



Morphological descriptors of young plants in *Acmella* spp.

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Abstract In young plants, the identification of descriptors guarantees both genotype conservation and early differentiation. Therefore, the aim of this study was to propose and validate the morphological descriptors for young plants of local *Acmella* spp. accessions to contribute to the assessment of genetic variability. To this end, testing was performed based on 19 *Acmella* spp. Accessions 36 days after emergence (DAE) for the descriptors: stem color (SCOL), leaf color (LCOL), leaf shape (LSHP), margin color (MCOL), leaf margin (LMAR), and number of leaves (NL). Multivariate analyses were performed to validate the results. NL and SCOL contributed the most to data variance. Jamb-16 had the largest genetic distance as a function of LSHP and MCOL. The tested descriptors provided genotype differentiation through the formation of three groups. MCOL, LSHP, and SCOL made it possible to differentiate between the different accessions and even the separate species of *Acmella* spp. They can also help producers easily

identify *A. oleracea* (L.) R. K. Jansen and *A. ciliata* (Kunth) Cass. in the field.

Keywords *Acmella oleracea* (L.) R. K. Jansen · *Acmella ciliata* (Kunth) Cass · Jambu · Amazon · Multivariate analysis

Introduction

The genus *Acmella* spp. is considered pantropical, belongs to the family Asteraceae, and is found in tropical and subtropical regions (Silva and Santos 2011). *A. oleracea* (L.) R. K. Jansen, *A. bellidioides* (Smith in Rees) R. K. Jansen, *A. brachyglossa* Cass., *A. ciliata* (Kunth) Cass., *A. decumbens* R. K. Jansen, *A. pusilla* (Hooker and Arnott) R. K. Jansen, *A. marajoensis* G.A.R. Silva, and J.U.M. Santos have been described in Brazil's Legal Amazon (BLA) region (Silva and Santos 2011). In addition, a new species, *Acmella kalelii* was recently discovered in Santarém, a municipality in Pará State, Brazil (Campos et al. 2018).

Species of the genus *Acmella* are well known for their considerable production of secondary metabolites, with spilanthol being the most widely studied because of its pharmacological functions that help treat diseases (Ramachandran and Radhakrishnan 2020; Grymel et al. 2022). Commonly referred to as jambu, *A. oleracea* is widely used in cuisine, both fresh and cooked (Lalthanpuui et al. 2020).

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As representatives of *Acmella* spp. are easily confused with other genera and species, previous research investigated pollen morphology (Ramachandran and Radhakrishnan 2019) and the anatomical characterization of the leaves, petioles, peduncles, stems, and roots (Ramachandran and Radhakrishnan 2020) of different species from the genus. However, further research is still needed on intra- and interspecific morphologies (Ramachandran and Radhakrishnan 2019).

To obtain the discriminatory characteristics between different plant material, the phenotype in question must be characterized using easily visualized qualitative and quantitative traits known as morphological descriptors (Burle and Oliveira 2010). Adequate knowledge of the morphology of local accessions enables the conservation and better economic use of these materials for agronomic purposes (Somma et al. 2021).

Since morphological traits can change from the early to adult stage (Alves et al. 2013), identifying morphological descriptors in young plants ensures early evaluation and differentiation of genetic material (Reategui-Betancourt et al. 2021). Thus, characterization should be conducted during the initial stages of development to correctly identify the taxa.

Martins et al. (2012) studied the morphological characteristics of *A. oleracea* and *A. ciliata*, however, they evaluated adult plants and provided no information on their leaf morphology patterns. Thus, there is still a lack of information on the diversity of species from the genus *Acmella*, especially those with agronomic potential. Moreover, according to Vargas et al. (2018), research on locally grown plant accessions using unimproved species makes it possible to explore greater variability owing to their broad genetic base.

Thus, given the absence of descriptors to support the correct morphological identification of the representatives of the genus *Acmella*, this study has aimed to propose and validate morphological descriptors of young local *Acmella* accessions to contribute to genetic variability assessment.

Material and methods

Acmella spp. seeds were collected from vegetable-growing areas in the northeast of Pará State and the metropolitan areas of the capital Belém, including

the municipalities of Ananindeua, Marituba, Santa Bárbara, Igarapé-Açu, Benevides, Santo Antônio do Tauá, Castanhal, Capanema, and Bragança (Fig. 1).

For the botanical identification of local *Acmella* accessions, plants at peak bloom were collected when the inflorescences were pale yellow to brown. The inflorescences were then removed from the plants and placed in brown paper bags for subsequent drying and processing. The collected plants were dried in a forced-air oven at 65 °C for 48 h. To obtain the seeds, the inflorescences were dried, threshed to remove achenes, and manually processed by eliminating the paleas and associated impurities. The seeds were weighed, placed in hermetically sealed packages, and then stored at 5 °C until sowing.

To assess the characteristics of the young plants, an experiment was conducted in an arched-roof greenhouse (30 m long × 16 m wide and 4.5 m high) at the Igarapé-Açu Teaching Farm of the Federal Rural University of Amazônia (UFRA). The climate in the region is classified as Am according to the Köppen-Geiger system, with an average annual temperature and rainfall of 26 °C and 2500 mm, respectively (Alvares et al. 2013).

A randomized block design (RBD) consisting of 19 treatments and three repetitions was used, with the treatments represented by the local *Acmella* spp. accessions collected. The plots consisted of beds measuring 1.0 m × 2 m and 0.20 m high. Each plot contained 40 plants spaced 0.20 m apart, with the 16 center plants in each plot considered the experimental unit.

Seedlings were produced in 200-cell expanded polystyrene cells filled with Carolina Soil® commercial substrate. Two seeds were sown in each cell and the trays were placed in a greenhouse and irrigated twice daily. Thinning was performed after full seedling emergence, with one seedling per cell. At 36 days after sowing (DAS), when the seedlings had three pairs of fully developed true leaves, they were transplanted to the beds in medium-textured yellow latosol with the following chemical attributes: $\text{pH}_{\text{H}_2\text{O}}=6.0$; organic matter = 1.7 g kg⁻¹; $\text{K}^+ = 29.0 \text{ mg dm}^{-3}$; $\text{P} = 10.0 \text{ mg dm}^{-3}$; $\text{Ca}^{2+} = 1.8 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Mg}^{2+} = 0.9 \text{ cmol}_c \text{ dm}^{-3}$; $\text{V}\% = 57.3$; $\text{B} = 0.05 \text{ mg dm}^{-3}$; $\text{Cu} = 0.5 \text{ mg dm}^{-3}$; $\text{Fe} = 84 \text{ mg dm}^{-3}$; $\text{Mn} = 4.6 \text{ mg dm}^{-3}$; $\text{Zn} = 2.0 \text{ mg dm}^{-3}$. Fertilization was performed according to the recommendations of Brasil et al.

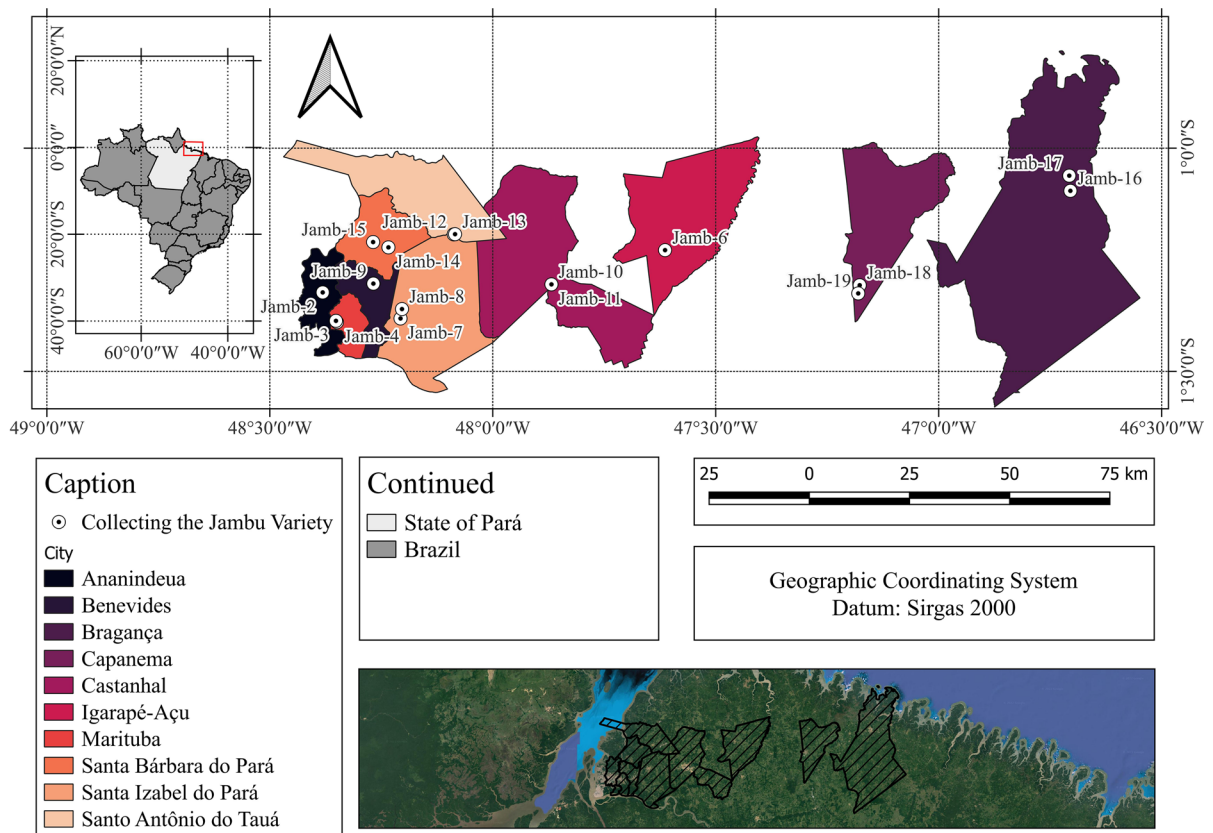


Fig. 1 Collection sites of local *Acemella* spp. accessions in Pará state (Brazil) for the application of morphological descriptors in young plants

(2020) for leafy greens in Pará, Brazil. To ensure water availability for the plants, drip irrigation was used via two self-compensating hoses, with drippers spaced 0.20 m apart.

Seven days after transplanting (DAT), when the plants were young, the following descriptors were assessed in 12 plants from each plot: stem color (SCOL; green, reddish green, purplish green); leaf shape (LSHP; elliptical, deltoid, and ovate); leaf color (LCOL; light green, dark green, and purplish green); leaf margin (LMAR; dentate and serrate); and margin color (MCOL; present and absent). The proposed and evaluated qualitative descriptors were based on the recommendations of Bioversity International (2007), the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), Munsell (1975), Vidal et al. (2021), and a list of descriptors for species from the family Asteraceae, with adaptations for the genus *Acemella* spp. The quantitative descriptor number of fully

developed leaves was also evaluated for 12 plants per plot.

Principal component analysis (PCA) was performed in order to validate and quantify the contribution of the descriptors using the *factoextra* package (Kassambara et al. 2020) and is depicted in a biplot.

The qualitative and quantitative descriptors were analyzed as multicategorical because two and three predefined categories were used to depict each descriptor. For the cluster analysis, the dissimilarity matrix was obtained using the arithmetic component of the index of coincidence based on the arithmetic component ($I_{jj}' = 1 - C_{jj}'$), where $C_{jj}' = CP/(CP + D)$, CP is the agreement, and D is the disagreement between values.

The dissimilarity matrix was subjected to cluster analysis using the unweighted pair-group method with arithmetic mean (UPGMA). Both analyses were performed using a Multivariate Analysis package

(Azevedo 2021). The cut-off point was defined using the method proposed by Mojena (1977), considering $k=1.25$, as suggested by Milligan and Cooper (1985), as the stopping point in defining the number of groups. Additionally, the clustering consistency was analyzed using the cophenetic correlation coefficient (CCC) (Sokal and Rohlf 1962), which assesses the goodness of fit between the original distance matrix and that resulting from UPGMA clustering, considering the three groups formed. All analyses were performed using R software (R CORE TEAM 2023).

Results

The collected specimens were deposited in the herbarium of Museu Paraense Emílio Goeldi, where the species *Acmella oleracea* (L.) R. K. Jansen (Fig. 2a) and *Acmella ciliata* (Kunth) Cass. (Fig. 2b) were identified. The *Acmella oleracea* specimens were registered as follows: MG249013 (Jamb-1), MG249014 (Jamb-2), MG249017 (Jamb-3), MG249020 (Jamb-4), MG249023 (Jamb-5), MG249024 (Jamb-6), MG249026 (Jamb-7), MG249027 (Jamb-8), MG249029 (Jamb-9), MG249031 (Jamb-10), MG249033 (Jamb-11), MG249035 (Jamb-12), MG249038 (Jamb-13), MG249039 (Jamb-14), MG249041 (Jamb-15), MG249042 (Jamb-17), MG249044 (Jamb-18), and MG249045 (Jamb-19). Only Jamb-16 was described as belonging to the species *Acmella ciliata*, and was deposited in the museum under the code MG249043.

The Principal component analysis (PCA) demonstrated that the first two principal components (PC1 and PC2) explained 92.7% of the total data

variance, indicating an adequate decline in data with the lowest possible data loss (Fig. 3).

The descriptors NL and SCOL contributed the most to explaining the data variance as a function of the longer vectors, whereas LMAR and LCOL explained little, which was evident in shorter vectors close to the origin (Fig. 3).

A dissimilarity matrix was obtained to evaluate the ability of the descriptors to group accessions according to genetic distance (Fig. 4), demonstrating that Jamb-16 and Jamb-17 were the furthest from the other accessions.

Greater distances were observed for Jamb-16 than for Jamb-1, Jamb-2, Jamb-5, Jamb-6, Jamb-7, Jamb-8, Jamb-12, Jamb-13, and Jamb-17, largely because of class variations in LSHP and MCOL, with Jamb-16 exhibiting ovate leaves with color along the margin (Table 1).

Distances for the Jamb-17 accession were smaller than those observed for Jamb-16, but were still considerable when compared with the accessions Jamb-1, Jamb-2, Jamb-5, Jamb-6, Jamb-7, Jamb-8, Jamb-12, Jamb-13, and Jamb-16 (Fig. 4).

The descriptors capable of distinguishing Jamb-16 and Jamb-17 from the other local accessions analyzed were MCOL, LSHP, SCOL, and NL, and these varied according to the class observed (Table 1).

When tested using UPGMA clustering, these descriptors could discriminate between the groups. The following groups were formed: Group I (Jamb-16); Group II (Jamb-17), and Group III (Jamb-7, Jamb-6, Jamb-5, Jamb-2, Jamb-13, Jamb-1, Jamb-12, Jamb-9, Jamb-4, Jamb-3, Jamb-19, Jamb-18, Jamb-15, Jamb-14, Jamb-10, and Jamb-11) (Fig. 5).

Fig. 2 Representative *Acmella oleracea* **a**, and *A. ciliata* **b**, at the young plant stage grown in a greenhouse in the eastern Amazon

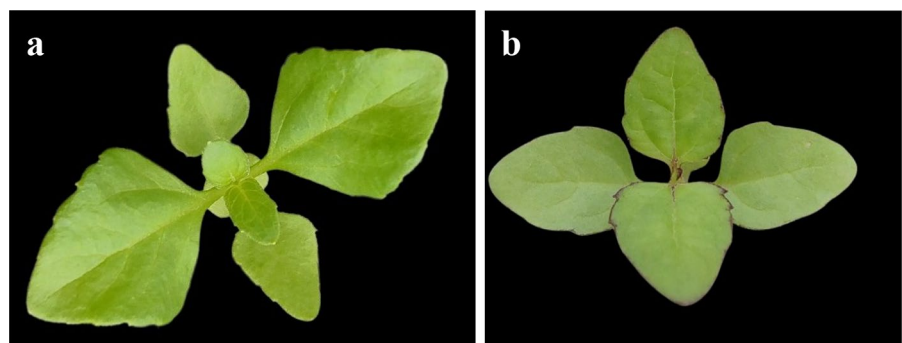


Fig. 3 Biplot depicting the contribution of each variable in explaining the descriptor importance.¹

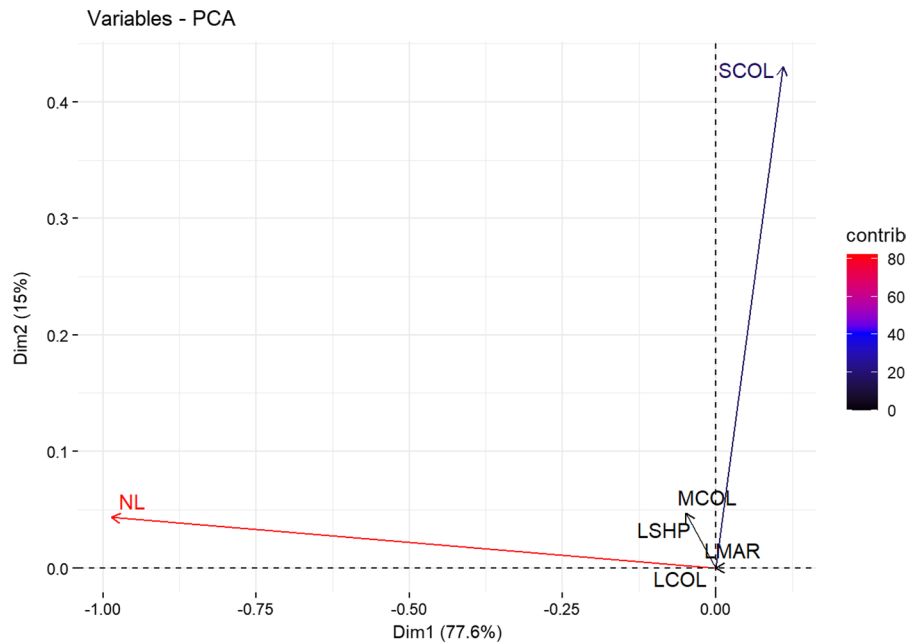


Fig. 4 Dissimilarity matrix obtained by the frequency of discrepancy for local *Acmella* spp. accessions, based on the multicategorical descriptors number of leaves (NL), margin color (MCOL), leaf color (LCOL), leaf margin (LMAR), leaf shape (LSHP), stem color (SCOL). *Darkblue indicates longer distances. Dark red indicates shorter distances (near zero)

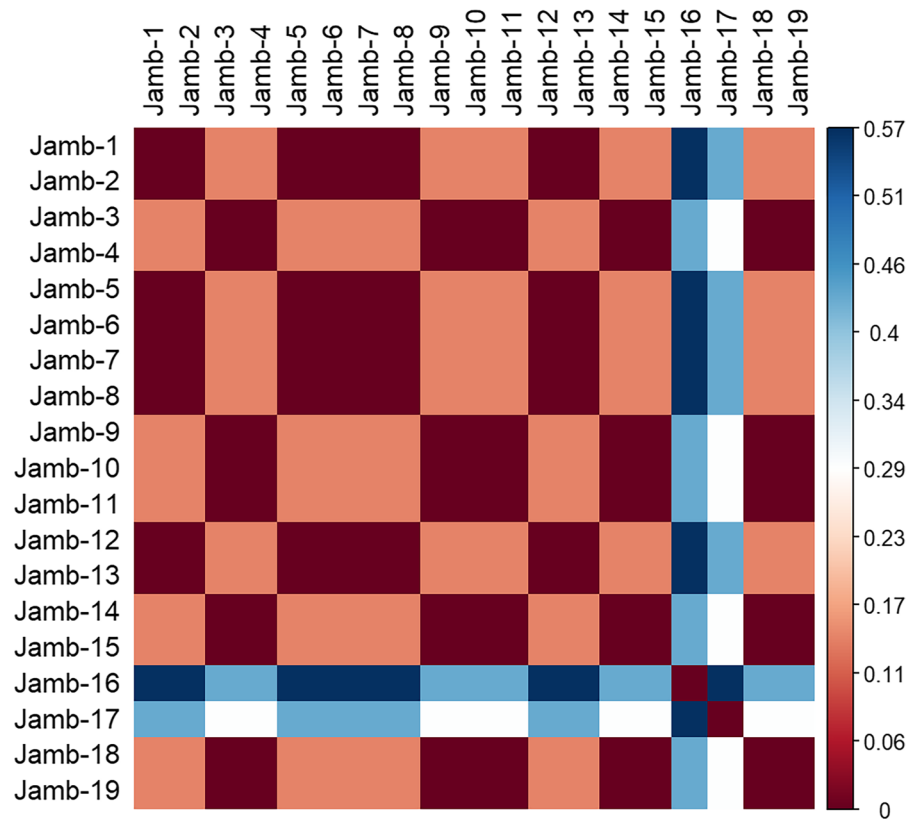
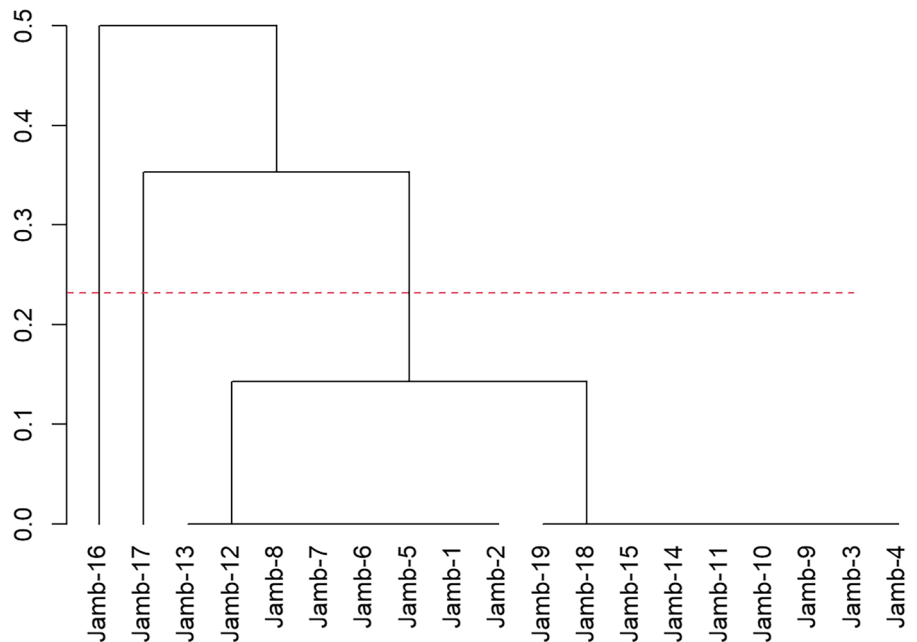


Table 1 Minimum descriptors of local *Acmella* spp. accessions for the variability research in young plants

Accession	MCOL ²	LCOL	LMAR	LSHP	SCOL	NL
Jamb-1	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-2	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-3	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-4	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-5	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-6	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-7	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-8	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-9	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-10	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-11	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-12	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-13	Absent	Light green	Serrate	Deltoid	Purplish green	6
Jamb-14	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-15	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-16	Present	Light green	Serrate	Ovate	Purplish green	8
Jamb-17	Absent	Light green	Serrate	Deltoid	Green	8
Jamb-18	Absent	Light green	Serrate	Deltoid	Purplish green	8
Jamb-19	Absent	Light green	Serrate	Deltoid	Purplish green	8

Fig. 5 Dendrogram obtained from the unweighted pair group method with arithmetic mean (UPGMA), representing the genetic dissimilarity between 19 local *Acmella* spp. accessions, considering the frequency of discrepancy for NL: number of leaves; MCOL: margin color; LCOL: leaf color; LMAR: leaf margin; LSHP: leaf shape; SCOL: stem color

Discussion

The PCA analysis was efficient in reducing the dataset, with more than 90% of the variance concentrated in the first two principal components (PC1

and 2), making it possible to assess the variability between the materials (Fig. 3).

According to Cruz et al. (2020), when PC 1 and PC 2 retain at least 80% of the total data variation, PCA can be used to explain the genetic

diversity of the accessions and the importance of their descriptors.

The greater contribution of NL and SCOL (Fig. 3) demonstrated their key roles in the morphological differentiation and variation found in young plants of local jambu accessions, indicating that these descriptors can be considered discriminatory in research on *Acmella* spp.

In contrast, based on the dissimilarity matrix (Fig. 4), LMAR and LCOL could not identify significant morphological differences in *Acmella* spp., which may indicate the need to disregard these descriptors in research on these species. Similarly, for Amazon chicory (*Eryngium foetidum* L.), a nonconventional leafy vegetable from the family Apiaceae, Gomes et al. (2022) observed a monomorphic pattern in most of the leaf morphological descriptors of the materials analyzed, indicating a low contribution to explaining the genetic variability.

The dissimilarity matrix indicated that there were greater distances when one of the pairs was Jamb-16 or Jamb-17 (Fig. 4), whereas the remaining accessions were genetically close. The high proximity between *A. oleracea* accessions demonstrates the low genetic variability between the collected materials.

Although MCOL was expressed only in Jamb-16 (Table 1), this descriptor is an important biological marker for differentiating between *Acmella* spp. in young plants. Melo et al. (2009) studied cilantro (*Coriandrum sativum* L.) and found that the presence of anthocyanins varied among the materials analyzed in both the young and adult stages, making this an efficient descriptor for differentiating between cultivars.

MCOL, LSHP, SCOL, and NL were found to vary in terms of class (Table 1) and played key roles in separating the accessions, particularly in terms of the genetic variability of the genotypes. They can also help jambu producers easily identify *A. oleracea* and *A. ciliata*. Thus, it can be inferred that these descriptors can identify differences inherent to local accessions after being summarized and represented as a measure of genetic dissimilarity, which is vital for establishing morphological descriptors and applying them in future research on genetic divergence.

Regarding the genetic diversity among the 19 *Acmella* spp. accessions studied (Fig. 5), the formation of three groups demonstrated the efficiency and discriminatory power of the proposed descriptors,

as they were able to distinguish *A. ciliata* (Jamb-16) from *A. oleracea* (the remaining accessions). In relation to the quality of clustering, it is important to note that the higher the CCC, the less the distortion caused by the clustering *Acmella* spp. accessions.

Thus, the characteristics of Group I, which contained only Jamb-16, were the presence of color along the margin and ovate leaves, which are descriptors responsible for the dissimilarity in relation to Groups II and III.

Group II, which included Jamb-17, was further from the remaining groups owing to the lack of stem color (completely green stem). As such, SCOL was capable of separating accessions, even within *A. oleracea*, and formed a single group. This intraspecific variation may be associated with mutation events that may coordinate the absence of stem color. However, in-depth research is needed on the heritability of this trait and its dispersion in the local jambu populations in Pará state.

Finally, Group III contained only *A. oleracea* accessions, which were found to be dissimilar to Groups I and II because they shared the same morphological descriptors, thus forming a highly similar group.

It is important to note that the local accessions used in this study were collected from the metropolitan mesoregions of Belém and northeastern Pará. Campos (2020) investigated the distribution of Spilanthinae subtribes in the Brazilian Legal Amazon and found widespread occurrence of *A. oleracea* in the metropolitan mesoregion of Belém, which is the capital of the state of Pará, Brazil. The same authors reported that despite *A. oleracea* occurrence in northeastern Pará state, *A. ciliata* predominated, specifically in the Guamá microregion, bordering the Bragançana microregion in the municipality of Bragança, where Jamb-16 and Jamb-17 were collected.

By considering the number of collections that were carried out, this number represented only a small fraction of Pará State, Brazil. Thus, accessions should be collected from other locations representative of the remaining microregions in the state and analyzed to obtain a broader view of the expansion of these descriptors.

Although the local *Acmella* spp. accessions showed low genetic variability, the study identified morphological descriptors with a discriminatory power that could be used to differentiate between the

species identified (*A. oleracea* and *A. ciliata*), contributing to both the protection of the cultivars and the conservation of this genetic resource.

Conclusion

The qualitative descriptors margin color (MCO), leaf shape (LSHP), number of leaves (NL), and stem color (SCOL) can distinguish between accessions and even species of *Acmella* spp. and they can contribute to the future genetic variability research from the young plant stage onwards.

We can conclude that multivariate analyses are efficient in validating the proposed morphological descriptors for young *Acmella* spp.

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Author Contributions DLS, FLNA, AOS, RMM, and RFG contributed to study conception and design. Material preparation and data collection were performed using DLS, FLNA, AOS, RMM, and RFG. Data analyses were performed using LSS and RFG. FLNA, ICBR, RFG and LSS wrote the first draft of the manuscript. All the authors have read and approved the final version of the manuscript.

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Data Availability Data supporting the findings of this study are available from the corresponding author [RFG] upon request.

Declarations

Competing interests The authors declare no competing interests.

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