Genetic Divergence and Importance of Traits in *Luffa cylindrica* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author CCAP was responsible for tabulating and analyzing the data, besides writing the article. Authors MGM and JSSSN participated in the management of the experiment from the implantation to the data collection. Author APBJ participated in designing the idea and management of the experiment and participated in the elaboration of the research project. Authors LMS and MAQ participated in the elaboration of the research project, conduction of experiments and writing of the article. All authors read and approved the final manuscript.

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ABSTRACT

This work aimed at estimating the genetic divergence between luffa accessions (*Luffa cylindrica* (L.) M. J. Roem) by means of multivariate analysis techniques and at verifying the importance of the assessed traits for the study of genetic divergence. Two trials were carried out in the municipality of Mossoró, in the state of Rio Grande do Norte, Brazil. The trials were carried out in incomplete block design, comprising two experiments in completely randomised blocks with four regular treatments and two treatments common to both experiments, amounting to a number of ten

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treatments, with three replications. Each luffa accession composed one treatment. Eleven descriptors (three quantitative and eight qualitative) were measured in order to quantify genetic divergence, using Gower's algorithm as a measure of dissimilarity, and one joint analysis was carried out considering quantitative and qualitative descriptors simultaneously. The UPGMA hierarchical method was adopted to group the genotypes, and the Singh criterion was elected in order to quantify the contribution of the traits to genetic divergence. It could be verified that there are accessions presenting great divergence from one another. Accessions BUCHA06 and BUCHA22 were the most divergent from the others, and accessions BUCHA06 and BUCHA10 were noted to be the most similar. The median portion fiber texture, the upper portion fiber texture, and fruit mass were found to be the most important traits for estimating genetic divergence.

1. INTRODUCTION

The genus *Luffa* comprises a diversified group, having probably originated in Tropical Asia, gathering eight species [1]. The most frequent harvests are *Luffa acutangula* (L.) Roxb and *Luffa cylindrica* (L.) M. J. Roem, the latter being the most cultivated variety, popularly known as luffa or loofah [1,2].

Luffa is a herbaceous climber, bearing axillary tendrils for support. In India, unripe fruits are commonly prepared and eaten as vegetables, and upon reaching maturity they are commercialised as sponges [3]. In Brazil, luffa is commonly grown in small family-run farms, and it exerts an essential socio-economic role as a source of income [4,5]. In the Northeast region of Brazil, luffa also grows spontaneously in nature and may be found as roadside and riverside vegetation, and in the cities, it can be found in vacant lots as well [6].

A significant amount of research has been carried out in order to investigate the different functionalities of luffa, particularly in an attempt to pinpoint the technological properties of its fibers, as they have clear physical, mechanical, thermal and chemical properties [7,8,9]. Among the attractive features of luffa is the fact that they are low-cost, biodegradable materials, in addition to being a renewable natural resource and being subject to a lesser degree of manufacturing equipment deterioration when compared to other synthetic fibers [10].

The medicinal properties of luffa have also been exploited due to its containing bioactive compounds with antibacterial, antifungal and antioxidant effects [11]. Considering the aspects above and the search for sustainable products, luffa production has expanded on account of the increasing demand for its derived products [12].

Notwithstanding the significant increase in production, most of the Brazilian harvests do not stem from breeding programs, but rather arise from family agriculture fields that perform phenotype selection of luffa fruits year after year [13]. Consequently, the fruits present some limitations, such as the lack of uniformity in size, coloring, diameter, and thickness, among other traits [14].

The existence of genetic variability is notorious among the kinds preferred by growers, as there is genetic diversity within the species itself, this being a key factor for success in a breeding program [15]. The Active Curcubit Germplasm Bank for the Brazilian Northeast, housed by Embrapa Semiárido, stores luffa accessions collected in the states of Bahia, Ceará, Maranhão, Minas Gerais, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul and Rondônia, from such varied locations as grower fields, free markets and by the verges of stopping lanes. These were primarily *Luffa cylindrica* (51 accessions) and there were also *Luffa opperculata* accessions (a total of 6) [16]. The Active Curcubit Germplasm Bank at Embrapa Clima Temperado currently stores 21 Luffa cylindrica accessions collected in Espírito Santo, Mato Grosso, Rio Grande do Sul, Paraná and São Paulo [17]. The collection stored at UFERSA also gathers Luffa cylindrica accessions, but these were exclusively collected in the state of Rio Grande do Norte and stem from the same species [6].

Furthermore, although *L. cylindrica* was domesticated in India [1] and has been grown in several parts of the world, the New World (and Brazil as well) saw greatly diverse varieties cultivated in different regions. Samples from said varieties were extracted in order to comprise the basis of the germplasm stored in the aforementioned banks. Thus, germplasm banks

play an important role in the study of genetic divergence and in the identification of important breeding traits, as they guide superior work strategies that are to be adopted since they will define the most divergent groups for the traits of importance and direct crossings to obtain segregating populations [18,19].

This study aimed at estimating the genetic divergence in luffa by means of multivariate analysis techniques, and at verifying the importance of the assessed traits for genetic divergence studies.

2. MATERIALS AND METHODS

Ten luffa accessions (*Luffa cylindrica* L.) were assessed (Table 1) – these were collected in the state of Rio Grande do Norte and belong to the luffa germplasm bank of the Federal Rural University of the Semi-Arid Region (UFERSA).

The experiments were carried out at the Experimental Vegetable Garden of the Agronomic and Forest Sciences Department at UFERSA, Mossoró/RN Campus, at 5º11' S, 37º 20' W and 18 m altitude. The climate, according to the Köppen classification, is 'BSWh', with a dry season (from June to January) and a rainy season (from February to May) and temperatures varying from 21.3 to 34.5ºC. The average annual rainfall is about 625 mm [20].

The seeds underwent a cutting process in the portion opposite the hilum – with pruning shears – to overcome dormancy [21]. They were then sown in 128-cell polystyrene trays and remained in the greenhouse until the emergence of the second definite expanded leaf, whereupon they were transplanted to the field.

The experimental design was done with incomplete blocks [22], with two completely randomised blocks, with four regular treatments and two treatments common to both trials (BUCHA19 and BUCHA20) comprised of three replications, and each plot consisted of four plants. The trials were conducted in the vertical-type cultivation system, spaced 3.0 m wide and 5.0 m long. The cultivation techniques were carried out as per luffa requirements [5]. Controlled pollinations were also carried out in order to yield endogamic progenies. The first trial was achieved from January to August 2012, and the second trial from February to August 2013.

The traits assessed were fruit mass - FRM (g.plant^{-1}), obtained from weighing whole fruits; fiber mass - FBM (g), by removing the peel and the seed; peel mass - PLM (g), post removal off fruits; lower portion fiber texture - LPT, median portion fiber texture – MPT, and upper portion fiber texture - UPT (with figures ranging from 1 to 3, where 1 corresponds to a softer texture and 3 to a rougher texture); ease of seed release - ESR (figures obtained in a scale of scores ranging from 1 to 3, where 1 corresponds to easy release, 2 to average easy release, and 3 to hard release); fruit length - LOF (cm), the measurement of the floral scar at the peduncle insertion, measured with a graduated ruler (cm); the diameter of the lower portion - DLP (mm), measuring the diameter of the fruit near the floral scar, with a digital caliper; diameter of the median portion - DMP (mm), with a digital caliper; diameter of the upper portion - DUP (mm) measuring the fruit near the peduncle insertion, with a digital caliper. For the assessment of the traits, at least one fruit per plant was utilised, amounting to a total of twelve fruits per accession [23].

Table 1. Luffa accessions (*Luffa cylindrica* L.)

Accession	Collection site	Geographic coordinates
BUCHA01	Mossoró-RN	05°12.548'S 37°20.186'W
BUCHA03	Mossoró-RN	05°12.386'S 37°19.779'W
BUCHA06	Mossoró-RN	05°09.770'S 37°20.480'W
BUCHA10	Mossoró-RN	05°09.143'S 37°20.493'W
BUCHA12	Mossoró-RN	05°09.707'S 37°20.493'W
BUCHA19	Apodi-RN	05°32'S 37°52'W
BUCHA20	Apodi-RN	05°39'S 37°47'W
BUCHA21	Apodi-RN	05°32'S 37°52'W
BUCHA22	Apodi-RN	05°39'S 37°47'W
BUCHA25	Apodi-RN	05°39'S 37°47'W

The obtained data were submitted to analysis of variance, by the F test, with the aid genetic-statistical software Genes [24]. The estimation of the genetic distance matrix was performed considering quantitative and qualitative descriptors simultaneously, as per Gower's algorithm [25]:

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

where

K = number of variables (k = 1,2,…,p); **i** and **j** = two individuals representing the accession; W_{ijk} = weight given to the **ijk** comparison, attributing a value of **1** to valid comparisons and a value of **0** to invalid comparisons (when the value of the variable is absent in one or both individuals); S_{ijk} = contribution of the variable **k** in the similarity between individuals **i** and **j**, ranging from **0** to **1**. For a qualitative (nominal) figure, if the value of **k** is the same for both **i** and **j**, then S_{ijk} = 1, if not, then it equals 0; for a quantitative (continuous) variable, S_{ijk} = 1 - | x_{ik} − xjk | / R_k where x_{ik} and x_{jk} are the values of **k** for **i** and **j**, respectively, and R_k is the interval (maximum value minus minimum value) of **k** in the sample. Dividing by R_k eliminates the differences between variables' scales, yielding a figure within the interval [**0, 1**] and equal weights.

The hierarchical groupings based on the genetic distance matrix were organised by using the Unweighted Pair Group Method with Arithmetic Mean method (UPGMA) as a measure of dissimilarity. With a view to identifying the significance of the traits for genetic divergence, the relative contribution of each trait was estimated by analysing the participation of the Euclidean distance (D^2) components in the totality of the observed dissimilarity [26]. The multivariate analyses were accomplished by utilising the genetic-statistical software Genes [24].

3. RESULTS AND DISCUSSION

According to the F test at 1% probability there was a significant difference between the experiments for the descriptors FRM, FBM, PLM, LOF and DMP. For the LPT descriptor there was a significant difference at the 5% probability level. The MPT, UPT, DLP and DUP descriptors did not present a significant difference at 5%

probability. The results of the F test detected significant differences between the accesses for the evaluated characters, except for LPT and ESR, evidencing, therefore, the presence of genetic variability between the accesses studied (Table 2).

The measurements of genetic dissimilarity (Table 3) estimated by means of Gower's algorithm featured a variance of 0.1755 to 0.5501. A total of 45 combinations were formed, but accessions BUCHA06 and BUCHA22 were identified as the most dissimilar from one another (D^2= 0.5501), followed by BUCHA01 and BUCHA20 (D^2=0.5356) – both combinations were made up of accessions extracted in different municipalities. Accessions BUCHA06 and BUCHA10 were considered the most similar as they bore a shorter distance (D^2= 0.1755), having originated in the same municipality. This also held true for the following combinations: BUCHA20 and BUCHA21, BUCHA21 and BUCHA22, and BUCHA22 and BUCHA25. Conversely, the combination of BUCHA01 and BUCHA03 (Table 3), in spite of having been collected in the same municipality, featured the highest divergence – similarly to the accession combination of BUCHA19 and BUCHA 25.

In fact, accessions BUCHA06 and BUCHA25 featured longer distances in relation to others in three combinations, being the most divergent. It is therefore deducible that the biparental crossings between these pairs are the most promising, because the more divergent the parents are, the more segregating populations can be obtained, intensifying allele combinations in order to yield favorable combinations [18]. It is highly important, however, that the traits contributing to this divergence be considered. The fruits from BUCHA06 present higher fruit mass, fiber mass, peel mass, and higher fruit diameter near the floral scar vis-à-vis BUCHA25. With regard to lower portion (LPT) and upper portion fiber textures (UPT), BUCHA06 bears a soft and smooth texture, and a median texture, respectively, whereas BUCHA25 shows a median texture (LPT) and a rough texture (UPT) (Table 4). Thus, one of the main objectives of the breeding programs is to obtain superior populations, but one of the primary steps to achieve such a result is the proper selection of breeders, which are commonly dissimilar vis-à-vis traits of interest [27].

Table 2. Summary of the pooled variance analysis for the descriptors used in ten accessions of vegetal bush (*Luffa cylindrica* L. Roem.). Mossoró, UFERSA, 2018

Source of variation	GL	FRM (g.plant-1)	FBM (g)	PLM (g)	LPT (Note)	MPT (Note)	UPT (Note)	ESR (Note)	LOF (cm)	DLP (mm)	DMP (mm)	DUP (mm)
Blocks	4	32.07	6.24	6.89	0.08	0.20	0.12	0.50	4.23	20.85	7.763	5.29
Experiments	1	1203.97**	382.33**	77.70**	1.79*	0.04^{ns}	0.12^{ns}	0.15^{ns}	270.38**	3.29^{ns}	257.9**	23.85^{ns}
Accesses adjusted	9	126.07**	36.79**	11.87**	0.37^{ns}	0.45*	0.76**	0.31^{ns}	18.88**	84.32*	65.65**	91.19**
Residue	21	34.54	4.71	3.41	2.10	0.13	0.21	0.14	1.86	26.51	16.01	11.30
Overall average		28.96	8.04	10.40	2.15	2.17	2.12	1.48	17.93	46.70	55.58	39.83
Average accesses		27.74	8.29	10.17	2.00	2.15	2.18	1.49	17.60	47.31	54.93	40.27
Mean of witnesses		31.41	7.54	10.85	2.10	2.22	2.20	1.46	18.59	45.50	56.90	38.95
CV (%)		20.29	27.00	17.78	26.18	17.19	20.83	25.45	7.61	11.02	7.20	8.44

** – Significant difference at 1% probability by the F test; * - Significant difference at 5% probability by F test; ns - Not significant at 5% probability by the test F. Fruit mass (FRM); Fiber mass (FBM); Peel mass (PLM); Lower portion fibers texture (LPT); Median portion fibers texture (MPT); Upper portion fibers texture (UPT); Ease of seed release (ESR); Length of the fruit (LOF); Diameter of the lower portion (DLP); Diameter of the median portion (DMP) and Diameter of the upper portion (DUP)
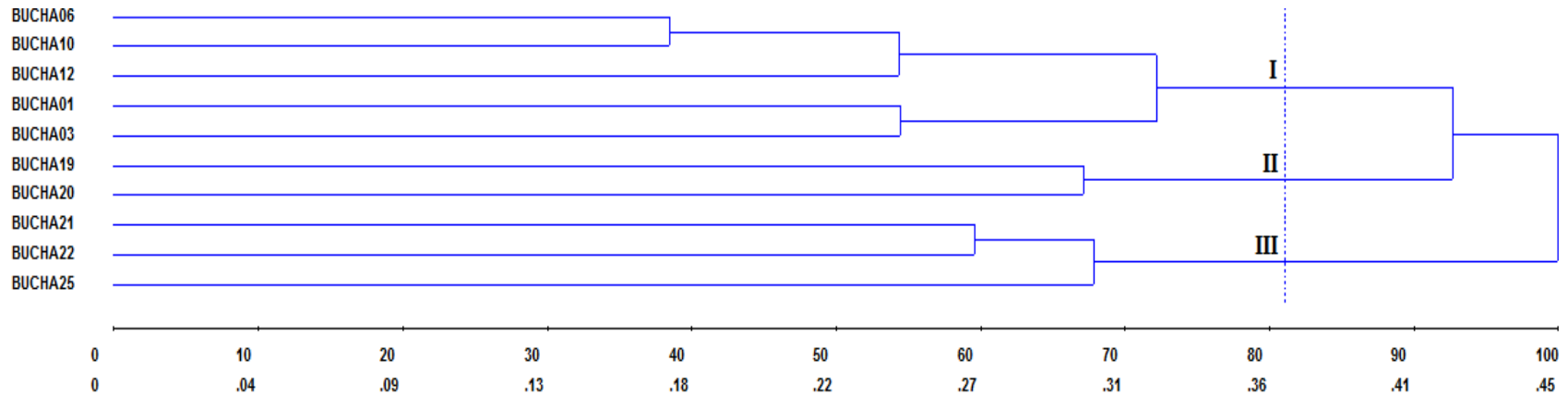

Fig. 1. Dendrogram illustrating the dissimilarity pattern obtained by means of the UPGMA method, based on Gower's distance algorithm in luffa accessions. Mossoró-RN, UFERSA, 2018

Table 3. Distance estimates of the closest and most distant accessions of luffa, based on Gower algorithm distances. Mossoró, UFERSA, 2018

Accession	Shortest D²	Closest accession	Longest D²	Most distant accession
BUCHA01	0.2482	BUCHA03	0.5356	BUCHA20
BUCHA03	0.2334	BUCHA10	0.4601	BUCHA19
BUCHA06	0.1755	BUCHA10	0.5501	BUCHA22
BUCHA10	0.1755	BUCHA06	0.4501	BUCHA25
BUCHA12	0.2073	BUCHA10	0.4826	BUCHA25
BUCHA19	0.2662	BUCHA12	0.5266	BUCHA25
BUCHA20	0.3044	BUCHA21	0.5356	BUCHA01
BUCHA21	0.2717	BUCHA22	0.4886	BUCHA06
BUCHA22	0.2717	BUCHA21	0.5501	BUCHA06
BUCHA25	0.3002	BUCHA22	0.5300	BUCHA06

Table 4. Characterisation of luffa accessions based on quantitative (mean values) and qualitative descriptors of the fruits. Mossoró, UFERSA, 2018

Accession	FRM (g)	FBM (g)	PLM (g)	LOF (cm)	DLP (mm)	DMP (mm)	DUP (mm)	LPT	MPT	UPT	ESR
BUCHA01	24.88	5.62	8.07	15.52	48.44	48.47	37.44	1	2	1	1
BUCHA03	24.18	5.17	9.25	15.32	44.80	53.20	37.31	3	2	2	1
BUCHA06	25.20	6.98	9.92	17.52	48.39	53.93	37.40	1	2	3	2
BUCHA10	32.31	10.62	9.47	18.25	53.23	55.48	42.14	2	3	2	2
BUCHA12	32.19	9.13	14.00	19.37	61.36	64.32	55.12	3	3	3	1
BUCHA19	33.12	7.47	11.01	19.58	47.07	59.95	42.03	2	2	2	1
BUCHA20	29.69	7.62	10.69	17.61	43.42	53.84	35.86	2	2	2	2
BUCHA21	30.48	9.23	12.31	21.28	42.27	56.64	37.88	2	2	2	2
BUCHA22	37.62	16.08	11.07	19.81	43.49	56.05	37.92	3	3	3	1
BUCHA25	15.02	3.52	7.26	13.43	36.47	51.32	36.97	2	2	2	2

FRM = fruit mass (g = gram); FBM = fiber mass (g = gram); PLM = peel mass (g = gram); LOF = length of fruit (cm = centimeter); DLP = fruit diameter near the floral scar (millimeter); DMP = diameter of the median portion; DUP = fruit diameter near the peduncle insertion (millimeter); LPT = lower portion fiber texture (1 = smooth and soft texture, 2 = intermediate texture and 3 = rough texture); MPT = median portion fiber texture (1 = smooth and soft texture, 2 = intermediate texture and 3 = rough texture); UPT = upper portion fruit texture (1 = smooth and soft texture, 2 = intermediate texture and 3 = rough texture); ESR = ease of seed release (1 = easy release, 2 = average ease of release 3 = hard release)

Considering the interest in biological sponges, it seems to follow that BUCHA01 is the most adequate accession in that it presents a soft and smooth texture in both the lower and upper portions while also having an intermediate texture in the median section (Table 4), and it could be crossed with accession BUCHA03 due to the high dissimilarity index and also because the latter presents an intermediate texture in the median and upper portions, thus facilitating the yielding of segregating types for selection.

The distance observation between the ten accessions was possible using the Gower distance matrix in association with the UPGMA method, forming three distinct groups (Fig. 1). According to Quintal et al. [28], the distance proposed by Gower allows for a better estimation of genetic divergence, by jointly analysing quantitative and qualitative traits. The cut at roughly 89% distance was considered [29]. The first group was the largest, consisting of five accessions (50%), all of which had been collected in Mossoró-RN. It is important to note that this group produced three subgroups, one of which comprised BUCHA01 and BUCHA03. Upon examining fiber texture data (Table 4), these accessions unequivocally have the most adequate textures for biological sponges. The second group was comprised only of accessions BUCHA19 and BUCHA20. The third group comprised accessions BUCHA21, BUCHA22, and BUCHA25. The last two groups were formed by accessions extracted in Apodi-RN. Nevertheless, BUCHA25 makes up an isolated subgroup, presenting an intermediate fiber texture in all three portions of the fruit (Table 4).

Some advantages are feasible by employing multivariate analysis techniques, namely: one is able to provide evidence of the importance of each trait studied out of all the accessions under analysis [26]. In accordance with the criteria proposed by Singh [1], in terms of relative contribution to genetic divergence (Table 5), the most contributing traits were the median portion fiber texture (10.20%), the upper portion fiber texture (10.31%) and the fruit mass (10.05%). Conversely, the trait presenting the smallest contribution was the diameter in the upper portion of the fruit (7.22%).

Table 5. Relative contribution of the traits to genetic divergence in luffa, estimated by the method proposed by Singh (1981). Mossoró, UFERSA, 2018

Trait	S. j.	(%)
UPT	88.17	10.31
MPT	87.28	10.20
FRM	85.98	10.05
LPT	82.84	9.69
PML	80.90	9.46
ESR	80.50	9.41
DMP	79.42	9.29
LOF	75.04	8.77
DLP	69.61	8.14
FBM	63.83	7.46
DUP	61.77	7.22

In a study of genetic divergence in *Luffa cylindrica* accessions – in which twelve accessions collected in seven Brazilian states were examined – fruit mass was one of the traits with the lowest contribution to genetic variability (1.62%) [30]. In this research, however, fruit mass was one of the traits with the highest contribution to group formation. Such a change may have been prompted by environment-related factors and the "genotype v. environment" interaction that takes place when the studies are carried out in different places, as was the case with this study. Moreover, this study also found all of the traits to contribute toward divergence, with the first three (Table 5) contributing to 30.55% of the variance. The following four contributing to 37.85% and the remaining four contributing to 31.59%. One of the traits that contributed to the divergence was ease of seed release (9.41%), which in all likelihood facilitates seed dispersal, and, along with seed dormancy, also contributes toward the emergence of spontaneous populations – which have been identified in different collection sites [6].

When the relative contribution of a trait is low, researchers normally advise that such analyses will be discarded in later studies, as a way to reduce labour costs and time [31,32]. In spite of that, disposing of said traits is not advisable as they are assessed under different conditions, and their behaviour may therefore differ and their degree of contribution may change [6]. Furthermore, this study found all of the assessed traits to contribute to the diversity we have been able to identify.

4. CONCLUDING REMARKS

− There is verifiable genetic divergence between the examined luffa accessions.
− Gower's algorithm proved to be efficient, as it evidenced the possibility of simultaneous analyses of quantitative and qualitative descriptors, thus conferring greater efficiency on the studies of genetic divergence.
− The texture in the median portion and upper portion fibers, as well as fruit mass, figure among the most important traits for estimating genetic divergence. All of the traits indicate a relatively balanced contribution to the genetic divergence of the collected accessions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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