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Artigos

Characterization and differentiation of forest species by seed image analysis: a new methodological approach

Caracterização e diferenciação de espécies florestais por meio da análise de imagens de sementes: uma nova abordagem metodológica

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ABSTRACT

Biometric seed analysis can be used to characterize and differentiate forest species. However, forest species are generally studied using manual methods such as measurements with a digital caliper, which provides a limited amount of information on plant morphological characteristics, whereas agronomic species are analyzed using expensive and often inaccessible equipment. Thus, the objective of the present study was to demonstrate that seed image analysis and processing tools can help characterize and differentiate Brazilian forest species. For this purpose, the seeds of 155 forest species belonging to 42 families were photographed and analyzed to extract data on their morphometric descriptors using a new methodological approach. A total of 18 characteristics were assessed, namely eight dimensions, four shape characteristics, and six color characteristics. A set of approximately 1.827 million data was extracted from 101,521 seed images. Digital image processing efficiently characterized the studied seeds and the obtained characteristics allowed us to differentiate between species, including those belonging to the same botanical family and genus. Therefore, seed image analysis using the proposed methodology can be used to characterize, differentiate, and automatedly identify forest species in Brazil.

Keywords: Biometry; Forest seeds; Image processing; Seed analysis

RESUMO

A análise biométrica de sementes contribui para a caracterização e diferenciação de espécies florestais. Entretanto, os estudos com espécies nativas geralmente utilizam métodos manuais como o paquímetro digital, o qual extrai uma quantidade limitada de características, enquanto espécies agronômicas dispõem de equipamentos caros e pouco acessíveis. Assim, o objetivo deste trabalho é demonstrar que ferramentas de análise e processamento de imagens de sementes podem auxiliar na caracterização e diferenciação de espécies nativas brasileiras. Para isso, sementes de 155 espécies nativas, distribuídas em 42 famílias botânicas foram fotografadas e analisadas para extração de descritores morfométricos por meio de uma nova abordagem metodológica. Um total de 18 características foram geradas, sendo oito para dimensões, quatro para formato, e seis para cor. Um conjunto de aproximadamente 1,827 milhões de dados foram obtidos a partir 101.521 imagens de sementes. O processamento digital de imagens foi eficiente para a caracterização das sementes nativas, e as características utilizadas permitiram diferenciar as espécies, inclusive àquelas que estão contidas na mesma família botânica e gênero. Portanto, a análise de imagens de sementes pela metodologia proposta contribui para a caracterização, diferenciação e automatização na identificação de espécies florestais nativas do Brasil.

Palavras-chave: Biometria; Sementes florestais; Processamento de imagens; Análise de sementes

1 INTRODUCTION

Forest seed characterization is crucial for studying specialists in forest resources as it helps perform genetic studies, taxonomic identification studies, optimize germination conditions, and select suitable species and parent trees for restoring degraded environments (CORREIA, FELIX, ARAUJO, FERRARI, PACHECO, 2019; DUARTE, GONZALEZ-RONDAN, ROCHA, 2019; SANTOS, SILVA, COSTA, SANTOS, SILVA, SILVA, 2019; MOREIRA, NEVES, LOBO, 2021; BEZERRA, ZUZA, SILVA-BARBORA, AZEVEDO, ALVES, 2022). The seeds of forest species are usually analyzed using manual methods for measuring the length, width, and thickness of a limited number of seeds, most often fewer than 100 (MENEGATTI, MANTOVANI, NAVROSKI, SOUZA, 2017; CORREIA, FELIX, ARAUJO, FERRARI, PACHECO, 2019; BEZERRA, ZUZA, SILVA-BARBORA, AZEVEDO, ALVES, 2022). Thus, traditional approaches that most commonly use a digital caliper are ineffective for analyzing a high number of seeds of one or more plant species.

Alternative image processing methods have been widely explored in economically or ecologically important agricultural studies (BAGHERI, EGHBALI, SADRABADI-

HAGHIGHI, 2019; GONZALÉZ-CORTÉS, GODINA, REYES-VALDÉS, TORRES, RODRÍGUEZ, QUINTANILLA, BENÍTEZ, 2019; MARTÍN-GÓMEZ, GUTIÉRREZ DEL POZO, UCCHESU, BACCHETTA, CABELLO-SÁENZ DE SANTAMARÍA, TOCINO, CERVANTES, 2020; NAZARI, SHAKER, KARIMI, ROPELEWSKA, 2021). Therefore, combining digital image processing tools and seed analysis with an image acquisition method may be an alternative for the biometric analysis of forest seeds. However, little research on forest species has been conducted thus far, mainly because of the complexity and diversity of their characteristics, such as seed size, shape, color, and dispersal structures or diaspores.

Few studies on agricultural crops, weeds, and environmentally important species have used high-resolution scanners (FARRIS, ORRÙ, UCCHESU, AMADORI, PORCEDDU, BACCHETTA, 2020), optical (BAO, BAMBIL, 2021) and scanning electron microscopes (MAZUR, MARCYSIAK, DUNAJSKA, GAWLAK, KAŁUSK, 2022), computed tomography (JOSHI, BUTOLA, KANADE, PRASAD, MITHRA, SINGH, BISHT, MEHTA, 2021), infrared spectroscopy (CECCO, MUSCIANO, D'ARCHIVIO, FRATTAROLI, MARTINO, 2019), and spectral and hyperspectral colorimetry, even though these techniques have been proven to be effective in differentiating species and characterizing genotypes. They are useful in scientific research on species with small, homogeneous seeds, but are not applicable for studying the diversity of shapes and sizes observed in forest species.

In addition to the high cost of the aforementioned equipment, most computational resources for digital image processing require specialized knowledge of computing. For this reason, professionals from other areas often struggle to use these devices. Nevertheless, digital image acquisition and processing tools can be an alternative to expensive and inaccessible equipment. For example, the software ImageJ® can be used to characterize the seeds of some species, thereby overcoming this problem (FARRIS, ORRÙ, UCCHESU, AMADORI, PORCEDDU, BACCHETTA, 2020; FELIX, MEDEIROS, FERRARI, VIEIRA, PACHECO, 2020; ARAÚJO, ARAÚJO, SANTOS, PACHECO, ARAUJO, 2022). In addition, this software can also be used in forest sciences for processing X-ray images of seeds (MEDEIROS, ZAVALA-LEÓN, ARAÚJO, PEREIRA, DIAS, SILVA, 2019), measuring seedlings (SILVA, MEDEIROS, PEREIRA, RAMOS, SILVA, 2020), and counting seeds (FELIX, MOCELIM, TORRES, KRATZ, RIBEIRO, NOGUEIRA, 2021a). It can analyze aspects related to the size, shape, and color of elements of an image after masking and processing. Therefore, the potential uses of this software include forest seed characterization and species differentiation.

In this context, image acquisition by camera and digital processing may be an alternative for assessing seed biometric characteristics and color. Thus, the objective of the present study was to use seed analysis and image processing tools to differentiate and characterize forest species.

2 MATERIALS AND METHODS

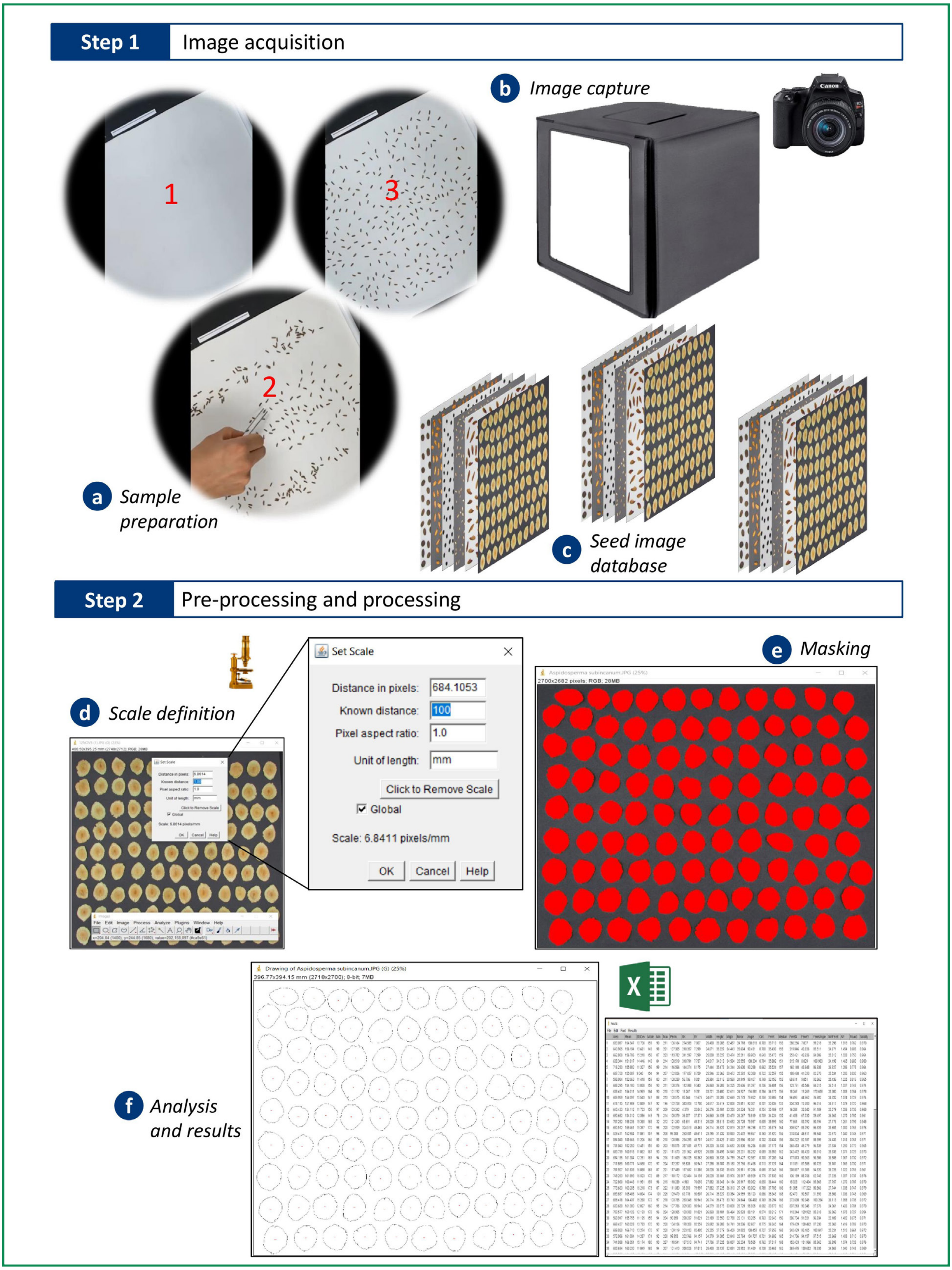
2.1 Seeds acquisition

The seed samples used in the present study were obtained from the seed banks of seven national and regional seed research, production, and teaching institutions: (i) Brazilian Agricultural Research Corporation - *Embrapa Florestas*, (ii) Environmental Institute of Paraná, (iii) Chauá Society, (iv) Forest Seed Laboratory of the Federal University of Paraná, (v) Seed Scholarship Program, a partnership between the Tobacco Growers' Association of Brazil and Federal University of Santa Maria, (vi) Biodiversity Conservation Center of the São Paulo State Environmental Research Institute, and (vii) Center for Ecology and Environmental Monitoring of the Federal University of São Francisco Valley. In total, 155 forest species belonging to 42 families were analyzed in the present study (Table 1, supplementary document). The evaluated species were checked according to the catalog contained in Brazil Flora (http://reflora.jbrj.gov.br).

2.2 Image acquisition and processing

The method proposed in the present study followed a sequence of tasks divided into two steps (Figure 1). Step 1 included sample preparation, image acquisition, and seed database generation, whereas step 2 included image processing and analysis.

Figure 1 – Forest seed image acquisition and processing steps



Source: Authors (2022)

In the sample preparation task, the seeds of each species were placed on ethylene-vinyl acetate (EVA) sheets (Figure 1A). Dark seeds were photographed on white EVA, and light seeds were photographed on matte black EVA to ensure contrast between seed color and background. Then, images were taken with a Canon PowerShot SX500 IS (f/4) digital camera, with a 12 MP lens, 50 cm away from the seeds, using a millimeter gauge. They were acquired in triplicates for each species using a mini photographic studio (50 x 50 x 50 cm), with artificial white light-emitting diode (LED) light to standardize the lighting conditions (Figure 1B). For each photograph, new seed samples were taken for each species, totaling 465 images, which formed the basis of seed images used in the present study (Figure 1C).

The obtained images (file with the extension .JPEG) were transferred to a microcomputer to determine the reference scale, in millimeters, using the ImageJ® software (version 1.53) (https://imagej.nih.gov/ij/) (Figure 1D). Then, the image components were compared by threshold masking (Figure 1E). Lastly, data on the size, shape, and color descriptors were exported to a spreadsheet (Microsoft Office Excel®) (Figure 1F).

2.3 Size, shape, and color descriptors

A total of 18 characteristics, which include eight dimensions, four shape characteristics, and six color characteristics, were analyzed per seed. Figure 2 shows an example of the size and shape characteristics of forest seeds.

Area: seed surface (mm^2), calculated from the boundary defined by the perimeter.

Perimeter: outer boundary of the selected seed image (mm), calculated from the centers of the boundary pixels.

Width and height: width and height measurements defined by the smallest delimiting rectangle surrounding the selected seed image (mm).

Major and Minor axes: major and minor axes of the ellipse surrounding the selected seed image (mm).

Feret and MinFeret diameters: the longest and shortest distance between two points along the seed selection boundary set to an angle of up to 180° (mm).

Circularity (Circ.): value in a scale ranging from 0.0 to 1.0; the closer this value is to 1.0, the closer the seed perimeter shape is to the perfect circle, and, conversely, the closer this value is to zero, the more elongated the seed perimeter.

Length-to-width ratio (LWR): ratio of the longest and shortest axes of an ellipse adjusted to the seed.

Roundness: value in a scale ranging from 0.0 to 1.0, which is the inverse of the seed length-to-width ratio.

Solidity: value in a scale ranging from 0.0 to 1.0, which is the ratio between the area of the seed captured in the image and the convex area of the seed. The convex area is a delimiter of the original shape of the image, tightly enveloping the area, without indentations.

Color: mean gray value between 0 and 255 calculated as the sum of pixel values of the selected seed surface image divided by the number of pixels, using the following formula: 0.229 × red + 0.587 × green + 0.144 × blue.

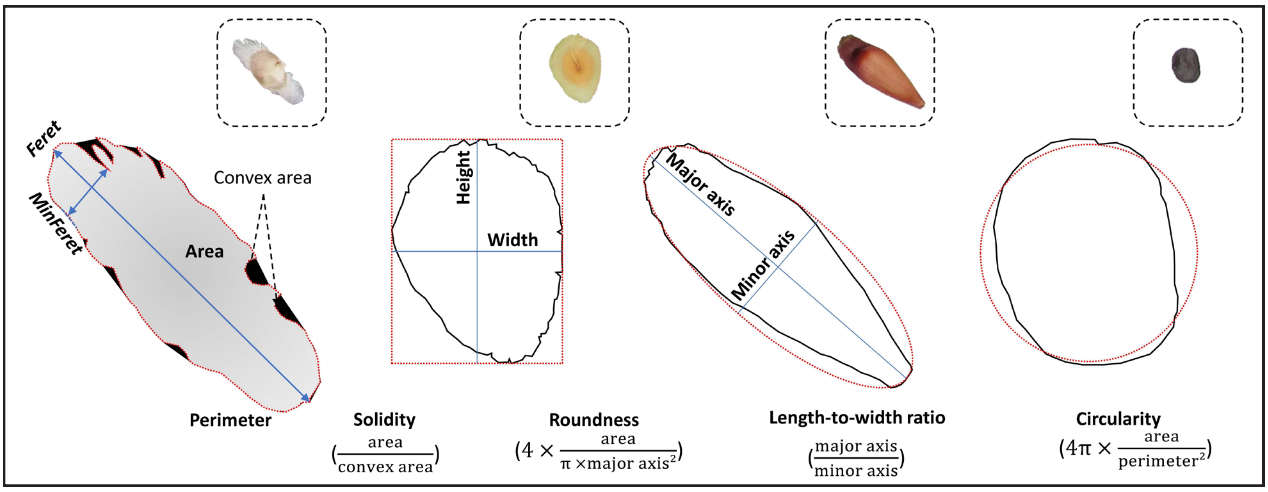
Standard deviation (StdDev): value of the standard deviation of the surface gray color values of seed images used to generate the average gray value.

Modal gray value (Modal): gray value of the most frequently occurring selected seed surface color, corresponding to the highest peak in the histogram.

Minimum and Maximum gray level (Min and Max): minimum and maximum values of the selected seed surface gray color.

Median: median pixel value of the selected seed surface color.

Figure 2 – Exemplification of the extraction of data on the size and shape characteristics of forest seeds after image processing



Source: Authors (2022)

2.4 Data analysis

A set of 1.827 million data points, consisting of 18 characteristics, was extracted by image processing for 155 species. These data were analyzed by descriptive statistics, and a dendrogram was constructed using the multivariate metric of Euclidean distance based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) model. Additionally, principal components analysis was performed using standardized data for the biplot representation of the characteristics. The statistical software programs Past® (version 3.18) and R® Core Team (version 4.1.3) were used for data analysis.

3 RESULTS AND DISCUSSIONS

In total, 101,521 seeds images of 155 forest species belonging to 42 families and occurring in different phytogeographic domains were analyzed in the present study. In Brazil, 33,978 angiosperm and 26 gymnosperm species have been cataloged in Brazil Flora (http://reflora.jbrj.gov.br), highlighting the high seed plant biodiversity of the country. Accordingly, the present study included the seeds of approximately 0.46%

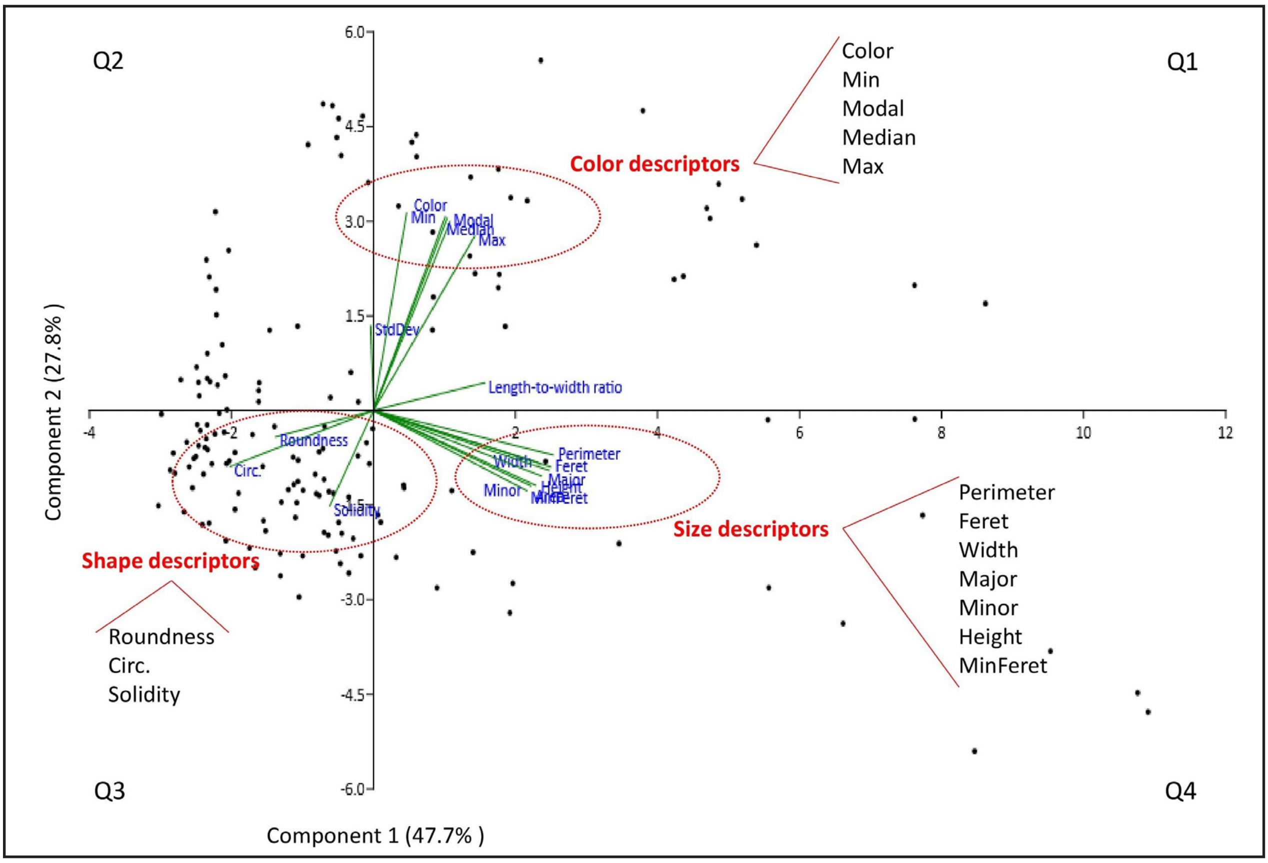
native spermatophytes (from 34,004 species) and 1.72% arboreal species (from 8,324 species) from Brazil. Considering our findings, we believe that the seeds of all species native to Brazil can be characterized by seed analysis using image processing tools.

Seed image analysis proved advantageous because it enabled us to measure more size, shape, and color characteristics than can be measured by manual approaches using a digital caliper or ruler, which is only possible by image processing. In addition, measurement time was optimized, and a higher number of seeds could be evaluated together, avoiding the need for manual measurements. Nevertheless, researchers must know how to use the chosen image processing software to extract seed data. The methodological details presented in the present study aimed at overcoming this need by showing the applicability of image-processing to seed biometric analysis.

According to the principal component analysis, 75.5% of the data variation was captured in the first two components (Figure 3), with component 1 accounting for 47.7% (eigenvalue 8.58) and component 2 for 27.8% of the data variation (eigenvalue 5.00). The characteristic that contributed the most to seed characterization was size (63.9%), followed by shape (18.9%) and color (17.2%) for component 1, which had the highest weight (47.7%), in contrast to color (59.1%), size (29.1%) and shape (11.8%) for component 2, which had the lowest weight (27.8%). Thus, the results showed that in the seeds of forest species, seed size, followed by seed shape, are the characteristics that most reflect the diversity of native species.

The species *Machaerium acutifolium* Vogel, *Platypodium elegans* Vogel, *Pterocarpus rohrii* Vahl, and *Terminalia mameluco* Pickel were found to have the largest seeds (area > 1.350 mm^2 and perimeter > 1.720 mm), whereas *Miconia theaezans* (Bonpl.) Cogn., *Pleroma sellowianum* (Cham.) P.J.F.Guim. & Michelang., and *Pleroma raddianum* (DC.) Gardner had the smallest seeds (area < 0.255 mm^2 and perimeter < 1.90 mm) (Table 1, supplementary document). Small seeds cannot be measured using manual tools such as a digital caliper or ruler, but they are measurable using the proposed method, thus demonstrating that research on small seeds does not necessarily require the use of devices such as scanners and microscopes (FARRIS, ORRÙ, UCCHESU, AMADORI, PORCEDDU, BACCHETTA, 2020; BAO, BAMBIL, 2021; MAZUR, MARCYSIAK, DUNAJSKA, GAWLAK, KAŁUSK, 2022). In addition, compared to other seed measuring techniques, imaging techniques such as those presented in the present study can be used to measure seeds more accurately and easily, reducing the possibility of error.

Figure 3 – Principal component analysis with biplot representation for size, shape, and color by digital processing of seed images of 155 forest species



Source: Authors (2022)

Circularity and roundness are shape measurements that can be used for seed differentiation, but they cannot be measured manually. The species whose seeds had the highest circularity indices (> 0.910) were *Miconia theaezans* (Bonpl.) Cogn., *Pleroma sellowianum* (Cham.) P.J.F.Guim. & Michelang., *Solanum viarum* Dunal, and *Zanthoxylum rhoifolium* Lam., whereas the seeds of *Cochlospermum orinocense* (Kunth) Steud., *Handroanthus impetiginosus* (Mart. ex DC.) Mattos, and *Handroanthus albus* (Cham.) Mattos had the lowest circularity indices (< 0.290). Regarding the roundness, *Vassobia breviflora* (Sendtn.) Hunz., *Sapindus saponaria* L., and *Cryptocarya aschersoniana* Mez seeds had the highest indices (> 0.930), whereas *Myrocarpus frondosus* Allemão, *Senna multijuga* (Rich.) H.S.Irwin & Barneby, and *Handroanthus albus* (Cham.) Mattos had the lowest indices (< 0.300). The species *Myrocarpus frondosus* Allemão stood out for having the greatest length-to-width ratio, being five times length-to-width ratio greater than that of the other species (Table 1, supplementary document).

Seed shape characteristics together with size characteristics can help characterize forest species, such as: rounded, elongated, elliptical, etc. As an example, in a previous study, the characteristics that were found to contribute the most to the divergence between the origins of *Mimosa scabrella* Benth. were seed length and width (MENEGATTI, MANTOVANI, NAVROSKI, SOUZA, 2017) while the seed shape and color measurements were not analyzed. Image processing can extract more data from seeds, avoiding the need for measuring few characteristics in studies such as the one cited above.

Standardizing the color on a gray scale (0–255) derived from the Red, Green, and Blue (RGB) scale enables us to differentiate seeds based on the colors of their images. For example, in the present study, the seeds of *Schinopsis brasiliensis* Engl., *Allophylus edulis* (A.St.-Hil. et al.,) Hieron. ex Niederl., *Psidium myrtoides* O.Berg, and *Handroanthus albus* (Cham.) Mattos seeds had a lighter color, whereas those of *Clitoria fairchildiana* R.A.Howard, *Erythrina speciosa* Andrews, *Matayba elaeagnoides* Radlk., and *Sapindus saponaria* L. seeds were darker (Table 1, supplementary document). Mean, median, minimum (Min), and maximum (Max) color values also help determine the seed color given their color variability and seed surface variation with the predominant color (StdDev), as in the seeds with two different colors (*Abrus precatorius* L. and *Ormosia arborea* (Vell.) Harms) and textures (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.).

Applying the proposed method to seed morphological analysis has major implications for silviculture, population genetics, botanical identification, and seed classification, as well as for understanding the ecological and physiological strategies of different species. For seed morphology, subjectivity related to seed shape and color was replaced by ranges of circularity, roundness, length-to-width ratio, solidity, and color values. For example, *Richeria grandis* Vahl seeds were visually described as ellipsoid with an orange-red sarcotesta (MOURA, COSTA, CARVALHO, TITON, PEREIRA, MACHADO, 2020), in contrast to the oval-oblong and irregularly rounded seeds of *Simira gardneriana* M.R.V. Barbosa & Peixoto (FELIX, OLIVEIRA, FREITAS, FREITAS, JILANI, TORRES, 2021b). These differences limit quantitative explorations in new studies or comparisons between species. In addition, color facilitates seed identification and species differentiation but should not be analyzed alone.

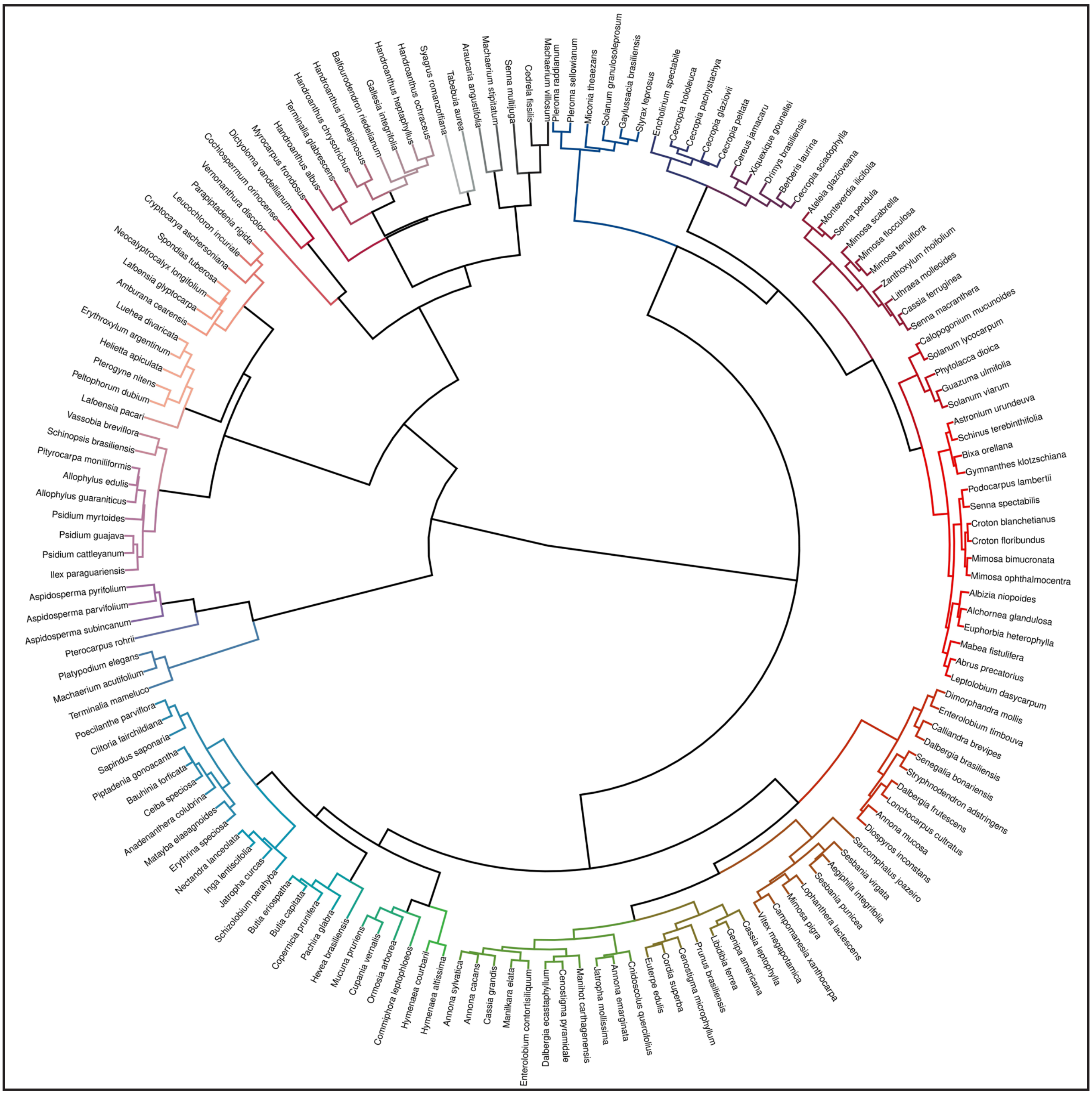
Size-based seed characterization can be used for intra- and inter-population genetic variability detection (BEZERRA, ZUZA, SILVA-BARBORA, AZEVEDO, ALVES, 2022), tree selection for seed production (CORREIA, FELIX, ARAUJO, FERRARI, PACHECO, 2019; GERBER, BRUN, TOPANOTTI, FERREIRA, PORRUA, GORENSTEIN, WAGNER-JÚNIOR, 2021), genetic improvement (YADAV, SINGH, SINGH, RAO, 2017), and progeny tests (LOVATEL, NAVROSKI, GERBER, OLIVEIRA, PEREIRA, SILVEIRA, 2021), as well as in studies on seed morphological traits (SOARES, SANTOS, SILVA, 2019) and ecological strategies of establishment, dispersal, and adaptation to different terrestrial environments (PATRÍCIO, TROVÃO, 2020; CONCEIÇÃO, MENDONCA, SOUZA, SOUZA, MOREIRA, 2019; DUARTE, GONZALEZ-RONDAN, ROCHA, 2019). Such studies usually require a long research period to assess a limited number of characteristics.

Considering the above, when aiming at extracting the key information about individuals or forest populations, the highest possible number of seeds should be evaluated. However, the methods used in the aforementioned studies are limited to measuring the length, width, and/or thickness/diameter one seed at a time in a limited number of species. As a result, these studies take a longer time to complete and are more prone to error because of the higher number of measurements needed for each seed. Therefore, using a camera and a mini studio for image acquisition, in addition to processing images in an open-access software, is a solution for various future studies on forest species.

Furthermore, in the present study, seed size, shape, and color descriptors enabled us to construct a dendrogram for forest species (Figure 4). The metric Euclidean distance groups all characteristics, demonstrating the similarity or dissimilarity between species. The descriptors differentiated the species (0.908 correlation coefficient), including those belonging to the same botanical family and genus, such as the species of the genera *Allophylus*, *Annona*, *Aspidosperma*, *Butia*, *Cassia*, *Cecropia*, *Cenostigma*, *Croton*, *Dalbergia*, *Enterolobium*, *Handroanthus*, *Hymenaea*, *Jatropha*, *Lafoensia*, *Machaerium*, *Mimosa*, *Pleroma*, *Psidium*, *Senna*, *Sesbania*, *Solanum*, and *Terminalia*. The characteristics evaluated by our seed image analysis were also effective in grouping species by proximity or genetic similarity, such as the genera *Aspidosperma*, *Butia*, *Cecropia*, *Croton*, *Handroanthus*, *Hymenaea*, *Mimosa*, *Pleroma*, and *Psidium*, which are strongly connected based on their Euclidean distance.

The biometric characteristics of seeds and fruits have diagnostic values in differentiating forest species (PEREIRA, FERREIRA, 2017; DUARTE, GONZALEZ-RONDAN, ROCHA, 2019; BARROS, CRUZ, PEREIRA, SILVA, 2020), as shown in the present study in the differentiation of species of the same genus as well as those of different botanical families. Compared to the traditional measurement methods, such as measurements with a digital caliper, seed image analysis is a more effective and precise method for the characterization of forest species. Even though the present study provides many advances, its scope should be expanded in future studies by including seeds of more forest species to expand the Brazilian flora database. Moreover, the use of automated classification tools by artificial intelligence and machine learning based on biometric seed analysis is an alternative for characterization the thousands of forest species that make up native plant biodiversity, contributing to seed production, species identification, trade, and environmental surveillance.

Figure 4 – Circular dendrogram based on the Euclidean distance of 155 forest species assessed by digital seed image processing



Source: Authors (2022)

4 CONCLUSIONS

Seed image analysis using the method proposed in the present study helps to better characterize and differentiate forest species. Using seed biometric analysis by image processing has direct implications for silvicultural, genetic, and ecological studies. The method promotes economic gains since it optimizes the seed evaluation time by the analyst. Furthermore, the proposed methodology can also be used for futures studying the seeds of exotic species.

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