of accessions, in addition to its predominance of spontaneous occurrence. These results corroborate those obtained by Zhang, Hu and Xu (2008) and Silva, Ranil and Fonseka (2012), who found a high degree of kinship in accessions of sponge gourd in different regions of Asia.

Accessions A2 and A18 (Table 1) were collected on the same property in different years, according to information from the germplasm collection. However, they are not in the same group, and the pair with the greatest similarity (0.706) is not represented in the dendrogram (Figure 3). This may be related to the allogamy of this species, so gene exchange may have occurred at some point.

Accession A1 was the least similar to the other accessions, as shown in Table 1. Accession A25 showed a unique genetic profile (Figure 3), as indicated by the dendrogram. These results suggest that both accessions may be crucial in preserving genetic diversity. In addition, because they are distant parents, it suggests the possibility of a significant heterotypic effect on their progeny. Therefore, these specific accessions can be considered important in future genetic improvement strategies (CRUZ; FERREIRA; PESSONI, 2011).

CONCLUSION

The accessions evaluated showed variability for the morphological descriptors evaluated.

The ISSR marker proved to be efficient in estimating the genetic diversity present in the *Luffa cylindrica* accessions evaluated.

The most divergent accessions were A1 and A25.

Accessions A19, A20, A22 and A32 were the most suitable for the cosmetic, domestic and industrial markets.
For the vegetable sponge market, accessions A19 and A23 are indicated.

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